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INNO-SAFE-LIFE

# CURRICULUM

## Partnership for innovation on the exchange of best practices and the design of joint collaborative initiatives at European level related to the awareness of the effects of contamination on human health.

Erasmus+ Project – Partnership for Coperation

Eds. Pinzaru Iulia Andreea, Dehelean Cristina Adriana

**INNO-SAFE-LIFE**

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INNO SAFE LIFE

# CONTENT

# CURRICULUM DESCRIPTION

**Project title:** Partnership for innovation on the exchange of best practices and the design of joint collaborative initiatives at European level related to the awareness of the effects of contamination on human health

**Project acronym:** INNO-SAFE-LIFE

**Project no:** 2023-1-RO01-KA220-HED-000164767

Curriculum – contributes to the construction of a healthy environment as the basis of human health, starting from ecological soil, the source of food and support of human health, to the production and consumption of safe and adequate food and food supplements. It includes proper data related to the protection of healthy soils with an essential role in the production of food, medicines, supplements etc., identification of contaminants, antimicrobial resistance, obtaining natural products and preservation of active principles and valorisation of plant resources.

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| | |
|---|---|
| **Objectives** | This curriculum aims to equip learners with the capacity to:<br>Explain the interdependence between healthy soils, environmental quality, food safety, and human health.<br>Detect and evaluate the origin, transmission, and effects of environmental pollutants, including antimicrobial resistance (AMR).<br>Apply eco-friendly and innovative techniques to prevent, limit, or remove contamination.<br>Identify, process, and optimise the use of plant-based resources for safe foods, medicines, and dietary supplements.<br>Combine expertise from multiple fields to address environmental health challenges in a sustainable way.<br>Lead awareness and education initiatives for varied audiences at local, national, and European levels. |

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| **Cognitive skills** | In-depth knowledge of soil ecosystems and their significance in food production and environmental stability.<br>Ability to analyse and interpret data on contaminants and their health implications.<br>Understanding of AMR mechanisms, pathways, and sustainable countermeasures.<br>Research competence in sourcing, preserving, and applying bioactive natural products.<br>Capacity to integrate agricultural, environmental, and public health information for decision-making.<br>Familiarity with EU legislation and global standards for environmental protection, food safety, and dietary supplements. |
| **Professional skills** | Collecting and analysing soil samples to assess health and contamination levels.<br>Performing laboratory tests for identifying pollutants and AMR indicators.<br>Designing and implementing green strategies for contamination prevention and remediation.<br>Developing, testing, and ensuring the safety of nutritional and medicinal products derived from plants.<br>Creating effective communication materials for public outreach and risk awareness.<br>Applying recognised standards in agricultural and manufacturing practices to ensure product safety. |
| **Competence units** | Soil Health and Human Survival – principles of soil ecology, prevention and monitoring of degradation.<br>Contaminants and AMR Control – detection methods, risk evaluation, and mitigation strategies.<br>Natural Products and Bioactive Compounds – sustainable sourcing, processing, and preservation techniques.<br>Sustainable Use and Consumption – optimal application forms, market integration, and waste reduction measures. |
| **Elements of innovation** | Merging knowledge from agriculture, environmental sciences, health care, and industry into a unified learning approach.<br>Introducing new content on emerging threats such as microplastics and climate-related contamination shifts.<br>Using plant-based and microbial agents as natural alternatives for remediation and AMR prevention.<br>Incorporating advanced tools such as remote sensing, GIS, and digital platforms for environmental monitoring and awareness.<br>Providing a dedicated experimental methods guide to connect theoretical concepts with hands-on practice.<br>Designing interactive training formats suitable for both academic and community settings. |
| **The impact** | Immediate Outcomes<br>Enhanced technical expertise in environmental health and contamination control among students and professionals.<br>Greater public understanding of environmental risks and sustainable prevention practices.<br>Mid-Term Benefits<br>Wider adoption of environmentally responsible practices in agriculture, food production, and public health. |

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| | Stronger links between universities, industries, and policy-making bodies within the EU.<br>Long-Term Results<br>Healthier ecosystems and communities with reduced exposure to environmental hazards.<br>Food systems better equipped to withstand environmental and health-related challenges.<br>Establishment of a lasting European network dedicated to fostering healthy environments and safe living conditions. |

***Activities hours - curriculum***

| ***Total hours*** ***80*** | **Theoretical** **40** | **Practical** **20** | **Individual study** **20** |
|---|---|---|---|

***Curriculum INNO-SAFE-LIFE***

| ***Technical and scientific data*** | **No of hours** | **Obs.** |
|---|---|---|
| *Chapter 1. The Impact of Soil Contaminants and The Innovative Green Methods for Contaminants Reduction* | 10 | Technical and scientific part consists in 8 hours x 5 days.<br>In total 40 hours |
| *Chapter 2. The Antimicrobial Resistance Resulting from Natural Selection Exacerbated by Human Factors and The Innovative Green Methods to Fight Against It* | 10 | |
| *Chapter 3. The Toxic Effects of Contaminants on the Human Body and Innovative Green Methods to Reduce Them* | 10 | |
| *Chapter 4. The Role of Healthy Nutrition and The Approved Consumption of Safe Food Supplements. Innovative Methods of Approach and Awareness* | 10 | |
| *The practical component of the program will be carried out in specialized laboratories within the partner universities.*<br>*Activities are designed around experimental approaches, using up-to-date techniques and standardized protocols applied to:* | | Practical part consists in 5 hours x 4 days.<br>In total x hours |
| *(i) safeguarding soil resources that are vital for the production of food, medicinal products, and dietary supplements, thereby contributing to food security,* | **5** | |
| *(ii) detecting and assessing contaminants, including studies on antimicrobial resistance,* | **5** | |
| *(iii) producing natural products with a focus on maintaining the integrity and stability of active compounds, and* | **5** | |
| *(iv) optimizing the use of plant resources to ensure safe and effective consumption.* | **5** | |

***Assessment Methods***

*Students will be evaluated through a variety of methods, including multiple-choice and dual-choice tests, short-answer tasks, structured or complementary questions requiring problem-solving, and essays addressing key subject areas.*

***Competence Certification Assessment***

*Certification of competencies will rely on tools and methods that align with professional and cognitive standards, taking into account both performance indicators and application conditions. The evaluation will measure the degree to which students develop skills in soil conservation for agricultural and medicinal purposes, contaminant and antimicrobial resistance analysis, preservation of bioactive components in natural products, and plant resource valorisation techniques for human health applications.*

***Study and Research Materials***

*The program promotes active engagement of students and early-career researchers in continuous learning. Throughout the project and in follow-up activities, modern and adaptable teaching–learning methods will be applied, incorporating international best practices. The program materials will be systematically structured and made available in five languages—English, Romanian, Italian, Slovak, and Croatian—customized to the context of each participating country.*

# EXTENDED CURRICULUM

# Chapter 1. THE IMPACT OF SOIL CONTAMINANTS AND THE INNOVATIVE GREEN METHODS FOR CONTAMINANTS REDUCTION

## 1.1 Introduction

Healthy soils are the foundation of food security, human health, and environmental stability. They serve not only as the medium for crop growth but also as a vital source of raw materials for medicines, food supplements, and other products essential to human well-being. Soil functions as a living system, supporting complex biological communities that regulate nutrient cycles, filter water, and maintain ecological balance. When soil health is compromised—through contamination, degradation, or imbalance—these functions are disrupted, jeopardising both food safety and public health.

This chapter focuses on three critical aspects of soil protection. The first addresses soilborne pathogens, which can persist for years, affecting plant health, reducing yields, and introducing harmful toxins into the food chain. Understanding their life cycles, modes of transmission, and management strategies is essential for sustaining agricultural productivity.

The second part examines the impact of pesticides on soil health, highlighting the delicate balance between controlling harmful organisms and preserving soil biodiversity. While pesticides play a role in safeguarding crops, their excessive or improper use can lead to persistent soil contamination, accumulation of toxic residues, and long-term environmental harm. Sustainable use and effective remediation practices are therefore essential.

The final section explores phytoremediation as an innovative, environmentally friendly solution for restoring contaminated soils. By harnessing the natural ability of plants, algae, and fungi to absorb, transform, or immobilise pollutants, phytoremediation offers a low-cost and sustainable method to address trace element contamination, including heavy metals.

Together, these sections present a comprehensive understanding of the threats to soil health and innovative approaches to its protection. By integrating biological knowledge, sustainable management, and green technologies, we can preserve soils as a secure foundation for food production, medicinal resources, and human survival.

## 1.2 Soilborne Pathogens as Soil Contaminants

Soil contains the most diverse biological communities on Earth (Nielsen et al., 2015). It serves as a reservoir for various pathogens capable of causing diseases in plants. Diseases caused by soilborne pathogens can lead to substantial yield losses in many crops (Katan, 2017). These pathogens include fungi, oomycetes, viruses, bacteria, and nematodes. Typical symptoms of infection include visible lesions, rots, and wilts.

Pre-emergence damping-off occurs when young seedlings decay beneath the soil surface before emerging. This typically happens under unfavourable germination conditions, such as cold, hot, or excessively wet soils, poorly drained or compacted soils, and the presence of undecomposed organic matter.

Post-emergence damping-off occurs when the stems and roots of seedlings are attacked at the soil line, causing them to collapse. High salt concentrations in the soil can also contribute to damping-off.

Root rots can affect plants beyond the seedling stage, as fungi invade internal root tissues, disrupting the supply of water and nutrients. Aboveground symptoms include reduced vigour, leaf yellowing, premature leaf drop, wilting from the growing tip, twig dieback, and sudden plant death. Vascular wilts are characterised by wilting and discolouration of the vascular system in stems, trunks, or branches.

Soilborne pathogens survive in the soil for at least part of their life cycle. Soil is a heterogeneous environment where microbial growth is often limited by the availability of organic substrates. Consequently, pathogens are strongly influenced by both abiotic and biotic soil factors, as well as by agricultural practices such as irrigation, tillage, manure application, and fertilisation. These organisms typically enter plants through belowground structures but can also spread to aboveground parts. Transmission occurs via soil, contaminated water, plant debris, or agricultural tools (Friberg et al., 2005).

Common fungal soilborne pathogens include species from the genera Fusarium, Rhizoctonia, and Phytophthora, which cause diseases such as root rot, damping-off, and wilt in various crops. These pathogens are challenging to manage because they can persist in the soil for extended periods, surviving as spores, cysts, or other resilient structures (Alegbeleye et al., 2018). Many fungal pathogens produce highly resistant resting spores—such as chlamydospores, oospores, microsclerotia, or sclerotia—that can survive in soil for more than 10 years (Jurković et al., 2017).

When root exudates from a susceptible host or other suitable nutrient sources are present in the rhizosphere, these structures germinate and infect plants under favourable conditions. Once established, soilborne pathogens can cause chronic infections, reducing plant health and crop productivity.

Mycotoxins—secondary metabolites produced by certain soilborne fungi—can be harmful to humans and animals. They contaminate food directly during cultivation or indirectly through contaminated animal feed (Juraschek et al., 2022).

Management strategies for soilborne pathogens include crop rotation, soil sterilisation, resistant plant varieties, and the use of biological control agents. Practices that enhance soil microbial diversity and biomass can promote antagonistic interactions, thereby helping regulate harmful organisms (Samaddar et al., 2021).

### 1.3 Influence of Pesticides on Soil Health

Sustainable pesticide use means applying pesticides in ways that do not endanger human health or natural resources such as soil, air, water, and biodiversity. Harmful organisms in agriculture—such as pathogens, pests, and weeds—can cause significant yield losses.

Without adequate plant protection measures, global crop losses can reach approximately 50% (Öerke, 2005).

A major challenge in agriculture is producing enough food for a growing global population while minimising environmental and biodiversity impacts. Awareness of the potential negative effects of pesticides is central to the concept of sustainable agriculture and sustainable plant protection (Barić et al., 2019).

Pesticides contain active substances—chemical compounds, elements, or microorganisms—that act against harmful organisms. After application, these substances undergo decomposition and transformation under the influence of light, temperature, moisture, and plant enzymes. The rate and nature of decomposition depend on environmental conditions and chemical properties.

Pesticide residues are typically small amounts of active substances measured in milligrams per kilogram of plant product. Such residues may be present in foods of plant origin (fruits, vegetables, cereals), foods of animal origin (meat, milk, eggs), drinking water, surface water, soil, and indoor air where pesticides are used (Baličević & Ravlić, 2014).

For each approved active substance, a maximum residue level (MRL) is established to ensure the safety of food, water, soil, or air. To avoid exceeding MRLs, it is essential to follow the application guidelines provided on plant protection product labels (Mešić et al., 2018).

Large quantities of pesticides enter the environment and soil through adsorption, leaching, evaporation, and runoff (Tudi, 2021). Their persistence is influenced by soil type, pesticide solubility, degradation rate, soil microbial activity, and climatic conditions. Pesticide residues can cause moderate soil degradation, making regeneration more difficult.

Common soil pollutants include heavy metals, polycyclic aromatic hydrocarbons (PAHs), and persistent organic pollutants (POPs). Sources of POPs include intensive use of mineral fertilisers and plant protection agents, as well as industrial emissions. These compounds are persistent, toxic, bioaccumulative, and capable of long-range atmospheric transport. Examples include polychlorinated biphenyls (PCBs), organochlorine pesticides (OCPs), and polychlorinated dibenzofurans (PCDFs) (Sofilić, 2014).

Soil remediation for pesticide residues can involve low-temperature desorption, incineration, bioremediation, or phytoremediation. The choice of method depends on the pesticide type, soil characteristics, and climatic conditions. Ideally, remediation should fully degrade contaminants without producing harmful intermediates (Đokić et al., 2012). However, some methods only stabilise or redistribute substances rather than removing them entirely.

Although pesticide application is strictly regulated, ongoing farmer education is vital to promote sustainable agriculture. This includes awareness of personal protective measures and adherence to manufacturer instructions regarding quantities, preparation, and application methods.

### 1.4 Phytoremediation as a Solution for Trace Element Contamination in Soil

Plants have developed multiple mechanisms to tolerate high, toxic concentrations of heavy metals. Soil contaminated with heavy metals can be treated using physical (soil leaching), chemical, or biological (bioremediation) methods (Singh et al., 2003).

Phytoremediation—alongside stabilisation, rhizofiltration, phytovolatilization, and soil leaching—is a biological method in which plants, algae, and fungi reduce pollutant concentrations in soil, water, or air (Tangahu et al., 2011). These organisms absorb, degrade, or transform harmful substances, helping restore ecosystems and improve environmental quality.

Key phytoremediation mechanisms include:

- Phytoproliferation – plants grow in contaminated areas and absorb pollutants through their roots.
- Phytorrhizostasis – plants accumulate pollutants in their tissues without significant damage, allowing easier removal.
- Phytodegradation – plant-produced enzymes break down or transform pollutants into less harmful compounds.
- Phytoextraction – plants absorb pollutants, which are then removed when the plants are harvested.
- Phytorhizofiltration – plant roots filter pollutants from water.

The term phytoremediation derives from the Greek *phyto* (plant) and Latin *remedio* (to treat or restore) and refers to the use of plants and their associated microorganisms to isolate, transport, detoxify, or mineralise pollutants in soil, thereby reducing their concentration, mobility, or toxicity (Prasad, 2003).

Heavy metal uptake by plants occurs via root absorption, root-to-shoot transport, and sequestration. Many metals are soluble and easily absorbed, while others require root-secreted chelating agents to increase their availability (Dalvi & Bhalerao, 2013). Absorption can occur via:

- Apoplastic pathway – passive diffusion through non-living tissues.
- Symplastic pathway – active transport through living tissues.

Once absorbed, heavy metals form complexes with chelators in root cells and may be immobilised or transported to aerial parts through the xylem (Ali et al., 2013; Thakur et al., 2016; Kumar et al., 2022).

Plants with exceptionally high metal uptake are called hyperaccumulators (Trapp & Legind, 2010). Many species can absorb contaminants such as lead, cadmium, chromium, arsenic, and radionuclides. Phytoextraction is particularly effective for removing both essential (Fe, Mn, Zn, Cu, Mg, Mo, Ni) and non-essential (Cd, Cr, Pb, Co, Ag, Se, Hg) metals (Vamerali et al., 2009).

Phytoremediation offers several advantages: it is effective, low-cost, applicable to a wide range of pollutants, environmentally friendly, and well-suited for large areas with low to moderate contamination. It requires minimal equipment, specialised personnel, or high energy input, making it an attractive alternative to conventional remediation methods, particularly for large-scale or low-level contamination sites.

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# Chapter 2. THE ANTIMICROBIAL RESISTANCE RESULTING FROM NATURAL SELECTION EXACERBATED BY HUMAN FACTORS AND THE INNOVATIVE GREEN METHODS TO FIGHT AGAINST IT

## 2.1 Introduction

A critical step in safeguarding public health and environmental integrity is the systematic analysis and accurate identification of contaminants, including the detection and monitoring of antimicrobial resistance across diverse ecosystems.

Antimicrobial resistance and pollution have emerged as major global health and environmental issues. Due to the widespread use of antimicrobials in medicine and animal husbandry, residual antimicrobials are constantly released into the environment, damaging ecosystems and promoting the development and spread of antibiotic resistance. Often overlooked is the contribution of environmental variables to antimicrobial pollution and the development of resistance. Humans carry a high number of antimicrobial-resistant genes and bacteria, raising the risk of pathogenic bacteria developing resistance and increasing the likelihood of human contact with antimicrobial-resistant pathogens. Since their discovery in the 20th century, antimicrobials have been widely used in disease prevention, treatment, and livestock rearing due to their effectiveness against pathogenic bacteria (Hao et al., 2014; Bacanli and Basaran, 2019; Hutchings et al., 2019).

The development of antimicrobials, of which over 100,000 tons are used annually worldwide, has saved millions of lives (Danner et al., 2019). However, the widespread application of antimicrobials has unavoidably resulted in environmental leakage and residue (Chen et al., 2020). Antimicrobials are released into the environment either directly or through host metabolism, particularly in agricultural and aquaculture applications (Bilal et al., 2019). Although antimicrobials have relatively short half-lives, often lasting from a few hours to several days, misuse and ongoing release have led to their presence in wastewater, groundwater, and surface water (Wei et al., 2011; Gothwal and Shashidhar, 2015; Chen et al., 2019). Furthermore, antimicrobial residues have been detected in vegetables, dairy products, eggs, and other foods, exposing people to antimicrobials over time. Recent research has shown that antibiotic residues may have harmful effects on living organisms (Petersen et al., 2021; Qian et al., 2021). As a result, antimicrobial contamination has become an international issue (Wang et al., 2021).

Antimicrobial-resistant bacteria (ARB) and antimicrobial resistance genes (ARGs) are primarily generated and transmitted by antibiotics (Wang et al., 2020; Shen et al., 2021). Through horizontal gene transfer (HGT), microorganisms, particularly clinical pathogens, acquire ARGs from their surroundings, reducing their susceptibility to antibiotics (Guo et al., 2022). Antimicrobial resistance can spread more easily in the environment due to the rapid and unrestricted growth of microorganisms. Over the past decade, ARGs have increased the risks posed by microbes to human health (Zhang et al., 2022). An estimated 6.22 million deaths globally in 2019 were attributed, either directly or indirectly, to antimicrobial-resistant illnesses (Murray et al., 2022). Antimicrobial resistance has already become a significant threat to public health worldwide (Lin et al., 2021). This is because antimicrobials, ARGs, and ARB—commonly considered emerging environmental pollutants—are difficult for wastewater treatment plants to eliminate (Zhang et al., 2018; Cerqueira et al., 2019). ARBs, ARGs, and antimicrobials have been reported in numerous settings (Li et al., 2021), including atmospheric environments, raising the risk of human exposure (Wang et al., 2019; Zhao et al., 2022).

The stomach is the primary organ in the human body for absorption, digestion, and hosting the microbiome. The significance of the gut microbiome for human health has recently come to light (Jin et al., 2017; Yuan et al., 2019). Diseases such as obesity and chronic kidney disease can be triggered by disruptions in the gut microbiota (Gerard, 2016; Nallu et al., 2017). However, antimicrobials and ARGs primarily affect gut bacteria, increasing human health risks (Duan et al., 2022). In environments with a high microbial population, such as the gut, HGT is more likely to occur (McInnes et al., 2020). Consuming food or water contaminated with residual antibiotics and ARGs is concerning because it reduces gut bacterial diversity and enhances ARB colonization and ARG amplification, lowering resistance to pathogen infiltration (Anthony et al., 2021).

In addition to the numerous pollutants caused by human activity, antimicrobials also interact with other elements or pollutants in the complex natural environment, posing greater risks to ecosystems and public health (Qin et al., 2022; Wang et al., 2023). These contaminants impact the development of multidrug-resistant bacteria and the enrichment and transmission of ARGs (Xia et al., 2019; Li et al., 2022). Antimicrobials and ARGs may cause more serious harm due to these unpredictable and variable circumstances.

## 2.2 Antimicrobial Resistance's Destiny in the Environment

The majority of antimicrobial compounds are produced naturally and have the ability to either kill or inhibit microorganisms (Kumar et al., 2019). One of the most significant medical advances of the 20th century was the discovery of antibiotics (Hutchings et al., 2019). Since the discovery of penicillin, the development of antibiotics has experienced a golden era. Numerous antimicrobials have been identified, used to prevent disease, and have become a vital component of modern medical care (Laws et al., 2019). Currently, over 150 antimicrobials are in use, which can be categorized based on their chemical structure into groups such as β-lactam, macrolide, quinolone, and aminoglycoside antimicrobials (Tasho et al., 2016).

Antimicrobials function by inhibiting the production of bacterial cell walls, interacting with the cell membrane to alter permeability, interfering with protein synthesis, and preventing nucleic acid replication and transcription (Hutchings et al., 2019). In addition to their therapeutic uses, antimicrobials have been extensively employed in agriculture to control diseases and pests, as well as in animal feed to promote growth (Hao et al., 2014; Zhou et al., 2018). Antimicrobial use for medical purposes is actually more common in China and the United States than in agriculture, aquaculture, and livestock production (Zhang et al., 2019).

Antimicrobials are produced in large quantities and used globally to meet the demands of medical care and disease management. Fortunately, awareness is increasing that improper and excessive use of antibiotics can harm both the environment and human health. As a result, there is growing momentum to reduce unnecessary antibiotic use and to develop safer alternatives.

## 2.3 Penetration of Antimicrobials into the Environment

There are many pathways through which antimicrobials can enter the environment and affect both ecosystems and human health. The main reservoirs of antimicrobials are soil and water. Sulfonamides have low bioavailability; therefore, after being digested by pigs, their concentrations in urine ranged from 4.54% to 69.22%, and in faeces from 15.03% to 26.55% (Qiu et al., 2016). According to a related study, when patients took solimycin orally, the drug concentration in their urine and faeces was 14.1% and 76.5%, respectively (MacLauchlin et al., 2018). Chen et al. (2020) reported that human and animal wastes accounted for 42.6% and 57.6% of antimicrobial release, respectively.

Cattle and poultry waste have primarily been used as fertilizer in agriculture, and any residual antibiotics have leached into the soil and reached surface water, groundwater, and other aquatic ecosystems via runoff (Bombaywala et al., 2021). In contrast, human waste typically enters wastewater treatment plants (WWTPs) through household sewer systems. However, WWTPs have only been partially effective in removing antibiotics (Aydin et al., 2019; Behera et al., 2011).

Surface waters have been exposed to treated wastewater from WWTPs, and activated sludge from these facilities has been recycled as biological fertilizer, enabling antimicrobials to enter the soil and natural water cycles (Langbehn et al., 2021). Wastewater, surface water, drinking water, groundwater, and soil have all been found to contain antimicrobials (Yao et al., 2021), with concentrations typically ranging from ng/L to mg/L (Kovalakova et al., 2020).

## 2.4 Antimicrobial Effects on Human Health and the Environment

Aquatic environments are rich in cyanobacteria, and red tides and blooms are caused by cyanobacterial overgrowth. Antimicrobials are sometimes applied, in addition to environmental residues, to eradicate cyanobacterial blooms. Nonetheless, it has been reported that antimicrobials can stimulate cyanobacterial growth and bloom formation at environmental concentrations as low as 300 ng/L (Xu et al., 2021). Similarly, Jiang et al. (2021) found that certain cyanobacteria produce more microcystin in response to low concentrations of antimicrobials.

The presence of antimicrobials in the environment increases the likelihood of microcystin release and cyanobacterial blooms, which disrupt ecological balance and indirectly threaten the health of humans and animals (Lei et al., 2021; Wan et al., 2021). Moreover, phytoplankton, as primary producers in ecosystems, are susceptible to certain antimicrobials (Guo et al., 2012). Antimicrobials can suppress photosynthesis and affect phytoplankton growth (Wan et al., 2015). Due to the detection of multiple antimicrobials in the environment, research has shifted from examining the effects of individual antimicrobials to investigating the effects of combined antimicrobial exposure (Kovalakova et al., 2020).

Although fish are generally less susceptible to antimicrobials than algae, they are still important targets in the aquatic environment (Yang et al., 2020). Zebrafish, a widely used model organism, have been extensively employed in toxicological investigations (Yang et al., 2021). Numerous studies on the toxicity of antimicrobials to zebrafish have been conducted recently. It has been shown that macrolide antibiotics negatively affect zebrafish growth, liver, and heart function (Yan et al., 2019; Zhang et al., 2020). Acute antibiotic exposure in zebrafish can cause behavioural changes and cognitive decline. Prolonged oxytetracycline exposure may alter thyroid hormone and serotonin homeostasis in the zebrafish brain (Li et al., 2020).

Environmental antimicrobial doses can also lead to intestinal microbiota disorders, damage the intestinal barrier, and impair fish physiological functions (Zhao et al., 2021). Antimicrobial residues have been detected in fish eggs, and antibiotics have been shown to affect zebrafish growth during reproduction and reduce the survival of offspring (Qiu et al., 2020). Furthermore, some negative effects of antibiotics have also been observed in mammals and amphibians (Peltzer et al., 2017; Kwon et al., 2020).

## 2.5 The Joint Impact of Antimicrobials and Environmental Pollutants

Numerous pollutants, including pesticides, heavy metals, fluorinated chemicals, and microplastics (MPs), have been generated by human activity and discharged into the environment. MPs have garnered significant attention as newly recognized environmental contaminants and are frequently detected in water (Dobrijevic et al., 2016). Researchers have recently discovered that antimicrobials may be absorbed by these plastic particles through hydrogen bonding, hydrophobic interactions, van der Waals forces, and electrostatic interactions—particularly in particles smaller than 5 microns (Guo et al., 2019; Guo and Wang, 2019; Li et al., 2018). This adsorption may increase the toxic effects on aquatic organisms.

Azithromycin and clarithromycin, at 50 mg/mL MPs (terephthalate, polylactic acid, polyoxymethylene, and polystyrene), were reported by Gonzalez-Pleiter et al. (2021) to be highly inhibitory to cyanobacterial growth and chlorophyll content. Furthermore, it was found that the addition of 0.26 mg/L polystyrene microplastics to the mixture caused clams to accumulate more oxytetracycline and fluoropyrimidine (Wang et al., 2020). Similarly, the addition of 500 nm-sized polystyrene microplastics at a concentration of 26 mg/L significantly enhanced the immunotoxicity of flufenicol and tetracycline to clams (Zhou et al., 2021). More importantly, there may be health risks if antimicrobial concentrations in humans become further enriched through the food chain.

Different metal ions have been found in soil and water, indicating that heavy metal pollution is another global environmental issue (Liu et al., 2017). The adsorption of antimicrobials by microplastics can be either facilitated or inhibited by certain metal ions (Yu et al., 2020). According to a recent study, interactions between heavy metals and antimicrobials can influence their toxic effects. For instance, complexing $Cu^{2+}$/$Cd^{2+}$ with chlortetracycline can cause antagonistic toxicity to cyanobacteria (You et al., 2022).

## 2.6 The Environmental Distinction of Antimicrobial Resistance

The incidence and transmission of antimicrobial resistance genes (ARGs) and antimicrobial-resistant bacteria (ARBs) are at the core of growing public health concerns regarding antimicrobial resistance. In nature, long-term microbial selection under environmental stress leads to the formation of ARBs and ARGs, and the misuse and overuse of antimicrobials have exacerbated this situation (Santos-Lopez et al., 2021).

The first mechanisms of bacterial resistance to antimicrobials include the expression of efflux transport proteins or the reduction of membrane permeability to prevent antimicrobial entry. The third and fourth mechanisms involve the development of enzymes that alter or break down antimicrobials, the modification or protection of antimicrobial targets to decrease antimicrobial affinity, and the production of proteins that can substitute the target function but are not inhibited by antimicrobials (Khan et al., 2018). Without a doubt, this poses a serious threat to both the environment and human health.

ARB and ARGs have recently been frequently detected in natural environments, with environmental media such as soil and water providing stable hosts for the exchange of ARGs between ARBs. The primary habitats for high levels of ARBs and ARGs include hospital wastewater, aquaculture wastewater, and wastewater treatment plants (WWTPs). Mobile genetic elements (MGEs) may play unexpectedly large roles in the spread of antibiotic resistance (Wang et al., 2018). ARGs, MGEs, and ARBs in the air have often been overlooked. The abundance of airborne ARGs is influenced by seasonal factors such as temperature, humidity, and air circulation, and studies have reported that bioaerosols are a major pathway for ARG propagation on cattle farms (Cabello et al., 2016).

Through vertical gene transfer and horizontal gene transfer (HGT), ARBs and ARGs in these habitats have been continually enriched, giving rise to various antimicrobial-resistant pathogenic bacteria that eventually contaminate fruits, vegetables, and other foods consumed daily by humans (Pintor-Cora et al., 2021). Unknowingly, humans are surrounded by ARBs and ARGs. Remarkably, human activities such as travel and the international trade of food have also contributed to the global spread of ARGs (Hu et al., 2008). Humans may, to some extent, act as potential carriers of ARGs, releasing them into the environment and contributing to their dissemination (Zhou et al., 2018).

## 2.7 The Influence of Various Environmental Factors on Antimicrobial Resistance

Heavy Metals

Numerous studies have examined the mechanisms influencing the spread of antibiotic resistance, and these factors show similarities to those affecting antimicrobial contamination. Residual antimicrobials are reported to be the primary cause of bacteria acquiring ambient ARGs and increasing bacterial mutation rates (Gu et al., 2021). Apart from antibiotics, heavy metals are also critical for the emergence and propagation of antibiotic resistance. The abundance of ARGs has been shown to correlate strongly with the concentration of heavy metals in contaminated water sources (Komijani et al., 2021). Although not the primary driver of ARG transfer, reactive oxygen species (ROS) are believed to play a role (Ren et al., 2021). One possible mechanism by which heavy metals promote the development of ARBs is through inducing oxidative stress, which increases intracellular ROS levels (Bombaywala et al., 2021).

Furthermore, research has demonstrated a cross-protection mechanism in bacteria, where microbial resistance to antimicrobials is linked to metal resistance (Bergholz et al., 2012). Under heavy metal stress, certain proteins may be expressed more frequently in bacteria, enhancing their resistance to antimicrobial agents (Zhong et al., 2022). Several genes encoding resistance to both metals and antibiotics also exhibit genetic associations with some MGEs (Imran et al., 2019). As a result, heavy metals may play a crucial role in the development of antibiotic resistance by facilitating the acquisition of MGEs and ARGs by bacteria. Additionally, heavy metals have long-term impacts on promoting the horizontal spread of ARGs due to their persistence in the environment and resistance to degradation (Buledi et al., 2021).

Microplastics (MPs)

The presence of plastics in the environment provides microorganisms—particularly on MPs, which are receiving increasing attention—with a novel biological niche (Feng et al., 2021). The surface of MPs is colonized by microorganisms, facilitating the transfer and exchange of ARGs through close cell-to-cell contact. A recent study reported that the majority of bacteria resistant to multiple antibiotics originate from MPs, with the detection rate of ARGs being 14.7% higher on MPs than in water. In the same study, ARBs were found to be 100–5000 times more abundant in MP samples than in water samples (Zhang et al., 2020).

Additional Elements

Another factor influencing the spread of antibiotic resistance is polyaromatic hydrocarbons (PAHs). In soils contaminated with PAHs, high abundances of tetracycline, sulfonamides, aminoglycosides, ampicillin, and fluoroquinolones—along with ARGs—were detected. In these conditions, MGEs and integrons increased the prevalence of HGT (Maurya et al., 2021). Fungicides, which are commonly used pesticides, can enrich ARGs and alter the composition of the gut microbiome of soil invertebrates such as *Enchytraeus crypticus,* in addition to killing fungi (Zhang et al., 2019).

These findings highlight the crucial role of the environment in the development of superbugs and other multi-antimicrobial-resistant bacteria that pose serious threats to human health. Environmental contaminants, in particular, have a significant influence on the spread of antimicrobial resistance. Some compounds commonly used for soil remediation and water treatment also impact the spread of antimicrobial resistance in addition to other environmental pollutants.

For example, sewage and drinking water can be treated with chlorine disinfection; however, chlorine disinfection releases MGEs and ARGs into the environment after killing ARBs. These genetic materials may then be taken up by microorganisms naturally resistant to chlorine, due to genetic mutations, thereby promoting the spread of ARGs (Jin et al., 2020). Although humic-acid-like substances in dissolved components have been shown to induce the transmission of bacterial ARGs, biochar remains a promising option for soil remediation (Liu et al., 2021).

## 2.8 Antimicrobial Resistance's Possible Risk to Human Health and the Ecological System

The environment can provide resistance factors to pathogens, making them less susceptible to the effects of antimicrobials and, in turn, making it more difficult to prevent and treat microbial diseases. ARBs affect people and the environment more directly than ARGs. Antimicrobial-resistant human pathogenic bacteria (ARHPB) have been detected in nearby soil and water, and one study revealed that animal farms are a significant source of ARBs and ARGs, which contaminate the surrounding environment (Fang et al., 2018). Although this should serve as a warning sign, there is currently no practical way to fully prevent the spread of antibiotic resistance.

## 2.9 Techniques for Reducing Antibiotic Resistance and Contamination

One of the most crucial steps in addressing the global issue of antimicrobial contamination is the degradation of antimicrobials in the environment. At the same time, considering the threat that antimicrobial resistance poses to global health, it is imperative to understand the efficacy of current removal procedures and develop novel treatments for ARGs and ARBs. Currently, WWTPs are primarily responsible for the removal of antimicrobials, ARBs, and ARGs. However, while conventional WWTPs can significantly reduce bacterial loads, their ability to diminish ARGs is limited (Hassoun-Kheir et al., 2020).

Advanced Oxidation Processes (AOP)

Strong oxidants, such as those used in AOP technologies like Fenton, photocatalysis, and activated pyruvate, can break down hazardous materials in water (Wang et al., 2020). Antimicrobials, as organic pollutants, have demonstrated significant degradation following AOP treatment. Distinct oxidation mechanisms result in different antimicrobial breakdown pathways (Li et al., 2023). However, the removal of complex pollutants by AOP alone is not very effective. Therefore, to achieve better elimination, AOP can be combined with other techniques such as membrane treatment, photolysis, and biological methods (Leng et al., 2020).

While AOP technologies are effective at eliminating pollutants, there are notable drawbacks. Practical application requires consideration of operation and maintenance costs. A single AOP process often cannot completely eliminate ARGs or fully mineralize antimicrobials; therefore, understanding the toxicity of intermediate products formed during degradation is essential. Additionally, enhancing AOP's capacity to remove ARGs is crucial for combating antibiotic resistance. Currently, AOP is mostly applied to wastewater treatment and is rarely used for the removal of resistant genes and antimicrobials from soil and animal waste (Phoon et al., 2020).

Constructed Wetlands (CW)

Due to their low cost and potential effectiveness in removing organic contaminants, constructed wetlands have already been applied in wastewater treatment (Fang et al., 2017). Previous studies have shown that CW can remove ARBs, ARGs, and antimicrobials through mechanisms such as macroplant absorption and degradation, microbial degradation, and substrate adsorption (Sharma et al., 2016). However, plant type, substrate type, ambient temperature, and CW design all influence removal efficiency (Chen et al., 2016).

Although CWs are generally less effective than AOPs in pollutant removal, their sustainability and low construction and maintenance costs make them an excellent green technology. Intermittent aeration has been shown to improve contaminant removal and overall water quality. Given their removal potential, CWs could be used as a secondary treatment for municipal wastewater after WWTP processing. Future research should focus on the interactions between CWs and other treatment technologies, as well as the persistence and degradation of pollutants within CW systems (Chen et al., 2016).

Microbial Fuel Cells (MFC)

Another promising form of green biotechnology, MFCs offer both environmental and economic benefits by breaking down pollutants while generating electricity. In treating swine wastewater containing sulfonamides, removal efficiencies for sulfamethoxazole, sulfadiazine, and sulfamethazine were reported at 99.46–99.53%, 13.39–66.91%, and 32.84–67.21%, respectively (Cheng et al., 2020). In a recent study, Long et al. (2021) developed MFC-Fenton and air-cathode MFC systems that successfully degraded tetracycline.

A combined system, CW-MFC, has been developed for wastewater treatment, achieving ciprofloxacin removal above 90% and sulfadiazine removal above 80%, while significantly reducing methane emissions (Xu et al., 2021). Further improvements, such as using novel iron–carbon fillers, have enhanced microbial adherence and boosted micro-electrolysis efficiency, leading to higher ciprofloxacin removal compared to conventional CW-MFC systems (Dai et al., 2022). Notably, MFCs have also been applied for *in situ* remediation of antimicrobial-contaminated soils, reducing ARG release risk and antimicrobial residue levels in the soil (Song et al., 2022).

Other Technologies

Microalgae-based treatment has been widely studied for wastewater remediation. This process removes antimicrobials from water primarily through adsorption, hydrolysis, accumulation, and indirect photodegradation (Leng et al., 2020). It is often combined with bacteria, UV light, or activated sludge to improve antimicrobial removal rates. Microalgal methods have demonstrated over 90% removal efficiency for tetracycline, amoxicillin, cephalosporins, and florfenicol (Song et al., 2022).

Most of these technologies focus on removing antimicrobials from sewage. However, veterinary antimicrobials in animal manure can also be treated using composting, a biological remediation technique. The effectiveness of composting depends on the type of antimicrobial compound (Ezzariai et al., 2018).

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# Chapter 3. THE TOXIC EFFECTS OF CONTAMINANTS ON THE HUMAN BODY AND THE INNOVATIVE GREEN METHODS TO REDUCE THEM

## 3.1 Introduction

The sustainable production of natural products, through careful selection of plant resources and preservation of their active principles, offers promising pathways to mitigate the toxic effects of environmental contaminants on the human body, forming a cornerstone of innovative green strategies for pollution reduction.

Across the globe, environmental toxicants and chemical contaminants have emerged as a serious and expanding public health challenge. They originate from a wide range of human activities — including industrial manufacturing, intensive farming, fossil fuel combustion, and poor waste management — and are now present in air, soil, water, and even in the food supply. The World Health Organization (WHO 2023) reported that in 2016, nearly a quarter of all deaths and disease burdens worldwide were linked to environmental factors that could be prevented, such as chemical pollution and hazardous waste.

The consequences of these pollutants for human health range from short-term effects like respiratory distress and skin irritation to chronic and life-threatening conditions. Air pollution alone is believed to claim over five million lives each year (Augusta University, 2023). Certain chemicals can act as carcinogens, teratogens, or mutagens, causing permanent biological harm.

Exposure occurs through multiple routes — breathing contaminated air, consuming polluted food and water, or skin contact (Sokan-Adeaga et al., 2023). Once absorbed, these substances can damage vital organs such as the lungs, heart, liver, kidneys, brain, and the reproductive organs, with children, the elderly, and pregnant women being the most vulnerable (Balbus et al., 2013).

The problem is not only medical but also economic. Lead poisoning alone is estimated to cost the global economy approximately US $6 trillion annually — about 6.9% of the world's GDP (WHO 2023). In 2019, children under five were estimated to have lost 765 million IQ points collectively due to lead exposure (World Bank, 2023), with lasting consequences for human development and productivity.

Climate change is expected to make matters worse by altering patterns of pollutant release, distribution, and human vulnerability (Balbus et al., 2013). These shifts could change risk profiles, making it essential to re-evaluate current strategies for assessing and managing environmental health hazards.

This chapter examines how environmental contaminants affect the human body and presents innovative green approaches aimed at reducing their impact, supporting the global vision of moving toward a pollution-free future (UNEP, 2023).

## 3.2 Toxic Effects on the Human Body

The influence of environmental pollutants on human health is broad and complex, forming the central focus of environmental toxicology—a discipline dedicated to understanding how chemicals, whether acting alone or in combination, may harm living organisms over time (Shetty, 2023). Even at low concentrations, many substances can interact in ways that amplify their toxic potential, making long-term exposure particularly hazardous.

General Toxicological Impacts

Pollutants have been linked to a wide spectrum of illnesses, including cancer, ischemic heart disease, chronic obstructive pulmonary disease (COPD), stroke, neurological and mental disorders, and diabetes (Shetty, 2023). Some contaminants accumulate selectively in certain organs, reaching internal concentrations that exceed those found in the surrounding environment. This bioaccumulation process can, over years, cause significant and sometimes irreversible organ damage (Alharbi, 2018).

Notable examples of harmful substances include:

- DDT – a pesticide historically used against agricultural pests and still applied in some regions of Africa, Asia, and Latin America.
- Furans – compounds arising from high-temperature cooking, certain chemical processes, and some consumer products such as packaging materials.
- Dioxins – toxic by-products of industrial processes or natural events like forest fires and volcanic eruptions.
- Volatile organic compounds (VOCs) – easily evaporating chemicals found in paints, solvents, fuels, building materials, and cleaning agents.
- Aldehydes – for instance, formaldehyde, used in pressed wood products, textiles, and cosmetics.
- Volatile heavy metals – such as mercury vapor from industrial activities, coal burning, or waste decomposition.
- Chlorinated hydrocarbons – present in some solvents and cleaning products.

In certain cases, substances are harmless in their original form but become toxic after metabolic conversion inside the body. These resulting metabolites can be even more damaging, sometimes possessing carcinogenic properties.

Impacts on Specific Organ Systems

Respiratory System

Airborne pollutants—such as carbon monoxide, ozone ($O_3$), nitrogen dioxide ($NO_2$), sulfur dioxide ($SO_2$), particulate matter, and heavy metals—can lead to both acute and chronic respiratory illnesses, including bronchitis, pneumonia, COPD, and asthma (Shetty, 2023). Prolonged exposure may cause permanent structural damage to the lungs, hinder lung growth in children, and elevate lung cancer risk (Schraufnagel, 2019).

Cardiovascular System

Fine particulate matter ($PM_{2.5}$) and similar pollutants can constrict blood vessels, weaken cardiac muscle, and promote inflammatory processes that contribute to atherosclerosis, hypertension, and heart attacks (Dai, 2024). Globally, air pollution is estimated to be responsible for 19% of cardiovascular deaths and 21% of stroke fatalities (Schraufnagel, 2019).

Nervous System

Pollutants in the air are associated with reduced cognitive performance in children and heightened risks of dementia and stroke in older adults (Dai, 2024). These effects are believed to result from oxidative stress and inflammation in neural tissue, which may accelerate neurodegenerative processes.

Endocrine System

A specific group of contaminants known as endocrine-disrupting chemicals (EDCs) can interfere with hormone production, metabolism, and receptor binding (Kumar, 2020). Exposure has been associated with reproductive and developmental issues, immune system changes, and an increased risk of hormone-sensitive cancers.

Chronic Diseases and Disorders

Respiratory diseases: long-term pollution exposure is a major risk factor for COPD and asthma and is linked to increased mortality from these conditions.

Metabolic disorders: pollutants can induce oxidative stress and inflammation, leading to insulin resistance and a greater likelihood of type 2 diabetes (Dai, 2024).

Neurological disorders: continuous exposure is connected to Alzheimer's disease, dementia, and other degenerative brain conditions through inflammatory and oxidative mechanisms.

Cancer: fine particulate matter ($PM_{2.5}$) is recognized as a carcinogen, contributing to lung, bladder, and certain paediatric cancers (Schraufnagel, 2019).

Endocrine disruption: EDCs can alter puberty timing, impair fertility, reduce semen quality, and increase risks of hormone-sensitive malignancies (Kumar, 2020).

In summary, environmental contaminants affect human health through multiple pathways, often targeting more than one organ system at a time. The interplay between various pollutants can intensify their harmful effects, underlining the urgent need for integrated environmental health policies and pollution control strategies.

### 3.3 Pathways of Exposure

Understanding how environmental contaminants enter the human body is essential for accurately assessing and mitigating associated health risks. This section outlines the primary mechanisms through which environmental toxicants are released, transported, and ultimately reach human populations.

## Environmental Release and Transport

Contaminants may be introduced into the environment through industrial processes, agricultural activities, waste disposal, and other anthropogenic actions. Once released, they can move through different environmental compartments:

- Air - pollutants may be directly emitted into the atmosphere or volatilize from soil and water. Air currents can transport these substances over long distances (United States Environmental Protection Agency, 2024a).
- Water - contaminants can enter rivers, lakes, and oceans via direct discharge, surface runoff, or leaching into groundwater. Water bodies not only act as transport systems but also serve as points of human exposure (United States Environmental Protection Agency, 2024b).
- Soil and Sediment - pollutants may settle and accumulate in soils through atmospheric deposition or irrigation with contaminated water. Soil can act as a long-term reservoir, later releasing contaminants back into air or water (Alharbi et al., 2018).

## Routes of Human Exposure

Humans can be exposed to environmental toxicants via three primary routes:

- Inhalation - breathing in contaminated air that contains gases, vapours, aerosols, or particulate matter. Indoor air can also be affected when outdoor pollutants infiltrate buildings (United States Environmental Protection Agency, 2024c).
- Ingestion - swallowing contaminants present in food, drinking water, or soil and dust particles. This includes accidental soil ingestion in children and hand-to-mouth transfer of residues (United States Environmental Protection Agency, 2024b; New Hampshire Department of Environmental Services, 2024).
- Dermal Contact - direct skin contact with contaminated soil, water, or consumer products containing hazardous chemicals. Occupational exposure is a frequent contributor to dermal uptake (New Hampshire Department of Environmental Services, 2024).

## Factors Influencing Exposure

The extent and severity of exposure depend on several factors:

- Duration - short-term (acute) vs. long-term (chronic) exposure affects health outcomes differently.
- Intensity - higher concentrations of contaminants increase potential risk.
- Frequency - repeated exposure can compound health effects.
- Individual Susceptibility - certain groups, including pregnant women, children, the elderly, and immunocompromised individuals, are more vulnerable (New Hampshire Department of Environmental Services, 2024).

Bioaccumulation and Biomagnification

Persistent organic pollutants and heavy metals can build up in body tissues over time through bioaccumulation. When these contaminants pass along the food chain, they may become more concentrated at higher trophic levels-a process known as biomagnification (United States Environmental Protection Agency, 2024b).

Exposure Assessment

Evaluating human exposure involves:

- Identifying contamination sources
- Tracing environmental transport and fate
- Determining exposure points and routes
- Defining at-risk populations

These assessments allow classification of exposure pathways as completed, potential, or eliminated, providing the basis for effective risk management strategies (Agency for Toxic Substances and Disease Registry, 2024).

## 3.4 Evaluation of Contaminants

Climate change, environmental pollution, biodiversity loss, and the unsustainable use of natural resources collectively pose significant risks to human, animal, and ecosystem health. These threats include infectious and non-communicable diseases, antimicrobial resistance, and water scarcity. Ensuring a healthy planet for all requires more effective monitoring, reporting, prevention, and remediation of pollution affecting air, water, soil, and commodities (European Commission, 2021).

The scale of the pollution problem can be greatly reduced through strong governmental action, advanced infrastructure, and the application of modern technologies. However, achieving the goal of a clean environment is hindered by several challenges, including insufficient public engagement in pollution control initiatives and inadequacies in ecological management systems.

Addressing pollution effectively requires the use of the latest technological solutions and targeted research to better understand the mechanisms by which contaminants accumulate in the environment.

Food contamination remains a critical concern, as elevated chemical concentrations in edible products pose serious health hazards. Contaminants in food may be naturally occurring in the environment or introduced through human activities. Furthermore, contamination can occur at multiple points along the food chain - during production, processing, packaging, transportation, and storage (Rather et al., 2017).

Food safety challenges can be grouped into four key categories:

- Microbiological safety
- Chemical safety
- Personal hygiene
- Environmental hygiene

With the globalization of the food trade, food has become a major route of human exposure to pathogenic microorganisms responsible for foodborne illnesses, potentially entering at various stages of the value chain. Tracking and detecting these pathogens - particularly bacteria - back to their sources remains a challenge for producers, processors, distributors, and consumers alike.

Food safety and nutrition are closely interlinked. Unsafe food can trigger a vicious cycle of disease and malnutrition, disproportionately affecting infants, young children, the elderly, and individuals with compromised health. Because food supply chains now span multiple national and regional borders, ensuring food safety in the 21st century will require strong collaboration between governments, producers, suppliers, distributors, and consumers (Fung et al., 2018).

In recent years, various analytical methods have been developed for detecting contaminants in different matrices. Since contaminants are often present at extremely low concentrations, a very low detection limit is required, and sample preparation becomes essential to reduce matrix effects in food analysis. Sample preparation may involve multiple steps - such as filtration, pH adjustment, extraction, clean-up, and enrichment - to ensure that analytes are detected at suitable concentration levels.

A wide range of sample preparation techniques is now available, including supercritical fluid extraction, solid-phase extraction, solid-phase microextraction, microwave-assisted extraction, liquid-liquid extraction, liquid-phase microextraction, pressurized liquid extraction, and stir bar sorptive extraction (Guo et al., 2019).

### 3.5 Innovative Green Methods to Reduce the Toxic Effects of Contaminants

Green chemistry, also known as sustainable chemistry, is a modern approach in the chemical sciences that has evolved significantly since the 1990s. It is defined as "the use of chemical techniques and methodologies that reduce or eliminate the use or generation of raw materials, products, by-products, solvents, and reagents that are hazardous to human health or the environment" (United States Environmental Protection Agency). At its core, this philosophy is based on sustainability, encapsulated in twelve fundamental principles:

- Prevention – avoid generating waste rather than treating or disposing of it after creation.
- Atom economy – design synthesis methods to incorporate as much of the starting materials as possible into the final product.
- Less hazardous synthesis – employ processes that use and generate substances with minimal toxicity.
- Designing safer chemicals – preserve chemical functionality while minimizing toxicity.
- Safer solvents and auxiliaries – avoid auxiliary substances (e.g., solvents) where possible or make them non-hazardous when required.
- Energy efficiency – minimize energy demands; perform reactions at ambient temperature and pressure when feasible.
- Renewable feedstocks – use renewable rather than depletable raw materials when are technically and economically viable.
- Reduce derivatives – avoid unnecessary derivatization steps that require extra reagents and generate waste.
- Catalysis – use catalytic reagents, which are more efficient than stoichiometric ones.

- Design for degradation – ensure that products break down into harmless substances at the end of their lifecycle.
- Real-time analysis – develop monitoring techniques to detect and prevent hazardous substances during processing.
- Inherently safer chemistry – select substances and process forms that reduce the risk of accidents such as explosions or releases.

Sustainable Alternatives to Conventional Pollutant Removal

The growing presence of pollutants — including heavy metals, organic compounds, pharmaceuticals, and emerging contaminants — poses significant environmental and public health risks. Traditional removal methods such as chemical precipitation, ion exchange, and membrane filtration often face limitations, including high costs, high energy demand, and the generation of secondary pollutants.

Recent research highlights the potential of non-conventional adsorbents as more sustainable alternatives. Materials such as nanocellulose, chitosan-based nanocomposites, and metal–organic frameworks (MOFs) have shown superior performance in terms of adsorption capacity, selectivity, and reusability, making them attractive for environmental applications (Akhtar et al., 2024).

Green Chemistry Solutions

1. Safer solvents and reaction conditions

Replacing hazardous solvents with safer alternatives is a major innovation in green chemistry. For example, water-based paints have replaced solvent-based coatings, eliminating toxic fumes and reducing air pollution without compromising performance. Running chemical reactions at ambient temperature and pressure further reduces energy consumption and minimizes hazards.

2. Renewable feedstocks

Green chemistry prioritizes feedstocks derived from renewable resources, such as agricultural by-products, over fossil fuels or mined materials. This reduces environmental impact, conserves non-renewable resources, and often results in more biodegradable products.

3. Catalysis and atom economy

Catalysts enable reactions to occur efficiently with minimal waste, often replacing stoichiometric reagents that are used in excess. At the same time, designing reactions for high atom economy ensures that the majority of input materials are incorporated into the final product.

4. Designing for degradation

Products are increasingly designed to degrade into harmless substances after use, reducing persistence in the environment and lowering hazardous waste management costs.

Biological Remediation of Heavy Metals

Rapid industrialization has intensified heavy metal contamination of soils worldwide, posing serious ecological and health threats. Removal and neutralization of these contaminants is now a global priority. Bioremediation — using microorganisms such as bacteria, microalgae, yeast, and fungi — is gaining attention as an eco-friendly and cost-effective alternative, particularly effective at low metal concentrations (Maqsood et al., 2022).

Often, integrated methods combining physicochemical and biological processes are used to achieve optimal results across the heavy metal treatment cycle. These approaches restore contaminated environments into healthier, life-supporting systems while minimizing environmental side effects.

Green Toxicology

Green toxicology merges the principles of green chemistry with toxicology to ensure chemical safety from the earliest stages of product design (Maertens et al., 2024). It employs modern, non-animal testing strategies, including *in silico* computational models, artificial intelligence predictions, and human cell-based assays, allowing for faster and more cost-effective hazard assessments compared to traditional animal testing.

Key elements of green toxicology include:

- Applying alternative, validated test methods.
- Incorporating safety considerations early in chemical design.
- Assessing life cycle impacts across supply chains.
- Prioritizing prevention over remediation.

Benefits of green chemistry and toxicology

For human health:

- Cleaner air and water through reduced hazardous emissions.
- Improved workplace safety in chemical industries.
- Safer consumer products and food.

For the environment:

- Reduced greenhouse gas emissions, smog formation, and ozone depletion.
- Minimized ecological disruption from chemical pollution.
- Lower need for hazardous waste disposal.

For economy:

- Higher reaction yields and lower raw material costs.
- Reduced waste disposal expenses.
- Increased plant efficiency and energy savings.
- Competitive advantage through eco-friendly product labelling.

Challenges and Future Directions

While green chemistry and toxicology offer clear benefits, their adoption faces barriers such as validation of new methods, regulatory acceptance, and institutional resistance to change. Future priorities should include:

- Expanding the portfolio of validated alternative test methods.
- Embedding green chemistry concepts into education and industrial practice.
- Creating policy incentives for sustainable innovation.
- Strengthening collaboration between chemists, toxicologists, and environmental scientists.

Innovative green methods represent a transformative pathway for reducing the toxic effects of contaminants on both human health and the environment. By integrating the principles of green chemistry, embracing biological remediation strategies, and applying green toxicology frameworks, we can design and implement safer, more sustainable chemical processes. This approach supports a long-term vision of a cleaner, healthier, and more resilient planet.

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# Chapter 4. THE ROLE OF HEALTHY NUTRITION AND THE APPROVED CONSUMPTION OF SAFE FOOD SUPPLEMENTS-INNOVATIVE METHODS OF APPROACH AND AWARENESS EVALUATION

## 4.1 Introduction

Nutrition plays a crucial role in maintaining health and preventing disease. Maximising the potential of plant resources—by selecting species rich in bioactive compounds and developing optimal forms for administration and consumption—offers a sustainable pathway to enhance human nutrition and prevent disease. Harnessing these natural assets in ways that preserve their active principles while ensuring bioavailability can complement modern dietary strategies and contribute to long-term health.

Nutrition remains a cornerstone of human health, influencing growth, immune function, cognitive performance, and the prevention of both infectious and chronic diseases. Yet, the global burden of malnutrition continues to be a pressing challenge, with one in nine people experiencing hunger and one in three classified as overweight or obese (Global Nutrition Report, 2020). This "double burden" of malnutrition—where undernutrition coexists with overweight and obesity—is now observed in many regions worldwide.

A balanced diet is essential for maintaining optimal physiological function. Key nutrients such as vitamins, minerals, dietary fiber, essential fatty acids, and amino acids are indispensable for health and well-being. However, modern dietary patterns, often dominated by processed foods, make it difficult for many individuals to meet these requirements through food alone. This gap has driven the increasing use of dietary supplements.

Dietary supplements—available as pills, capsules, powders, or liquids—can provide concentrated sources of nutrients, either derived from natural sources or synthetically produced. The global dietary supplement market was valued at approximately USD 151.9 billion in 2021, with more than 50,000 products available in the United States alone. While supplements can help correct specific nutrient deficiencies, they should not be considered a substitute for a balanced diet based on whole foods. Whole foods offer a synergistic combination of nutrients, phytochemicals, and dietary fiber, which cannot be replicated by isolated compounds.

The safety and regulation of dietary supplements vary globally. In the United States, the Food and Drug Administration (FDA) oversees labelling and safety but does not require pre-market approval, unlike pharmaceutical products. This regulatory gap raises concerns about the quality, efficacy, and potential adulteration of some supplements.

Addressing global nutrition challenges demands a multifaceted approach: improving access to affordable, nutrient-rich foods; reducing diet-related health inequalities; and ensuring consumer awareness regarding the safe and effective use of supplements. The economic costs of malnutrition are staggering—estimated at USD 3.5 trillion annually due to undernutrition and an additional USD 2 trillion linked to overweight and obesity (World Bank, 2023).

This chapter explores the pivotal role of healthy nutrition and the responsible consumption of safe dietary supplements. It will also highlight innovative strategies for awareness and education, integrating modern nutrition science with sustainable resource use to promote optimal health outcomes worldwide.

## 4.2 Fundamentals of Healthy Nutrition

The optimal capitalization of plant resources-through careful selection, preservation of bioactive compounds, and using effective forms of administration and consumption-anchors a dietary pattern that supports long-term health (WHO, 2023).

Essential Nutrients and Their Roles

A healthy diet supplies the six fundamental nutrient groups-proteins, carbohydrates, fats, vitamins, minerals, and water-each with distinct physiological functions such as tissue repair, energy provision, cellular structure, and metabolic regulation (Delight Medical & Wellness Center, n.d.). Water underpins thermoregulation and enables core biochemical reactions (Delight Medical & Wellness Center, n.d.).

Principles of a Balanced Diet

Dietary balance emphasizes variety and quality while matching energy intake to need:

- Plant-forward eating with fruits, vegetables, legumes, nuts, and whole grains boosts fiber and micronutrient intake (Heart and Stroke Foundation of Canada, n.d.; WHO, 2023).
- Whole grains (e.g., oats, brown rice, whole-wheat breads) are preferred over refined grains to increase fiber and micronutrients (Heart and Stroke Foundation of Canada, n.d.).
- Varied protein sources, prioritizing plant proteins and including fish and lean animal options, help meet amino-acid needs while supporting cardiometabolic health (Heart and Stroke Foundation of Canada, n.d.).
- Minimizing highly processed foods lowers excess sodium, added sugars, and trans fats (Heart and Stroke Foundation of Canada, n.d.; WHO, 2023).
- Portion awareness helps align calories with expenditure (Delight Medical & Wellness Center, n.d.).

Nutrient Density

Choosing foods that deliver high nutrients per calorie-such as vegetables, fruits, legumes, nuts, seeds, eggs, plain yogurt, and fatty fish-improves overall diet quality; some of these foods are energy-dense yet remain nutritionally advantageous in sensible portions (Healthline, 2024; Harvard T.H. Chan School of Public Health, n.d.).

Evidence-Based Dietary Guidelines

Consensus recommendations include:

- ≥400 g/day of fruits and vegetables (about five portions) (WHO, 2023).
- Free sugars <10% of total energy (WHO, 2023).
- Fat quality over quantity: limit saturated and trans fats; favor unsaturated fats (WHO, 2023; Cena and Calder, 2020).
- Make whole grains a plate quarter as a practical cue for meals (Heart and Stroke Foundation of Canada, n.d.).
- Diversify proteins with an emphasis on plant-based options (Heart and Stroke Foundation of Canada, n.d.).

Impact on Health

Adhering to these principles is linked with lower risk of cardiovascular disease, type 2 diabetes, and certain cancers, as well as reduced all-cause mortality (Harvard T.H. Chan School of Public Health, n.d.; Cena and Calder, 2020; World Cancer Research Fund, 2024). Diets modelled on these guidelines also improve intermediate markers such as blood pressure, lipids, and inflammation (Cena and Calder, 2020).

Challenges and Future Perspectives

Persistent barriers-food cost and access, marketing of ultra-processed foods, and limited nutrition literacy-impede adoption. Progress will require improving affordability and availability of healthy foods, tailoring advice to cultural and individual contexts, and using digital tools for personalized guidance and behavior change support (Harvard T.H. Chan School of Public Health, n.d.; WHO, 2023).

In summary, a nutrient-dense, minimally processed, plant-forward pattern-guided by evidence-based targets for fats, sugars, and food groups-offers a pragmatic foundation for disease prevention and lifelong health (WHO, 2023; Cena and Calder, 2020).

### 4.3 Food Supplements: Regulation, Safety, and Scientific Evaluation

Food supplements, also called dietary supplements, are products designed to complement the diet and provide nutrients that may not be consumed in sufficient amounts from regular food. The global dietary supplement market was valued at approximately USD 151.9 billion in 2021, reflecting their widespread use (NIH, 2013). Despite their popularity, the safety and efficacy of these products remain subjects of ongoing scientific investigation and regulatory oversight.

Regulatory Framework

In the United States, dietary supplements are regulated under the Dietary Supplement Health and Education Act (DSHEA) of 1994, which defines them as products containing one or more dietary ingredients such as vitamins, minerals, herbs, amino acids, enzymes, or metabolites (ODS, 1994).

Unlike pharmaceutical drugs, dietary supplements do not require pre-market approval from the U.S. Food and Drug Administration (FDA). Instead, manufacturers are responsible for ensuring that their products are safe before they are marketed (FDA, 2023). The FDA can take action to remove unsafe products only after they reach the market (NIH, 2013).

## Safety Considerations

While many dietary supplements are considered safe when used appropriately, potential risks exist:

- Quality Control - manufacturers must follow current Good Manufacturing Practices (cGMPs) to ensure product identity, purity, and strength, although contamination and mislabelling can still occur (FDA, 2023).
- Interactions - certain supplements can interact with prescription medications, potentially leading to harmful effects (NIH, 2013).
- Overuse - excessive intake of some nutrients can cause toxicity; for example, high vitamin E consumption has been associated with increased prostate cancer risk in men (NIH, 2013).
- Lack of Evidence - many supplements have limited or inconclusive clinical data supporting their claimed health benefits (Harvard Health Publishing, 2023).

## Scientific Evaluation

Research has explored the potential preventive and therapeutic roles of dietary supplements, focusing on micronutrients, bioactive plant compounds, probiotics and prebiotics, polyunsaturated fatty acids (especially omega-3 EPA and DHA), phytosterols, polyphenols, and dietary fibers (CorCon International, 2023).

Evidence-based supplements-those supported by rigorous clinical studies-provide more reliable information regarding safety and efficacy (CorCon International, 2023).

## Regulatory Measures for Safety

Regulatory agencies have implemented measures to improve supplement safety:

- Adverse Event Reporting - manufacturers must report serious adverse events to the FDA (FDA, 2023).
- New Dietary Ingredient Notifications - new ingredients must be reported to the FDA at least 75 days before marketing, with safety data provided (FDA, 2023).
- Labeling Requirements - labels must list all ingredients and include safety warnings (FDA, 2023).
- Health Claim Restrictions - only certain health claims are allowed on product labels (FDA, 2023).

Dietary supplements can help address nutrient deficiencies and support health when used appropriately. However, they should not replace a balanced diet and must be chosen carefully to avoid safety risks. Consulting a healthcare professional is essential, especially for individuals with chronic conditions or those taking medications. Continued research and strong regulatory oversight remain crucial to ensuring product safety and consumer protection.

### 4.4 Innovative Approaches to Nutrition Education

Addressing the global challenges of malnutrition and poor dietary habits requires creative and engaging nutrition education strategies. Traditional lecture-based models often fail to inspire lasting behavioral change, making it necessary to adopt approaches that are more interactive, participatory, and tailored to diverse audiences. In recent years, several innovative methods have gained attention for their ability to improve knowledge retention and encourage healthier food choices.

Gamification and Interactive Tools

Gamification-the integration of game-like elements into educational activities-has shown particular promise in nutrition education, especially for children and young adults (Gkintoni 2024). By making learning enjoyable and competitive, gamification increases engagement and reinforces positive behaviors. Examples include:

- Interactive challenges and quizzes on healthy eating habits
- Simulations demonstrating the outcomes of different dietary choices
- Food label reading games and nutrition-themed bingo
- Role-playing activities that mimic real-life nutrition scenarios

These interactive formats can transform nutrition education from a passive learning experience into an active, memorable process that supports long-term healthy eating habits (Gkintoni 2024).

Hands-on and Experiential Learning

Experience-based learning has been shown to produce greater improvements in dietary behaviors compared to traditional classroom instruction (Jung et al., 2015). Practical activities create opportunities for participants to directly engage with food and nutrition concepts. Such methods include:

- Action-learning activities and interactive games
- Sensory-based workshops inspired by the SAPERE approach
- Cooking demonstrations and skill-building sessions
- Food tasting activities to increase exposure to healthy options

Research indicates that children exposed to these hands-on programs are more likely to try new foods, particularly fruits and vegetables, and adopt healthier eating patterns (Jung et al., 2015).

Community-Based Approaches

Community-driven nutrition education focuses on empowerment and participation, integrating local knowledge and cultural practices into dietary improvement strategies (FAO 2024). Common practices include:

- Training community leaders to deliver nutrition messages
- Incorporating indigenous food traditions into health promotion
- Addressing social and economic barriers that influence food choices

Such initiatives build local capacity, enhance program relevance, and contribute to sustainable, community-wide improvements in nutrition.

Technology-Enhanced Learning

Digital tools and online platforms have expanded the reach and adaptability of nutrition education. Innovations include:

- Mobile apps that host virtual nutrition challenges
- Online leaderboards to track personal or group progress
- Interactive e-learning modules designed for health professionals (BMJ 2020)

Technology-based solutions are particularly effective for engaging younger, tech-oriented audiences and can provide personalized, real-time feedback to support dietary changes.

Innovative Educational Tools

Some specialized resources have been developed to make nutrition learning both fun and impactful:

- Nutricartes®: An interactive card game teaching core principles of healthy eating
- SAPERE Method: Sensory-focused workshops that help children explore and accept a wider variety of foods (Jung et al., 2015)

Both approaches have demonstrated success in improving nutrition knowledge and encouraging healthier dietary choices.

In summary, modern nutrition education is evolving towards strategies that actively engage learners through play, participation, community involvement, and technology. When combined, these innovative approaches can create comprehensive programs capable of influencing both individual behaviors and broader public health outcomes.

### 4.5 Evaluating Nutritional Awareness

Evaluating nutritional awareness is essential for measuring the effectiveness of nutrition education initiatives and identifying areas for improvement in public health interventions. This section outlines established, and emerging tools used to assess nutrition knowledge, ranging from validated questionnaires to technology-driven innovations.

Standardized Questionnaires

General Nutrition Knowledge Questionnaire (GNKQ)

The GNKQ, developed by Parmenter and Wardle (1999), is a widely used instrument assessing knowledge in four domains: dietary recommendations, nutrient content of foods and food groups, healthy food choices, and the links between diet, disease, and body weight. This questionnaire has been validated and adapted for various cultural contexts. A revised version (GNKQ-R) was created by Kliemann et al. (2016) to align with updated dietary guidelines and improve applicability across populations.

Nutritional Knowledge Test (NKT)

The NKT, developed by Feren et al. (2011), evaluates knowledge in areas such as energy intake and metabolism, nutrient composition, sweeteners and oral health, food knowledge, and nutrition-related terminology. It has been applied in diverse populations, including healthcare professionals and students, to measure baseline knowledge and the impact of training programs.

Item Response Theory (IRT) Analysis

Item Response Theory (IRT) provides a refined approach to nutrition knowledge evaluation by selecting questions with high discriminatory power, analyzing question difficulty, and generating precise scores that can be correlated with dietary behavior outcomes. Matsumoto et al. (2017) used IRT to design a validated scale assessing shokuiku (food and nutrition education) knowledge among Japanese elementary school children, resulting in more accurate evaluations compared to traditional scoring methods.

Comprehensive Surveys

FAO Guidelines for Assessing Nutrition-Related Knowledge, Attitudes, and Practices (KAP)

The Food and Agriculture Organization (FAO, 2014) developed standardized guidelines for conducting KAP surveys to measure not only knowledge but also attitudes and behaviors related to nutrition. These guidelines provide a robust framework for designing, implementing, and analyzing assessments, ensuring comparability across regions and programs.

Biomarkers and Dietary Intake

To validate nutrition awareness assessments, researchers often compare knowledge scores with objective health and dietary indicators. Biomarkers can include serum vitamin and mineral levels, anthropometric measures such as BMI and waist circumference, and biochemical markers like lipid and glucose profiles. Dietary intake is frequently measured using 24-hour recalls, food frequency questionnaires (FFQs), and diet diaries. Spronk et al. (2014) found that higher nutrition knowledge was associated with greater fruit and vegetable consumption and lower fat intake among Australian adults.

Innovative Approaches

Modern methods are expanding the ways nutritional awareness is evaluated:

Technology-Enhanced Assessments

Mobile applications, such as Nutricise (Hsu et al., 2018), offer interactive quizzes and healthy eating tips, while web-based platforms provide real-time scoring and personalized feedback.

Gamified Evaluation Tools

Quizzes incorporating points, leaderboards, and badges can increase engagement, and scenario-based challenges requiring virtual food choices reinforce practical skills.

Virtual Reality Simulations

Immersive supermarket or kitchen environments assess the real-world application of nutrition knowledge. Mack et al. (2020) demonstrated that gamified mobile applications can improve both dietary habits and knowledge retention in young adults.

Factors Influencing Nutritional Awareness

Research indicates that nutritional knowledge is shaped by several factors, including sociodemographic variables such as age, gender, income, and occupation; education level; prior exposure to nutrition education programs; and cultural and environmental influences. Studies by Spronk et al. (2014) and Hendrie et al. (2008) highlight the significance of these factors in determining baseline awareness and the success of nutrition interventions.

Evaluating nutritional awareness requires a multi-method approach, combining validated questionnaires such as the GNKQ and NKT with modern innovations including gamification, mobile technology, and biomarker validation. This integrated strategy offers public health professionals a more complete understanding of nutrition literacy, enabling them to design targeted interventions that can positively influence dietary behavior and health outcomes.

### 4.6 Effectiveness of Innovative Nutrition Interventions

The effectiveness of innovative nutrition interventions has been widely studied, with research exploring diverse strategies aimed at improving nutritional knowledge, behaviors, and health outcomes in different populations. Findings indicate promising benefits in some contexts, while also highlighting areas where further investigation is needed.

Technology-Enhanced Interventions

Digital and gamified strategies have shown considerable potential in enhancing nutrition education and promoting healthy behaviors. For example, Han and colleagues reported that health promotional board games achieved a large improvement in nutrition knowledge (Cohen’s d = 0.82) and a moderate improvement in healthy behaviors (Cohen’s d = 0.38). Their research also found that digital games such as Fit Food Fun and ETIOBE Mates led to short-term gains in nutrition-related knowledge among children, while the Kaledo board game maintained improved nutrition knowledge even 6 and 18 months after the intervention (Han et al., 2020).

However, the overall effect of technology-assisted programs on actual food choices remains uncertain. A scoping review and meta-analysis by Chew and colleagues (2023) found that while interactive, technology-based interventions supported weight loss goals, they did not consistently improve dietary choices.

Targeted Interventions for Specific Populations

Pregnant Women and Early Life Interventions

The Nutrition Now project focuses on pregnant women and parents of infants aged 0–2 years, using technology to implement evidence-based early life nutrition interventions. As highlighted by Benajiba and colleagues (2022), such targeted approaches can yield substantial benefits; for example, in The Gambia, distributing locally produced nutritional biscuits during pregnancy reduced the prevalence of low birth weight by 39% and increased mean birthweight by 136 g.

Adolescents

Mancone and colleagues (2024) evaluated a multifaceted food literacy program for adolescents that combined workshops, interactive activities, and digital tools. This intervention significantly enhanced nutrition literacy, reduced emotional eating, and improved self-regulation in eating behaviors.

Adults with Chronic Conditions

A systematic review and meta-analysis by Barnett and colleagues (2023) examined dietary interventions delivered through digital health platforms to adults with diet-related chronic diseases. Their findings showed modest but significant improvements in Mediterranean diet adherence, fruit and vegetable consumption, sodium reduction, waist circumference, body weight, and hemoglobin A1c levels.

Nutrition Social Behavior Change Communication (NSBCC)

NSBCC strategies have proven effective in improving infant and young child feeding practices. A meta-analysis by Mahumud and colleagues (2022) demonstrated a significant increase in exclusive breastfeeding rates (odds ratio = 1.73, $p < 0.001$) and positive effects on key child growth indicators, including height-for-age, weight-for-height, and weight-for-age z-scores.

Combined Exercise and Nutrition Interventions

For older adults who are frail or pre-frail, combined exercise and nutrition interventions can be highly beneficial. Han and colleagues (2020) reported significant reductions in frailty scores

(SMD = 0.25) and improvements in short physical performance battery scores (MD = 0.48), indicating better functional health.

Overall, innovative nutrition interventions—especially those leveraging technology—hold promise for improving nutritional knowledge, promoting healthy behaviors, and enhancing health outcomes. However, their effectiveness varies by target population, program design, and intended outcomes. Further research is required to evaluate long-term impacts, refine implementation strategies, and address variations in results across different settings.

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