Novel Technologies and Beneficial Microorganisms in Food Decontamination

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By

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PREFACE

Food is essential for human health and provides energy and nutrients, playing vital roles in the human body, tissues, growth, and organ development, functioning normally, and sustaining metabolism. In addition to nutrients, food may contain trace amounts of various toxins that occur naturally or result from food processing or storage. Typically, these levels are undetectable, and adverse effects are not observed. Food toxins, such as mycotoxins, heavy metals, and pesticide residues, pose a risk to human health by increasing the likelihood of dysbiosis, mutagenesis, and carcinogenesis. Mycotoxins have been a threat to humanity for millennia, and their presence in food has been linked to various acute and chronic toxicities, including cancer induction, mutagenicity, and other harmful effects, ranging from mild discomfort to fatality. Extensive heavy metal contamination in the food industry poses a significant threat to human health. The intake of heavy metals heightens the possibility of developing cardiovascular, kidney, and neurological diseases.

It is challenging to completely avoid food contamination. Nevertheless, through government regulations and routine monitoring of the food value chain, there has been a significant reduction in the risks of toxins contaminating our food supplies. However, toxins generally withstand common food cooking and processing methods, which implies that the current food processes are incapable of mitigating toxins in food. The rising incidence of toxins in food is resulting in the global economy losing billions of dollars annually. Consequently, the need for inventive approaches and procedures to combat the menace of food toxins is of utmost importance. One promising solution is food detoxification employing biological methods, specifically employing health-giving microorganisms, like probiotics, in mitigating the negative effects of toxins. In recent decades, probiotics have garnered attention due to their expansive properties, which not only affect the digestive system but also impact *in vivo* and *in vitro* bio-detoxification.

The focus of this book is to look into the most recent advances related to the decontamination of toxins in foodstuffs using different strains of probiotics and potential probiotics. The authors have provided many insights into the development of food detoxifications and addressed certain critical challenges in the applications of such strategies. We hope that this

book will further stimulate research interest in this field and speed up the development of food decontamination using probiotics.

Alaleh Zoghi
Ramona Massoud

INTRODUCTION

Food plays an important role in a human beings' health and energy and seems vital for growth, body organs, tissues, metabolism, and functions. Along with nutrients, food may contain toxins (naturally produced during the processing and storage of food), mostly in low levels and not in adverse effects levels. Food and water are being contaminated by industrial activities increasingly and they affect human and food safety. Food and water contaminants cause ecological unbalance. Therefore, decontamination is necessary to reduce the contaminants of foodstuffs to guarantee their safety.

Food contamination from various sources, such as chemicals, remains one of the most prevalent global food safety concerns. Typically, food technology is designed to prevent the occurrence of microbial contamination in food products. This involves assessing the potential risk of spoilage in the raw materials and processing stages to deliver safe foodstuffs to consumers. However, the rise in pollution due to human activities, particularly in the use of essential food ingredients and water, can lead to ecological imbalances (and even disasters), have toxic effects on entire ecosystems, and impact food safety. Unfortunately, the prolonged use of agrochemicals, excessive exploitation of agricultural lands, monoculture farming, genetic modifications to plants, ecological imbalances in farming practices, and the use of microorganisms in agricultural production have all resulted in the food industry facing additional challenges to ensure the safety standards of food products. Furthermore, quality standards related to food safety have risen in recent decades. The impact of agricultural additives and food processing ingredients on consumer health highlights the pressing need for changes in the food industry's practices.

Mycotoxins are fungal metabolites that are of major concern due to their strong toxicity, mutagenic, carcinogenic, and other adverse effects. They can contaminate crops or foodstuffs during processing and storage. It has been reported that almost 30% of crops worldwide are contaminated with mycotoxins annually. Also, human activities such as mining, construction, pesticides, chemicals, and industrial effluents are found to contaminate the environment and food chain by introducing various heavy metals and toxins. Chemical contamination adversely affects foodstuffs and produces a group of chemical combinations. The sources of the contamination can be natural from volcanic activity, rock weathering, mines, and earthquakes or

industrial and anthropogenic like using antibiotics, pesticides, and fertilizers as well as all chemical sprays. Therefore, decontamination seems to be crucial in reducing specific pollutants in food products accessible to consumers to ensure safety.

Current chemical and physical detoxification methods can be time-consuming, and costly, and result in a loss of nutrition. These techniques exhibit extensive specificity and effectiveness, with different microorganisms used in this detoxification process. As opposed to these, there are potential alternatives utilizing biological techniques that have proven effective in detoxifying hazardous pollutants in food. *Some studies have reported the bio-removal of contaminants such as mycotoxins (patulin, aflatoxins, zearalenone, ochratoxin A, and microcystin-LR), heavy metals (lead, cadmium, and arsenic), and chemical substances (bisphenol A and benzo(a)pyrene) by potent microorganisms.* Different microorganisms are used to remove contaminants from water and food; among them bacteria are a large useful group. Probiotics are a suitable option for bio-decontamination in the food industry since they are on the generally recognized as safe (GRAS) list. Their health benefits include eliminating lactose intolerance, supporting immunity, reducing cholesterol levels, preventing diarrhea, inhibiting colon cancer, inhibiting intestinal and gut pathogens, and having anticarcinogenic and antimutagenic effects. Probiotics can decontaminate food using viable or non-viable microorganisms or their specific enzymes. Certain probiotics can even adhere to intestinal cells to form complexes with toxins or heavy metals, which then rapidly pass through the gastrointestinal tract.

In general, after the introduction of food contaminants and beneficial microorganisms for bio-decontamination, this book reviews the role of probiotics and potential probiotics, as beneficial microorganisms, in the reduction and inactivation of different toxins and heavy metals in various food products, such as dairy products, drinking water, cereals and nuts, fruits and fruit-based products. For a better understanding of bio-decontamination, the last chapter describes the mechanisms of toxins bio-decontamination by beneficial microorganisms.

CHAPTER ONE

FOOD CONTAMINANTS

The contamination of drinking water and food is a primary concern in both developing and developed countries. Many contaminants such as heavy metals, mycotoxins, and chemicals are released into the environment from natural and anthropogenic sources contaminating foodstuff. In this chapter, food contaminants, which can be eliminated by using beneficial microorganisms, are evaluated.

1.1. Mycotoxins

Mycotoxins are compounds that fungi creates and are harmful to both humans and other animals. Key mycotoxigenic fungi include *Claviceps, Aspergillus, Penicillium*, and *Fusarium* species that are either saprophytes or parasites of cereal and pulse crops. Many epidemics in the human populations of various nations have been brought on by mycotoxins generated, particularly trichothecenes and aflatoxins, throughout history and in the present. They may hurt our diet. Most review publications on the scale of contamination cite Food and Agriculture Organization of the United Nations (FAO) data from 25 years ago that "up to 25% of world food crops are significantly contaminated with mycotoxins". Human diets contain natural carcinogens such as mycotoxins because of contaminated source materials or their creation during food processing and/or storage. The main factors affecting mycotoxin formation are temperature, water activity (a_w), relative humidity (RH), pH, fungal strain, and substrate. Humidity and temperature are closely related and have a critical impact on mold and mycotoxin production. Production of mycotoxins may happen during the process of production, harvesting, storage, or processing, under suitable temperatures between 20 and 30 °C; and humidity above 13% (1).

Mycelial growth and mycotoxin production are influenced by osmotic pressure within the substrate. Growth is restricted within specific low or high osmotic pressure ranges. To support their development and provide energy, filamentous fungi have the inherent capability to break down a range of carbon sources. *Aspergillus niger* can utilize sugars as the exclusive

carbon and energy source for cellular development and metabolism. A commonly acknowledged fact is that *Aspergillus niger* responds to saccharides by growing and spreading, which leads to overall colony growth, an increase in biomass, and a reduction in the amount of carbohydrates present in the environment. Therefore, the mere presence of fungi does not necessarily indicate future mycotoxin contamination, since the conditions needed for mycotoxin formation differ from those that facilitate fungal growth. The absence of fungus from food does not guarantee the absence of mycotoxins, as they possess chemical and thermal stability. Mycotoxin formation in crops is also facilitated by various environmental stresses, including insect infestation, drought, mechanical damage, nutrient deficiencies, erratic temperatures, precipitation, and humidity. Good agricultural practices that reduce plant stress can limit fungal invasion and consequently, mycotoxin contamination (2).

Due to their immediate and long-term toxic, carcinogenic, teratogenic, mutagenic, and immunosuppressive effects, mycotoxins are a major health concern. About 25% of human foods and animal feed include their contamination, which is particularly common in tropical and subtropical nations. Food that contains toxicogenic molds or tainted plant and animal raw materials may transmit disease. Six kinds of mycotoxins are regularly found in various food systems: ochratoxins, patulin, trichothecenes, fumonisins, and zearalenone (3).

Direct or indirect exposure to mycotoxins may cause teratogenic, mutagenic, estrogenic, hemorrhagic, carcinogenic, immunotoxic, nephrotoxic, hepatotoxic, neurotoxic, and immunosuppressive impacts on the health of humans. The severity of the outcome is influenced by the mode of exposure (i.e., how the substance enters the body), the dose (i.e., how much of the substance is there), and the duration (i.e., how long the mycotoxin is present for). Mycotoxins cause cancers as well as several other conditions of the gastrointestinal, urogenital, vascular, renal, and neurological systems. Certain mycotoxins impair immunity, which lowers resistance to infectious diseases. A quarter of the world's crops, including many staple foods, are thought to be affected by fungi that produce mycotoxin (4).

Many developing countries have not yet established regulatory guidelines for mycotoxins, and in those that have, regulations are frequently only enforced in the context of international trade with developed nations. In such instances, mycotoxins not only adversely impact the health of local communities but also impede their access to profitable markets. In developing nations, surveys on mycotoxins have offered momentary perspectives on mycotoxin pollutants and have frequently been concentrated on regions at higher risks of exposure. Limited research has

evaluated the dynamics of mycotoxins over extended periods and/or vast regions, which will provide a more comprehensive insight into mycotoxin pollution across diverse food systems. In the future, it is increasingly feasible to create monitoring systems that examine the intricate spatial and temporal changes of mycotoxins within them. These systems will also scrutinize the interplay of biological, environmental, and socio-cultural factors. Such processes are vital in the quest to produce effective mycotoxin surveillance systems and innovative mitigation schemes (5).

There are more than 500 identified mycotoxins, and this number is growing. Mycotoxins, which can be created before, during, or after harvest or at any point along the food chain, are secondary metabolites produced by fungi that contaminate agricultural goods as a result of fungal deterioration. Mycotoxins can have a wide variety of molecular pathways and cellular targets that can mediate their biological effects, making them challenging to categorize as a group. One difficult element of the pathogenesis of diseases caused by mycotoxins is that different fungal species may produce different mycotoxins, and different fungal species may simultaneously be present in food products. The idea of "hidden mycotoxins," compounds made from mycotoxins as a result of plant metabolism, is particularly significant since their existence can be easily overestimated by physicochemical analytical procedures, which makes the danger they pose to both animals and people easy to ignore (6).

Mycotoxin-producing fungi have a wide range of origins and food sources, which are frequently still being determined (Table 1.1). Mycotoxicosis is the disease brought on by these chemicals; however, not all mycotoxins cause disease, and some have even been employed for their beneficial effects (7).

Table 1.1. Occurrence of mycotoxins in food commodities (7)

Mycotoxin	**Fungi of origin**	**Commodities affected**
Aflatoxin	*Aspergillus flavus*; *A. parasiticus*	Corn, peanuts, tree nuts, cottonseed, cereals, some spices, and milk
Citrinin (citreoviridin)	*Penicillium citrinin*; and other spp. from the *Aspergillus* and *Monascus* genera	Cereals

Ergot alkaloids (ergotamine, etc.)	*Claviceps* spp.	Cereals and grasses
Fumonisins	*Fusarium verticillioides*; *F. proliferatum*	Corn, other cereals
Ochratoxin A	*Aspergillus ochraceus*; *A. niger*; *Penicillium verrucosum*	Legumes, grapes, cereals, coffee beans
Patulin	*Penicillium expansum* and other spp. from the *Aspergillus* and *Byssochlamys* genera	Apples, grapes, pears, other fruits
Penicillic acid	*Penicillium martensii*; *P. puberulum*; *P. palitans*	Corn, legumes
Psoralens	*Sclerotinia sclerotiorum*; *S. rolfsii*; *Rhizoctinia solani*; *Erwinia aroideae*	Celery, figs, parsley, parsnip, lime, cloves
Trichothecenes	*Fusarium graminearum*; *F. sporotrichioides*; *F. poae*; *F. culmorum*, and spp. from the *Myrothecium*; *Phomopsis*; *Stachybotrys*; *Trichoderma*; and *Trichothecium genera*	Wheat, corn, barley, oats
Zearalenone	*Fusarium graminearum*; *F. culmorum*; *F. cerealis*; *F. equiseti*; *F. crookwellense*; *F. semitectum*	Corn, sorghum, wheat
Alternaria toxins	*Alternaria alternata*	Fruits, tomatoes, olives, sunflower seeds, oil seed rape meal and pecans

- **Identification and Quantification of Mycotoxins**

The challenge of identifying and accurately quantifying masking mycotoxins poses a significant hurdle in the sectors of food processing and preservation. Masked mycotoxins are a type of mycotoxin that is not easily detected by

standard analytical techniques as they result from mycotoxins binding with other compounds in the food matrix, including proteins or sugars, making their detection and quantification challenging. However, these masked mycotoxins can be converted back into their active form during digestion, leading to possible adverse health effects. Numerous methodologies are available for identifying masked mycotoxins in food commodities. Several methods are available for detecting mycotoxins, including immunoassays, chromatographic methods, and mass spectrometry. Although immunoassays are sensitive and relatively easy to perform, they can also generate false positive results. Chromatographic methodologies, for instance, high-performance liquid chromatography (HPLC), are frequently employed in mycotoxin detection. These techniques can isolate the components of a sample and pinpoint specific mycotoxins via their retention time and spectral characteristics. Mass spectrometry is a potent analytical method utilized for identifying mycotoxins. It offers great precision, selectivity, and sensitivity, making it beneficial to uncover concealed mycotoxins (8).

Numerous chromatographic techniques have been used to detect both individual and combined mycotoxins, while analytical protocols ensure rapid and precise quantification. Liquid chromatography (LC) with specialized detectors such as MS, DAD, and FLD are commonly utilized methods. Gas chromatography (GC) has been widely used for quantifying mycotoxins, while HPLC–MS/MS is regarded as the most accurate and sensitive method. Additionally, bespoke methods such as HPLC–FLD, HPLC–DAD, and LC–PDA have also gained widespread use. Alternatively, instrumental-based protocols have shown a preference for GC using flame ionization detection (GC-FID) and GC with tandem mass spectrometry (GC-MS/MS), along with the adoption of immunoaffinity columns to effectively identify and quantify masked mycotoxins.

The chemical composition and complex structures of mycotoxins present a challenge in selecting a single detection technique. A combinational approach that is sensitive, cost-effective, flexible, routine, broad-based, and accurate is necessary. Customized protocols have been developed, tested, and executed to identify even the smallest concentrations of mycotoxins in food. HPLC and GC-MS are commonly used for identifying prevalent mycotoxins. Nevertheless, integrated analytic methods, including reversed-phase HPLC, microemulsion electrokinetic UV-HPLC, ultra HPLC-MS, HPLC in comparison to ELISA and TLC, GC-MS combined with the electronic nose, and LC-MS/MS, have demonstrated successful results (9). Figure 1.1 represents an outline of several analytical techniques adopted in mycotoxin identification.

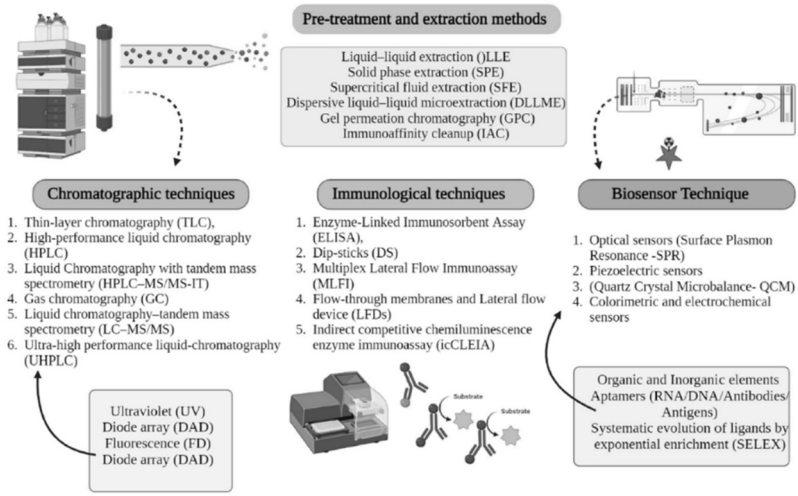

Figure 1.1. Schematic representation of analytical methods applied for the detection of mycotoxins in food matrices (9)

1.1.1. Aflatoxins

Aflatoxins are a class of mycotoxins that are frequently discovered in a range of foods and feeds and are responsible for increased veterinary care expenditures, decreased animal productivity, and losses to the industry. Certain *Aspergillus* species, particularly *A. flavus, A. parasiticus*, and *A. nomius*, produce these toxins as secondary metabolites. The generation of this toxin is influenced by several variables, including water activity, temperature, nutrients available, the growth of competing bacteria, and pH level. Aflatoxin contamination can occur in a variety of agricultural products, including cereal grains like rice, corn, maize, wheat, soy, rye, oats, barley, and sorghum, nuts like almonds, peanuts, and pistachios, as well as oily seeds like cottonseed. Aflatoxin exposure in humans can occur directly or indirectly through the consumption of contaminated goods or meals produced from them, such as dairy products and meats from contaminated livestock. Aflatoxin exposure has detrimental effects on both human and animal health, including chronic poisoning and malignancies of the liver and kidney. Due to their low molecular weight, once aflatoxins are consumed by animals, they are swiftly absorbed in the gastrointestinal tract (GIT) and promptly show up in the blood and milk after 15 minutes and 12 hours of post-feeding, respectively (10).

18 different varieties of aflatoxins have been discovered through toxicological research, however, aflatoxin B_1 (AFB_1), aflatoxin B_2 (AFB_2), aflatoxin G_1 (AFG_1), and G_2 (AFG_2) are the most significant aflatoxins (Figure 1.2). Their designations refer to their fluorescence under UV light, which is either blue (B) or green (G), and their chromatographic migration patterns in thin-layer chromatography (TLC). *A. flavus* typically creates the B group of aflatoxins, but *A. parasiticus* use a variety of metabolic mechanisms to produce both the B and G groups. AFB_1 is the most prevalent and toxic of the four aflatoxins described, and exposure to it causes both acute and chronic hepatocellular injury. AFB_1 and AFB_2 are metabolically derived from each other to become aflatoxin M_1 (AFM_1) and aflatoxin M_2 (AFM_2). When livestock consume feed containing AFB_1, it may bio-transform into AFM_1 (4-hydroxy-AFB_1) in the liver and be expelled in their milk, tissues, and urine. All dairy processing steps, including pasteurization and sterilization, are resistant to AFM_1. All dairy processing steps, including pasteurization and sterilization, are resistant to AFM_1. The amount of ingested AFB_1 by livestock that manifests as AFM_1 in milk ranges from 0.3 to 6.2%. The rate at which AFB_1 transforms into AFM_1 can be influenced by the diet type, quantity of milk produced, breed, health, and rate of digestion (7).

AFB_1 and AFM_1 have been classified as category 1 human cancer-causing agents by the International Agency for Research on Cancer (IARC). AFM_1 has been shown to cause cancer in numerous species, but it is roughly ten times less carcinogenic, mutagenic, and genotoxic than AFB_1. AFM_1 is cytotoxic, and in animal cells, it can also result in DNA damage, gene mutation, chromosomal abnormalities, and cell transformation. The Food and Drug Administration (2005) suggested that 0.5 μg/kg of AFM_1 be the highest permissible level in milk, and the European Commission (2006) established this limit at 0.05 μg/kg. The maximum levels for AFB_1 and AFB_2 in fruits and their processed products are 2.0 and 4.0 μg/kg, respectively. Also, the maximum level for AFB_1 in processed cereal-based foods and baby foods for infants and young children is 0.1 μg/kg (7).

Kenya experienced an intense aflatoxicosis outbreak in 2004. With 317 reported cases and 125 fatalities, it was noted as one of the worst aflatoxin poisonings in recorded history. This epidemic was brought on by eating maize infected with aflatoxins, which had an average content of 354 ng g1. Human milk has also been discovered to have AFM_1. For instance, AFM_1 was found in 248 of 443 (56%) test samples from a human milk survey in Egypt, implying a correspondingly high rate of exposure for unweaned infants (11).

Detection of AF in minuscule quantities in food is a crucial and intricate process that's reliant on sophisticated sampling, sample preparation, and analytical techniques. AF analysis can be performed using various strategies. Immunoaffinity columns (IACs) for AF sample clean-up have become nearly ubiquitous and are more common than liquid-liquid partitioning and solid-phase extraction (SPE). A comparison of these clean-up methods reveals that IACs demonstrate the highest selectivity. Liquid chromatography-fluorometric detection (LC-FLD) is the preferred chromatographic method for detecting AFs. However, AFs possess low native fluorescence, thereby necessitating pre- or post-column derivatization to improve their detection rates. Enzyme-linked immunosorbent assay (ELISA) is an immuno-based technique that offers several advantages over other methods as no clean-up is required. However, the ELISA's use of antibodies can lead to false-positive results due to cross-reactivities being a major drawback (12).

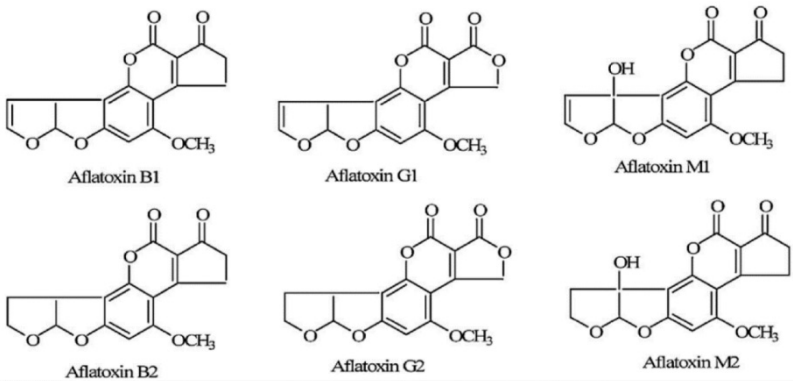

Figure 1.2. Chemical structures of aflatoxins

1.1.2. Fumonisin

Fusarium species, primarily *Fusarium verticillioides*, and *F. proliferatum*, produce fumonisins, which are harmful secondary metabolites. Fumonisin B_1 (FB_1) and fumonisin B_2 (FB_2) are the most significant and prevalent (70%) fumonisins known as of yet. FB_1 is the diester of 2-amino-12,16-dimethyl-3,5,10,14,15-pentahydroxyeicosane and propane-1,2,3-tricarboxylic acid (tricarballylic acid, TCA), in which the C14 and C15 hydroxyl groups are esterified with the terminal carboxyl group of TCA. The equivalent stereo genic units on the icosane backbone have identical structures in FB_2, which is the C10 deoxy counterpart of FB_1 (Figure 1.3). Both of these carcinogens are

phytotoxic to corn and cytotoxic to several mammalian cell lines, and FB_1 causes cancer in the kidney and liver of rats. FB_2 is the cause of two illnesses in domestic animals, including horse leukoencephalomalacia and porcine pulmonary edema syndrome. Fumonisin's hydrolyzed or N-acetylated derivatives are less harmful than simple forms. Toxins called fumonisins are typically connected to maize and other crops. Fumonisins' biological toxicity is linked to their ability to stop protein synthesis, alter RNA and DNA, and exacerbate immune-suppressive conditions. Direct and indirect ELISA methods have been developed for the detection of *Fusarium* toxins in cereals (13).

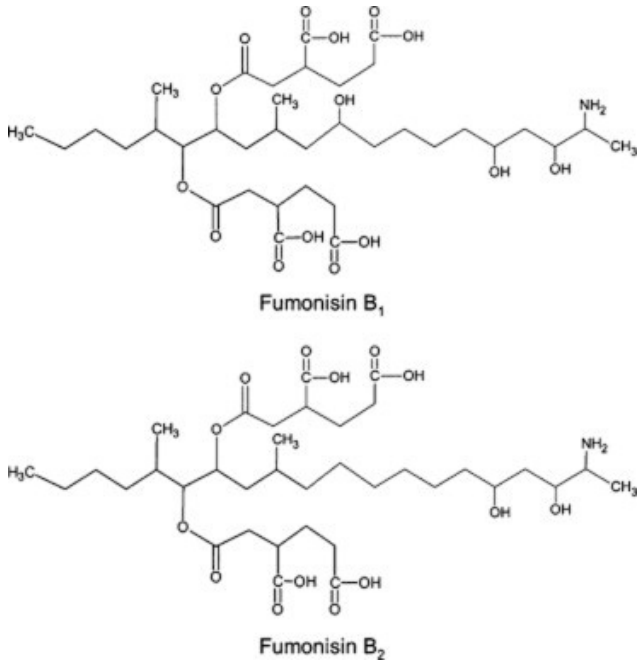

Figure 1.3. Chemical structures of fumonisins

1.1.3. Patulin

One of the mycotoxins most frequently discovered in human foods is patulin (PAT), which has detrimental impacts on both human and animal health. It is a 4-hydroxy-4H-furo[3,2-c]pyran-2(6H)-one unsaturated heterocyclic lactone toxin (Figure 1.4) that is produced as a secondary metabolite by a variety of fungal species from the genera *Penicillium* (*P.*), *Eupenicillium*,

Paecilomyces, *Aspergillus*, and *Byssochlamys*. Apples and associated goods, cereals, bread, pears, apricots, peaches, grapes, and derivatives have all been found to contain PAT. *P. expansumis*, one of the fungi described, is the most frequent pre- and post-harvest contamination species in apples, whereas *Byssoclamynivea*, a possible source of PAT in pasteurized fruit juices, is the most heat resistant. *P. expansum* can frequently grow on the skin of the fruit that is healthy, but in post-harvest situations, it can cause damage to infected fruit (10). It is noteworthy that PAT can undergo degradation when subjected to food processing or storage, thereby leading to the formation of other harmful compounds. Furthermore, PAT may undergo interaction with other compounds present in food, which can have an impact on its bioavailability and toxicity. PAT appears to pose a significant hazard during the postharvest life of fruits, from single grains to the contamination of whole fruit, ultimately resulting in the spoilage of the entire stored fruit (14).

The biosynthesis of PAT comprises numerous enzymatic reactions that take place in the fungal cell. The initial stage involves the amalgamation of two acetyl-CoA molecules to produce 6-methyl salicylic acid (6-MSA), an action catalyzed by the polyketide synthase (PKS) enzyme. Subsequently, a sequence of reactions, involving oxidation, decarboxylation, and esterification, converts 6-MSA into PAT. PAT is a secondary metabolite produced from poly acetate. Its metabolic pathway has been extensively studied using cell-free extracts and kinetic pulse-radiolabelling systems (8).

PAT is a member of the third group of carcinogens according to the International Agency for Research on Cancer. The PAT toxification's acute symptoms (convulsions, anxiety, gastrointestinal distress, epithelial cell degeneration, and hemorrhage), as well as its chronic symptoms (neurotoxic, genotoxic, teratogenic effects, and immunosuppressive effects), are brought on by the inhibition of protein, DNA, and RNA synthesis. PAT has been demonstrated to cause cell effects including disruption of the plasma membrane, and inhibition of protein synthesis of Na+-coupled amino acid, transcription, translation, and DNA. Additionally, it inhibits the production of interferon from T-helper type 1 cells. PAT is toxic to several enzymes with a sulfhydryl group in their active site. Also, PAT has been found to facilitate intramolecular and intermolecular protein cross-linking, favoring cysteine's thiol group but also affecting the side chains and -amino groups of lysine and histidine. This process promotes the formation of cross-links between amino acids within and between proteins (15).

A provisional maximum tolerated daily intake for PAT of 0.4 mg/kg body weight/day has been set by the Food and Agricultural Organization

and World Health Organization Expert Committee on Food Additives. The maximum permitted level of PAT in juice, nectar, spirit drinks, cider, reconstituted juices, and other fermented apple juice derivatives is 50 µg/kg, according to the European Commission (16).

The main focus of managing PAT contaminations is developing reliable and sensitive assays for detecting PAT in various food matrices. Liquid–liquid extraction (LLE) has been the traditional method of sample preparation for patulin analysis in food samples. LLE with ethyl acetate has been successfully validated through a collaborative study for patulin determination in apple juice and apple puree and has been adopted by AOAC International as an official method. However, LLE is considerably expensive and time-consuming. To identify and quantify PAT in food, several methods such as thin-layer chromatography (TLC), gas chromatography-mass spectrometry (GC-MS), and high-performance liquid chromatography with ultraviolet detection (HPLC-UV) can be used. The use of high-performance liquid chromatography-tandem mass spectrometry (HPLC-MS), capillary electrophoresis (CE), fluorescence polarization, chemiluminescence assays, quantitative PCR assays, surface plasmon resonance (SPR), quartz-crystal microbalance (QCM), electrochemical reduction techniques, and so on, is prevalent in many scientific studies (17).

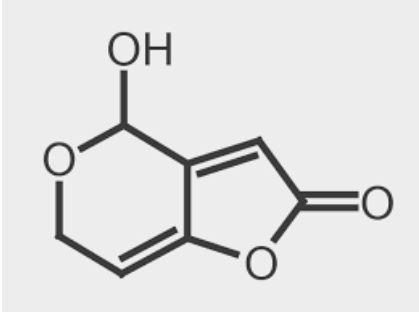

Figure 1.4. Chemical structure of patulin

1.1.4. Ochratoxin A

The three main recognized manufacturers of OTA (Ochratoxins A) are *Aspergillus carbonarius*, *A. ochraceus*, and *P. verrucosum* (Figure 1.5). Many foods, including dried fruits, grains, coffee, cocoa, grapes, wine, beer, cereals, nuts, and spices have been documented to contain OTA as pollutants. They are classified as extremely dangerous food pollutants

because OTA is heat stable and is well known to promote tumor growth in humans and other animals. The toxicity of OTA is fatal as it causes genotoxicity, hepatotoxicity, immunotoxicity, teratogenicity, and neurotoxicity. A chronic nephrotoxin that impairs kidney function is OTA. The bloodstream's half-life of OTA is prolonged, thus in regions where OTA is frequently found in foods, healthy people's blood regularly carries measurable levels of this toxin. However, the exact mechanism through which OTA causes cancer is still unknown. Based on enough evidence of carcinogenicity in investigations on experimental animals and insufficient data in human studies, the International Organization for Research on Cancer has categorized OTA as a potentially carcinogenic substance for people (Group 2B) (10). The tolerable threshold quantity of OTA for weekly intake is stipulated to be 120 ng/kg/body weight. The maximum level for OTA in different foodstuffs is shown below, according to the European Commission (2006).

Dried vine fruit (currants, raisins and sultanas)	10.0
Wine (including sparkling wine, excluding liqueur wine and wine with an alcoholic strength of not less than 15 vol%) and fruit wine	2.0
Aromatised wine, aromatised wine-based drinks and aromatised wine-product cocktails	2.0
Grape juice, concentrated grape juice as reconstituted, grape nectar, grape must and concentrated grape must as reconstituted, intended for direct human consumption	2.0
Processed cereal-based foods and baby foods for infants and young children	0.50

OTA is a colorless crystalline compound with blue fluorescence under UV light and weakly acidic characteristics. LC with FLD, following a clean-up method involving SPE with an IAC, is the most widely used technique to detect OTA in fruits and their processed products. Despite immune extraction's ability to increase yields and ease the analytical protocol, it suffers from several drawbacks. In recent years, there have been numerous attempts to replace antibodies with combinatorial peptides, low-mass synthetic ligands, aptamers, and molecularly imprinted polymers. Combinatorial peptides, specifically well-designed ones, show significant promise as capturing agents. They allow for superior recoveries (>95%) with limits of quantification of 2 µg/L (18).

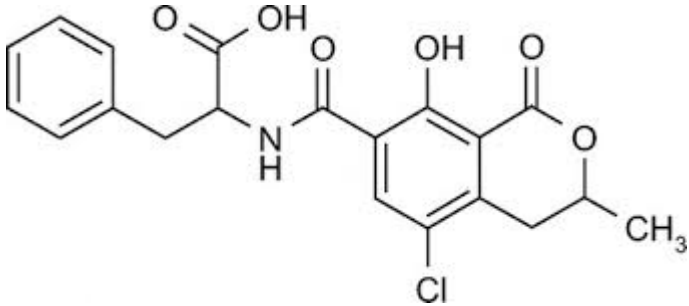

Figure 1.5. Chemical structure of ochratoxin A

1.1.5. Zearalenone

Many species of *Fusarium* fungi produce the nonsteroidal estrogenic mycotoxin zearalenone (ZEA) (Figure 1.6). *Fusarium graminearum* is the main source of ZEA in plants. *Fusarium culmorum*, *F. verticillioides, F. sporotrichioides, F. semitectum, F. equiseti*, and *F. oxysporum* are additional *Fusarium* fungus that can produce ZEA. Worldwide reports of ZEA contamination of cereal grains have been made, mainly in temperate settings. ZEA concentrations are typically low in grain that has been infected in the field, but they rise when it is stored with moisture levels of more than 30% to 40%. ZEA has been assessed to be a potent ROS influencer, a harmful genotoxic and carcinogenic substance, and classified under Group 3 carcinogens. Although ZEA primarily affects female reproduction (as seen by hypoestrogenism), it also has an impact on the male reproductive system. Endocrine disruptor ZEA is receiving attention due to some indications that it interferes with human growth and maturation and may be a factor in the rise in breast cancer cases (11).

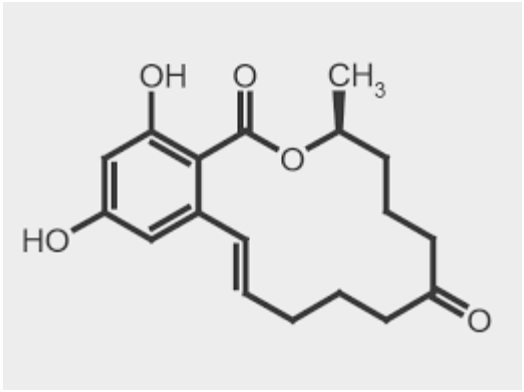

Figure 1.6. Chemical structure of zearalenone

1.1.6. Trichothecenes

Trichothecenes are one of more than 150 members of a sizable family of secondary metabolites. *Fusarium* species are mostly responsible for their production. Trichothecenes are found in grains used as food, including wheat, barley, maize, oats, and rice. The vast group of trichothecenes is often divided into the A to D subgroups. Type A and type B trichothecenes are frequently found in grain goods, whereas type C and the macrocyclic type D are of modest importance in food commodities. A keto group is present at the trichothecene ring's C-8 position in type B trichothecenes like deoxynivalenol (DON), making them more polar than type A trichothecenes like T-2 and HT-2, which have an ester bond in this location. *Fusarium graminearum* and *Fusarium culmorum* produce DON. DON (Figure 1.7) is particularly prevalent in foods made from earlier cereals, including bread, flour, pasta, malt, and beer, as well as grains including barley, maize, oat, and wheat. By the major interaction between DON presence and increased exposure compared to other body organs, it was shown that DON is capable of influencing and stopping protein synthesis as well as negatively affecting the GIT (11).

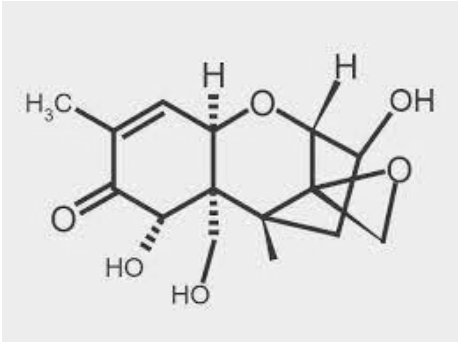

Figure 1.7. Chemical structure of deoxynivalenol

1.1.7. Cyanotoxins

Cyanotoxins are primarily thought of as pollutants for drinking water and are created by cyanobacteria. These are cyclic heptapeptide poisons made up of five nonproteins and two protein amino acids, seven peptide rings, and several species of freshwater cyanobacterial genera. Cyclo-(d-Ala-L-X-d-MeAsp-L-Z-Adda-d-Glu-Mdha) is the general structure of microcystins, where X and Z are the variable L-amino acids. The microcystin-LR (Figure 1.8) contains the amino acids arginine and leucine and is the most poisonous. In normal circumstances, microcystins are physically resistant to ultraviolet radiation, high temperatures, and enzymes. According to several reports, protein phosphatases 1 and 2A are inhibited by microcystins, which thus act as tumor promoters. Microcystin-LR in drinking water should not exceed a guideline value of 1 µg/L, according to the WHO (16).

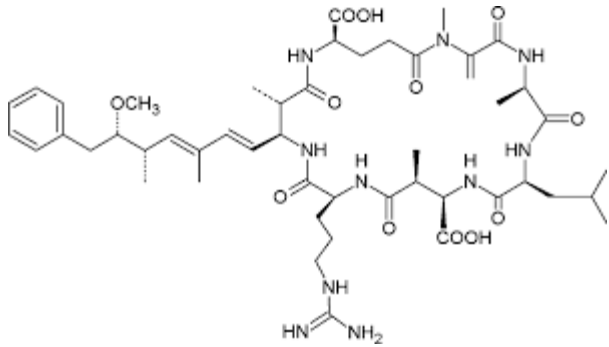

Figure 1.8. Chemical structure of microcystin-LR

1.1.8. Citrinin

The prevalent fungal species, such as *Penicillium*, *Aspergillus*, and *Monascusare*, are known to produce citrinin (CIT). It affects cereal crops, foodstuffs, and fruits, irrespective of the climatic conditions. These fungi contaminate the food commodities during harvesting, storage, and post-harvesting leading to spoilage of grains, spices, condiments, citrus fruits, herbs, and fruit juices. The production of CIT (Figure 1.9) is promoted by various factors such as the availability of oxygen, carbon sources, humidity, temperature, and storage conditions, as well as the addition of preservatives. However, the safe limit of CIT in food matrices varies across countries. Basic and advanced chromatographic techniques, including TLC, HPLC, LC-MS, ELISA, capillary zone electrophoresis, and immunochromatographic assays, are utilized for detection, identification, and quantification purposes.

The harmful effects caused by CIT include oxidative stress, changes to antioxidative responses, the promotion of oxidative stress generation, and increased synthesis of superoxide anions. These effects predominantly result in malfunctions associated with mitochondrial dysfunction, lipid peroxidation, and cell death (9).

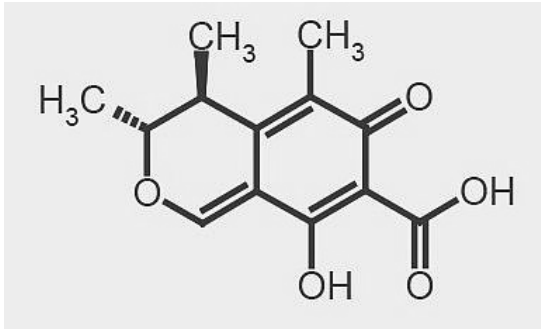

Figure 1.9. Chemical structure of citrinin

1.1.9. *Alternaria* toxins

The *Alternaria* genus was first described in 1816. *Alternaria* species belong to the phylum Ascomycota, commonly called sac fungi. *Alternaria* fungi are parasitic on plants and can cause spoilage of fruits and vegetables during transportation and storage. Different species of *Alternaria* can produce mycotoxins, including *A. scripinfestans*, *A. botrytis*, *A. oudemansii*, *A. leptenallea*, and *A. alternata*. The most common and important species is *A. alternata,* which produces several mycotoxins, including alternariol (AOH),

alternariol monomethyl ether (AME), altenuene (ALT), alter toxin I and II (ATX-I and -II), and tenuazonic acid (TeA), a tetramic acid (see Figure 1.10). AOH and AME were first isolated in 1953. The mycotoxicological analysis affirmed the presence of various morphotypes of *A. alternata* and *A. arborescens*, the causes of heart rot disease, in the fruit. It is noteworthy that the growth of *Alternaria* sp. doesn't directly correspond to the production of *Alternaria* toxins. Specifically, it has been demonstrated that AME, AOH, and TeA were found in the rotten regions of yellow peach fruits that were artificially inoculated with *Alternaria* sp. under monitored conditions. Significantly, TeA was also detected in the unrotten fleshy tissue of the fruit (9).

Alternaria species produce secondary metabolite toxins that can cause cancer and mutagenesis, resulting in health disorders among animals and humans. It is vital to note that *Alternaria* species toxins and allergens can pose a significant health risk, particularly in the food industry. Additionally, *Alternaria* spores are airborne allergens that can cause issues in certain environments. Pathogenic species of *Alternaria* can also cause leaf spot, blight, and leaf rot diseases in plants. These diseases are associated with host-specific and non-host-specific toxins that cause black spots in various fruits and vegetables during the post-harvest storage and marketing period. Exposure to these mycotoxins has been linked to a range of detrimental health effects, such as cancer, allergies, and other toxic reactions (8).

Among the mycotoxins that have been isolated, ALT and ATX-I exhibit the highest acute toxicity in mice with LD_{50} values of 50 and 200 mg/kg, respectively. On the other hand, AOH and AME are less acutely toxic in mice with an LD_{50} of 400 mg/kg, and TeA is sub-acutely toxic in mice with an LD_{50} of 115 mg/kg via intravenous administration. The culture extracts of *A. alternata* show mutagenicity in different microbial and cell systems and are also known to be carcinogenic in rats. Furthermore, it has been suggested that *A. alternata* might be one of the causative agents for oesophageal cancer in humans. It is noteworthy that ATX-1, AOH, and AME demonstrate mutagenicity. Furthermore, it is significant to note that AOH produces androgenic effects, which could have a more severe impact on children (19).

The *Alternaria* toxins AOH and AME are produced across a temperature range of 5-30 °C and an a_w range of 0.90-0.98. However, at marginal temperatures and an a_w of 0.90, little of any mycotoxin was produced. The minimum a_w that allows germination of *A. alternata* conidia is 0.85, whereas a minimum a_w of 0.88 is necessary for growth on wheat extract agar at 25 °C. The limiting a_w for detectable mycotoxin production is thus

slightly greater than that for growth, with optimum production occurring above an a_w of 0.95 (19).

The biosynthesis of *Alternaria* toxin metabolites is a complex process involving numerous enzymes and pathways. A well-documented instance of *Alternaria* toxin metabolite biosynthesis is the production of the mycotoxin AOH. AOH derives from the precursor AME and is hydroxylated. The synthesis ensues through a chain of enzymatic reactions. The biosynthesis of AOH in *Alternaria* toxin entails a PKS gene cluster encompassing the genes for a PKS, a ketoreductase (KR), an enoyl reductase (ER), and a cytochrome P450 monooxygenase (CYP). The PKS gene cluster generates a polyketide intermediate that the KR and ER enzymes subsequently convert into the AOH precursor, AME. Eventually, the CYP enzyme catalyzes the hydroxylation of AME, producing AOH (8).

Alternaria mycotoxins, specifically AOH and AME, have been detected in various fruit beverages using TLC, GC, and LC techniques, primarily with ultraviolet detection. Although fluorescence and electrochemical detectors have also been used in detection. In addition, two ionization techniques, atmospheric pressure chemical ionization (APCI) and electrospray ionization (ESI), were examined for the LC-MS detection of AOH and AME. Both methods provide superior sensitivity and specificity compared to the standard UV detection technique. The preferred course of action is to employ a combination of ESI with negative ion detection and tandem mass spectrometry (MS/MS). This procedure makes it simple to detect sub-µg/L quantities of AOH and AME in fruit juice samples (20).

Alternaria mycotoxins can exist as "masked" mycotoxins, which are conjugates formed when mycotoxins bind to other molecules, such as sugars or amino acids, in plant material. These conjugates are not typically identified through conventional mycotoxin analysis methods, as the mycotoxin is masked and not present in its free form. Nevertheless, the conjugate can be divided down during digestion, thus releasing the free mycotoxin. Masked *Alternaria* mycotoxins have the potential to pose a health risk, as their toxicity may not be fully understood, and they may not be detectable via standard testing methods. The European Food Safety Authority (EFSA) has recognized AOH and AME as potentially masked mycotoxins of concern (8).

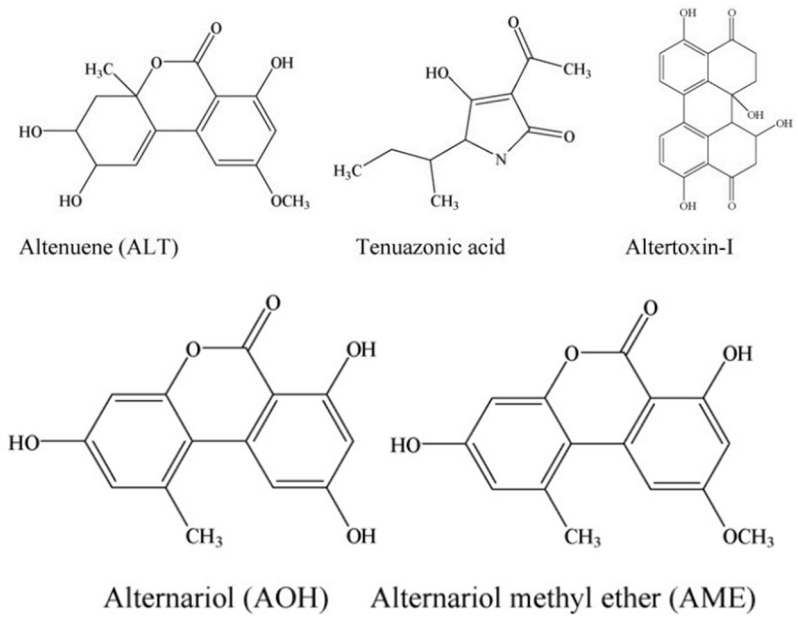

Figure 1.10. Chemical structures of *Alternaria* toxins

1.2. Heavy Metals

Recently significant interest has been focused on the investigation of the metal content of foods. Heavy metals are naturally occurring elements with high atomic numbers; most heavy metals occur in the Earth's crust, where they are entangled with various natural and anthropogenic activities. Metals with a density of more than 5 g/cm^3 are referred to as heavy metals. These are unbreakable substances that can take on various inorganic and organic forms. Heavy metals are classified as essential and non-essential. While lead (Pb) and cadmium (Cd), which have no beneficial biological functions and are harmful even in very small doses, are among the heavy metals, some are necessary trace elements, including Fe, Cu, Mn, and Zn. However, when the concentration surpasses the tolerated limit for organisms, essential heavy metals can be hazardous to living beings.

Due to the toxicity of heavy metals and the potential damage to human health, their contamination in the food chain is one of the biggest global problems. Pollution with various heavy metals has become more and more important as various human activities, such as construction, transportation, agro-pesticides, and the chemical and mining industries, have developed.

The biological toxicity of heavy metals is supported by several facts, including the ones that can accumulate and spread through the food chain and are harmful at even extremely low concentrations. We are exposed to the hazardous effects of heavy metals because of the expansion in industrial population brought on by the development of several disciplines of industrial chemistry over the past 200 years. The toxic metals Pb, Cd, arsenic, and mercury (Hg) are regarded as being more dangerous. According to studies, between 40 and 60 percent of ingested metals that get through the intestinal barrier cause oxidative stress, tissue damage, inflammation, and gastrointestinal problems. Because they can bind to hormone receptors like steroids (estrogen and androgen receptors), and glucocorticoid and mineralocorticoid receptors, some heavy metals like lead, mercury, and arsenic are categorized as endocrine disruptors. Potential targets for endocrine-disrupting substances include corticosteroid homeostasis, mineralocorticoid receptors, and glucocorticoid receptors (21).

1.2.1. Cadmium

Cd is a highly toxic metal with primarily anthropogenic sources of contamination. Cd is not naturally found in its pure form but is present in zinc ores and commonly utilized in batteries. The chemical composition of Cd can affect its absorption and distribution throughout various organs within the body. Phosphate fertilizers used in agriculture may contain high levels of Cd as well. Acidification of soil and water may release Cd bound to soil and sediments and thereby cause contamination. Cd is found in grains, cereals, vegetables, starchy roots, tubers, meat, and seafood. Nutrient deficiencies, specifically iron, can increase the absorption of Cd through food consumption. However, dietary zinc supplementation can decrease the absorption of this element in the gastrointestinal tract. Additionally, inhalation of high levels of Cd can lead to lung damage, pneumonia, and pulmonary edema. Long-term exposure to even small amounts of this element in the air can cause lung cancer and accumulation of Cd in the kidneys eventually leading to kidney disease. Exposure to Cd may cause several diseases including renal damage possibly kidneys, prostate, renal cancer, osteoporosis, as well as intellectual impairment in children. Cd may also cause increased bone desorption and lung cancer, as well as decreased activation of vitamin D3 precursors in the kidney and blood calcium concentration. A preliminary weekly tolerable intake of only 3 µg/kg body weight has been established. As Cd is not degradable and tends to accumulate in exposed organisms, chronic exposure to even low levels of cadmium could also lead to adverse renal and negative bone effects. Cd