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Sustainable and nutrition-sensitive food systems for healthy diets and prevention of malnutrition in Europe and Central Asia

Editors
Cheng Fang and Mirjana Gurinović

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Table of contents

Foreword

Globally, we have not been on track to meet our commitments to end world hunger and malnutrition in all its forms by 2030. Now, the COVID-19 pandemic has made this significantly more challenging. In the past two decades in the Europe and Central Asia (ECA) region, many countries have made significant progress in combating undernourishment. However, despite that hunger is not a major issue at the country level in the region, the prevalence at the moderate or severe level is quite high. A lack of regular access to nutrition and sufficient food has put many people at greater risk of malnutrition and poor health – not only in low- or middle-income countries, but also in developed countries. A large majority of countries in the region risk not meeting the targets set by the 2030 Agenda for Sustainable Development. In particular, there are alarmingly high – and increasing – rates of adult obesity in most countries in the region.

Healthy and nutritious foods are expensive to many households in the region, resulting in poor diets that contribute to obesity, malnutrition and diet-related non-communicable diseases. This nutrition transition poses a major challenge for the public sector in terms of its ability to anticipate and mitigate possible negative nutritional and health impacts. Major drivers of transformation in food consumption and diets include rising incomes, urbanization, trade liberalization and market integration of food chains, and changes in food cultures and consumer preferences. Considering the complexity of the underlying causes of malnutrition, these challenges need to be addressed in a multisectoral approach. However, malnutrition is still considered a health sector issue in the ECA region, and there is a lack of supportive policies – particularly in Central Asia and the Caucasus – in sectors such as agriculture, food processing and distribution, food marketing and trade, environment, and education.

A sustainable food system is a system that delivers food security and nutrition for all in such a way that the economic, social and environmental bases that help ensure food security and nutrition for future generations are not compromised. A key outcome of sustainable food systems is the provision of sufficient, safe, healthy, nutritious and affordable food that meets the nutritional needs of all, including children and adolescents. Countries will need to reorient their food and agricultural policies towards more nutrition-sensitive investments and social protection to adapt and transition to healthy dietary production and trade systems. Specific policy interventions can promote mindset and behavioural changes among all food systems actors, bring about desirable shifts in food production and consumption patterns, and lead to more inclusive and equitable agrifood systems.

The roles of agriculture and the rural sector remain unclear, largely because of a limited understanding of the nutritional impacts of food systems. A comprehensive situation and policy analysis of the region is needed as a basis for planning effective policy responses. This book aims to contribute knowledge and understanding regarding the nutritional impacts of food systems. The ECA region contains great diversity in income levels and in food insecurity, malnutrition and other socioeconomic deprivations. This book supports countries suffering from various forms of malnutrition (undernutrition, micronutrient deficiencies and overnutrition) in strengthening their evidence base for addressing nutrition-related challenges from the food system perspective. In so doing, this book can serve as a basis for multistakeholder engagement and stakeholder dialogue.

This book aims to promote a holistic agrifood systems approach to achieving food security and improved nutrition in all its forms, including the promotion of nutritious food and increasing access to healthy diets for better nutrition.

Foreword-do we need signature from:

Vladimir Rakhmanin

Assistant Director-General and Regional Representative for Europe and Central Asia

Food and Agriculture Organization of the United Nations

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Editors’ note and acknowledgments

The nature of food insecurity and malnutrition have considerably changed in the ECA region during recent decades. In three main sections, this book addresses the role of food systems in the transition of diets and the prevention of malnutrition in the ECA region. **Part I** provides an introduction and executive summary of the book and shares key points for policy actions that can help bring about sustainable and nutrition-sensitive food systems for healthy diets and the prevention of malnutrition. **Part II** shares normative studies and a regional-level review, and **Part III** shares case studies from selected ECA countries.

Sustainable and nutrition-sensitive food systems for healthy diets and prevention of malnutrition in Europe and Central Asia was produced under the direction and guidance of editors **Cheng Fang** and **Mirjana Gurinović**, who jointly developed and decided on the outline and content of the publication and coordinated the preparation of the book.

Several studies reported in this book were carried out by the FAO Regional Office for Europe and Central Asia for a regional project on food system analysis titled “Programme support for the role of food systems in the transition of diets and prevention of malnutrition in the ECA region.” Under this project, a workshop titled “Awareness raising on the food system approach to address malnutrition” was held 27 October 2020 in Belgrade, Serbia.[1] During the workshop, drafted papers were presented and awareness-raising activities carried out to promote a better understanding of the food system approach in the prevention of malnutrition in the region. The event served as a platform for multistakeholder engagement and dialogue, including the sharing of methodologies, tools and lessons learned to strengthen beneficiary countries’ evidence base for addressing nutrition-related challenges from the food system perspective.

A great many people have contributed to this book. Special thanks go to all of the authors for their excellent collaboration and valuable contributions to this book. Their names are listed under each chapter and here in alphabetical order: Meline Beglaryana, Eleonora Dupouy, Cheng Fang, Maria Glibetić , Mirjana Gurinović, Ewa Halicka, Raimund Jehle, Mary Kenny, Youngseo Kim, Marija Knez, Hanna Lienivova, Aleksandra Martinovska Stojcheska, Jelena Milešević, Dragan Milićević, Marina Nikolić, Keigo Obara, Tamara Ostashko, Davit Pipoyana, Jelka Pleadin, Marija Ranić, Pieter van’t Veer and Milica Zeković.

Special thanks also go to Lily G. Blain, FAO consultant, for her high-quality English editing during the preparation of the draft, and to FAO consultant Matthew D. Anderson, who provided excellent final editing and proofreading. Data collection and analysis were provided by FAO assistants Junying Lin, Jiawen Zhao, Pavel Kiparisov and Fanni Zsilinszky.

Editors
Cheng Fang and Mirjana Gurinović

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[1] For more information on this workshop, which was held online, see https://www.capnutra.org/events/.

List of tables, figures and boxes

List of acronyms and abbreviations

ADB	Asian Development Bank
AL	Albania
AMR	antimicrobial resistance
BA	Bosnia and Herzegovina
BG	Bulgaria
BMI	body mass index
CA	Central Asia
CAP	Common Agricultural Policy
CAPNUTRA	Capacity Development in Nutrition Network
CFS	Committee on World Food Security
CHD	coronary heart disease
CIS	Commonwealth of Independent States
CIS FTA	Commonwealth of Independent States Free Trade Area
COICOP	Classification of Individual Consumption by Purpose
COSI	European Childhood Obesity Surveillance Initiative
COVID-19	Coronavirus Disease 2019
CSO	civil society organization
CZ	Czechia
DALY	disability-adjusted life years
DAP	Diet Assess and Plan tool
DES	dietary energy supply
DRVs	National Dietary Reference Values
EAEU	Eurasian Economic Union
ECA	Europe and Central Asia
ECO	Economic Cooperation Organization
EE	Estonia
EFSA	European Food Safety Authority
ERC	FAO Regional Conference for Europe
EU	European Union
EuroFIR	European Food Information Resource Network
FAO	Food and Agriculture Organization of the United Nations
FAOSTAT	Food and Agriculture Organization Corporate Statistical Database
FBDGs	food-based dietary guidelines
FBS	Food Balance Sheet
FCDB	Food Composition Data Base
FDI	foreign direct investment
FF	food fortification
FfA	Framework for Action
FIES	Food Insecurity Experience Scale
FLW	food loss and waste
FNH-RI	Food, Nutrition and Health Research Infrastructure
FS	food systems
G	gram
GBD	Global Burden of Disease study

GDP	gross domestic product
GHG	greenhouse gas emissions
GNI	gross national income
GSP	Global Soil Partnership
HBS	Household Budget Survey
HFSS	high fat, sugary and salty foods
HLPE	High-Level Panel of Experts
HR	Croatia
HU	Hungary
ICN2	Second International Conference on Nutrition
IFAD	International Fund for Agricultural Development
I-TF	industrially produced trans fats
KGS	Kyrgyz som
LT	Lithuania
LV	Latvia
MD	Republic of Moldova
ME	Montenegro
MSB	monthly social benefit
NCD	non-communicable disease
NMK	North Macedonia
NoU	number of undernourished
OECD	Organisation for Economic Co-operation and Development
PHN	public health nutrition
PL	Poland
PoU	prevalence of undernourishment
PPP	purchasing power parity
PRIs	population reference intakes
R&I	research and innovation
RIs	reference intake
RO	Romania
RS	Serbia
SPIs	science–policy interfaces
SDGs	Sustainable Development Goals
SFN	school food and nutrition
SFS	sustainable food systems
SHD	sustainable and healthy diets
SL	Slovenia
SK	Slovakia
SMART	specific, measurable, achievable, relevant, time-bound
TFA	trans-fatty acids
TR CU	Technical Regulations of the Customs Union
UN FSS	United Nations Food Systems Summit
UN	United Nations
UNICEF	United Nations Children’s Fund
USD	United States dollar
WFP	World Food Programme
WHA	World Health Assembly
WHO	World Health Organization
UNICEF	United Nations International Children’s Emergency Fund
USAID	United States Agency for International Development
WTO	World Trade Organization

General introduction and context of the book

Cheng Fang and Mirjana Gurinović

Global agrifood systems are facing a range of challenges to healthy diets and the prevention of malnutrition from climate change, resource scarcity, biodiversity loss, soil degradation, a growing and ageing population, urbanization, food waste and food poverty, and conflict. The *State of Food Security and Nutrition in the World* report has shown that the number of undernourished has been slowly increasing for several years, and at the same time the number of overweight and obese people all over the world is increasing at an alarming rate (FAO *et al.*, 2020).

The *Regional Overview of Food Security and Nutrition in Europe and Central Asia 2021* report indicated that the ECA region has a low prevalence of undernourishment and severe food insecurity when compared with the worldwide state of food insecurity (FAO, 2021). However, reductions in the numbers of people affected by hunger and severe food insecurity in some countries of the region have slowed since 2014. The COVID-19 pandemic has worsened food security and nutrition, adding more than 10 million people in 2020 alone to those who are moderately or severely food insecure (HLPE, 2017).

The situation is more challenging in reducing various forms of malnutrition in the region. In general, the ECA region is making progress in reducing malnutrition, but it is not on track regarding childhood overweight, adult obesity, anaemia and exclusive breastfeeding. The triple burden of malnutrition – undernutrition, overweight and obesity, and micronutrient deficiencies – is present to varying degrees in all countries of the region. Poor dietary diversity, inadequate dietary patterns and frequent consumption of foods of high energy density and minimal nutritional value are contributing to malnutrition and non-communicable diseases in later life. The alarming trend towards overweight and obesity in the region needs to be reversed. Similar to other regions, large sections of the population in vulnerable groups (including women, children and adolescents) can neither afford sufficient nutritious foods nor access healthy diets.

Changes are happening in the nutrition-related policy environment, with an increasing number of countries taking regulatory action to improve the food environment promote healthy diets and better nutrition, but current progress in achieving the related Sustainable Development Goals (SDGs) and the global nutrition- and diet-related non-communicable disease (NCD) targets for 2025 and 2030 is not sufficient. Given the complexity of agrifood systems, dietary behaviours, and the wide range of factors that influence diets, improving diets requires the active collaboration of a variety of actors throughout agrifood systems, along with policies targeting multiple sectors. Recent major international policy process in nutrition has been generated by FAO and the World Health Organization (WHO) in the Second International Conference on Nutrition (ICN2) Rome Declaration on Nutrition and its Framework for Action (FfA) and policy recommendations, which acknowledged that "current food systems are being increasingly challenged to provide adequate, safe, diversified and nutrient-rich food for all that contribute to healthy diets" (FAO/WHO, 2014a, 2014b).

The way food is produced and consumed is taking a toll on the environment and natural resource base, with concerns over the loss of biodiversity, pressures on water, deforestation, increase in greenhouse gas emissions, and one-third of all food being lost or wasted. Food systems need to be transformed for food to be produced sustainably and inclusively.

Sustainable, healthy diets are essential to the achievement of all of the SDGs. The SDG vision for nutrition is to end all forms of malnutrition, address nutritional needs throughout the life course, give universal access to safe and nutritious food that is sustainably produced, and ensure universal coverage of essential nutrition actions. Beyond that, the High-Level Panel of Experts on Food Security and Nutrition has stated that "a food system gathers all the elements (environment, people, inputs, processes, infrastructures, institutions, etc.) and activities that relate to the production, processing, distribution, preparation and consumption of food, and the output of these activities, including socioeconomic and environmental outcomes" (HLPE, 2017). Food supply chains, food environments and consumer behaviour are the key connection points for nutrition and health.

As stated in the United Nations Decade of Action on Nutrition 2016–2025 and the Decade of Action to achieve the SDGs by 2030, many of the world's food systems are fragile and not fulfilling the right to adequate food for all. The United Nations Food Systems Summit 2021 was intended to mark a decisive step in the Decade of Action towards achieving the SDGs by 2030. A growing number of stakeholders, including governments, policymakers and corporate businesses, acknowledge the crucial role played by food systems in delivering sustainable growth and food systems transformation.

The current COVID-19 pandemic and the measures taken to reduce its spread have disrupted food environments around the world. Unhealthy diets leading to overweight and obesity are the leading cause of NCDs, including diabetes, heart disease, cancer and chronic respiratory disease. Lockdowns, policy responses and COVID-19 itself are showing an evolving impact on both external and personal food environment domains. There is a need for the development of effective pandemic policy responses and actions to mitigate changes, improve food environments and build resilient food systems that incorporate healthy nutrition.

To improve contributions to healthy diets and ensure the transformation of food systems, it is necessary to identify and evaluate the state of various elements of food systems and related needs at national, regional and global levels. This understanding can aid in evidence-based policy- and decision-making and help create mechanisms for monitoring and evaluating the progress in food systems transformation. The Food Systems Dashboard is one example (Fanzo *et al.*, 2020).

The roles of agriculture and the rural sector in addressing malnutrition remain unclear, largely due to a limited understanding of the nutritional impacts of food systems globally and in the ECA region. A comprehensive situation and policy analysis of the region is needed as a basis for planning effective policy responses.

To see future progress in strengthening food and nutrition security, we also need to create adequate targets and indicators for monitoring – indicators that cover the whole food system and reflect overall outcomes. Measuring progress will demonstrate momentum towards future-proofing European food systems in a sustainable, resilient, responsible, diverse, competitive and inclusive manner.

The chapters of this book represent a normative and empirical contribution to the development of sustainable and nutrition-sensitive food systems for healthy diets and the prevention of malnutrition in Europe and Central Asia.

Book contents

The book is divided into three parts.

Part I starts with executive summary of the book and then shares key points for policy actions towards food systems transformation for healthy diets and the prevention of malnutrition in Europe and Central Asia, by Mirjana Gurinović, Jelena Milešević and Cheng Fang. This section shares an introduction to food systems and offers a summary of regional-level analysis regarding the development of sustainable and nutrition-sensitive food systems, including findings from Albania, Armenia, Bosnia and Herzegovina, Bulgaria, Czechia, Estonia, Hungary, Kyrgyzstan, Latvia, Lithuania, Republic of Moldova, Montenegro, North Macedonia, Poland, Romania, Serbia, Slovenia and Ukraine. This executive summary is an overview of food security and nutrition, food availability for consumption, and pathways towards sustainable and nutrition-sensitive food systems transformation for healthy diets and the prevention of malnutrition in the selected case countries of Armenia, Kyrgyzstan, North Macedonia, Poland, Ukraine and Western European countries. It concludes with key points for policy actions based on the comparative analyses of the situations in the case countries.

Part II contains four chapters:

Chapter 1, by Mirjana Gurinović, Jelena Milešević, Milica Zeković, Maria Glibetić, Marina Nikolić, Raimund Jehle, Eleonora Dupouy, Cheng Fang, Keigo Obara, Marija Ranić, Marija Knez and Pieter van't Veer, discusses challenges and opportunities related to support for food systems transformation for healthier diets in central and southeastern Europe. The objective of this review is to assess, evaluate and recommend priorities in policy development that are most relevant to sustainable food systems for healthy diets in the countries of central and southeastern Europe.

Chapter 2, by Eleonora Dupouy, describes food safety in the context of accelerating the flow of the food supply and the dynamic change of food systems. This chapter highlights the polyvalent importance of food safety and the enhancements in three food safety-supportive areas that may benefit the ongoing accelerations in global megatrends and dynamic changes in food systems, bringing into focus food safety governance, emergency response preparedness, and the professional training and education of agrifood system specialists.

Chapter 3, by Dragan Milićević and Jelka Pleadin, studies the incidence of mycotoxins in southeastern European countries and the implications for the food supply chain. This chapter provides insight into fungal sources of major mycotoxins, their ecology, occurrence in foodstuffs, toxicity, significance to human health, methods of analysis, governing regulations, and strategies to manage pertinent risks. In addition, Chapter 4 reviews the impacts of climate change on mycotoxin contamination of feed and food.

Chapter 4, by Keigo Obara, provides a detailed analysis of the nutrition transition in Central Asia from food systems perspectives. This chapter provides an overview of the changing nature of food and nutrition security in the countries of Central Asia. A food systems framework was used for understanding the dynamic transitions in diets, food affordability, and food environments experienced in Central Asia.

Part III contains six country case studies, with reviews of national food security and nutrition, food availability, composition of food consumption, and lessons learned to strengthen the countries' evidence base for addressing nutrition-related challenges from the food system perspective.

The ECA region is heterogeneous in terms of the composition of countries and their economic structures, climate conditions, and rates of economic growth and food systems transitions. This book covers case studies from countries with various food insecurity and malnutrition situations, with some countries suffering from the triple burden of malnutrition (undernutrition, micronutrient deficiencies and overnutrition) and others mainly with obesity issues. The results will be useful in strengthening countries' evidence bases for addressing nutrition-related challenges from the food system perspective in various situations. The countries were selected from four country groups:

- Group 1: undernutrition and micronutrient deficiencies: Kyrgyzstan
- Group 2: triple burden of malnutrition: Armenia, North Macedonia and Ukraine
- Group 3: overnutrition: Poland
- Group 4: good practices: Western Europe

The case studies examine the potential effects of various policy options throughout the food system, in particular policies in agriculture, food marketing and trade, social protection, gender, foreign direct investment in the food industry, nutrition, environment, education and more. Good practices and lessons learned are summarized, and the results will serve as a basis for multistakeholder engagement and stakeholder dialogue.

The six case studies are presented as separate chapters:

- Armenia, by Meline Beglaryana and Davit Pipoyana
- Kyrgyzstan, by Kanat Tilekeyev, Michael Onah, Mariia Iamshchikova and Zalina Enikeeva
- Ukraine, by Tamara Ostashko and Hanna Lienivova
- North Macedonia, by Aleksandra Martinovska Stojcheska
- Poland, by Ewa Halicka
- Western European countries, Youngseo Kim, Cheng Fang and Mary Kenny

Each country case study chapter covers the following areas:

- an introduction and background of food insecurity and malnutrition, with a review of the country's historical trends and statuses in all forms of malnutrition;
- analyses of the transformation of dietary change and food systems based on the FAO country food balance sheets and based on household survey data, identifying the gaps and challenges of dietary changes and sharing good practices;
- a review of the market and trade structure and policies to identify how policies may address the key food systems issues identified;
- a review of the agricultural and food production systems structure and identification of the elements of existing production that contribute to less desirable nutritional outcomes, undernutrition, overweight, obesity and diet-related NCDs and how have they evolved over time;
- a review of the food processing and marketing structure and how it has changed over time, and an overview of the policies and drivers for the change (such as food additives, food fortification, advertising practices, taxation on foods of high energy density and minimal nutritional value, food price policies for promoting healthy diets, and food labelling and public procurement);
- a review of consumer demand, awareness, school food, education and social protection, food-based dietary guidance and rural–urban food systems; and
- a review of cross-cutting issues, such as nutrition-sensitive value chains, pesticides and soil health.

The case study for Western Europe provides an overview of the nutritional approach of countries of lesser concern in food security and nutrition (but with rising overnutrition issues) to mitigate the predominant nutritional problem of the ECA region. This chapter analyses the successful nutritional policies, reflecting on good practices for the future in high-income countries. Among the less-concerned group, Finland was selected regarding school food and nutrition education, Denmark and Austria for public food procurement, and France and the Netherlands for urban food systems. Last, the city of Rome, Italy, was chosen for an in-depth analysis of integrated nutritional policy.

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Chapter 3

Mycotoxin incidence in southeastern European countries: implications for the food supply chain

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Table of Contents

Abstract

Mycotoxins are chemical hazards of microbiological origin, produced mainly by filamentous fungi such as secondary metabolites. Fungal and mycotoxin food contamination results in lower product quality and nutritional losses and can significantly compromise food safety, especially in low- and middle-income countries. Analyses of food and feed carried out in southeastern European countries in recent decades have revealed the presence of mycotoxins, such as aflatoxins (AFs), ochratoxin A (OTA), fumonisins (FUMs), type A trichothecenes (TCTs), T-2 and HT-2 toxin (T-2/HT-2), type B TCTs, deoxynivalenol (DON), patulin (PAT) and Alternaria toxins, with varying distribution patterns and in variable concentrations, dependent on the affected region and agricultural season. Thus, mycotoxin contamination of food and feed has a considerable impact on food safety, regional and international trade, national economies and public health. The role of mycotoxins has been recognized in the etiological background of several diseases, particularly non-communicable ones, including malignancies. Furthermore, mycotoxin contamination directly reduces food availability and increases its costs, contributing to hunger, malnutrition and growth impairment. Due to its negative impacts on public health and economy, mycotoxin contamination will continue to be a large public health challenge that must be addressed by producers, regulatory agencies, the research community and consumers. This review aims to provide an up-to-date overview of the prevalence of mycotoxins in food and feed in the countries of southeastern Europe and to discuss their impacts on food supply chains and food system sustainability in these countries.

Keywords: *mycotoxins, southeastern European countries, prevalence, public health, sustainable food systems*

Introduction

Food safety assurance, as a prerequisite for the prevention of hunger and malnutrition, is one of the major issues that still needs to be addressed to protect human health and ensure global economic development. According to data released by the World Health Organization (WHO), the number of undernourished people reached 821 million in 2017, and the rate of undernourished people continues to be exacerbated, particularly in the Global South (WHO, 2011). Undernourished adults and children may contract multiple acute or chronic diseases, such as developmental disorders, unmet genetic potential, overall poor health and shorter life expectancy as a result of poor dietary quality and low nutrient intake. Therefore, food safety was declared as the key policy objective of the 2030 Agenda for Sustainable Development (Sustainable Development Goal 2) adopted by the United Nations in 2015 (Dupouy and Gurinović, 2020). The problem of food and feed contamination by food-borne fungi and the hazard of consuming contaminated foods has been recorded worldwide throughout the history of humankind. Based on data provided by numerous food safety authorities, mycotoxin contamination of food and feed is more common in low- and middle-income countries and has a considerable impact on food safety systems, regional and international trade, and the global economy (Milićević and Nedeljković-Trailović, 2021). Mycotoxins are defined as a structurally heterogeneous group of secondary metabolites produced by a high number of filamentous fungal genera of microscopic size, which can contaminate staple foods and feeds during growth, after harvesting, during storage, during transportation, and during processing. Their common low-level co-occurrence in food and feed jeopardizes human and animal health and causes major economic losses, which can be felt by the likes of farmers, the food industry, international supply chains and society as a whole. On top of the most important mycotoxins, such as aflatoxins (AFs), ochratoxin A (OTA), trichothecenes (TCTs) of type A and B, including T-2 and HT-2 toxins (T-2/HT-2), fumonisins (FUMs), zearalenone (ZEA), deoxynivalenol (DON), patulin (PAT), and Alternaria toxins (AT), some other mycotoxin groups, such as "modified," "masked" and newly emerging mycotoxins (moniliformin, enniatins, beauvericin and fusaproliferin) have also attracted a lot of attention from the research community (Jajić *et al.*, 2019). The term "modified mycotoxins" refers to any mycotoxin whose structure has been changed during the chemical/biochemical reaction taking place in herbal or animal fungi or during food processing (Rychlik *et al.*, 2014). "Masked mycotoxins" are a group of mycotoxins produced during detoxication reactions run by plants in an attempt to neutralize native mycotoxins (Khaneghah *et al.*, 2018). Given that modified mycotoxins are usually undetectable by commonly used analytical methods, only limited data on their occurrence in crops is available. Thus, their impact on food safety may be even more relevant than current research suggests. It is also important to point out that most mycotoxins are relatively stable during conventional food processing, such as cooking, baking, frying, roasting, etc. and thus remain in the final product (Carballo *et al.*, 2019; Udovički *et al.*, 2018). Exposure to mycotoxins primarily comes from ingestion, either directly via the consumption of contaminated foods or indirectly (*carryover*) through the consumption of mycotoxins and/or their metabolites residing in animal tissues, milk and eggs (Milićević *et al.*, 2009; Pleadin *et al.*, 2015a; Vasiljević, M., Marinković, D., and Milićević, D.;, 2021). Nevertheless, other routes of exposure, such as dermal and inhalation routes, cannot be dismissed either. Ingestion of mycotoxin-contaminated food/feed induces, subclinically, a disease known as mycotoxicosis. Depending on the toxicity of the culprit mycotoxin(s), the concentrations in foods, the duration of exposure and the age and nutritional status of the affected individual, the health risks can range from acute to chronic (mutagenic, teratogenic, and carcinogenic) in both humans and animals (Datsugwai *et al.*, 2013; Milićević, Škrinjar and Baltić, 2010). Based on the data presented by the Rapid Alert System on Food and Feed (RASFF), in the last ten years, mycotoxins, particularly aflatoxins, represent the most frequently reported type of food safety hazard. The data show that 93 percent of the overall mycotoxin notifications contain aflatoxins (EC Directorate General for Health and Consumers, 2019). Due to the high risk of mycotoxin exposure, especially for infants and children, and their significant impact on food quality and safety, mycotoxins-focused research and preventive activities have been declared a high priority by the Food and Agriculture Organization of the United Nations and the WHO (FAO *et al.*, 2018). It is encouraged that competent authoritative bodies establish an effective food safety system that includes integrated and well-coordinated longitudinal "field-to-table" monitoring (Gurinović *et al.*, 2016). In 1993, the International Agency for Research on Cancer (IARC) evaluated the carcinogenic potential of AFs, OTA, TCTs, ZEA and FUMs. The IARC re-evaluated the AFM1 carcinogenicity in 2012 (IARC, 2012; Ostry *et al.*, 2017). Naturally occurring aflatoxins (AFB1, B2, G1, G2, M1) were classified as human carcinogens (Group 1), while OTA and FUMs were classified as potential carcinogens (Group 2B). TCTs, ZEA, and PAT, however, were not classified as human carcinogens (Group 3) (Table A4- 1). Currently, there are no regulations governing the presence of *Alternaria* toxins in food and feed in Europe. The European Food Safety Authority (EFSA) is currently investigating the relative hazard posed by mycotoxins produced by *Alternaria* species across the European Union to obtain information on the levels of *Alternaria* toxins in different food products. Although based on limited data, the results show that vegetarians seem to have a higher dietary exposure to *Alternaria* toxins than the general population (EFSA, 2016). Recently, several papers have assessed the impact and consequences of climate change on food safety, food production and availability, the levels of mycotoxin contamination, and the levels of exposure of humans and animals consuming mycotoxin-contaminated food and feed (Milićević, Nastasijevic and Petrovic, 2016; Paterson and Lima, 2011). These data clearly show that mycotoxin contamination is becoming a more severe problem because of its hazards to public health, affecting feed and food safety, food security and international trade, especially in low- and middle-income countries. The EFSA has investigated the potential impact of climate change in Europe and has suggested that effects will be regional and detrimental or advantageous depending on geographical region (Battilani *et al.*, 2012). In southern and southeastern Europe (i.e. Portugal, Spain, southern France, Italy, Slovenia, Greece, Malta, Cyprus, Bulgaria and southern Romania), temperature increases of 4–5 °C and reduced water availability during the summer months are predicted. If these predictions come to fruition, they could decrease agricultural yields by 10–30 percent in many regions, sustain droughts, cause heat waves, catalyse soil and ecosystem degradation, and initiate desertification.

In this overview, we aim to review and discuss the prevalence of mycotoxins in southeastern European countries, review the key aspects of the impacts of mycotoxin contamination on the food supply chain, understand the effects of mycotoxins on human and animal health and the applicable regulations governing the subject matter, analyse the latest trends in mycotoxin reduction activities and forecasts of their success, and discuss necessary support measures for sustainable food systems. The Scopus and the Medline electronic databases were searched to identify articles reporting on mycotoxin contamination of food or feed in the countries of southeastern Europe.

Background of toxicogenic moulds and major mycotoxins

Fungi belong to natural microflora and may be found across the entire food production chain: before harvesting, between harvesting and drying, and during storage. The production of secondary metabolites is not essential for normal fungal growth, but it allows moulds to rapidly colonize, compete with other organisms or inhibit competitor growth and reproduction, thus giving them a "head start" within complex ecosystems (Raffa and Keller, 2019). Mycotoxins are synthesized from biochemically simple interim products of the primary metabolism, such as acetates, malonates, mevalonates, and certain amino acids, through a chain of enzyme-catalysed reactions. The major reactions implicated in mycotoxin biosynthesis are condensation, oxidation/reduction, alkalinization and halogenation, through which a unique range of secondary compounds is formed (Bennett and Klich, 2003). The major pathways included into their synthesis are polyketone (aflatoxins), terpene (trichothecenes), amino acidic (gliotoxins) and tricarbone acid pathways (rubratoxins). Some of the mycotoxins (e.g., cyclopiazonic acid) are synthesized through two or more major pathways detailed above.

Mycotoxins embrace a variety of forms, spanning from simple C4 compounds (e.g., moniliformin) to complex substances such as tremorgenic mycotoxins or phomopsins (Brase *et al.*, 2009). The growth conditions of a fungal species may be different in the field as compared to postharvest stages, but various factors operate interdependently to affect fungal colonization and/or the production of secondary metabolites, such as mycotoxins. Toxicogenic moulds, known as mycotoxin producers, grow on substrates of plant and animal origin in areas of constant high relative humidity and moderate to high temperatures and can be divided into three groups (Fleurat-Lessard, 2017):

1. moulds in natural habitats growing on cereal grains in the field before harvest, mainly species of the *Alternaria, Fusarium*, *Rhizopus* and *Cladosporium* genera;
2. moulds growing on stored cereals during postharvest storage, mainly species of the *Penicillium*, *Aspergillus* and *Mucor* genera; and
3. advanced spoilage moulds growing on grains damaged by other microorganisms, mainly species of the *Fusarium* and *Chaetomium* genera.

Although moulds dangerous to human health mostly belong to the group of postharvest colonizers predominately found in storage facilities, some biologically active mould metabolites that have formed in natural habitats also have been shown to be toxic to humans and animals. Toxicogenic moulds present in food and feed do not necessarily imply the presence of mycotoxins, but it should be noted that the absence of visible mould from biological material does not vouch for the absence of mycotoxins (Milićević et al., 2010b). Since natural substrates almost never contain pure but rather mixed mould cultures and given that mixed cultures are often more biochemically active than pure ones, the problem of biosynthesis of various metabolites during mixed cultures' mould growth becomes even more significant. The main mycotoxin-producing mould genera include the *Aspergillus*, *Penicillium, Fusarium, Claviceps, Alternaria, Pithomyces, Phoma, Stachybotrys* and *Diploidia genera*, with the most common among them being those of the *Aspergillus*, *Penicillium* and *Fusarium* genera (Bennett and Klich, 2003; Marin *et al.*, 2013).

Although 400 toxigenic secondary metabolites of the above kind have been discovered, only about 50 of them have been studied in detail due to their important role in jeopardizing food safety. From the standpoint of agricultural economics and public health, the most important classes of mycotoxins are AFs (produced by *Aspergillus flavi* species), OTA (*Aspergillus circumdati* species, *Aspergillus nigri* species, *Penicillium nordicum*, *P. verrucosum*), FUMs (*Fusarium verticillioides, F. sporotrichioides*), T-2/HT-2 (*F. langsethiae*, *F. sporotrichioides*), DON (*F. graminearum* and related species), PAT (*P. expansum*), and *Alternaria* toxins and ergot toxins (Liew and Mohd-Redzwan, 2018; Rai, Das and Tripathi, 2019). Some of the classes may produce more than one mycotoxin, and some mycotoxins are produced by more than one fungal species. Such a contamination may affect different types of cereals, widely used as raw materials in the production of food and feed. The most important mycotoxin-producing moulds and the mycotoxins they produce are shown in Table A4-1.

Given that the presence of mycotoxins produced by both preharvest (plant-pathogenic) and storage-associated (saprophytic) fungi depends on the climate, climate change and increased climate variability will lead to a slight shift from traditional occurrence areas of the *Aspergillus* and *Fusarium* species to areas offering the optimum climatic conditions these species require for their growth, distribution and mycotoxin production. For example, in Serbia, the co-occurrence of high air temperatures (up to 40 °C) and heavy rainfall with high relative humidity (up to 80 percent) within the same year presents a novel weather pattern for this country. Under volatile conditions, the presence of toxigenic fungi and consequent mycotoxin co-contamination has become a rising issue. It is important to note that Serbia's economy relies heavily on its agricultural sector, and thus mycotoxin contamination of agricultural products has had an adverse impact on Serbian trade, especially on the European Union markets. Due to the long-reaching effects on global trade and food systems, it is extremely important to determine trends in the occurrence of toxicogenic moulds and mycotoxins depending on differing climate conditions. The control of mycotoxins in food and feed samples can be adjusted appropriately for different regional contexts.

Toxic effects of mycotoxins on humans and animals

The toxic effects of mycotoxins on humans and animals are summarized under the term "mycotoxicoses." Mycotoxicoses are defined as food- or feed-related, non-transferable, non-immunogenic diseases and are untraceable to microorganisms other than fungi (Zain, 2011). Clinical symptoms usually subside on removal of contaminated food or feed. Symptoms depend on the type of mycotoxin; the level and duration of exposure; the age, health status, sex and genetic characteristics of the host; and the synergistic effects of mycotoxins and/or other toxins to which the organism has been exposed (Bennett and Klich, 2003). If mycotoxicosis occurs in combination with malnutrition, vitamin deficiency, excessive alcohol consumption and/or infectious diseases, the health consequences may be severe. Mycotoxicosis can make the host more susceptible to other diseases and intensify toxic effects. The direct effects of mycotoxins on humans and animals are slowed growth, weakened immunity and proneness to infections. In cases with high levels of exposure to mycotoxins, the outcome might even be fatal. In animals, decreased egg and milk production can be witnessed. On top of acute toxicity, chronic diseases, including tumours seen after prolonged exposure to small amounts of mycotoxins, can be developed (Council for Agricultural Science and Technology, 2003). A report compiled by the WHO's Food-borne Diseases Burden Epidemiology Reference Group used global estimates of incidence to calculate illnesses, deaths and disability-adjusted life years lost (DALYs) to reveal the highest global DALYs to be attributable to liver cancer (Gibb *et al.*, 2015). The chemical associated with the greatest number of DALYs was reported to be aflatoxin (636 869). Aflatoxins have been identified as highly toxic substances (IARC, 1993) that exhibit teratogenic, mutagenic, carcinogenic, and immunosuppressive properties. The clinical symptoms of aflatoxicosis include fever, vomiting, anorexia, tachycardia and oedema. The most common route of intake of almost all aflatoxins is the ingestion of contaminated foodstuffs, while dermal exposure results in slow and insignificant absorption. However, the most toxic representative of this group, aflatoxin B1 (AFB1), can also enter the body through the skin or via inhalation of contaminated dust. Inhalational exposure in humans has not been studied because of its irrelevance for food toxicology (Fung and Clark, 2004). Other types of aflatoxins are several times less toxic than AFB1 (Lee *et al.*, 2014) and rarely present health hazards. *In vitro* metabolism studies have shown AFB1 to undergo the following metabolic pathway: reduction resulting in the nascence of aflatoxicol (AFL), hydroxylation resulting in the nascence of aflatoxin M1 (AFM1), hydration resulting in the nascence of AFB2a, and epoxidation resulting in the nascence of AFB1-2,3-epoxide. The latter epoxide is the most reactive metabolite and is thought to be responsible for both acute and chronic toxic (carcinogenic) effects of AFB1, especially in the liver (WHO, 2011).

Although AFB1 is more toxic than AFG1, if metabolic activation of AFG1 occurs through epoxy formation, the toxicity of these two is almost equal, while AFB2 and AFG2 are far less toxic because of their inability to produce epoxides. AFM1 is a hydroxylated metabolic product of AFB1, formed during oxidation in the body after the ingestion of contaminated food, while AFM2 is a hydroxylated metabolic product of AFB2 that is formed during oxidation in the milk of mammals that received contaminated food. Both fall into the group of less toxic mycotoxins (Council for Agricultural Science and Technology, 2003). The average conversion was found to be 2.5 percent, although a direct relationship between the carryover rate and the milk yield, with a maximal 6.2 percent carryover rate, was found (Walte *et al.*, 2016). AFB1 is the most predominant and most potent hepatocarcinogen and has been classified as Group 1 of proven human carcinogens (IARC, 1993). Therefore, no tolerable daily intake can be defined for this type of carcinogen, and a different approach is needed to evaluate the risk of this carcinogenic mycotoxin. The EFSA recommended using the margin of exposure (MoE) approach, concluding that MoE values higher than 10 000 (based on the $BMDL_{10}$ from animal studies) are of low concern from the point of view of public health (Schrenk *et al.*, 2020a). In other words, only a zero level of exposure will result in no risk.

OTA, as the most important representative of the ochratoxin group, shows nephrotoxicity, carcinogenicity, teratogenicity, hepatotoxicity, and neurotoxicity, and, to a lesser extent, it acts as a mutagen and an immunosuppressant (Doi and Uetsuka, 2011). It is easily absorbed through the gastrointestinal tract, mainly through the duodenum and the jejunum. There have not been enough studies done on skin or inhalation absorption. When absorbed, OTA has a high binding affinity for plasma protein. It can be found in decreasing order of concentrations in the kidneys, liver, fat, and muscle tissue (Milićević *et al.*, 2008; Pleadin *et al.*, 2016). In humans, it is excreted in two phases, the first being the rapid excretion phase and the second being the purification phase, which determines the rate of final elimination of the compound. OTA toxicity involves several mechanisms. It inhibits protein synthesis by competing with the phenylalanine aminoacylation reaction catalysed by Phe-tRNA synthetase. This results in the inhibition of protein, DNA and RNA synthesis. OTA also disrupts hepatic microsomal calcium homeostasis by impairing the endoplasmic reticulum via lipid peroxidation. The main target affected by OTA is the kidney; OTA can cause endemic nephropathy associated with urothelial cancer, known as Balkan endemic nephropathy and witnessed in some endemic areas in Bulgaria, the former Yugoslavia and Romania (Pavlović, 2013; Peraica, Lucić and Pavlović, 1999). The first signs and symptoms of the disease are fatigue, headache and pale skin, while after prolonged exposure, symptoms of low molecular weight proteinuria develop, without concomitant hypertension but in combination with aplastic or normochromic anaemia. Historically, the consumption of pork has been a significant source of human exposure to OTA in these regions. Unlike other monitored mycotoxins, OTA has the potential to bioaccumulate in the organism and resides in edible tissues, particularly in the kidneys and liver, often used by the meat industry (Milićević *et al.,* 2014). Due to high OTA-induced health risks, OTA toxicity has been studied more often and more in depth than that of other mycotoxins encountered in the southeastern Europe region. OTA is classified as a possible human carcinogen (Group 2B) by the IARC (1993) based on sufficient evidence of carcinogenicity in animal models but insufficient evidence from human studies. Based on the last assessment of the Scientific Committee on Food (SCF), the tolerable weekly intake (TWI) of 120 ng per kg of body weight established by the CONTAM Panel in 2006 (EFSA, 2006) is no longer valid. Following EFSA guidance for substances that are both genotoxic and carcinogenic, an MoE of 10 000 or higher between the reference point and the estimated dietary exposure would be of low health concern. However, in the absence of elucidated modes of action for the genotoxicity/carcinogenicity of OTA, the panel concluded that an MoE of 10 000 needs to be applied to the $BMDL_{10}$ of 14.5 μg per kg of body weight per day for neoplastic effects (kidney tumours) in rats (Schrenk *et al.*, 2020b).

The co-occurrence of nephrotoxic mycotoxins OTA and citrinin (CTN) is commonly reported in grains grown in moderate climatic regions (Pleadin *et al.*, 2018). Studies have shown that citrinin exposure increases liver and kidney weight, while prolonged exposures lead to the formation of smaller adenomas (Wen, Mu and Deng, 2016). It is thought that the increase in kidney weight should probably be attributed to the accumulation of toxins within the renal tissue after their transport to the proximal renal tubules. In addition, CTN has been found to significantly affect the mitochondrial respiratory chain, as it leads to changes in calcium ion fluxes through the cell membrane and alters its permeability, thereby disrupting the electron transfer chain. This mycotoxin has been associated with "yellow rice syndrome," but there has been no systematic investigation into the actual mycotoxin or agent responsible for this poorly defined illness. Other possible toxic effects of CTN exposure include decreased cytokine production, inhibition of DNA and RNA synthesis and decreased gene expression, induction of oxidative stress, and activation of cell apoptosis (Rašić *et al.*, 2018; Sugiyama *et al.*, 2013).

Trichothecenes are classified into four groups. Group A includes T-2 toxin and diacetoxyscirpenol (DAS), and Group B includes 4-deoxynivalenol (DON) and nivalenol (NIV). Many *Fusarium* species produce Group A and Group B trichothecenes. The plant species *Baccharis megapotamica* produces the Group C trichothecene baccharin that is the least common of them all. Group D mycotoxins include roridins produced by *Mycothecium roridum*, verrucarin produced by *M. verrucaria*, and satratoxins produced by *Stachybotrys atra*. It is important to point out that more common and more potent trichothecenes are produced by the *Fusarium* species. In animals, DON causes severe nausea, vomiting and diarrhoea, resulting in food rejection (Valenta and Dänicke, 2005). The direct toxicity of DON is catered by the epoxy part of its structure and results in impaired immunity. It manifests itself in the supraregulation of mRNAs, thus modulating the expression of several cytokines, chemokines, and immune-related proteins (Pestka *et al.*, 2008). Other important negative impacts are those on immunoregulatory processes responsible for cell proliferation, differentiation, and apoptosis (Bae and Pestka, 2008; Rocha, Ansari and Doohan, 2005). Other trichothecenes, such as T-2 toxin and diacetoxyscirpenol, also show immunosuppressive effects. In addition, they are cytotoxic, resulting in an increased susceptibility to infectious microbes. In humans, these two trichothecenes cause toxic alimentary aleukaemia, the early symptoms of which are skin inflammation, vomiting and hematopoietic tissue damage. In the acute phase, necrosis of the oral cavity and profuse bleeding from the nose, mouth and vagina occur, and neurological disorders have been observed. Since T-2 toxin has an epoxy ring and side chains containing several acetyl and hydroxyl substituents, it is characterized by high biotoxicity manifested in the form of inhibition of DNA and RNA synthesis and consequent induction of apoptosis. T-2 toxin is rapidly metabolized to HT-2 toxin, which is its major metabolite (Wu *et al.*, 2014). In 2003, the IARC added DON, NIV, T-2 and HT- 2 toxins to Group 3 of non-classifiable human carcinogens due to inadequate evidence of animal carcinogenicity and a lack of human studies. Tolerable daily intakes of 1 µg per kg^{-1} of body weight per day^{-1} and 1.2 µg per kg^{-1} of body weight per day^{-1} were established for DON and NIV, respectively (EFSA, 2014a, 2014b). Recently, the SCF concluded that the full tolerable daily intake of 0.1 µg per kg^{-1} of body weight per day^{-1} for the sum of T-2 and HT-2 toxins could be established (EFSA, 2014b). In domestic animals, the toxic effects of ZEA can lead to infertility, low offspring birth weight and hypoestrogenism. In addition, research has shown that in humans, ZEA disrupts hormonal balance, decreasing male fertility due to xenoestrogen accumulation and causing gynecomastia (Kowalska, Habrowska-Górczyńska and Piastowska-Ciesielska, 2016). The reason for this is that this compound acts as a competitive substrate for hydroxysteroid dehydrogenase (HSD), an enzyme involved in steroid synthesis and metabolism. HSD works by reducing the 6-keto group of ZEA to a hydroxyl group to form α-zearalenol and β-zearalenol. Of these two metabolites, zearalenone is a potentially active α-form that exhibits enhanced oestrogenic activity (Fink-Gremmels and Malekinejad, 2007; Minervini and Dell'Aquila, 2008). After conjugation with glucuronic acid and sulfonation reactions, both enol metabolites form adducts (Guerre, 2015). Other important zearalenone derivatives are zearalanaol and zearalanon, which have been associated with immunotoxicity. Zearalanaol is used as a growth promoter for fattening cattle and has an anabolic effect in the body. In humans, changes in different immune responses can occur, resulting in the dysfunction of lymphoid organs, thymic atrophy, changes in the phenotype of lymphocytes produced by the spleen, and decreased peroxidase production (Hueza *et al.*, 2014). The IARC found limited evidence of ZEA carcinogenicity in animal models, classifying it together with DON into Group 3. In 2000, the SCF established a tolerable daily intake of 0.2 µg per kg^{-1} of body weight per day^{-1} for ZEA. However, in 2011, the SCF concluded that a tolerable daily intake of 0.25 µg per kg^{-1} of body weight per day^{-1} should be established based on recent data gathered across the most sensitive animal species (EFSA, 2014a).

The primary biological activity of FUMs mirrors in the initial stages of sphingolipid synthesis because of the inhibition of ceramide synthetase (sphingosine N-acetyltransferase). Other signalling pathways regulating the production of early sphingolipid products, such as ceramides, sphingoid bases and phosphorylated sphingosine-1-phosphate, are disrupted as well (Stockmann-Juvala and Savolainen, 2008; Wen, Mu and Deng, 2016). Apoptosis caused by the disturbance of cellular balance and mitosis leads to carcinogenesis. In animals, FUM toxicity has been linked to diseases such as equine leukoencephalomalacia, which was recently recorded in Serbia (Jovanovic *et al.*, 2015), and porcine pulmonary oedema syndrome. Given that some studies have linked human oesophageal cancer with high levels of FUMs ingested through a corn-based diet, it was thought that in developing countries whose residents mostly consume corn as a staple of the diet, the risk of cancer would be higher than in the rest of the world. However, further research has refuted this by proving that there is no statistically significant correlation between serum sphingolipid levels and cancer risk (Sydenham *et al.*, 1991). The consumption of food contaminated with FUMs during pregnancy is considered to be the key factor responsible for the occurrence of neural tube defects (anencephaly and *spina bifida*) attributed to sphingolipid deficiency. This deficiency damages folate receptors and reduces folate (Vitamin B9) levels. The latter vitamin is the main nutrient used to create new cells and synthesize DNA and RNA (Imbard, Benoist and Blom, 2013). Hence, FB1 is listed as a Group 2B carcinogen (IARC, 1993), while recent evaluation by the EFSA (2018) established a group tolerable daily intake of 1 µg per kg^{-1} of body weight per day^{-1} based on the increased incidence of megalocytic hepatocytes found in a chronic mice exposure study.

PAT is a mycotoxin produced by a wide range of fungal species of the *Penicillium, Aspergillus* and *Byssochlamys* genera (Frisvad, 2018). Due to the high affinity of PAT for sulfhydryl groups, this toxin can inhibit enzymes, resulting in acute toxic effects such as gastrointestinal symptoms, neurotoxic effects, pulmonary symptoms, ulceration and oedema. Other toxic effects include teratogenicity, genotoxicity and carcinogenicity (Vidal *et al.*, 2019; Wen, Mu and Deng, 2016). On top of the above, PAT has a mutagenic effect and is classified as clastogenic, as well as immunotoxic in terms of reducing cytokine production and increasing the production of neutrophils and T-lymphocytes in the spleen (Puel, Galtier and Oswald, 2010). Regarding its carcinogenicity to humans, the IARC included PAT in Group 3 of non-classifiable compounds. The Joint FAO/WHO Expert Committee on Food Additives (JECFA) established a provisional maximum tolerable daily intake for PAT of 0.4 µg per kg^{-1} of body weight per day^{-1} (JECFA, 1995).

Emerging mycotoxins are defined as mycotoxins that are neither routinely determined nor legislatively regulated. However, the evidence of their incidence is rapidly increasing (Gruber-Dorninger *et al.*, 2017). The most relevant and frequently occurring emerging mycotoxins are *Fusarium* toxins. *Fusarium*-generated mycotoxins include enniatins (ENNs), beauvericin (BEA), moniliformin (MON) and fusaproliferin (FUS). Emerging fusariotoxins were mostly investigated in the Mediterranean countries. Moreover, their presence was recently reported in maize from Serbia (Jajić *et al.*, 2019; Janić Hajnal *et al.*, 2020). The authors found that MON, BEA and FUS had the highest presence of all emerging mycotoxins and were present in all the investigated regions. Emerging mycotoxins also include citreoviridin, gliotoxin, griseofulvin, mycophenolic acid, b- nitropropionic acid, kojic acid, tremorgenic mycotoxins (penitrems, janthitrems, lolitrems and paspalitrems), penicillic acid, viomellein, vioxantin, xan-thomegnin and walleminols. They are not classifiable as to their carcinogenicity to humans by the IARC. Due to the lack of research showing direct human and animal health effects, no regulations governing their presence in food or feed have been enacted.

In grazing livestock, such as sheep, cattle, poultry and pigs, the risk of toxic effects evoked by ergot alkaloids is high. Common clinical symptoms of ergotism are hypersensitivity, convulsions, gangrene, muscle contractions, abortion and ataxia (Bennett and Klich, 2003; Bennett and Bentley, 1999). The convulsive form manifests itself in epileptic seizures, diarrhoea, paraesthesia, itching, nausea and effects on mental health, such as psychosis, headaches and drowsiness. The effect on the central nervous system is manifested after gastrointestinal symptoms. Dry gangrene usually occurs as the result of vasoconstriction caused by the ergot alkaloids (ergovaline, ergotamine, ergocryptine, ergocristine, ergonovine, ergocornine, and lysergic acid). The main target tissues are those less perfused (fingers and toes). Symptoms include desquamation, loss of peripheral sensation, oedema and, ultimately, the death of target tissues (Eadie, 2003; Tudzynski, Correia and Keller, 2001). In medicine, ergot alkaloids are used in low concentrations to reduce menstrual bleeding or miscarriage, to stimulate placental abruption after childbirth due to uterine wall muscle contractions, and to treat acute migraines, Parkinson's disease, cerebrovascular insufficiency, and prolactin-Ca^{+2} inhibition (Bennett and Bentley, 1999).

The occurrence and the significance of mycotoxins in southeastern Europe

Data on the occurrence of mycotoxins are extremely important to determine the risk to both humans and animals. This report is a valuable risk assessment input that contributes to the enforcement of new and effective regulations, the upscaling of laboratory facilities, etc., particularly in vulnerable countries prone to mycotoxin contamination, such as the countries of southeastern Europe. Dietary habits observed by the southeastern Europe countries differ depending on the region (Mediterranean or continental), religion (Christianity, Judaism, or Islam), and socioeconomic and cultural factors. When it comes to mycotoxins, the riskiest foodstuffs are wheat, maize, rice, beans, coffee, grapevines, wine, fruits, nuts, spices, dried food and animal products, such as meat and eggs. Various groups of toxigenic moulds and related mycotoxins have been reported to be associated with the contamination of these commodities. From 2010 to 2020, the ingestion of mycotoxin metabolites through dairy products also has been observed. However, the highest level of contamination is still that of cereals, especially maize, since the nutritional composition of this cereal is particularly conducive to mould development and mycotoxin production (Chulze, 2010). It is important to point out that consumers also can be exposed to mycotoxins through foods whose spoilage has not been recognized, i.e. mouldy foods. While the data released by FAO in 1999 indicate that 25 percent of cereals were contaminated with mycotoxins, new data show a significantly higher level of contamination (Eskola *et al.*, 2019). The authors point out that global mycotoxin prevalence in food crops varies largely and is dependent on many factors, such as the mycotoxin of concern and the analytical and reporting methods used, but, nevertheless, it appears that the correct percentage is as high as 60 to 80 percent. Additionally, an extensive, multiyear research project on cereals and animal feed, which included data from nearly 100 countries around the world collected between 2008 and 2017, proved a strong association between mycotoxin occurrence and certain climatic conditions. Mycotoxins were detected in most of the samples, with 88 percent of the samples containing at least one mycotoxin and 64 percent of the samples containing at least two mycotoxins (Gruber-Dorninger, Jenkins and Schatzmayr, 2019). Data provided by this study for the countries of southeastern Europe (separately for countries of southern and eastern Europe) show a high incidence of *Fusarium* contamination, primarily with FUMs (74.9 and 33.6 percent, respectively), ZEA (36.3 and 42.5 percent, respectively) and DON (52.9 and 59.9 percent, respectively), but also an increased incidence of AFB1 presence, especially in southern Europe (28.9 percent). Mycotoxin representation in agricultural crops and foods evident for some countries of southeastern Europe through individual research is shown in Table A4-2. Significant advances have been made in the past few decades in the development of methods for the detection and qualification of mycotoxins. The most frequently used was the semi-quantitative screening, called the enzyme-linked immunosorbent assay (ELISA), a rapid and sensitive assay suitable for mycotoxin determination. Other methods of mycotoxin quantification that require sophisticated laboratory equipment include high-performance liquid chromatography (HPLC), gas chromatography (GC), liquid chromatography/mass spectrometry (LC/MS), and gas chromatography/mass spectrometry (GC/MS) (Rahmani, Jinap and Soleimany, 2009).

Reports on mycotoxin contamination of these staple foods in countries of southeastern Europe have mainly focused on the *Fusarium*, *Aspergillus* and *Penicillium* mycotoxins, such as AFs, OTA, ZEA, DON, T-2, FUM and PAT. Authors from Serbia and Romania were the first to provide the mycotoxin profile of wheat and maize with respect to newly emerging mycotoxins (moniliformin, enniatins, beauvericin and fusaproliferin) (Jajić *et al.*, 2019; Stanciu *et al.*, 2017). Data concerning mycotoxin levels in staple foods from Albania, Bulgaria, Bosna and Herzegovina, Montenegro, Kosovo[23] and North Macedonia is sparse. Given that recent decades have seen an increasing occurrence of extreme weather, a high level of AFs was detected in maize followed by a high incidence of AFM1 in milk coming from the southeastern European countries. Therefore, several Rapid Alert System for Food and Feed (RASFF) notifications related to AF levels above the maximum levels found in maize delivered by the southeastern European countries were issued at the end of 2012 and the first months of 2013 (EC Directorate General for Health and Consumers, 2013).

A recent report on the occurrence of mycotoxins in maize harvested in northern Serbia in seasons with an extreme drought (2012), hot and dry conditions (2013 and 2015), and extreme precipitation (2014) revealed significant differences in the incidence of AFs, OTA, ZEA and FUMs among the sampling seasons. The results showed that in each study year, the samples were fumonisins-positive in high numbers (from 76 percent to 100 percent). AFB1 was detected in 94 percent and 90 percent of maize samples sampled in 2012 and 2015, respectively, while in 2014, DON, ZEA and their derivatives were detected in 100 percent of the samples. The study revealed OTA to be the most predominant contaminant of the 2012 samples (25 percent positive samples). Weather conditions recorded throughout the four-year study period had a significant influence on the occurrence of the above mycotoxins in maize (Kos *et al.*, 2020).

In the past decade, data from Croatia indicate a high incidence of cereal and animal feed contamination, primarily with DON (up to 85 percent in cereals and 97 percent in feed), ZEA (up to 88 percent in cereals and 93 percent in feed), and FUMs (up to 90 percent in cereals and 88 percent in feed) (Pleadin *et al.*, 2012a, 2012b, 2012c, 2013). The contamination of feed also resulted in a high incidence of ZEA contamination of cow milk (94 percent) (Pleadin, 2017). High AFB1 contamination of maize was observed during 2013 due to the tropical and subtropical climatic conditions seen in 2012 during maize cultivation (Pleadin *et al.*, 2014, 2015b), although earlier Croatian research indicated more frequent contamination of maize and other cereals with Fusarium mycotoxins. The contamination of maize and feed with AFB1 resulted in higher incidence and higher concentrations of AFM1 in cow milk (Bilandžić *et al.*, 2014). Studies also show that mycotoxins can be present in dry-cured meat products overgrown by surface moulds during ripening, as well as consequent to a carryover effect or the use of contaminated spices (Markov *et al.*, 2013; Pleadin *et al.*, 2015a; Pleadin, Kovačević and Perši, 2015).

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[23] All references to Kosovo should be understood to be in the context of United Nations Security Council resolution 1244 (1999).

The impact of climate change on mycotoxin incidence and food safety

Research shows that environmental stress has a significant impact on the occurrence of mycotoxins (Medina *et al.*, 2015; Medina, Rodríguez and Magan, 2015). Although moulds are mainly resistant to elevated carbon dioxide concentrations, combined with other factors (drought, plant damage by insects and changes in crop phenology, such as changes in flowering time and grain ripening), this gas can have a pronounced indirect effect on mycotoxin production (Medina *et al.*, 2017; Van der Fels-Klerx, Liu and Battilani, 2016). Recent findings suggest that a two- or threefold increase in atmospheric carbon dioxide concentration, from 350–400 to 800–1200 ppm, and temperature increases of 2–5 °C, followed by dry periods, can be expected in the coming decades (Bebber and Gurr, 2015; Bebber, Holmes and Gurr, 2014; Gregory *et al.*, 2009; Medina *et al.*, 2017; Medina, Rodríguez and Magan, 2014).

Increased levels of contamination of food and feed may be caused by the presence of the existing mycotoxins or their "relocation" to new, still-unaffected or only mildly affected geographical regions. Stress caused by climate change can result in the emergence of new mycotoxins, and their occurrence can seriously impair the availability of food and feed, especially in developing countries (Miraglia *et al.*, 2009). It is believed that AFs produced by the moulds of the *Aspergillus* genus, present in areas with hot and humid climates that became even hotter and more humid due to climate change, caused an increased contamination in the early 2000s across Europe. In southern Europe, extremely hot summers already have resulted in changes in maize growing ecosystems, leading to changes in the occurrence patterns common to *Fusarium* species and *Fusarium* mycotoxins. This has led to a more frequent occurrence of *A. flavus* and contamination with highly toxic AFB1 (Milićević *et al.*, 2019a). During the year, temperatures have been above the annual average (more than 35 °C). Nevertheless, drought has been the factor causing the greatest damage, especially to maize. In comparison to 2016, the total 2017 maize production decreased by 45.5 percent. The next two years (2018 and 2019), particularly 2019, were the hottest years in recorded Serbian meteorological history. According to the report of the Republic Hydrometeorological Service of Serbia, 13 of the 15 hottest years on record have occurred after 2000 (Republic Hydrometeorological Service of Serbia, 2020). Due to the severity of maize contamination, elevated concentrations of AFM1 have been found in milk countrywide, with 31 percent of milk samples non-compliant to the European Union maximum levels (0.05 µg kg^{-1}) (Milićević *et al.*, 2019b).

Research conducted in Croatia indicated high contamination of maize with aflatoxins during 2013, influenced by tropical and subtropical climatic conditions witnessed during maize cultivation in 2012 (Pleadin *et al.*, 2014, 2015b). It is important to point out that previous research in this part of Europe indicated a more frequent contamination of maize and other cereals with *Fusarium* mycotoxins and puts Croatia among countries for which *Fusarium* contamination is now considered characteristic (Binder *et al.*, 2007; Pleadin *et al.*, 2012a, 2012b, 2012c, 2013).

Globally, recent research has focused on studying the impact of a number of environmental variables, such as temperature, pest attack and nutrient availability, on mycotoxin production and their occurrence in food (Paterson and Lima, 2011). Research on predictive models is important in order to identify geographical regions in which interactive climatic factors may have the highest impact on toxicogenic mould infection and consequent mycotoxin contamination. It is important to point out that mycotoxins often occur together and have synergistic effects in the body, and that, therefore, intensive research is being conducted to develop models for risk assessment in case of contamination with multiple mycotoxins. The findings from these various research projects have been linked to food chains and the impact on food availability in the future. The EFSA predicts that the effects of climate change on the occurrence of mycotoxins will be regional and mostly detrimental, but possibly also beneficial for a particular geographical area (Battilani *et al.*, 2012; EFSA *et al.*, 2020). Climate change is also expected to affect global changes in the occurrence and geographical distribution of insects (Bebber and Gurr, 2015; Bebber, Holmes and Gurr, 2014; Medina, Rodríguez and Magan, 2015), which are known to have a significant impact on crop infection caused by toxicogenic moulds. Research shows that climate change may be responsible for one-third of the variability of basic food yields globally and that it negatively affects the nutritional value of food (EFSA *et al.*, 2020; Ramos, 1998).

Climate change and predictions of mycotoxin contamination

In the last century, temperatures have risen by 0.7 °C globally and by 1 °C in Europe. The amount of precipitation during the same period has increased by 10–40 percent in northern Europe and decreased in southern Europe. The frequency of droughts, heat waves and extreme rainfall in Europe has increased, while the occurrence of cold extremes has decreased. Projections show that in the next century, the increase in annual average global temperature could be between 1.4 °C and 5.8 °C, and between 2.0 °C and 6.3 °C in Europe. It is projected that cold winters will almost completely disappear by the end of the century, while warm summers will be even more frequent. It can be expected that climate change will have a strong impact on mycotoxin contamination of various cereals. Changes in the production of mycotoxins, as a consequence of temperature increases by 3 °C and 5 °C under different water activity conditions, are shown in Table A4-3.

Due to increased contamination with aflatoxicogenic moulds, their most toxic representative AFB1 will become a significant food safety threat in the next century, primarily in eastern Europe, the Balkans and the Mediterranean (Battilani *et al.*, 2012; Milićević *et al.*, 2020). Literature shows that the southern part of Europe will be particularly affected by climate change in terms of increased carbon dioxide in the atmosphere, rising temperatures, and the occurrence of extreme precipitation and drought. Similar impacts are expected in parts of North America, South America, Africa and Asia (Paterson and Lima, 2011). Predictive models suggest that northern Europe could benefit from these changes, while the Mediterranean region could be the "hot spot" in which numerous extreme climatic changes could take place. The maturation of crop cultures in southern and central Europe is expected to arrival much earlier than usual. In some countries in southern and southeastern Europe, the temperature is expected to rise by 4 °C to 5 °C, followed by reduced water availability, especially in summer. Western and Atlantic European countries can expect temperatures to rise by 2.5 °C to 3.5 °C, with drier and warmer summers (Paterson and Lima, 2011). Due to higher amounts of precipitation, strong storms and floods are predicted to be more frequent, especially in winter.

Perspectives for risk management and controlling strategies

Due to the considerable impacts on food safety, regional and international trade, the economy and public health, mycotoxin contamination remains a significant challenge for the food industry, regulatory agencies, researchers and consumers. Past food crises, such as AFs contamination, have confirmed the importance of rapid identification and disposal of unsafe foodstuffs prior to consumption. Therefore, several food standards, guidelines and codes of practices have been developed by the Codex Alimentarius to prevent harmful effects of mycotoxins throughout the food chain (CAC, 2003). Likewise, many studies are focused on different processes and strategies for the reduction of mycotoxins in food and feed, giving detailed information about implementable decontamination approaches (Cheli, 2020). These recommendations are divided into two parts: preharvest practices adopted based on good agricultural practices (GAP), postharvest practices such as good manufacturing practices (GMP), and good hygiene practices (GHP) implemented in the hazard analysis and critical control points (HACCP) systems. However, GAP coupled with prediction models that integrate the most important field parameters and weather input are the best options to prevent fungal colonization and mycotoxin production in the field.

Storage conditions are crucial for the postharvest prevention of mycotoxin contamination. Among many factors within the storage ecosystem, temperature and humidity are critical for fungal infection and mycotoxin contamination. Improved storage management, especially at the farmer and the small trader level, will prevent fungal growth and mycotoxin contamination in stockpiled commodities (Milićević *et al.*, 2019b). An organization dealing with food production and grain storage will develop a formal Food Safety Management System (FSMS) to ensure that food is safe for consumption. Furthermore, organizations need to establish and implement control measures appropriate for specific hazards and the risks they pose to the final consumer.

If mycotoxin contamination has occurred, contaminated feed and food must be managed through postharvest decontamination or detoxifying treatment to convert mycotoxins into non-toxic or less toxic products. Traditional detoxifying methods include physical, chemical, and biological methods (Wan, Chen and Rao, 2020).

It is important to protect the consumer from the effects of a mycotoxin detected within the food supply chain, assuming the risk assessment indicates a level of exposure to be unacceptable. This study highlights that the population of the southeastern Europe countries could be exposed to mycotoxin-contaminated food, particularly due to the consumption of food staples, such as cereals, dried fruits and animal products, including meat and dairy. The presence and range of concentrations of mycotoxins are correlated with the location of production and seasonal weather. Because this southeastern Europe subregion is more susceptible to climate change than northern Europe, further research and implementation of new monitoring principles is required. The challenges to be addressed include:

- Development and implementation of new national strategies for monitoring and prediction of agroclimatic changes that could affect fungi development and mycotoxin production in specific foods/feeds in given geographic regions.
- Designing of a map of mycotoxin incidence in correlation with climatic conditions.
- Identification of high-risk commodities, and the periodic surveillance and risk assessment of those commodities.
- Strengthening and improvement of analytical capacities, investment into targeted research, and sharing of results to reduce economic and health-related costs.

- Wider education on GAP and GMP practices, upgrading of GAP and GMP facilities oriented towards farmers and small-scale industries, and integration of these practices into mycotoxin management systems that can readily be adopted at critical points along the entire food chain, from producer to final consumer (Logrieco *et al.*, 2018).
- The use of information and communications technology (ICT), artificial and business intelligence, cloud systems, sensors and algorithms for the generation, storage, interpretation and distribution of all data relevant for mycotoxin management.

The results presented in this report should be seen as the first step in the establishment of a regional mycotoxin risk assessment centre in charge of the appropriate dissemination of available information (Milićević *et al.*, 2020), among other responsibilities. Since it is impossible to fully eliminate the presence of undesirable substances and contaminants from food and feed, the applicable legislation is a persistent pending issue. Many countries have stipulated the maximum levels or given recommendations for mycotoxin content in food and feed, depending on the intended use of food or feed in question. These limits and recommendations are guided by food-regulating bodies, such as FAO, the WHO and the European Union. Factors influencing mycotoxin-related regulations include the availability of toxicity data, the availability of data on mycotoxin occurrence in different commodities, analytical data, methods of sampling and analysis, and economical and political factors, such as the commercial interests of each country (Van Egmond, Schothorst and Jonker, 2007). The strictest standards are those laid down under the European Union legislation. Most of the non-European Union countries in the southeastern Europe region have harmonized regulations for sampling protocols, analytical performance criteria, and the MLs according to European Union guidance. However, countries with mycotoxin problems might not always be able to adopt these regulations due to capacity and capability issues.

In Serbia, the MLs of 11 food mycotoxins have been stipulated in accordance with the European Union regulation, as follows: AFB1 and AFM1 alone, as well as the sum of AFs (AFB1, B2, G1, and G2), FUMs (FB1, FB), OTA, patulin, DON, ZEA, and ergot sclerotia (Serbian Regulation, 2019). In comparison with the European Union legislation, which applies to the southeastern Europe European Union Member Nations, some MLs remain high or are not enacted (as is the case with AFM1, FUMs, and TCTs), which calls for the amendments of the existent laws and regulations. Surprisingly, the Serbian regulatory authorities failed to establish the MLs for FUMs in feed despite their widespread occurrence and their animal health hazards (Serbian Regulation, 2016).

Monitoring and control systems are an integral component of the food safety system that have been established to obtain reliable information about the real exposure of human populations to mycotoxins and consequential public health risks. Official food control laboratories in the countries of southeastern Europe are mostly accredited by national accreditation bodies according to ISO 17025 and participate in interlaboratory proficiency testing schemes. Given the often-restricted budgets, national programmes of monitoring, prevention and control of mycotoxin contamination in food and feed do not entirely serve their purpose, calling for evidence-based reinforcement and upscaling.

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