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Environmental and Food Safety and Security for South-East Europe and Ukraine

Edited by
Ksenija Vitale

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Environmental and Food Safety and Security for South-East Europe and Ukraine

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Series C: Environmental Security

Environmental and Food Safety and Security for South-East Europe and Ukraine

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Preface

Food and drinking water represent fundamental human needs and access to them constitutes a basic human right. Safe and secure food and drinking water supply became the priority and challenge of modern society as well as important issues determining global and regional security and stability.

The workshop relates to these problems with a novel approach, emphasizing the intimate interrelationship between the food safety/security and the state of the environment. Observation of the food safety/security and preservation of the environment are strategic issues for every country, as reduced access to the clean environment and associated with that scarcity and poor quality of food and water, causing adverse health effects, could create sense of insecurity among population, political and social tension and, in extreme cases, violence or even war.

Although the above conditions indicate the global trends, the problems with environmental and food safety and security are most vividly manifested in condensed form in South-East Europe and Ukraine. Having one of the best climates in the world suitable for diversified agriculture (including subtropical zones in Crimea, Adriatic and Mediterranean) and agriculturally-minded population, South-East Europe and Ukraine could significantly contribute into the wellness of global population.

Also, this ARW was strategically scheduled to be one of the events commemorating 25th Anniversary of Chernobyl nuclear reactor accident, which took place in Ukraine in April 1986 and had an enormous regional and global environmental and public health impact.

The primary focus of the workshop was on maintaining the food and drinking water safety and security through the improving of environment and implementation of the clean products and processes principles. The main discussed topics were:

– Environmental preservation and improving of ecological situation in South-East Europe and Ukraine for sustainable food and drinking water production; safety and sustainable development of regional agro industrial complexes;

- Policy making in the areas of environmental and health protection and crisis response;
- Biomonitoring as a tool for reliable risk assessment;
- Contamination as a threat to environmental safety, food quality and public health;
- Best practice in use of advanced methods and tools for cleaning, purification and special treatment of food, water and soil;
- Novel methods and technologies of water treatment for use in various areas of agriculture, industry and public health;
- Prevention of and emergency responses to adverse public health conditions and outbreaks of diseases;
- Progress in modern biology, especially in biosafety and biosecurity issues; cooperation in science and security in South-East Europe and Ukraine.

The above described topics are covered in the book, but unfortunately book can never reflect the passionate discussion and exchange of ideas during the workshop and informal meetings. Still we believe that book will become important source of information for the scientific community and policy makers.

NATO Advanced Research Workshop "Environmental and Food Security for South-East Europe and Ukraine" was held in Dnipropetrovs'k, Ukraine on May 17–19, 2011. The location of the ARW was chosen quite deliberately. The city of Dnipropetrovs'k was located on the banks of the Dnieper River, the Ukrainian main water transport artery and a major source of water for industrial, agricultural and household use. Being the third largest city in Ukraine with population of one million people, Dnepropetrovsk is a pivotal political, industrial, agricultural and educational centre of Ukraine. It shares all the environmental and public health problems, which are characteristic to modern Ukraine. The workshop brought together scientists and specialists with extensive expertise within environmental and food technologies and public health from a range of NATO and Partner countries. Forty eight participants from 15 countries including Bulgaria, Croatia, France, Germany, Georgia, Greece, Lithuania, Poland, Russia, Serbia, Slovak Republic, Turkey, Ukraine and USA attended the meeting. Invited key speakers, specialists and young scientists discussed problems related to food and drinking water safety and security crating at the same time collaborative links between scientists and specialists from NATO and Partner countries, and establishing a network of stakeholders to foster further collaboration and the exchange of ideas.

In the end the editor, on her behalf and on the behalf of all the authors, express their deep gratitude to NATO Science Affairs Division for the moral, professional and no less important financial support, which made possible, organization of this event.

The Editor

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Chapter 1
Defending the Safety of the Global Food System: Advances in Food Security and Safety

Hami Alpas and Taylan Kiymaz

Abstract The recent food and financial crises developed from different underlying causes but intertwined in complex ways through their implications not only for financial and economic stability, food security, political security; but also for greater diligence in food defense against deliberate contamination with either economic or terrorist motives. Food security is a hot topic therefore; its disruption via environmental breakdown is an obvious cause for terrorism. The intentional contamination of the food supply poses a real threat to society. It has the potential to disrupt food distribution, loss of consumer confidence in government and the food supply, business failures, trade restrictions, and adverse effects on the economy. The global food system is very vulnerable, both structural and social. The bulk production and need for rapid production, sourcing and distribution at both national and international level is beyond the limits of routine food safety measures of the industry; especially against high-impact deliberate contamination. Adapting to the additional threats to food security arising from major environmental changes requires an integrated food system approach – strengthening the sector's infrastructure against deliberate contamination – thereby making the food system less vulnerable to attack(s) or destructive economic outcomes. In this respect vulnerability assessment arouses as an alternative to address food supply-chain security by determining the selection of countermeasures to minimize or eliminate vulnerabilities as well as enhancing the capability to identify, respond and recover from intentional contamination and emergency responses.

Keywords Food safety • Food security • Global food system

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1.1 Introduction

"Food Safety", "Food Security" and, more recently, "Food Defence", are commonly used terms but not always with correct meaning. Especially, food security is very often misused: depending on the context, it could be related to food access or to food terrorism. It is, thus, important to remind the agreed definitions of these expressions.

Food safety is the assurance that food will not cause harm to the consumer when it is prepared and/or eaten according to its intended use [1]. It is the systems reliability to reduce the exposure to natural hazards-errors-failures; and it is *unintentional*. Routine food safety measures, in place throughout the food system, are not designed to prevent or mitigate deliberate contamination of food.

Food chain security can be breached by contamination at any point in the food chain. Using the FAO definition of food chain security:

"Food security exists when all people at all times have physical and economic access to sufficient, safe and nutritious food to meet their dietary needs and food preferences for an active and healthy life style" [2]. This means that there should be supply sufficiency – access to nutritionally adequate and safe food, it goes even further when having an asset that food safety should be a requirement and that this food should meet the dietary needs and food preferences. Therefore, this is a more inclusive concept.

Food defence on the other hand is the "*system resiliency*" – *reducing the impact* of system attacks, it is *intentional*. It mainly found use after the events of September 11th to avoid the misleading "food security" term. Yoe et al. [3] reviewed the way this expression has been used. They concluded that there has been a pervasive and growing usage, without a definition widely agreed upon.

Food protection on the global food supply system should act as an "umbrella" consisting of food safety and food defence.

A contamination incident that makes the food unsafe will breach the security of the food chain. Such contamination incidents have been identified and recorded and can be accidental or deliberate. The vast majority is accidental and includes microbial, physical and chemical contamination. Contamination incidents occur accidentally during food production and include physical contaminants such as glass, metal or plastic and microbial contamination by pathogens [4]. Chemical contamination may also occur but appears to be less common [5]. Microbial contamination is well documented and can result in major outbreaks e.g. Cowden [6]. Occasionally a deliberate contamination incident occurs. These are typically carried out for commercial gain, for example the adulteration of cooking (rapeseed) oil with aniline in Spain in 1981 [7, 8]. Also very occasionally a deliberate contamination incident can be attributed to political or social concerns [9]. In many countries the food industry implements food safety management systems to minimize the number of accidental contamination incidents. These safety systems may

be proactive (e.g. HACCP based systems) and reactive (e.g. Recall systems). Although designed to address the issues of accidental contamination, these systems tend to also offer some protection against deliberate contamination incidents. The interrelationship between food safety and security measures in protecting the food supply has been recognized by both the World Health Organization (WHO) and United States Food and Drug Administration (US FDA). The WHO in its report on "Terrorist Threats to Food" [10] noted that "outbreaks of both unintentional and deliberate food borne disease can be managed by the same mechanism. The key to preventing food terrorism is establishment and enhancement of existing food safety management programmes and implementation of reasonable security measures. Prevention is best achieved through a cooperative effort between government and industry, given that the primary means for minimizing food risks lie with the food industry". As it is often stated in FAO studies, approximately one billion people worldwide are currently undernourished. This situation is likely to worsen in future as a result of climate change, as food insecurity in the lower latitudes, i.e. in many developing countries, will increase with a temperature rise of just 2°C (relative to the 1990 baseline). With global warming of 2–4°C, a drop in agricultural productivity is anticipated worldwide. This trend will be substantially reinforced by desertification, soil salinization or water scarcity. In most Less Developed Countries (LDCs) the areas suitable for agriculture are already largely exploited and this may trigger regional food crises and further undermine the economic performance of weak and unstable states, thereby encouraging or exacerbating destabilization, the collapse of social systems, and violent conflicts [11]. As climate change will affect the availability of water and impact ecosystems, it creates new and competing demands for water and land [12]. For populations that depend on subsistence farming, or do not have sufficient income to buy food, this situation is expected to translate directly into wider prevalence of malnutrition. In turn, malnutrition and under-nutrition increase the severity of many infectious diseases, particularly among children. Effects on infectious disease will not be restricted to developing tropical regions or to other poor. For example, climate change is also expected to change distributions of diseases such as Lyme disease and tick-borne encephalitis, and to increase rates of Salmonella and other food borne infections in Europe and North America [10].

1.2 What Has Changed?: Challenges to the Global Food System in the Twenty-First-Century

The global food system is very vulnerable, both structural and social. The bulk production and need for rapid production, sourcing and distribution at both national and international level is beyond the limits of routine food safety measures of the industry; especially against high-impact deliberate contamination. Adapting to the additional threats to food security arising from major environmental changes requires an integrated food system approach – strengthening the sector's infrastructure

against deliberate contamination – thereby making the food system less vulnerable to attack(s) or destructive economic outcomes.

In the future, it is expected that individual consumption of plant products will continue to decline in favor of an increase in meat and dairy products but also products derived from processed animal products. This change in demand from vegetable to animal products is accompanied by a growth in demand for plant products for animal feed as well as for the production of biofuels. This biomass competition with the effects of the climate change and the proceeding rise in energy and transport costs leads to a sustained rise in agricultural prices, the effects of which are exacerbated by their instability and volatility. The 2008 food crisis and its aftermath in 2009 and 2010 clearly reflect this instability appearing by a sudden and excessive increase in prices followed by an increase in volatility in the short term.

The changing climate will inevitably affect the basic requirements for maintaining health: clean air and water, sufficient food and adequate shelter. According to WHO [10] each year, about 1.2 million people die from causes attributable to urban air pollution, 2.2 million from diarrhea largely resulting from lack of access to clean water supply and sanitation, and from poor hygiene, 3.5 million from malnutrition and approximately 60,000 in natural disasters. A warmer and more variable climate threatens to lead to higher levels of some air pollutants, increase transmission of diseases through unclean water and through contaminated food, to compromise agricultural production in some of the Less Developed Countries (LDCs), and to increase the hazards of extreme weather.

1.2.1 Food- A Potential Victim of Direct Contamination

The contamination and disruption of components of the food supply system may have adverse effects on:

- Public health through injury or death;
- The viability of the food supply;
- Public confidence in the safety of the food we eat; and
- Social and political instability.

Therefore, the safety and security of the food supply is a priority in any comprehensive counter terrorism strategy and threats to the safety of the food supply are already a reality. Remembering that in the modern food supply system:

- Many of the agricultural materials used by processors are seasonal and are held either in bulk storage or as processed product;
- The processing steps generally, but not always, control or remove dangerous organisms and contaminants;
- Food is generally processed as branded products in large scale plants;

- Food is moved through the supply chain as quickly as possible with minimal inventory, particularly of short-shelf life products, to ensure low, internationally competitive prices to consumers;
- An increasing proportion of fresh food produced in large scale plants and is sold through major retail chains;
- A significant proportion of food is sold directly to the consumer through restaurants and other food service outlets; and
- Water is a significant ingredient throughout the food chain – in irrigation, processing and as an ingredient.

It would not be wrong to claim that "food is both a potential victim of direct contamination and a likely casualty of disruption to basic infrastructure services" [13].

1.3 Instability in Agricultural Production and its Effects on Less Developed Countries (LDCs)

The instability in agricultural production also leads to unforeseen price effects. For instance, wheat prices soared in spring 2010 and late summer 2010 due in particular to production losses in Russia and the Ukraine which were undergoing a prolonged period of drought and harvest fires. The conditions for the emergence of new crises are again present with a potentially more risky situation: agricultural products have become the financial assets that are most speculated on. This potential situation of tension is a reality for many Less Developed Countries (LDCs) that share common characteristics in the evolution of the dynamics of their agricultural and food products. The rapid fluctuation in the price increase is a primary trigger for the riots which have occurred in the Mediterranean since the early 2005 [14]. While producers benefit (or at least those who are net producers and whose asset base and knowledge enable them to respond effectively), consumers, especially poor consumers, are severely adversely affected by high prices. Food accounts for a very high share of the total budget of the poorest households. Poor households often consume foods that are less processed so that the effect of rises in commodity prices is felt more strongly. When looked at in the long term there is little or no evidence that price volatility in international agricultural commodity prices, as measured using standard statistical measures, is increasing and this finding applies to both nominal and real prices. However, volatility has been higher during the decade since 2000 than during the previous two decades and this is also the case of cereal (like wheat and rice) prices in the most recent years (2006–2010) compared to the 1970s. Another conclusion that emerges from the study of long term trends in volatility is that periods of high and volatile prices are often followed by long periods of relatively low and stable prices. Domestic price movements can be different. The extent to which global prices are transmitted to domestic markets depends on how strongly integrated the latter are with the former.

Measures such as import duties, export taxes, non-tariff barriers or domestic policies such as price support all influence the extent to which price changes in domestic markets mirror those on international markets. Market structure is also important. In monopsonistic markets, whether private or state controlled, higher international prices may not always result in better prices for producers [15]. In the long-term, climate change and increased biofuel production represent major risks for food security. Although countries in the Southern hemisphere are not the main originators of climate change, they may suffer the greatest share of damage in the form of declining yields and greater frequency of extreme weather events. Agriculture will have to adapt to climate change, but it can also help mitigate the effects of climate change, and useful synergies exist between adaptation and mitigation. Biofuel production based on agricultural commodities increased more than threefold from 2000 to 2008. In 2007–2008 total usage of coarse grains for the production of ethanol reached 110 million tones, about 10% of global production. Increased use of food crops for biofuel production could have serious implications for food security [16]. Recent decades show that land degradation, water shortage and resource competition, when combined with other conflict-amplifying factors, have indeed caused violence and conflict. The review of 73 empirically well recorded 'environmental conflicts' which occurred between 1980 and 2005, however, also showed that these were limited to a regional scope and did not present any serious threat to international security. On the other hand, in the future, climate change can become an international security risk. Climate change is only just beginning, but its impacts will mount steadily in coming decades if global warming is not slowed by effective climate policy. It is thus apparent that large-scale disruptions relevant to security are only to be anticipated in future decades. Unabated climate change could increasingly undermine human security in many regions of the world. WBGU identifies four conflict constellations that may be driven by climate change: 'Climate-induced degradation of freshwater resources', 'Climate-induced decline in food production', 'Climate-induced increase in storm and flood disasters' – and 'Environmentally induced migration', which is triggered by the former three constellations [11]. According to the latest revision of the UN population prospects, the world population is projected to grow from approximately 7.0 billion by 2010, to 7.5 billion in 2020, 8 billion in 2030 and 9.1 billion in 2050. When the nutrition needs of the increasing population are considered along with the climate change, it is believed that especially a change in diet of developed world is going to be needed. The food and agriculture sector contributes about 10–12% of global greenhouse gas emissions, with additional contributions from associated land-use change. Moderating meat consumption and favoring foods that are lower in the food chain has the potential both to enhance health and to reduce impacts on the global climate [10]. Low-income food deficit countries need to reduce their vulnerability to international market shocks not through erection of new trade barriers but through investment in productive capacity and risk management. So long as they do not succeed in improving their overall economic and socio-political stability, they are likely to remain dependent on short-term external assistance [16].

1.4 Policy Options and Recommendations

In order to cope with the adverse effects of climate change in agriculture, some policy options can be given as [11]:

- Strengthening and reorienting rural development: In view of the anticipated drop in agricultural yields, development cooperation should focus to a greater extent on the development of rural regions. However, it is not enough simply to invest more resources in strengthening the agricultural sector. Instead, a new qualitative focus is required in agricultural development strategies in light of climate change.
- Reforming world agricultural markets: The reform of world agricultural markets should be pursued vigorously in order to generate opportunities for market access and production incentives in the developing countries. However, liberalization leads to price increases which can have an extremely adverse effect on Low-Income Food-Deficit Countries. For that reason, it is particularly important to establish compensation mechanisms for these countries through international institutions.

Investing in agricultural productivity growth and resiliency in low income countries is paramount. In the medium and longer term only investment in developing countries agricultural sectors will result in sustainable increases in productivity, healthy markets, increased resilience to international price spikes and improved food security. Investments in infrastructure and especially irrigation, extension services, education, as well as in research and development, can increase food supply in developing countries and improve the functioning of local agricultural markets.

For maintaining environmental security; agricultural policies which devastate the environment are to be replaced by rather controllable alternatives. Moreover, adapting to the additional threats to food security arising from major environmental changes requires an integrated food system approach, not just a focus on agricultural practices. Therefore it is very important to focus on existing research challenges for adapting food system to global environmental change (GEC) [17].

The technological solutions alone are not sufficient for adaptation of food systems to GEC for increasing agricultural yields as bulk of the research is still focusing on agriculture not food systems. Also; tradeoffs across multiple scales among food system outcomes are a pervasive feature of globalized food systems and failure to recognize these often results in food insecurity, ecological degradation and loss of livelihoods [18]. On the other hand; resolving these tradeoffs, particularly across multiple levels of organization and decision making, is crucial to reduce the vulnerability of food systems to GEC [19]. The climate variability and climate extremes are likely to increase in near future, and changes in variability in climate are likely to have greater impacts on agricultural production than changes in mean climate alone [20]. Therefore, much relevant information will need to come directly from the region(s) in question [20, 21], including regional climate models as well as stakeholders.

Taking account of many developing countries' growing dependency on food imports: The liberalization of the agricultural markets and short-term compensation payments will not solve the long-term supply and demand problems faced by many developing countries. A number of developing countries will experience major drops in agricultural yields and growing dependency on farm imports, not least as a result of climate change. For that reason, international climate policy should focus to a greater extent on this issue as well. One option which could be considered is whether those countries which are the main drivers of climate change should pay compensation to other adversely affected states for world market price increases and climate-related drops in agricultural yields.

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Chapter 2
Food Safety System in Croatia

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Abstract The article focuses on the strengths and weaknesses of the food safety system in the Republic of Croatia. Within the process of accession of the Republic of Croatia to the European Union (EU), *Acquis Communautaire* in Chapter 12 – Food Safety, Veterinary and Phytosanitary Policy has been transposed into national legislation and food safety system has become more efficient. The Food Act (Official Gazette No. 46/07) is the basic framework law on food safety in Croatia. The Food Act transposes the provisions of Regulation (EC) No 178/2002 and provides the basis for the assurance of a high level of protection of human health and consumers' interest in relation to food. Food Act covers a general overview and requirements on food safety and hygiene. It lays down the obligations of food business operators and regulates official control system; laboratory operations; crisis and emergency situation management; the rapid alert system for food and feed; and food hygiene. Food Act also stipulates general food quality and labeling requirements, as well as general requirements for food made from, or consisting of, genetically modified organisms (GMO). Moreover, the Food Act is legislative framework for regulating the official control system, setting out the competencies of authorities responsible for carrying out official controls. Enforcement of laws and regulations is done on a central and regional/local level. Ministry of Agriculture, Fisheries and Rural Development (MAFRD) and Ministry of Health and Social Welfare (MHSW)

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are responsible for the enforcement of legislation both on the central and regional/local level. Integrated EU strategy on food safety: “from farm to fork” should improve efficiency of the whole food safety system and increase confidence of all interested parties in Croatian food safety policy, from farmers to consumers.

Keywords Croatia • Food safety • Official controls • Public health

2.1 Introduction

Food and nutrition are major determinants of health, disease and productivity. Safe food and adequate nutrition affect the survival, well-being and functioning of individuals and societies, and should thus be prominent components of policies addressing health, education, welfare, agriculture and fisheries, trade, environment and infrastructure [1]. Recent trends in global production, processing, distribution and preparation of food are creating an increasing demand for food safety research in order to ensure a safer global supply of food. Foodborne diseases pose a considerable threat to human health and the economies of individuals, families and nations. Foodborne diseases, particularly those of zoonotic origin, represent a considerable public health burden and challenge. In some parts of South-eastern Europe, the prevalence of foodborne and waterborne diseases such as hepatitis A, salmonellosis, campylobacteriosis, trichinellosis and echinococcosis are of particular concern. Antimicrobial resistance is an increasing public health problem that is partly related to non-human use of antimicrobial agents. Various chemical hazards also represent a public health risk, and food allergies are being increasingly recognized as a concern [2]. Changes in the world food economy are further reflected in changed dietary patterns, for example, increasing consumption of energy-dense diets high in fat, particularly saturated fat, and salt and low in unrefined carbohydrates. Because of these changes in dietary and lifestyle patterns, chronic non-communicable diseases (including obesity, diabetes mellitus, cardiovascular disease, hypertension and stroke) and some types of cancer are becoming increasingly significant causes of disability and premature death. Over 34 million disability-adjusted life-years (DALYs) (23% of total) are lost each year to cardiovascular disease in Europe [3]. A retrospective inquiry recognized that poor agricultural practices and bad policy-making, which did not take public health into account, led to BSE being transmitted to humans as a new fatal disease (new variant Creutzfeld-Jakob disease) [4]. Since BSE, policy-makers across Europe and worldwide have overemphasized food safety as the major health issue to be considered in agricultural and food policy, despite the relatively higher importance of food security, nutrition, and other risk factors in terms of global burden of disease [5, 6] (Fig. 2.1).

In 2000, the 53rd World Health Assembly unanimously confirmed food safety as an essential public health priority and committed World Health Organization (WHO) to expanding its responsibilities in food safety. The outcome of this has been a

Fig. 2.1 Factors driving changes in food safety systems. Source: FAO/WHO. Food safety risk analysis – a guide for national food safety authorities, 2006 [7]

global food safety strategy with the objective of reducing the health and social burden of foodborne disease [8].

It is intended that this objective will be achieved by:

- Advocating and assisting in the development of risk-based, sustainable, integrated food safety systems;
- Developing science-based measures along the entire food chain that will prevent exposure to unacceptable levels of chemical and microbial hazards; and
- Assessing, communicating and managing foodborne risks in cooperation with other sectors and partners.

To enable national authorities, particularly in developing countries, to improve their food safety systems and to ensure that the standards for imported foods conform to national requirements, WHO and the Food and Agriculture Organization (FAO) jointly published guidelines for strengthening national food safety systems [9].

This article presents the overview of the legislation in the food safety area together with a description of the organization of the food safety system in the Republic of Croatia. The special emphasize is given to the strengths and weaknesses of the existing food safety system.

2.2 Previous Efforts that Led to the Food Safety System in Croatia, as We Now Know

There are several documents that enabled the development of food safety system in Croatia [10]. The first document was the "Croatian Food and Nutrition Policy". The aims and priorities of future action plan, as well as proposed measures to improve feeding status, were based on an estimation of Croatians' eating habits. One of the main prioritized objectives in this document was the promotion of an

adequate diet and healthy lifestyle, together with a reduction of overweight and prevention of obesity [11]. Subsequently, many Croatian experts coming from different fields participated in the preparation of the government document "Croatia in twenty-first Century: Strategy for Development of the Republic of Croatia – IV Feeding: Food Safety and Food Security", which gave an overview on the current status within these two items, general aims, strategic measures, critical questions and recommendations [12]. In order to provide assistance in the field of cooperation between south-east European countries, WHO organized the workshop "Development of Food and Nutrition Action Plans in Countries of South-East Europe" in Brijuni – Croatia, where 13 participating countries presented their progress in developing action plans followed by a discussion on the priorities for implementing national food and nutrition action plans, the Stability Pact Initiative and United Nations International Children's Emergency Fund (UNICEF) work [13]. The biggest encouragement for the development of the Croatian national Food Safety Strategy came from the European Commission's Food and Veterinary Office (FVO) mission to Croatia carried out in March 2005 with the task to provide a general assessment of control systems for food safety, animal health, animal welfare and plant health. One of the most important conclusions of the mission was that Croatia needs a clear and unique Food Safety Strategy that would prepare the food safety system's adjustments to European Union (EU) requirements. Improvement of coordination and collaboration between different food safety institutions in Croatia was stressed as the main precondition to start working on the Strategy [14].

The first international project that encouraged Croatia to improve and upgrade its national food safety system was the project "Strengthening Food Safety and Nutrition Services in South-East Europe", launched in May 2002. This Project aims to strengthen the public health approach to food safety and nutrition in South-eastern Europe (SEE) countries and to increase the effectiveness of activities to promote health and reduce the burden of foodborne and nutrition-related diseases. Since food moves between countries and populations, the Project also aims to build the essential network to ensure safety along the food chain. The SEE Health Network provides a forum to ensure a safe, healthy, accessible and sustainable food supply, and to assess the effectiveness of governments in addressing these issues from production to consumption. The Network is further developing recommendations for change and for movement towards more effective food safety and healthy nutrition systems [1].

The second international project that helped to speed up the process of harmonization of Croatian legislation with the EU standards and to establish harmonized and integrated food safety system was project "Capacity building in the area of agriculture, live animals and food products" with partners from Italy and Great Britain that was implemented during 2005–2006. This project aimed to support the Croatian Ministry of Agriculture, Fisheries and Rural Development (MAFRD) and the Croatian Ministry of Health and Social Welfare (MHSW) and other organizations involved in food safety to develop and upgrade control system, to draft a Food

Safety Strategy and build the institutional and legislative framework necessary to implement this Strategy [15]. These two projects were coordinated by the Croatian authorities in the way that activities in the first one were used to support the activities of another. Both projects aimed to prepare data in a form of country profiles: the first one by the national expert and second one by the international expert. The benefit was in having two independent food safety system analyses that revealed both the national and international points of view [10].

2.3 Food Safety System in the Republic of Croatia

Food borne and nutrition-related diseases represent a significant public health burden and challenge throughout the European Region, affecting all age groups and all socio-economic classes. Today, the food chain has become longer, more complex and globalized. As a consequence, food contamination is very common. Globally an estimated 2.2 million people die from food- and waterborne diarrheal diseases per year, 1.9 million of whom are children. In industrialized countries, nearly one in three persons per year suffers from a foodborne illness. These figures represent only the tip of the iceberg, as the estimated number of unreported cases is very high. Furthermore, the figures refer to gastrointestinal illness only and do not include disease caused by chemical contamination in the food chain. Foodborne diseases of zoonotic origin, i.e. transmitted from animals to humans, represent a particular public health burden and challenge. Salmonellosis and campylobacteriosis are the most common, with several hundred thousand cases reported per year in the European Union alone. In some parts of the Region, brucellosis, and the parasitic zoonoses trichinellosis and echinococcosis are also of great concern. Foodborne viruses are increasingly recognized as a major cause of gastrointestinal illness throughout the Region. Foodborne staphylococcal intoxication is widespread, and in some countries botulism is still a significant threat. Antibiotic resistance caused in part by usage of antibiotics in food animals is becoming a significant public health problem. Foodborne infections caused by resistant bacteria like *Salmonella*, *Campylobacter* or *E. coli* pose a particular risk to human beings including possible treatment failure, prolonged disease and increased severity of disease, mortality and societal costs. Various chemical hazards also represent a public health risk and food allergies are increasingly recognized as a concern. The prevention of foodborne disease and the response to food safety challenges requires holistic, risk-based and timely policies and strategies [16]. The reported number of epidemics and individual cases of foodborne infections and intoxications in Croatia varies between 50 and 100 annually. While the number of registered cases ranges between 8,000 and 10,500 annually. The most common casual organisms of epidemics are *Salmonellae* sp., *Staphylococcal* toxin and, among parasites, *Trichinella*. Salmonellosis accounts for 50% of all registered cases of foodborne diseases [1].

2.3.1 Food Safety System

Food safety system in the Republic of Croatia includes the Ministry of Agriculture, Fisheries and Rural Development (MAFRD) as the Competent Authority, authorities responsible for drafting and enforcement of legislation (MAFRD, Ministry of Health and Social Welfare – MHSW) and other bodies and institutions involved in the food safety system (Croatian Food Agency – CFA, official and reference laboratories, control bodies). Pursuant to the provisions of the Food Act, the MAFRD has been designated as the Competent Authority, i.e. the central state administration authority responsible for food safety and hygiene, development of the food safety policy, organization of official controls and for ensuring efficient and effective co-ordination between all bodies and institutions involved in the food safety system and is a contact point for communication with the European Commission. In Croatia risk analysis is established as an integral part of a food safety system. Risk analysis, a systematic, disciplined approach for making food safety decisions developed primarily in the last two decades, includes three major components: risk management, risk assessment and risk communication. Risk analysis is a powerful tool for carrying out science-based analysis and for reaching sound, consistent solutions to food safety problems. The use of risk analysis can promote ongoing improvements in public health and provide a basis for expanding international trade in foods [7]. In Croatia risk assessment is institutionally separated from risk management. Risk management is under the responsibility of the MAFRD and the MHSW, while Croatian Food Agency (CFA) is responsible for risk assessment. The MAFRD and CFA cooperate to ensure the coherence of the risk communication process [17].

The mission of CFA includes providing scientific advice and technical support, collecting and analyzing data to allow the characterization and monitoring of risks which have a direct or indirect impact on food safety. The Agency does not have a role in the co-ordination of management activities (Fig. 2.2).

Communication on the risks in food has developed in about last 10 years into a special field as one of the three components of analysis of the risk in food (risk assessment, risk communication and risk management). Today's food safety risk analysis scheme recommended by the *Codex Alimentarius* commission in which two circles – risk assessment and risk management are encircled by the third circle – risk communication clearly emphasize that communication of the risks in food is considered to be very significant nowadays [18].

2.3.1.1 Food Safety Legislation

The Food Act is the basic framework law on food safety in Croatia. The Food Act transposes the provisions of Regulation (EC) No 178/2002 of the European Parliament and of the Council of 28 January 2002 laying down the general principles and requirements of food law, establishing the European Food Safety Authority

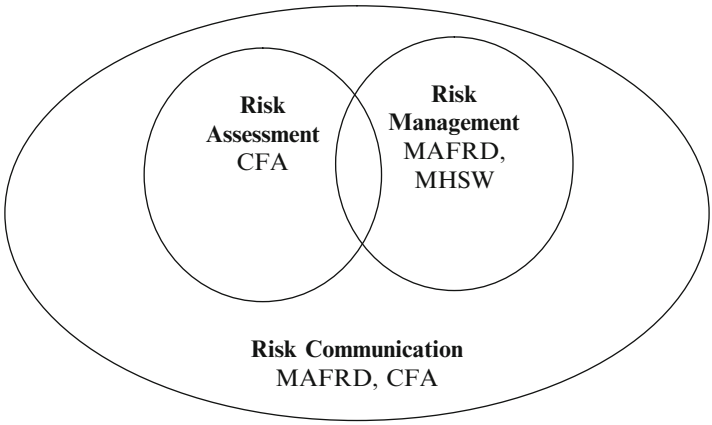

Fig. 2.2 Competent authorities involved in the food safety risk analysis in Croatia

and laying down procedures in matters of food safety. Food Act provides the basis for the assurance of a high level of protection of human health and consumers' interest in relation to food, taking into account the protection of animal health and welfare, plant health and the environment. Food Act covers a general overview and requirements on food safety. It lays down the obligations of food business operators and regulates official control systems; laboratory operations; crisis and emergency situation management; the rapid alert system for food and feed; and food hygiene. Furthermore, Food Act contains general provisions for traceability which cover all food and all food business operators. According to the Food Act primary responsibility for ensuring compliance with food law, and in particular the safety of the food, rests with the food business operators. The Food Act also stipulates general food quality and labeling requirements, as well as general requirements for food made from, or consisting of, genetically modified organisms (GMO). Moreover, the Food Act is legislative framework for regulating the official control system. In addition to the provisions transposed from the *Acquis*, the Food Act also contains certain national provisions relating to the CFA, competencies of authorities responsible for carrying out official controls, and penalty provisions for the infringement of certain provisions of the Act [19]. Legislation brought on the basis of the Food Act covers all stages of the production, processing, distribution and placing on the market of food intended for human consumption. Regarding the placing food on the market, following implementing regulations, which transpose the provisions of the *Acquis*, have been published under the Food Act: Ordinance on the hygiene of foodstuffs (Official Gazette No. 99/07, 27/08, 118/09), which transposes the provisions of the Regulation (EC) No 852/2004; Ordinance on the hygiene rules for food of animal origin (Official Gazette No. 99/07, 28/10), which transposes the provisions of the Regulation (EC) No 853/2004; Ordinance on official controls performed to ensure the verification of compliance with feed and food law and animal health and welfare rules (Official Gazette No. 99/07, 74/08), which

Table 2.1 Division of competencies for policy and legislation development and for official controls in the food safety area

Policy and legislation	Official controls
MAFRD	
Directorate for food safety and quality	Directorate for veterinary inspections
Veterinary directorate	Directorate for agricultural and phytosanitary inspection
MHSW	
Directorate for sanitary inspection	Directorate for sanitary inspection

transposes the provisions of the Regulation (EC) No 882/2004; Ordinance on official controls on food of animal origin (Official Gazette No. 99/07, 28/10), which transposes the provisions of the Regulation (EC) No 854/2004. The Ordinance on microbiological criteria for foodstuffs (Official Gazette No. 74/08, 156/08, 89/10), which transposes the provisions of Regulation (EC) No 2073/2005, has also been adopted. As regards food safety, responsibility for the adoption, alignment and interpretation of legislation under the Food Act is shared between the MAFRD and the MHSW. All legislation adopted by MHSW is subject to the consent of MAFRD (Table 2.1).

2.3.1.2 Food Safety Official Controls

Pursuant to the provisions of the Food Act, the MAFRD is central competent authority responsible for the organization of official controls and for ensuring efficient and effective co-ordination between all the authorities and their directorates responsible for carrying out official controls on food. The authorities responsible for enforcement of legislation in food safety area are the MAFRD and the MHSW, which are responsible for carrying out official controls to verify compliance with food law and for monitoring and verifying that all the requirements of food law are fulfilled by food business operators at all stages of production, processing and distribution. Moreover, the MAFRD is responsible for: developing a single multi-annual national control plan and for preparing and submitting to the European Commission annual reports on the implementation of that plan; managing the rapid alert system for food and feed (RASFF) at the national level (CRO RASFF) and has been designated as RASFF national contact point with the European Commission; designation of official and reference laboratories for food. MHSW co-operates with MAFRD as regards the preparation of multi-annual national control plan, annual control plans and reports, implementation of official controls, development of legislation, documented procedures, authorization of official laboratories and registration of establishments. In the event of a crisis or when food is found to pose a serious risk to human or animal health either directly or through the environment, MAFRD is responsible for setting up a crisis unit, while both MAFRD and MHSW are responsible for crisis management. Official controls are carried out by the MAFRD and the MHSW, or more precisely, by the Directorate for Veterinary

Table 2.2 Division of responsibilities for official controls in food safety area

Stage	Responsible administrative organization	Inspection service
Food of animal origin		
Primary production	MAFRD – Directorate for Veterinary Inspections	Veterinary inspection of the MAFRD
Production and processing	MAFRD – Directorate for Veterinary Inspections	Veterinary inspection of the MAFRD
Retail	MAFRD – Directorate for Veterinary Inspections, in establishments approved by MAFRD under special conditions	Veterinary inspection of the MAFRD
	MHSW – Directorate for Sanitary Inspection	Sanitary inspection of the MHSW
Import	MAFRD – Directorate for Veterinary Inspections	Border veterinary inspection of the MAFRD
Food of non-animal origin		
Primary production – food of plant origin	MAFRD – Directorate for Agricultural and Phytosanitary Inspection	Agricultural inspection of the MAFRD
Production and processing	MHSW – Directorate for Sanitary Inspection	Sanitary inspection of the MHSW
Retail	MHSW – Directorate for Sanitary Inspection	Sanitary inspection of the MHSW
Import	MHSW – Directorate for Sanitary Inspection	Border sanitary inspection of the MHSW
Food containing ingredients of animal and non-animal origin		
Production and processing	MAFRD – Directorate for veterinary inspections	Veterinary inspection of the MAFRD
	MHSW – Directorate for sanitary inspection	Sanitary inspection of the MHSW
Retail	MHSW – Directorate for sanitary inspection	Sanitary inspection of the MHSW
Import	MAFRD – Directorate for veterinary inspections	Border veterinary inspection of the MAFRD
	MHSW – Directorate for sanitary inspection	Border sanitary inspection of the MHSW

Inspections of the MAFRD, the Directorate for Agricultural and Phytosanitary Inspection of the MAFRD and the Directorate for Sanitary Inspection of the MHSW, while the Directorate for Food Safety and Quality of the MAFRD is responsible for co-ordination of official control activities or co-ordination of all the authorities responsible for carrying out official controls in food safety area (Table 2.2).

Official controls on food of animal origin are carried out, at the levels of primary production, production and processing and at the import by the Directorate

for Veterinary Inspections of the MAFRD i.e. the veterinary inspection and the border veterinary inspection. Official controls on food of plant origin are carried out, at the level of primary production, by the Directorate for Agricultural and Phytosanitary Inspection of MAFRD i.e. the agricultural inspection. Official controls on food of non-animal origin are carried out, at the levels of production and processing and at the import, by the Directorate for Sanitary Inspection of the MHSW i.e. the sanitary inspection and the border sanitary inspection. Official controls on food containing ingredients of animal and non-animal origin are carried out, at the levels of production and processing and at the import, by the veterinary inspection and the border veterinary inspection of the MAFRD and the sanitary inspection and the border sanitary inspection of the MHSW. At the level of retail, official controls on food of animal and non-animal origin and on food containing ingredients of animal and non-animal origin are carried out by the sanitary inspection of the MHSW, except in establishments approved by the MAFRD where official controls are carried out by the veterinary inspection of the MAFRD. Directorate for Veterinary inspections of the MAFRD carries out official controls in establishments that are registered and approved by the MAFRD. Directorate for Sanitary Inspection of the MHSW carries out official controls establishments that are registered by the MHSW.

2.3.1.3 Surveillance and Monitoring

Surveillance of foodborne diseases is part of the Communicable Disease Information System. It is based on the Population Protection from Infectious Disease Act, under which the first contact physician is obliged to report an infectious disease or epidemic to the epidemiological service in charge. Data on the incidence of foodborne diseases and intoxications are collected by the public health sector and a bi-monthly report is published. This part of work is mainly done by the Croatian National Institute of Public Health and County Public Health Institutes in the field. The network of county institutes of public health is responsible for analyses of food for human consumption, water and human transmissible diseases. Furthermore, several private laboratories carry out analyses of food samples taken in the framework of hazard analysis critical control point – HACCP plans and quality assurance samples implemented by the food industry. The Croatian Veterinary Institute is responsible for carrying out analyses of samples taken from animals, animal feed and food of animal origin. An increasingly important role for food safety systems is the delivery of information, education and advice to stakeholders across the farm-to fork continuum. These activities include the provision of balanced factual information to consumers, of information packages and educational programs for workers in the food industry, and of reference literature to extension workers in the agriculture and health sector. According to the Food Act, the CFA should do this with a high level of transparency [1, 19].

2.3.2 Resource Generation

MAFRD, MHSW and CFA provide information to the food business operators, consumers and other stakeholders concerning food risks. Education and health promotion are also activities of the Croatian National Institute of Public Health. According to the Food Act, as from 2009 food business operators should implement HACCP principles in all establishments involved in the production of animal and non-animal food. The main actors involved in the implementation of the health promotion policy are the MHSW and the subordinate Croatian National Institute of Public Health and institutes of public health in each of the 21 counties [1].

2.3.3 Financing

The public health system is financed by the State Health Insurance Institute and by the MHSW at central level and through the county budgets. Nutrition and food safety services are almost 100% financed from the market in laboratory services, part of which is also control of foodstuffs ordered by the sanitary inspection service. This control is financed from county budgets according to the Framework Plan of Food Inspection. As regards the financing of official controls, pursuant to the provisions of the Food Act funds are secured in the State Budget to provide for the necessary number of staff performing official controls and to cover all the costs for the implementation of such controls. Fees and charges are levied and collected to cover the costs occasioned by official controls. Moreover, Food Act stipulates that the minister of the MAFRD, by means of an implementing regulation, determines the criteria for setting the level of fees, the level of charges and the method of their calculation and payment [17]. In addition, the Veterinary Act (Official Gazette No. 41/07) stipulates that the inspections are carried out by state veterinary inspectors, county-city veterinary inspectors and border veterinary inspectors, who have the status of civil servants paid from the national budget [1, 20].

2.3.4 Laboratory Services: Food Monitoring and Epidemiological Data

The Ministry of Agriculture, Fisheries and Rural Development (MAFRD) is the central competent authority concerning food safety. At local level, veterinarians and sanitary inspectors carry out official controls of foodstuffs. Within the MAFRD there are several directorates that are involved in food safety control system, these are: the Food Safety and Quality Directorate, the Veterinary Inspections Directorate

and the Directorate of Agricultural and Phytosanitary Inspection. In the MHSW only the Sanitary Inspection Directorate is involved in food safety [1]. On the basis of the Food Act and Ordinance on authorization of official and reference laboratories for food and feed (Official Gazette No. 86/10, 7/11) the official and reference laboratories are authorized by the Minister of the MAFRD. Ordinance on authorization of official and reference laboratories for food and feed stipulates procedure and methods for authorization of official and reference laboratories for food and feed, conditions to be met by the official and reference laboratories for the performance of analysis of samples taken during official controls, obligations of official and reference laboratories, as well as areas for which is required to authorize reference laboratories. Areas for which is necessary to authorize the reference laboratories are in line with the Regulation (EC) 882/2004 with two additional areas that are of national interest for Croatia (olive oil, honey and honey products). The main requirement for obtaining authorization as an official or reference laboratory is accreditation according to the standard HRN EN ISO/IEC 17025. The laboratory network is organized through the Croatian National Institute of Public Health (CNIPH) and the Croatian Veterinary Institute (CVI) dealing, respectively, with food of non-animal origin and food of animal origin. Each national center coordinates a network of peripheral laboratories with different responsibilities. The Croatian National Institute of Public Health (CNIPH) provides laboratory services for food safety purposes (central and county laboratories) and takes part in preparing and implementing annual monitoring programs. The County Institutes of Public Health (21 of them, one in each County) form a national network. The Croatian Veterinary Institute (CVI) provides laboratory services in implementation of animal health programs as well as in the official controls of animal health, food and feed of animal origin. It comprises the central veterinary laboratory in Zagreb together with the Poultry Centre and four regional veterinary laboratories.

2.4 Discussion and Conclusion

In order to ensure high level of human health and protect consumers' interest in relation to food it is necessary to strengthen Croatian food safety system in terms of increasing efficiency and effectivity of the system. The first step in the process of strengthening Croatian food safety system is an effective and efficient organization of official controls which is the standard that must be met in order to protect human health and consumers' interest. Official controls should be implemented at all stages of production, processing, distribution and import of food. Efficient and effective organization of official controls can be achieved by improving the implementation of official controls in accordance with annual control plans, which have to be based on risk analysis and have to include clear deadlines and division of responsibilities. In order to achieve uniformity of the performance of official controls, competent authorities have to carry out official controls in accordance with documented procedures, i.e. detailed, written information and instructions for staff performing official

controls. Furthermore, Croatian competent authorities have to ensure sufficient number of suitably qualified and experienced staff so that official controls can be carried out efficiently and effectively. In order to strengthen official control system, Croatian competent authorities have to ensure effective coordination of activities and cooperation within and between competent authorities, especially in relation to issues which involve joint action or cooperation between different directorates within a competent authority or between different competent authorities. Special emphasis should be placed on establishing an effective system of official and reference laboratories for food. Competent authority should only designate laboratories that are assessed and accredited in accordance with the HRN EN ISO/IEC 17025 on 'General requirements for the competence of testing and calibration laboratories'. Accreditation according to the HRN EN ISO/IEC 17025 means a confirmation that the analysis are carried out in accordance with the highest standards. Protection of consumers' health is the primary task of all participants in the food chain which includes production, processing and distribution of food. Good inter-institutional cooperation and good communication is the basic prerequisite for rapid response regardless of whether the information coming through the rapid alert system or otherwise. It is also of particular importance properly developed system of information flow, accountability and decision-making. The institutional networking in the area of food safety, which is in Croatia established and coordinated by CFA aims to facilitate a scientific cooperation framework by the coordination of activities, the exchange of information, development and implementation of joint projects and exchanging of expertise. In order to successfully solve various food safety problems one always has to bear in mind that highly important tool for doing so is risk analysis, which should be constantly developed and improved in accordance with new scientific perceptions and socioeconomic trends. Consumer awareness of food safety issues is of the utmost importance toward healthy lifestyles and disease prevention. Thus, it is necessary to recognize the importance of communication about the risks arising from food between competent authorities and consumers. It is necessary to increase information exchange and consumer education in order to increase public awareness.

Improving the food safety system will increase the effectiveness of coordination and cooperation, communication and reporting, information and data exchange between all bodies and institutions involved in the food safety system, as well as communication with the public regarding the food safety.

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Chapter 3
Bioactive Compounds of Georgian National Food: Determination of Antioxidant Activity

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Abstract The diversity of the food in Georgia is directly connected with the biodiversity of the country consisting of 14 different soil climatic zones, beginning from subtropical and including steppe and alpine areas. The country is characterized by great amount of plants (the amount of endemic plants exceeds 1,100). Plants grown in the country contain hundreds of structurally different secondary metabolites the majority of which are characterized by definite physiological activity. Bioflavonoids, characterized by extremely high antioxidant activity, seem to be especially interesting. Other secondary metabolites presented in plants in high amount are: phenolics, organic and amino acids, vitamins of different groups, alkaloids, terpens, glycosides, etc. Antioxidant potential of Georgian wines and some Georgian food have been determined. All the food tested showed high antioxidant activity. Georgian wines (both red and white) produced by Kakhetian Technology are characterized by higher content of flavonoids and antioxidant activity than European type wines. The content of phenolic compounds and antioxidant activity is much higher in red wines than in whiter ones. On the base of Georgian flora biologically active biocomposites have been produced. These biocomposites show high antioxidant and antimicrobial activities. They are successfully used in food industry as biologically active food additives including food colorants.

Keywords Antimicrobial activity • Antioxidant potential • Biologically active compounds • Flavonoids • Georgian national food • Georgian wines

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3.1 Introduction

Georgia in spite of the small area (69,000 km^2) is characterized by extreme soil-climatic diversity. Geologically, the territory of Georgia belongs to the Alpine System of Eurasia. Its geological and geomorphologic structures reveal great diversity resulting from tectonic, volcanic, petrologic, gravitational, erosion, and other processes. There are 14 soil-climatic zones in Georgia from the humid, subtropical to the permanent snow and glaciers. The country is characterized by great amount of plants; the amount of endemic plants exceeds 1,100 and is determined by cultural and wild flora. The detailed investigation of the chemical composition of annual and perennial vegetation led us to the suggestion that plants grown in the country contain hundreds of structurally different secondary metabolites the majority of which are characterized by definite physiological activity. Bioflavonoids which are characterized by extremely high antioxidant activity are especially interesting. These compounds bind and eliminate free radicals (formed during stress situation) and heavy metals (penetrated into the body by food chain) from human body. Flavonoids are chemical constituents of great majority of vegetations (above 200 varieties) and fruits. The largest amount of flavonoids is found in red grape called "Saperavi", green tea, quince tree, etc. Other secondary metabolites presented in plants in high amount are: phenolics, organic and amino acids, vitamins of different groups, alkaloids, terpens, glycosides, etc. The diversity of the food in Georgia is directly connected with the biodiversity of the country. Different kinds of plants with their wide spectrum of bioactive compounds enable local people to produce diverse physiologically and pharmacologically active foods such as Matsoni cheese (containing probiotics). More than 500 varieties of grapevine are grown in different regions of Georgia. Technology of producing wine from these varieties of grapevines is different. Antioxidants of red wine are mainly catechins, proantocyanidins, antocyanins and flavonols that are localized in grape skin and seeds. The use of such food additives allows intensifying industrial processes, increasing nutritional value and shelf life of food products and preventing different kinds of diseases. *The aim* of this work was to investigate antioxidant potential of some Georgian national foods and plant multifunctional bioactive food composites.

3.2 Materials and Methods

White and red wines of Kakhetian and European types, prepared from the varieties of grapes (*Vitis vinfera* L.) cultivated in Georgia, were supplied to us by wineries and private producers. Green tea extract made from leaves of *Camellia sinensis* L. was obtained from the local plant extracts producing company, red wine lees was produced from Georgian variety of *Vitis vinifera* "Saperavi", lemons of Georgian variety of "Kartuli" were commercially available at local market. Green tea extracts with 23%, 60% and 80% total polyphenols content made from leaves of *Camellia sinensis* L. were obtained from the local plant extracts producing company. Georgian national

foods Churckela, specially treated vine leaves for making Tolma, Matsoni (Georgian yoghurt) Gebzhalia (rolled slices of cheese in mint sauce, Tkemali (wild plum sauce) were homemade. The Ferric Reducing Ability of Plasma (FRAP) assay was used to measure the concentration of *total antioxidants* [1]. *Antiradical* efficiency was determined by using 2,2-diphenyl-1-picrylhydrazyl (DPPH) [2, 3]. *Total phenolic* compounds was analyzed with Folin-Ciocalteu reagent [4] and in red wine lees and composite was investigated as described in ref. [5]. *Pectin substances* were quantitatively analysed by the method based on the reaction of galacturonic acid with carbasole in sulphuric acid medium [6]. Amino acids were determined by color reaction of free amino acids with nynhydrin reagent [7]. Mineralization of samples was done at 525°C in a combustion oven. The standard reducing-sugar assay was that of Nelson and Somogyi [8] with some modification described by Green et al. [9]. Composite 1 was prepared from green tea extract and red wine lees according to Georgian patent [10]. Composite 2 was prepared from green tea extract, red wine lees and lemon peels according to Georgian patent [10]. Agar well diffusion method was used to investigate *antimicrobial properties* of plant extracts in detail described elswhere [11]. The following microorganisms were used as tests in our experiments: Gram positive bacteria – *Staphylococcus aureus*, *Rhodococcus* sp., Gram negative bacteria – *Esherichia coli, Pseudomonas aeroginasa*; Yeasts – *Candida utilis*, *Saccharomyces fragilis*, *Sacharomyces cerevisiae*, Pathogenic fungi – *Fusarium solani, Rhizoctonia* sp. Detailed method is described elswhere [12]. Data were subjected to one way analysis of variance and/or *t*-test. When necessary, the "least squares" method was used to calculate a straight line that best fitted experimental data, and returned an array that described the line. All calculations were performed with Microsoft Excel (Version 4, statistical functions, Microsoft Corp., Redmond, WA, USA).

3.3 Results and Discussion

The content of total phenolic compounds and antiradical efficiency (AE) of wines of Kakhetian type are much higher than those of wines of European type. This must be caused by technology of production of wines of Kakhetian type, according to which the grape juice together with the skin and pulp is placed in a pitcher buried in the ground and allowed to fermentation. During alcoholic fermentation a large quantity of flavonoids is extracted by the grape juice from grape stems, skins and seeds. These compounds play an important role in the formation of some characteristics of wine of Kakhetian type. They determine the essence of this type of wine as well as its taste and aroma (as a result of interaction of flavonoids with oxidizing enzymes of stems and skins of grapes, the specific color, taste and aroma characteristic of Kakhetian wine is created) [13]. The average values of AE of white and red wines of Kakhetian type 2.8 and 5 times higher than those of European type white and red wines respectively. The comparison of white and red wines produced by the same technology showed that the content of phenolic compounds and antioxidant activity was much higher in red wines than in white ones.

Table 3.1 Antioxidant potential of some Georgian National Food (of 100 g dry matter)

Georgian food	Antioxidant potential (Fe^{2+}) mM
Churchkela	300
Matsoni	500
Tkemali	300
Gebzhalia	700
Boiled vine leaves for preparing Tolma	1,140

Table 3.2 Antioxidant potential of plant products

Plant product[a]	Antioxidant potential (Fe^{2+}) mM
80% green tea polyphenols	30.20 ± 0.7
60% green tea polyphenols	26.00 ± 0.6
(−) epicatechin	16.00 ± 0.3
23% green tea polyphenols	2.10 ± 0.1
L-ascorbic acid	9.40 ± 0.7
Composite 1	1.80 ± 0.3
Composite 2	2.30 ± 0.2

[a]Figures are calculated on 1 g/l concentration of the extracts

Antioxidant activities of some Georgian foods such as Churchkela, Matsoni, Gebzhalia, Tkemali and specially treated vine leaves for preparing Tolma are given in (Table 3.1). All the food showed high antioxidant activity but vine leaves revealed the highest antioxidant potential. Also antioxidant potential of some plant products are presented (Table 3.2).

3.3.1 Biologically Active Plant Composites on the Base of Georgian Tea and Red Wine

Biocomposites with new original formula (1) green tea extract + red wine lees (Composite 1); (2) green tea extract + red wine lees + extract of citrus peel (Composite 2) were created. They do not consist of any synthetic chemicals and additives, aromas and enhancers – that is are absolutely natural products. The biocomposites are produced in a dry powder ready to use form, easily soluble in hot/cold water; they have a pleasant, natural reddish and/or brownish colour, aroma and characteristic astringent taste. The main constituent of these composites is red wine lees, which is a by-product of the winery industry, its price is relatively low, but it is very valuable product because of bioactive compounds present in it. Abundant polyphenols and pectin substances were found in red wine ("Saperavi") lees (16.4% and 12.8% respectively). Red wine lees are rich in other bioactive compounds, such as sugars, amino acids – 14 and 19 respectively. Red wine lees consist of red pigments – anthocyanins, and therefore it can be successfully used for purpose of food colouring.

Table 3.3 Chemical composition of composites (% dry matter)

Composite	Polyphenols	Soluble pectin	Amino acids	Sugars	Minerals
Composite 1	17.3 ± 0.5	11.5 ± 0.3	15.3 ± 0.5	22.5 ± 0.7	8.5 ± 0.5
Composite 2	21.3 ± 0.5	14.0 ± 0.4	7.0 ± 0.4	30 ± 0.6	8.0 ± 0.3

In case of enriching red wine lees with polyphenolic compounds it can be used as an antioxidant food additive or physiologically active biocomposite. For this purpose green tea extract was selected, since it is known to consist of high content of polyphenols, about 58% of which is (−) epigallocatechin gallate, the one of the most antioxidant and anticarcinogenic compounds known so far. Green tea extract is also commercially available in Georgia. These biocomposites are rich in polyphenolics (15–22%), as well as in other biologically active compounds such as pectin, amino acids, etc. (Table 3.3). Antioxidant potential of the composites was compared with that of vitamin C, (−) epicatechin 60% and 80% green tea polyphenols. Composite 1 showed maximum antioxidant potential, assumingly due to synergetic effect (Table 3.3). Both composites showed approximately four times less activity than L-ascorbic acid, though the composite from green tea extract and red wine lees showed by 30% more antioxidant potential than the other composite. Antioxidant potential of 80% green tea polyphenols was three times as much as that of L-ascorbic acid. (−) Epicatechin showed by 50% more antioxidant potential than vitamin C. Differences between antioxidant potentials of 23% green tea polyphenols, Composite 1 and Composite 2 were not statistically significant at the 5% probability level. The composites were found to possess high antioxidant potential. They revealed only about four times less activity than ascorbic acid.

Antimicrobial activity of the composites was also investigated. As shown in Table 3.4, the composites, at a given concentration, were active against gram positive and negative bacteria, and against pathogenic fungi *Rhizoctonia* sp., *Streptomyces glaucus* 71 MD, but did not show any activity against yeasts and pathogenic fungus *Fusarium solani*. The composites expressed the highest activities against gram positive bacteria *Staphylococcus aureus*, *Rhodococcus* sp and pathogenic fungi *Streptomyces glaucus* 71MD. The differences between antimicrobial activities of the composites against these three species of microorganisms were not statistically significant ($\alpha = 0.05$ level). Statistically significant differences were observed between activities of the composites against gram negative bacteria and pathogenic fungi. Inhibition zones of the growth of gram negative bacteria *Esherichia coli* were 18.1 and 6.1 mm for Composite 1 and Composite 2 respectively. Composite 1 showed less activity against the other gram negative bacteria – *Pseudomonas aeroginasa* (inhibition zone was 6.1 mm) in comparison with Composite 2 (inhibition zone was 10.2 mm). *Rhizoctonia* sp's growth inhibition zones were 14.7 and 8.5 mm for Composite 1 and Composite 2 respectively.

Table 3.4 Antimicrobial activities of the plant composites

Microorganisms:	Inhibition zones (mm)		Antimicrobial titre numbers of plant products		
	Composite 1	Composite 2	Composite 1	Composite 2	80% green tea polyphenols
Gram positive bacteria					
Staphylococcus aureus 71 MD	16.1 ± 0.7	18.5 ± 1.2	54 ± 0	54 ± 0	486 ± 0
Rhodococcus sp.	24.1 ± 1.1	24.3 ± 1.5	18 ± 0	18 ± 0	486 ± 0
Gram negative bacteria					
Esherichia coli	18.1 ± 1.3	6.1 ± 0.7	54 ± 0	54 ± 0	54 ± 0
Pseudomonas aeroginasa	6.1 ± 0.7	10.2 ± 1.5	6d ± 0	18 ± 0	54 ± 0
Yeasts					
Candida utilis	No effect	No effect	No effect	No effect	162 ± 0
Sacharomyces cerevisiae	No effect	No effect	No effect	No effect	18 ± 0
Pathogenic fungi					
Fusarium solani	No effect	No effect	No effect	No effect	162
Rhizoctonia sp	14.7 ± 1.0	8.5 ± 0.4	54 ± 0	54 ± 0	54 ± 0
Streptomyces glaucus 71 MD	22.3 ± 1.4	20.3 ± 1.2	162 ± 0	486 ± 0	1458 ± 0

Antimicrobial activities, expressed in titre-numbers, of the composites and 80% green tea polyphenols against the pathogenic micro-organisms were determined. In most cases the titre-numbers of the 80% green tea polyphenols were about by one order higher in comparison with the composites, but for *Esherichia coli* and *Rhizoctonia* sp. the titre-numbers of 80% green tea polyphenols and the both composites were not significantly different ($p < 0.01$) and were equal to 54. The highest titre-numbers were found in the case of pathogenic fungus *Streptomyces glaucus* 71 MD. These numbers were – 162, 486, and 1,458 for Composite 1, Composite 2 and 80% green tea polyphenols respectively.

3.4 Conclusions

Georgian wines (both red and white) produced by Kakhetian Technology are characterized by higher content of flavonoids and antioxidant activity than European type wines.

The content of phenolic compounds and antioxidant activity is much higher in red wines than in whiter ones.

Red wine lees, a by-product of the winery industry are a good basis for production of multifunctional food additives of high physiological activity.

Plant composites from green tea extract, red wine lees and lemon peels are rich in polyphenols, pectin substances and other bioactive compounds. The composites show high antioxidant and antimicrobial activities and are used as bioactive composites and/or natural additives in food industry.

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Chapter 4
Eco-Friendly Food Processing: 21st Century Challenges

Mladen Brnčić

Abstract In past 30 years food has become a way of life". More educated with information's through media such as internet and TV, consumer's requirements for more convenient, diverse, less processed and healthier food products grow up exponentially. This kind of demands puts food manufacturers in position to find new innovative methods and technologies, mostly from fundamental science that could be applied as techniques for improved and enhanced production of various foodstuffs. So far, preservation technologies for foods were based on various unit operations such as pasteurization, sterilization, boiling, cooking, drying, extraction, homogenization mainly powered by traditional "fuels" such as steam and hot air that could provide enough heat important for thermal operations which includes already mentioned processes for the purpose of well processed raw material together with guarantee for microbiological safety, nutritional, textural and sensory attributes of final products. Therefore, ECO-FRIENDLY diverse improved technologies usually called "minimal processing technologies" have been developed at the end of twentieth and beginning of the twenty-first century to provide us with eco-friendly, healthier, nutritive enriched, less polluted and less processed foods, together or in combination with serious consideration concerning possible consequences of these technological applications from the processing point of view. However negative consequences of these applied technologies lies mainly in initial investment which could be quite expensive but there is no alternative for cleaner and safer production. Less energy usage, improvements in quality (textural, sensory, microbiological) and yield, heating efficiency, significantly decreased usage of emulsifiers and additives, decreased emission of pollutant gasses are just some of the positive "feedbacks"

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that gives us cleaner, safer and more acceptable foods. There is even greater need to combine novel technologies with results in way of large scale processing plants to provide an easier access to modern, eco-friendly, environmentally more appropriate and safer food technologies.

Keywords Energy • Environment • Food • Non-thermal technologies • Pollution

4.1 Introduction

Environment has been threatened today by a lot of factors. Complete industry in world today is based and dependent more or less on fossil fuels which have been main source of energy distribution. Hunt for a profit at most of the industries is still the main goal for owners of the large companies. During last 25–30 years it has become apparent how from the environment point of view the world paying "price" due to various factors is affecting industry resources. All general "fuels" for broad industry, if we do not take into consideration nuclear energy, goes to fossil fuels. In that kind of production there are two major concerns: Huge energy consumption and major release of heat. Those are the factors that in generally influence on human surroundings and quality of life which are by all means irreversible. Concerning energy we have a kind of equilibrium in our planet but it is changing now, and is directly tied with activities of human kind. Changes are negative, rapid and drastic and well known in past two decades as "greenhouse effect". In general "green house" effect has been "assisted" by human kind by overheating of earth surface (Fig. 4.1).

Solar radiation from space is "bombarding" our atmosphere. Some of the waves are reflected from our "protector" atmosphere, but almost half of the waves is penetrating our shield and warms up the earth's surface. Here we cannot do a thing because this is happening for a billion of years with an energy balance which goes few degrees up and few degrees down. Modern and contemporary technologies for many industries make that possible i.e. helps to the nature to increase and fall of the temperature. In first part of this phenomenon some of the infrared radiation passes through the atmosphere and some are absorbed and reflected in all directions by greenhouse gas molecules and clouds. This could be explained with small but very significant rise of CO_2 in earth's "protector" atmosphere which notably leads to increase of average temperature. On the other hand huge amount of so many particulate matter has been forwarded into our atmosphere from the fossil fuels combustion which affect as fall in average temperature and by all means poluttions of all kind. Thus this means how we are "helping" our planet to get negative consequence. It was completely understandable and unavoidable that this will actually happen. Industrial revolution, oil and refineries, mines, car industry, aviation, railroads and many other activities were in birth period. No one take care about possible pollutions like air, land, soil, water and noise as well are. In so many years of natural exchanges of resources, rise and fall of various living organisms, rapid climate

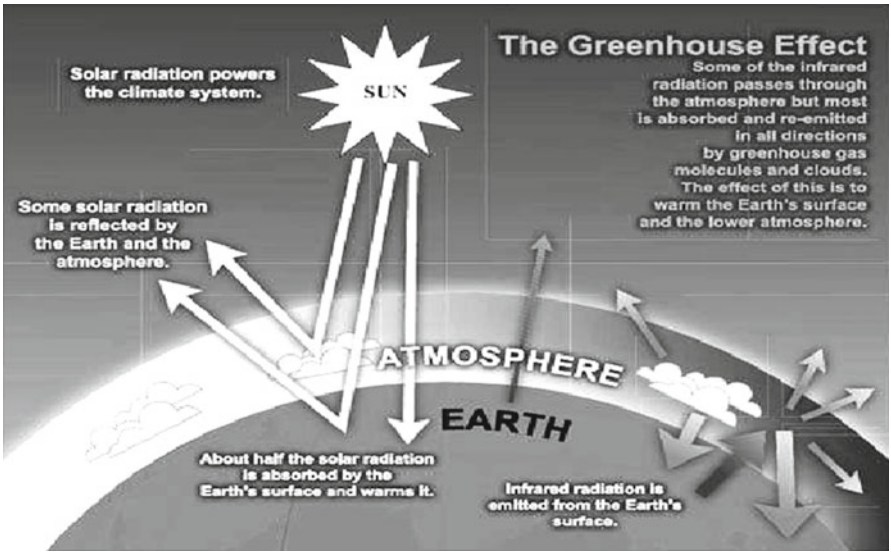

Fig. 4.1 Causes and consequences of "greenhouse effect"

changes and many other factors like air, land, soil and water changes it was a normal development. But, human kind "made" possible exponential warm up of our planet within past 100–150 years.

However, awareness of this kind of problem in past few decades becomes very considerable. Individuals at the beginning, small groups of activists and organized organisation for environment protection start to warn mankind about global contamination and environmental pollution. Very important backup for "healing" of our surroundings came from growing accessibility for a single person to use world wide web (internet) facilities. It is almost impossible to hide anything from the public. Various browsers made possible to anyone "to be" at a various places at the same time and to witness anything almost anytime.

4.1.1 Traditional Food Processing Technologies and Influence on Environment

World population grown up in past 100 years up to four billion people which apparently leads to needs and demands for more foods and water production as well. With industrial revolution and possibilities of better and safer processing food became not just necessary merchandise essential for life but also challenge in various ways. Traditional processing of foods in most of the applications supposes usage of heat intake during thermal processing like evaporation, drying, sterilization, cooking, and pasteurization [5, 15]. Those are still methods or techniques among food

processing itself which gives us necessary requirements for semi food products or final food products that should be placed on the market. Basic principle of such food production depend on heat creation which should than be propagated into food material. Existing methods of food processing are still irreplaceable in a way of large industrial scale. As mentioned above, classic procedures like sterilization, pasteurization, cooking and others imply well known heat exchange technologies whether is it about processing of foodstuffs which allude to temperature rise that leads to final product or cooling and even freezing as well to get preserved food. Heat in this case enables energy which is in industrial scale enormous. Heat that is created outside of food material is than propagated into system mostly by two mechanisms: convection and conduction. Convections stand on propagation of hot air throughout food system and conduction lean on direct contact of foodstuff with the hot surface of the heater. This is general case scenario which maintain in industry in past 100 years and still is. It is well known how existing technologies of modern food industry have devastating influence on environment and human kind like:

- Pollution
  - Air
  - Water
  - Soil
  - Noise
  - Land
- Climate changes
- Problems with energy
- Lack of fuels
- Health issues

However, rigorous demands placed in front of producers of foods are microbiological safety which is first condition, but also chemical and physical analysis must be according to the legislator's regulations. Positive consequence is rapid need of changes in way of easier approach to novel, versatile and modern eco-friendly technologies to be implemented in food processing today.

4.1.2 Demands of Eco-Friendly Food Processing Technologies

In food technology and biotechnology today there are great potential interests about new and novel non-thermal and thermal preservation and processing techniques that could improve or replace heat-based operations and methods which are usual in food processing. Now days incorporating of enhanced technologies from other fields that take into consideration various demands (consumers point of view and producer's benefits and obligations) are necessary but also trend towards eco friendly processed foods. Mostly those technologies are used as processing tools

but also as non-destructive applications for implementation of food safety issues. Usually named as minimal processing technologies each of these technologies is advanced over conventional methods of treatment based on prolonged heating. Flavor, color, less usage of chemicals and emulsifiers, enhanced textural properties together with improved physical and chemical properties, nutritional quality, and shelf life are just some of characteristics that can be improved or preserved during and after processing. Indeed, rapid requirement for all aspects of food manufacturing to get safer, healthier, eco cleaner and environmental friendly foods using novel food processing technologies exists. Moreover it is not easy to encircle new processing issues. Every part of production chain must be contented. Besides just saved energy and cleaner production, achieved merchandise should be microbiologically acceptable; pollution should be reduced to minimum; waste and toxic compounds as well. Obviously there is need for "cluster" of complex and extremely hard labor work which should be performed both by industrial investors and universities groups that are doing research and practical applications implemented within industry in this area. Final result should be not just eco friendly and environmentally more acceptable foods compared with foods processed by traditional food processing technologies, but also new products on the market arise by novel thermal and non-thermal food processing technologies. It is important to mention how some of those applications are in small scale already in use within food processing, but the need for large industrial scale-up still exists. Various of novel applications are translated from fundamental sciences and applied for manufacturing of foodstuffs with processing regard which is narrowly tied with smaller energy consumption, safer food production, environmental safety, lower or none pollution of any kind, lower emulsifier and additives usage as well. Also, there is strong requirement "dropped as a glove" from the final consumer point of view like: better nutritional properties, enhanced texture and flavour, fresh look of the novel foods and even more desirable visual look of the food which includes packaging. The final consumer will almost always come back to the product with which he or she was pleased. Since consumer is well informed about ways how foods are produced there is strong need form the manufacturer of food stuffs to manufacture items in a way that consumer could be pleased.

4.1.3 Novel Eco-Friendly Food Processing Technologies and Influence on Environment

Novel minimal processing technologies could be divided in two main directions:

- Electrotechnologies or novel thermal technologies: radio-frequency dielectric heating (RFDH), pulsed electric fields (PEF), microwave heating (MH), high electric fields (HEF), infrared heating (IH), ohmic heating (OH). Those are just some of the mostly researched technologies that gives results on foods based on heat creation within foods.

- Non-thermal technologies: hi-intensity ultrasound (HUS), high hydrostatic pressures (HHP), oscillating magnetic field (OMF), pulse-light (PL), cold plasma (CP), ultraviolet light (UV).

Due to a different mechanisms of action given non-thermal technologies rely on changes within the foods. Characteristic of each of this applications is to make possible change in foods like traditional processed foods but with shortening of treatment time, more uniform coverage of whole sample, fresher look of final product and at the end but also very important to make environment protected.

4.2 Brief Overview of Some Novel Processing Technologies

Diverse demands from consumers and public create "new policy" of food manufacturing. There are significant number of distinguished scientists, researchers and industrial engineers who make possible novel eco friendly technologies to be implemented in real food processing. High hydrostatic pressure (HHP) is a method with which foods could be treated within few minutes of processing [17, 34, 37]. Foods undergo rapid pressures up to 1,000 MPa, although usual pressures are from 600 to 800 MPa. Just for understanding those are pressures more than six times higher than at the deepest point in the ocean. Such activities could destroy most of the microorganisms, retain physical and textural quality of foods, save energy and enhance products [9]. This is possible because most of the foods contain water which is relatively incompressible. Technology of HHP could be well combined with other non-thermal technologies ([2, 18]). Hi-intensity ultrasound (HIU), sound, hearing represents a completely normal phenomenon for the most of us of human kind, but human hearing can't go beyond 18,000 vibrations per second or 18 kHz (Fig. 4.2).

For over million years in past, certain animal species used an ultrasound as a tool or a weapon for their survival. Ultrasound waves as well as sound waves are mechanical vibrations in a solid or fluid [6, 11]. It has been used in a many different technologies for a various industries, and in biotechnology and food technology it can be divided globally in two main different directions, but also combined in complex research [20, 27]. More and more in biotechnology and food industry, this utilizable technology is taking place as an analytical tool (low-intensity), or for the material modification before, during and after the processing (hi-intensity). First, low-intensity ultrasound is diagnostic and non-destructive method that enables reliable data about physicochemical properties of food components and products such as structure, flow rate, thickness, composition, presence of unwanted foreign bodies, texture and particle size, whereas second use of ultrasound (hi-intensity) is used for promoting, modification and changes of physical or chemical properties of foodstuffs such as: filtration, degassing, extraction, drying, homogenization, emulsification, sterilization-inactivation of m.o., oxidation, enzyme reactions, deblinding-sieving, cleaning, meat tenderization, etc. In most of the reactions with this type of ultrasound changes are permanent [12, 23, 26, 30–32, 33].

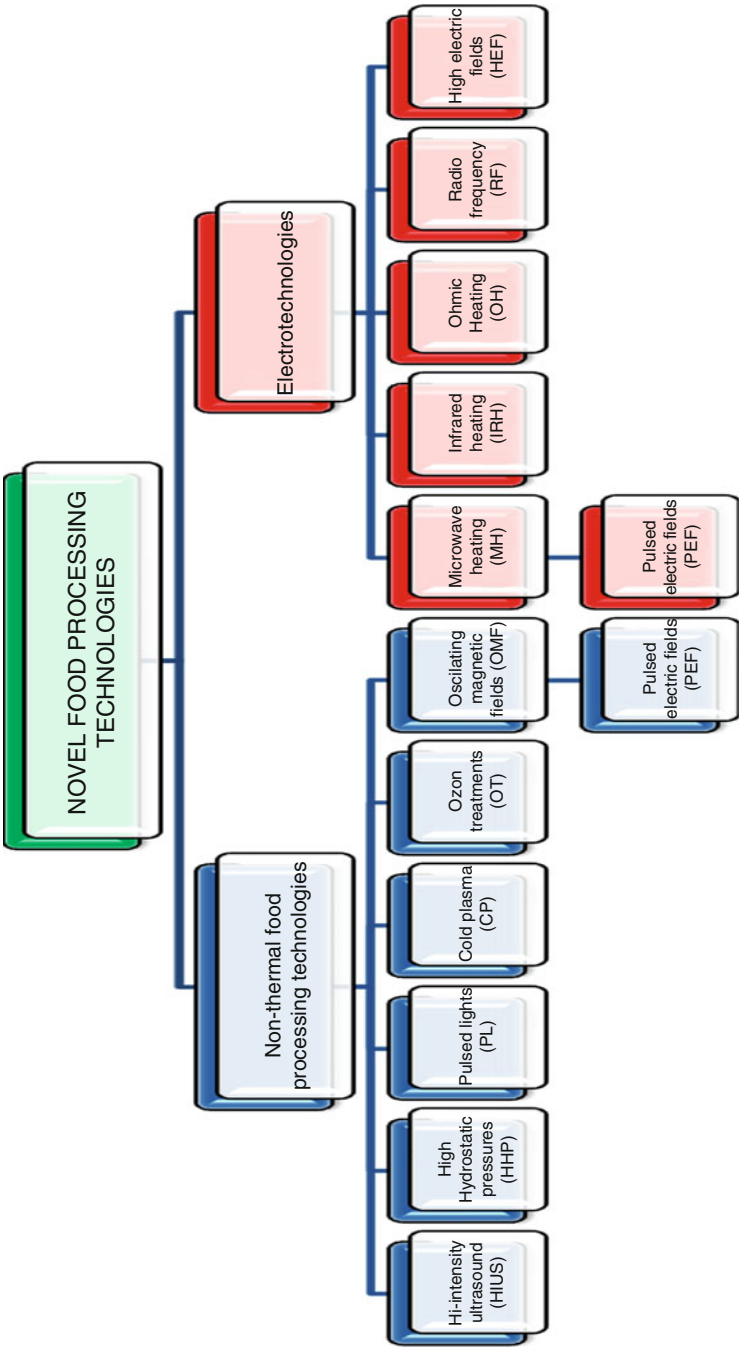

Fig. 4.2 Novel thermal and non-thermal processing technologies

One of the most emerging technologies for treatment of liquid foods is PEF. It uses brief time of treatments which significantly saves energy [40, 42]. Food preservation is achieved by a mechanism of short high voltage electric pulses propagated through foods [29, 38]. For typical inactivation of microorganisms in liquid foods during 2 μs 10 pulses are propagated. Field strength could reach up to 50,000 V/cm. This is so called electrical pasteurization or sterilization [1]. The damage that PEF produce on the microbes is tied to a cell wall. PEF could also be very useful for treatments of spores, yeasts and enzymes [13].

Cold plasma (CP) is generated using microwave fields or even electricity under low pressure in especially designed chambers. This process creates gas such helium comprise of oxygen with ions, electrons, photons and initiated molecules [8]. The mechanism of work is that when CP is propagated in foods and reaches microorganisms they remain without charges and microorganisms are inactivated. Important part is to avoid rise of temperature above 60°C, because of possible heat damage to nutrients and compounds within food samples [16]. Important novel thermal processing techniques for preservation of foods are ohmic heating (OH) and radio frequencies (RF). Those are both applications that could be used as replacement for traditional heating operations [3, 10, 28]. The main mechanism is environmentally almost perfect i.e. thermal energy converted into heat exchange is formed within food [21]. There is no spread of energy, loss of heat or other problems that are narrowly tied with classic convectional heating processes. For OH food sample must be placed between electrodes. Electricity circuit is formed with electrical resistance which causes generation of heat inside food. Temperature rise inside of food and inactivation of microorganisms occurs [2]. Radio frequency (RF) is often used as technology for meat preservation [24]. HEF is offers many advantages compared with traditional processing technologies. During treatment with HEF there are no significant changes in temperature. Food retains complete bioactive compounds in form that was before treatment which leads to “fresh” like treated foodstuff [2, 35, 36].

4.3 Environmental Effect and Benefits of Novel Food Processing Technologies

Development of food processing leads to various positive impacts on environment. Even in traditional food processing there is development in technological knowledge transfer. However, with such a technologies food engineers are limited in a way of changing things. Food technology is versatile and it has to be provided with new ideas seek to attain and develop technologies which will take into consideration energy usage reduction, decreased emissions of pollutants, enhanced preservation characteristics of products, improved nutritional quality and safer consumption as well. This should be aim and goal of total supply chain within food processing leading to eco-friendly processing and environmental concern which should include; water usage, pesticides, steam usage and release, packaging

and fertilizers. From processing point of view shortening of processing treatment is also of a great importance. This is issue in which both traditional and emerging novel technologies could be combined. For example: pre-treatment of fruits using HIU before drying is proven to decrease time of treatment in conventional and infra-red dryer up to 40% [6]. Concerning drying of various food there are positive drawbacks form other novel thermal and non thermal technologies. Various authors proved in their works how foodstuffs could be dried with ohmic heating, radio frequency and ultrasound as well [41, 21, 25]. In fact not just energy savings but also enhanced texture and sensory attributes were achieved. Eco friendly processing could be established during "simple" homogenization of milk. Hi-intensity ultrasound is shown to be promising technology for homogenization of milk. Dairy industry is using huge amount of energy to make primary homogenization of milk. Bosiljkov et al. [4] in their work made a research about how HIU could be applied on milk homogenization. The main goal was to reduce fat globules and save in energy. They used two in diameter different probes (7 and 10 mm) which were directly immersed in milk. After treatment samples were analyzed with optical microscope. Statistical analysis results and pictures taken with camera showed significant decrease in size of fat globules. That leads to prolonged shelf life of milk and improved milk sensory attributes. Pasteurization is one of the most commonly used processes in food industry. Using this kind of heat treatment or sterilization as well, most of the microorganisms are inactivated. However traditional pasteurization processing have a negative backdrop in a form of nutritional and sensory loses, increased unwanted softening and significant loss of energy which is released in surrounding. There are other negative effects like minerals, proteins and vitamins loss. On the other hand, pasteurization could be conducted with PEF. Respective scientists [7, 14, 19] shown how despite of delivered power needed for treatment using PEF this technology proven to be energetically more cost-effective. Notably, using PEF as tool for pasteurization natural gas savings were estimated at 100% because thermal process is completely eliminated. The basics of this application was studied and results in form of energy savings presented in study published by Lung et al. [22]. Authors showed how estimated electrical savings could be up to 18% in comparison with traditional technologies. One of the most promising technologies in food technology is usage of High pressures (HHP). As emerging non-thermal method HHP could and is already applied in many phases of food processing. Technology of HHP was developed in the beginning of the 1990s. The major benefit of HHP within food processing is in the shortening of processing time. Full treatment could be conducted from 1 min to 30 min. Processing of solid or liquid food products with or without packaging happens in the temperature interval 5–90°C, and pressures 50–1,000 MPa [39]. Applied high pressure is distributed uniformly through the whole food product independently of its quantity and shape. Such characteristics of processing and positive effects fulfilled in a way of improved food microbiological safety, less energy expenditure, low concentration of waste products and longer shelf life make high-pressure processing a very promising novel non-thermal food technology.

In addition with lower costs once when device is installed this HHP technology is very economically profitable [17]. In his work [38] showed how specific energy used for sterilization of cans is possible to be reduced up to 300 kJ/kg.

4.4 Conclusions

Complete or total supply chain of food processing is very complex from various points of view. Food engineering interventions should aim to achieve sustainable and enhanced production of already known food products or completely new products based on novel emerging technologies. This should and is followed with efforts to establish eco-friendly and environmentally cleaner and more acceptable food processing plants and products as well. Reduced production and processing cost; reduced energy usage; upgraded value addition technologies to achieve high quality products based on novel thermal and non-thermal processes; utilization of byproducts and waste; decrease of emulsifier and additives usage; improving food quality; utilization of novel packaging materials; less pollutants release and finally response to consumers demands are just some of the tasks placed in front of eco-friendly food processing. Hence, food technologists using novel technologies are working on development, semi industrial applications, industrial applications and implementation within industry as a final goal from the side of manufactures. They have to respond to community and ecology demands to produce better, safer and eco-friendly foods. Simultaneously, improving food quality, safety, and security remain critical issues in food engineering study. Put into operation of HHP, HIU, PEF, RF and other innovative and emerging technologies for food processing already makes this possible. From the eco-friendly food processing systems novel foods with benefits in microbiological, sensory, nutritive and textural properties are possible to be produced. Traditional individual components of preservative (kitchen salt, sugar, vinegar, etc.) are possible to be replaced with some bioactive compounds or with macrobiotic components, so there is increase in product functionality and value.

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Chapter 5
REACH Regulation – A Prerequisite for Health and Environmental Safety: Lithuanian Case

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Abstract The paper analyses in which way EU REACH regulation is capable to contribute to health and environmental safety in Lithuania. In this respect, the importance of improved knowledge on intrinsic properties of chemical substances, implementation of safety measures, and use of less hazardous substitutes are emphasized. The quantitative assessment of the possible benefits of REACH was carried out for a few occupational diseases – asthma, dermatitis, and cancer. REACH has a good opportunity to remind the necessity of prevention and protection measures. The study on the use and substitution of substances of very high concern showed a low awareness of industry on hazardous chemicals prior to REACH adoption.

Keywords REACH • Human and environmental safety • Occupational diseases • Substitution

5.1 Introduction

Sound chemicals management is essential to the sustainable development of countries all over the world. The European Union played an important role, when one of the international initiatives – the Strategic Approach to International Chemicals Management (SAICM) – was launched by UNEP in 2006. The SAICM Global Plan of Action foresees activities that will help countries achieve the sound management of chemicals throughout their life-cycle so that, by 2020, chemicals

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are produced and used in ways not compromising health and environmental safety. The EU is striving to meet the 2020 goal through its policies and legislation.

One of the important pieces of EU legislation is the Regulation on chemicals (EC Regulation 1907/2006), known as REACH regulation. It entered into force on 1 June 2007. The purpose of the regulation is to ensure a high level of protection of human health and environment as well as free circulation of substances on the internal market while enhancing competitiveness and innovation. The main elements of REACH are registration, evaluation, authorization, restrictions, and information in the supply chain. It is expected that through these elements REACH will close the knowledge gaps, will place a greater responsibility on industry to manage the risk of chemicals, will encourage the replacement of hazardous chemicals with safer ones, and will cause a phasing out of chemicals very dangerous to human health and the environment.

The current paper analyses ways in which REACH is capable to contribute to health and environmental safety, assesses the possible benefits of the REACH regulation on human health in Lithuania, and presents practical aspects of use of the most hazardous chemical substances and their substitution in industry in Lithuania.

5.2 REACH Regulation and its Capability to Influence Health and Environmental Safety

There were several reasons to introduce a new chemicals policy in EU and adopt REACH regulation. According to the White paper ("Strategy for a future Chemicals Policy", COM(2001) 88 final), among the main reasons were: a lack of knowledge on chemicals; too much responsibility for chemicals on state institutions rather than on industry itself; no reliable instruments to ensure the safe use of the most hazardous chemicals; and no encouragement to introduce new and possibly safer chemical substances.

5.2.1 Knowledge on Intrinsic Properties of Chemical Substances

When a difference between so called "existing" and "new" chemical substances was introduced in the 9th decade, the idea was to test comprehensively, depending on a volume placed on the market, all newly introduced chemical substances. Those chemical substances, which have been on European market before September 1981 ("existing" substances), could be further used and had to be tested according to a certain procedure. However, the system turned to be not really effective. Approximately 4,000 "new" substances have been quite rigorously tested till adoption of REACH regulation. Information on about 100,000 "existing" substances

was substantially poorer. For these chemicals, more or less exhaustive information was available for substances included into Annex I of Dir. 67/548/EEC, 10,500 substances from IUCLID database, 900 substances covered by Restrictions Dir. 76/769/EEC, and 70 substances assessed after the prioritisation under the Existing Substances Regulation 793/93. All the other tenth thousands of substances were supplied to the market and used in spite of lack of knowledge on their properties. However, it is not possible to assess properly a risk to human health and to the environment, and to take the necessary safety measures without knowing the intrinsic properties of a chemical.

Thus, generation and collection of information on intrinsic properties of chemical substances is one of the objectives of REACH. This is done via registration of chemical substances placed on the market in amounts 1 ton or more per year. Importer or manufacturer (the one who is placing a substance on the market) is obliged to provide information on the properties and classification of the substance as a part of the registration dossier to the European Chemicals Agency. The information can be collected from already existing data sources, can be obtained by different modelling techniques, or on the case by case basis needs to be obtained by performing new tests. Such collection of information leads to the findings about previously unknown properties of chemical substances. For example, White Paper predicted that 500 new carcinogenic, mutagenic and reprotoxic (CMR) substances will be identified during the whole registration process of phase-in substances (the previous "existing" substances).

REACH has three deadlines for registering substances: December 2010, June 2013 and June 2018. The deadline depends on the tonnage band and the hazardous properties of the substance. Although the number of pre-registered substances was around 143,000 of substances by 65,000 companies, around 30,000 substances are expected to be registered till the last registration deadline for phase-in substances in 2018.

5.2.2 Safe Use

In addition to information on the properties of a substance and on its classification, a registration dossier must contain information on application of the substance, as well as information on how to use a chemical safely. Available information will be improved and knowledge gaps closed, leading to the improved classification and labelling, completed safety data sheets (SDS), corrected limit values, established and specified restrictions, taken decisions for authorisation. All the mentioned is a precondition for a safer use of chemical substances. It is also important to note that REACH introduced an obligatory implementation of risk reduction measures indicated in safety data sheets and in exposure scenarios. Exposure scenarios are a part of the so-called chemical safety assessment and chemical safety report, and must be prepared for hazardous chemical substances manufactured or imported in quantities starting at 10 t per year.

5.2.3 Use of Less Hazardous Substitutes

The absence of different requirements for "existing" and "new" substances is expected to encourage the development of new substances, which shall be less hazardous.

Regarding the use of the most hazardous chemicals, certain substances were banned or restricted for all or some uses by Dir. 76/769/EEC, which is currently transferred to Annex XVII (Restrictions on the manufacture, placing on the market and use of certain dangerous substances, preparations and articles) of REACH regulation. However, such restrictions were limited only to certain substances and certain critical uses. At the same time there were many cases, when a substance having e.g. PBT properties (persistent, bioaccumulative, toxic) was placed on a market and used for various downstream purposes, while other less hazardous substitutes were available for the same usages. Downstream users were supposed to search for the safest possible alternatives by themselves, but in many cases they have less knowledge and experience with chemicals than producers or importers. Consequently, the choice was not always the proper one. REACH introduced a procedure of authorisation, which idea is to eliminate placing on the market substances of very high concern (carcinogenic, mutagenic, reprotoxic (CMR), classified in category 1 or 2; persistent, bioaccumulative and toxic (PBT) or very persistent and very bioaccumulative (vPvB); and/or identified as causing probable serious effects to humans or the environment of an equivalent level of concern as those before, e.g. endocrine disrupters) in cases when safer alternatives exist. Companies using or making available substances mentioned in Annex XIV (List of substances subject to authorisation) of REACH Regulation need to apply for an authorisation for each use of the substance, regardless of the quantity. It is the duty of the applicant to demonstrate that the risk from the use is adequately controlled (this route of adequate control is not applicable for those substances for which an effect threshold cannot be determined) or that the socio-economic benefits outweigh the risks and there are no suitable alternative substances or technologies.

5.3 Methods

Thus, human and environmental safety will be enhanced by reduced exposure to chemicals and by use of safer alternatives. The study was carried out to assess quantitatively the possible benefits of REACH on human health in Lithuania, targeting on occupational diseases.

Exposure to hazardous chemical substances might cause a number of diseases for skin, respiratory system, nervous system, eyes, cardiovascular system, digestive system, liver, kidney, malignant diseases (cancer), etc. Of all these, two different types of diseases were chosen for the assessment. One type is diseases, which have comparatively short time lag between exposure and effects, and which are quite clearly related to the exposure to chemicals. These are skin and respiratory system

diseases. Another type is represented by cancer. There may be several factors leading to the development of this disease, and there is a long time lag for consequences to be realised.

The potential number of occupational diseases that may be avoided through implementation of REACH was predicted following these steps:

- Overview of the number of respiratory, skin and malignant diseases in Lithuania;
- Evaluation of the burden of occupational respiratory and skin diseases, and occupational cancer by reviewing the number of recognised cases and estimating the actual number of cases;
- Estimation of the percentage of occupational respiratory and skin diseases, and occupational cancer related to chemicals exposure;
- Based on assumptions, prediction of the number of cases that may be avoided due to the implementation of REACH.

Then, the preparedness of industry to implement the substitution of hazardous chemicals by less hazardous ones was investigated. The target industry was furniture industry, as one of the most developing and successful branches of industry in Lithuania. A communication has been set up with one furniture company in order to establish substances for investigation and to research aspects of substitution. Questionnaires were sent out to other companies of the branch to survey the use of the selected hazardous chemicals. Risk assessments were carried out to investigate the suitability of the proposed alternative chemicals. Risk assessment at workplaces was carried out according to COSHH methodology (British Control of Substances Hazardous to Health Regulations: www. hse.gov.uk/coshh). Assessment of risk for the environment caused by chemical substances was carried out by screening method [1].

5.4 REACH for Health Safety: Avoided Occupational Diseases in Lithuania

5.4.1 Overview of the Number of Respiratory, Skin and Malignant Diseases

The incidence and prevalence of respiratory diseases was permanently increasing in Lithuania, and has more or less stabilised in recent years. The incidence and prevalence of skin diseases was rather constant already over the last 10 years. Table 5.1 presents information about ill adults (18 and more years old), having respiratory and skin diseases.

Prevalence and incidence of malignant diseases is increasing. Table 5.2 presents cancer statistics in Lithuania. The number of new cases is increasing by few hundreds every year. Fortunately, the number of death cases was rather stable over the recent years.

Table 5.1 Number of ill adults (18+ years old) by groups of diseases per 1,000 population[a]

	2009	2008	2007	2006	2005
Respiratory system diseases, of them:	194.5	189.1	211.9	188.0	203.0
Asthma	10.4	10.0	9.5	8.9	8.5
Chronic lower respiratory diseases	33.5	32.9	33.5	31.6	33.2
Skin diseases, of them:	60.3	60.8	61.3	61.0	59.3
Dermatitis and eczema	23.6	24.4	24.7	31.5	24.9

[a]Compiled of data from Health Information Centre of Institute of Hygiene (www.lsic.lt)

Table 5.2 New and fatal cases of cancer in Lithuania[a]

	New cases			Death cases		
Year	Men		Women	Men		Women
2008	9,854		8,376	4,547		3,769
		18,230			8,316	
2007	10,129		7,860	4,729		3,535
		17,989			8,264	
2006	9,420		7,852	4,607		3,541
		17,272			8,148	
2005	8,243		7,881	4,497		3,674
		16,124			8,171	
2004	8,181		7,764	4,574		3,611
		15,945			8,185	

[a]Compiled of data from Lithuanian Cancer Register (www.vuoi.lt) and the Institute Oncology, Vilnius University (www.vuoi.lt/index.php?-2043095342)

5.4.2 Burden of Occupational Diseases

A part of respiratory and skin diseases, and also a part of cancer are work-related. State Register of Occupational Diseases in Lithuania keeps a track on numbers of recognised occupational diseases. An overview is presented in Table 5.3. These are diseases arising from different risk factors at workplaces, not only from chemical agents.

There is a general opinion that the recognised cases are far beyond the real number of cases. Such a situation is determined by various reasons: peculiarities of recognition of occupational diseases and of compensation system, attitudes of employers and employees themselves, insufficient knowledge about the origin of diseases, difficulties to prove the causal link between effect and exposure. The estimation of the actual number of cases is important for assessment of possible REACH benefits. This was done by the analogy with other countries. The current study followed the same approach as in Sheffield's study [15]. Although a situation with underestimation of occupational diseases is similar in many countries, nevertheless there are some countries in Europe (e.g. Denmark, Finland, France,

Table 5.3 The recognised occupational respiratory and skin diseases, occupational cancer[a]

	Respiratory system diseases	Skin diseases	Cancer	All occupational diseases
2009	17	2	–	846
2008	26	6	–	1,030
2007	18	5	–	1,123
2006	19	3	–	1,447
2005	27	3	–	1,380
2004	31	4	–	939
2003	17	5	–	808
2002	15	4	1	801
2001	16	5	–	570
2000	16	1	–	572

[a]Compiled of data from the State Register of Occupational Diseases (www.hi.lt/content/prof_lig_stat.html)

Germany, Sweden, and the UK) having conducted a wide scale research and collected rather comprehensive data sets on occupational diseases [2, 15]. Both Sheffield's and the current study took an assumption, that incidence rates across the EU-25 are broadly the same. After the analysis of available data for the mentioned countries, the estimate of the incidence rate of occupational asthma was 200–400/million/year [15]. For the labour force of 1.5 million in Lithuania (according to "EU Labour Force Survey – Annual results 2007" from Eurostat), this results in 300–600 new cases of occupational asthma in Lithuania per year. Compared to the number of recognised cases (see Table 5.3), the estimate suggests at least 10–40 times more cases in reality (the recognised cases include different respiratory diseases, not just asthma).

Regarding occupational skin diseases, the analysis of available data concentrated on dermatitis and resulted in an estimate of about 400 new cases per million employers per year in the European Union [15]. This implies for 600 new cases of occupational dermatitis in Lithuania. Compared to the number of recognised cases, the real situation might be up to 100 times worse (the recognised cases include different skin diseases, not just dermatitis).

According to the Fourth European Working Conditions Survey, conducted in EU-27 in 2005, 14% of workers believe they have work-related respiratory problems, and 15.3% believe they have work-related skin problems [16].

There was only one recognised case of occupational cancer in Lithuania. Based on information from Lithuanian State Labour Inspection, the patient worked for 25 years as operator in an asphalt concrete factory. According to a safety data sheet, the road bitumen is a mixture of heavy hydrocarbons, benzopyrene among them. Occupational lung cancer was recognized for the employee in 2002. Obviously, occupational cancer is underestimated in Lithuania, as it was also with occupational respiratory and skin diseases.

Table 5.4 The recognised occupational diseases[a]

	Occupational diseases from exposure to chemical agents		Total number of occupational diseases	
	Number	%	Number	Per 10,000 employees
2009	11	1.3	846	6.0
2008	16	1.6	1,030	6.8
2007	11	1.0	1,123	7.3
2006	7	0.5	1,447	9.7
2005	16	1.2	1,380	9.5
2004	9	1.0	939	6.5
2003	9	1.1	808	5.6
2002	10	1.2	801	5.7
2001	6	1.1	570	3.75
2000	16	2.8	572	

[a]Compiled of data from the State Register of Occupational Diseases (www.hi.lt/content/prof_lig_stat.html) and from Health Information Centre of Institute of Hygiene (www.lsic.lt)

5.4.3 Estimation of the Percentage of Diseases Related to Chemicals Exposure

Six to sixteen cases of occupational diseases are recognised as arising from chemical agents every year since 2000 in Lithuania. This constitutes 0.5–2.8% from all occupational diseases (see Table 5.4).

Exposure to chemical agents is a significant factor leading or contributing to the development of work-related diseases. Labour force survey 2007 found out that a quarter of workers felt health effects from chemicals, dust, or aerosols in Lithuania [16]. For the diseases analysed in the current study, at least half of the cases of dermatitis might be due to REACH-affected chemicals [15]. This gives a figure of 300 cases of occupational dermatitis in Lithuania per year. Occupational asthma may develop due to chemical agents (e.g. isocyanates), but also due to other factors, such as wood or grain dust, biological factors [11]. Chemical agents might be responsible for up to about half of occupational asthma cases [15]. A lower number, 11%, is suggested by the World Health Organisation in the project "Global Burden of Disease". Using estimates from the EU, the result is 150–300 new cases of occupational asthma due REACH-affected chemical agents in Lithuania per year.

Development of cancer may be caused by numerous risk factors: environmental, occupational, life-style (e.g. diet, smoking etc.), genetic, and other [9]. Four hundred and fifteen known or suspected carcinogens identified by the International Agency for Research on Cancer are among important risk factors [3]. Although there was just one case of recognised occupational cancer in Lithuania, some estimates suggest that even 28% of employees in Lithuania might be affected by various carcinogens (chemicals as well as UV radiation, radon and others) [8]. A review of scientific articles and reports suggest, that about 5% of cancer in men and 1% of cancer in women result from exposure to carcinogenic substances in their work environment

[14, 17]. The most common cancers associated with occupational exposure are lung and pleura, bladder, skin, laryngeal, nasal cavity, leukemia. Around 3.5% of fatal cancer cases (5.6% for men and 1.1% for women) are due to exposure to chemical substances [4, 10, 12] at workplaces. The study in Great Britain estimated even 4.9% of deaths (8% for men and 1.5% for women) to be attributable to work-related carcinogens [13]. Mortality from cancer attributable to chemicals for most of the end-points is around 1% for men and 0.5–1% for women. However, some cancer end-points are more sensitive to chemicals. Chemical substances cause about 15% men and 5% women fatal lung cancer cases, 10% and 5%, respectively, fatal bladder cancer cases, and 10% and 5%, respectively, fatal leukemias. In this way, carcinogenic chemical substances could be blaimed for about 304 fatal cases of cancer (265 cases for men and 39 cases for women) in Lithuania in the year 2007, when REACH entered into force.

5.4.4 Prediction of the Number of Diseases that may be Avoided

The most optimistic assumption that can be accepted is the complete avoidance of occupational asthma and occupational dermatitis, which are assessed as being caused by REACH-affected chemicals. The precondition to achieve any reduction in incidence of the mentioned diseases is the implementation of the necessary risk reduction measures, which will be identified taking into account the intrinsic properties of chemical substances. It is likely that REACH will lead to the clarification of substances, which till now were not known to be sensitizing, irritant, or having acute toxicity by inhalation or in contact with skin.

Also for carcinogenic substances, it is likely that REACH will lead to the clarification of currently unknown carcinogens. Expert estimate predicts 20% of fatal cancer cases arising from currently unknown carcinogens [12]. Of course, hazardous properties (carcinogenic, sensitizing, or any other) of chemicals would be clarified sooner or later. The REACH regulation merits of speeding up this process. The more new properties will be clarified, the more pronounced consequences of REACH can be expected. It needs to be stressed that translation of knowledge of hazardous properties into the risk reduction measures are essential for the benefits to become visible. If experts estimated 20% of fatal cancer cases caused by unknown carcinogens, the other 80% were caused by already known carcinogens. Worker protection legislation is in place and lays down obligations for those dealing with chemicals. The real situation suggests that there are certain shortcomings in application of the requirements at workplaces. REACH has a good opportunity to remind the necessary prevention and protection measures. The obligatory implementation of safety measures indicated in safety data sheets and exposure scenarios are hopeful in this respect. For carcinogenic substances, authorisation is crucial element of REACH that is expected to have a positive impact on health and environmental safety. Although the speed of all the

authorisation process is not clear yet, the predictions were some 20–30 substances per year. There were six entries in Annex XIV (List of substances subject to authorisation) in the first half of 2011.

The following assumptions were taken for calculation of the potentially avoided fatal cancer because of REACH:

- Twenty percent of fatal cancer cases are from exposure to unknown carcinogens;
- One third to two thirds of deaths from cancer will be avoided due to the implementation of measures in response to new information.

Calculations (based on data from the year 2007) allow to expect 20–40 cases of deaths from cancer caused by chemicals at work-places to be avoided. That constitutes just 0.25–0.5% of all fatal cancer cases, and can hardly be noticeable in the statistics. But at the same time REACH will also add to the reduction of the number of non-fatal cases, not just to the mentioned death rates.

Implementation of preventive measures may start as soon as hazardous properties become known, that is not later than in 2018. Benefits related to acute effects shall become visible within the period of 11 years from the entry into force of REACH regulation (till the last registration deadline in 2018). The longer time span (up to some 30 years) will be needed to realise benefits for chronic diseases, such as cancer.

5.5 Health and Environmental Safety Due to Substitution of Hazardous Chemical Substances

The prevention of occupational diseases can be achieved by application of various control approaches, from process containment to the use of personal protection equipment or good practise. However, substitution of hazardous chemicals with safer alternatives is at the top of hierarchy of measures. It is one of the major principles of REACH regulation.

5.5.1 Substitution of DEHP in Lithuanian Furniture Industry

Investigation in the furniture industry has been carried out before the entry into force of REACH regulation. A candidate list of substances of very high concern for authorisation was not available yet. A communication has been set up with one of the biggest Lithuanian companies of furniture industry in order to survey chemicals used by the company. The majority of used chemicals were intended for furniture finishing, and the minor part were adhesives. Many components were classified as flammable, harmful or irritant. The most hazardous substance was di(2-ethylhexyl) phtalate (DEHP). It was selected for further investigation in furniture industry as a

substance regulated under the different frameworks (priority substance under HELCOM and OSPAR conventions, under the Water Framework Directive), and as a substance of interest in a view of REACH requirements.

DEHP (CAS number 117-81-7, EC number 204-211-0, molecular formula C24H38O4) is a dioctylester of the phthalic acid. Hazardous properties of DEHP are related to its reprotoxicity, its ability to bioaccumulate and accumulate in aquatic environments. DEHP was classified as being reprotoxic, category 2, with R60 (May impair fertility) and R61 (May cause harm to the unborn child) according to Dangerous substances directive (67/548/EEC). The classification of DEHP under the CLP regulation ((EC) 1272/2008) is category 1B reproductive toxicant. DEHP is a suspected endocrine disrupter. EU risk assessment of DEHP was finalized for the environment in 2001, and for human health in 2004 [5]. In recent years, some new reports on DEHP were prepared by the European Chemicals Agency due to ongoing work related to Annexes XIV and XVII of REACH regulation [6, 7].

DEHP is known to render polymers' plastic properties. Such chemicals are called softeners or plasticisers, and are used in a variety of polymers. One of the most widespread uses is in polyvinylchloride (PVC). Phthalates have also found widespread uses in paint production as softeners. Phthalates are also used as additives in adhesives, for the processing of rubber and for electronic purposes. The reason for inclusion of DEHP in furniture finishing chemicals is its plastifying properties. Plasticizer increases the elasticity and flexibility of the finishing films at extra tension (high stress) or at low temperatures. However, in 3–5 years, when the aging process of finishing coating starts, the plasticizers gradually migrate to the coating surface, thus being removed.

DEHP was manufactured in the European Union in a volume of approximately 340,000 t/year in 2007. DEHP use for formulation and processing for the same year was approximately 283,000 t [6]. The amounts decreased dramatically since 1997 [5]. Instead of the use of DEHP companies moved to the use of other high molecular weight phthalates [7]. However, even the decreased amounts can be considered as substantial ones, the downstream use of DEHP takes place in many sites, and there is a wide dispersive use of products containing DEHP, which has a potential for release. Having in mind all these circumstances, DEHP was included into the Annex XIV (List of substances subject to authorisation). Certain restrictions for DEHP have been introduced already earlier. Entry 51 of Directive 76/769/EEC (currently Annex XVII of REACH) implied that DEHP shall not be placed on the market or used as a substance on its own or in a preparation, at concentrations greater than 0.1% by mass of the plasticised materials, in toys and childcare articles. There were 15 major furniture manufacturing companies in Lithuania, which produced 59% of total furniture production, and had 50% of industry branch employees. These 15 companies were approached and inquired, whether they used chemicals containing DEHP. Twelve companies responded to the presented questionnaire; one refused to participate in the survey, and two did not reply. Three companies answered they do not use chemicals for furniture finishing at all. Three manufacturers used chemical preparations containing DEHP out of nine remaining companies. Two products containing DEHP were indicated by the companies: a polish of two modifications

(mat and semi-mat) and a prime. Polish contained 2–3% of DEHP, prime – 1–2% of DEHP. Based on the information provided by the companies, the consumption of these preparations was 56.5 t, and they contained 1.18 t of pure DEHP. Estimating that the major furniture producers manufacture 59% of total furniture produced in Lithuania, and presupposing that the usage range of chemical preparations with DEHP in other furniture manufacturing companies is similar, it was calculated that the whole Lithuanian furniture industry could have consumed approximately 94.2 t of these preparations. This would include about 1.9 t of pure DEHP. None of the companies using chemical preparations containing DEHP knew about its hazardous properties and about it being listed as a priority hazardous chemical. Companies, which didn't use DEHP containing products, seemed not to use them accidentally and not because of deliberate choice of chemicals. All this clearly showed insufficient awareness of downstream users dealing with hazardous chemicals. Information flow on the supply chain regarding the potential risk of chemicals was not satisfactory either. Both products were manufactured by the same EU company, and sold by the same local supplier. The supplier stated they were not selling DEHP containing products already since 2002, whereas downstream users confirmed they were purchasing chemicals regularly and not having stocks of chemicals in warehouses. Even more, safety data sheets of DEHP containing products presented wrong classifications of DEHP and of products themselves. Furniture producers trusted safety data sheets and thus were not properly informed about the risk and the necessary safety measures.

The contacted furniture company was encouraged to search for alternatives to substitute the preparations with DEHP. At the same time their important international customer presented the same requirement and refused to purchase furniture treated with chemical preparations having DEHP in their composition. Through the mutual co-operation between furniture producer and the supplier of chemicals, an alternative chemical was found. It could be used both for priming and polishing. Risk assessment at work places and for the environment confirmed the suitability of the proposed alternative. Instead of DEHP, the substitute contained hexane (small amount, less than 0.5%) and naphta hydrated light, which was assigned to rather low hazard group in risk assessment. Some more hazardous components, not just DEHP, were missing in substitute, and other components were present in different amounts compared to the original polish and prime.

One of the other companies using polish and prime with DEHP acknowledged they were not intending to look for substitutes. The company was satisfied with relation between the price and quality of these chemicals. Their customers did not present requirements regarding the use of hazardous chemicals. The following criteria were clarified to be important for the choice of chemicals:

- Customer requirements regarding the use of certain chemicals;
- Customer requirements regarding the furniture surface quality (e.g. brilliance of the surface);
- Relation between price and quality, when the company itself chooses a chemical.

REACH input to the analysed supply chain and use of the substance of very high concern will be via the authorisation procedure and via the improved information flow.

5.6 Conclusions

There are various ways in which REACH is capable to contribute to health and environmental safety. The main of them are the improved knowledge on intrinsic properties of chemical substances, measures (such as improved classification and labelling, completed safety data sheets, established restrictions) leading to the safe use of chemicals, and the incentives to use less hazardous substances. REACH is expected to contribute to 300–600 avoided asthma cases, and approximately 600 avoided cases of dermatitis caused by REACH-affected chemicals at workplaces if the set out risk reduction measures are properly implemented. Regarding chronic diseases – it might take a few decades to achieve some 20–40 cases of deaths from cancer avoided.

The use of less hazardous chemicals is essential for health and environmental safety, and REACH contribution in this respect is expected to be a significant one. The study conducted prior to REACH entering into force disclosed a low awareness of downstream users regarding the hazardous chemicals. DEHP, a substance which was a priority substance under the different frameworks, and which is now included into the List of substances subject to authorisation, was in use in furniture companies. Customer requirements was the main driving force for substitution.

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Chapter 6
Evolution, Efficiency and Challenges of Environmental Management in Bulgarian Agriculture

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Abstract Paper presents evolution of environmental management in Bulgarian agriculture during post-communist transition and EU integration; assess efficiency and major challenges of existing system of eco-governance; and suggest recommendations for improvement of public policies. First, it defines content, generic mechanisms, and efficiency of eco-governance. Second, it analyzes evolution of institutional environment, and market, private, public and hybrid modes of environmental management during transition and EU integration. Third, it assesses impact(s) of agricultural transformation on state of environment and identifies major eco-challenges, conflicts and risks. Finally, it suggests recommendations for public policies improvement for effective eco-management and security.

Keywords Eco-governance • Market, private, public modes • Bulgarian agriculture

6.1 Introduction

There has been a fundamental transformation of Bulgarian agriculture for the last 20 years. It has affected profoundly agricultural impact(s) on and from the environment. Paper presents evolution of environmental management in Bulgarian agriculture during post-communist transition and EU integration; assess efficiency and major challenges of existing system of eco-governance; and suggest recommendations for improvement of public policies. First, it defines content,

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generic mechanisms, and efficiency of eco-governance. Second, it analyzes evolution of institutional environment, and market, private, public and hybrid modes of environmental management during transition and EU integration. Third, it assesses impact(s) of agricultural transformation on state of environment and identifies major eco-challenges, conflicts and risks. Finally, it suggests recommendations for public policies improvement for effective eco-management and security.

6.2 Definition, Modes and Efficiency of Environmental Governance

Environmental management means governance of environment preservation and improvement activities of various agents. It requires an effective *social order* (governance) regulating agents' behavior and relations related to natural environment – a system of coordination and stimulation of eco-actions at individual, group, regional, national, and transnational levels [1].

- *Institutional environment* ("rules of the game") – that is distribution of rights between individuals, groups, and generations, and system(s) of their enforcement. Diverse rights are defined by formal or informal rules (laws, tradition, culture, religion, ideological and ethical norms), and enforced by state, convention, community pressure, trust, or self-enforcement.
- *Private modes* ("private and collective order") – diverse voluntary initiatives, and specially designed contractual and organizational arrangements of private agents as codes of behavior, contracts, cooperatives, associations, business ventures etc.
- *Market modes* ("invisible hand of market") – various decentralized initiatives governed by free market price movements and market competition (e.g. production and trade of organic products, origins etc.).
- *Public modes* ("public order") – various forms of a third-party public (Government, international) intervention in market and private sectors such as public information, regulation, assistance, funding, taxation, provision.
- *Hybrid forms* – some combination of above three.

Efficiency of individual governing modes is quite different since they have unlike potential to: induce eco-friendly behavior, reconcile eco-conflicts and coordinate eco-actions of different parties, impact environmental sustainability and mitigate eco-risks, minimize overall environment management (conservation, third-party, transaction) costs in the specific (socio-economics, agents preferences and capability, natural) conditions of each eco-system, community, industry, region, country. Depending on system of governance, individual societies achieve dissimilar results in eco-conservation and enhancement ("governance matters").

6.3 Evolution of Environmental Management in Bulgarian Agriculture

During most of transition, rights on agrarian resources (farmland, water) and diverse eco-rights (on clean, aesthetic nature; preservation of nature resources, biodiversity) were badly defined (Fig. 6.1). Inefficient public enforcement of laws, and absolute and contracted rights was common. That has negative consequences on development of farming structures and efficiency of eco-management [2]. For instance, privatization of assets of ancient public farms took 10 years. During a good part of that period, management of critical resources was in ineffective and "temporary"

Institutions	*Private modes*	*Market modes*	*Public modes*
Post-communist transition (1989-2000)			
Not well defined eco- and resource rights, bad enforcement; No sustainability concept	*Provisional lease in contracts on natural resources; Unregistered farms; Agri-firms; Cooperatives*	*Trade with informal brands, origins, and ecosystem services; Free (monopoly) agricultural water pricing*	*State and cooperative farms; Organization under privatization, liquidation and reorganization; Outdated system of eco-regulations, monitoring and information*
Pre-accession to EU (2001-2006)			
Better defined and badly enforced rights on agrarian and eco-resources, and contracts	*Unregistered farms; Agri-firms; Cooperatives; Water User Associations; Vertical integrated modes*	*Trade with formal brands, origins, organic products, and ecosystem services; Free (monopoly) agricultural water pricing*	*Special Accession Program for Agrarian and Rural Development (SAPARD); Cross-compliance; Environmental regulations, standards, and agencies; Regulations for organic farming; Agricultural Advisory Service*
EU membership (since January 1, 2007)			
Well-defined rights, and better enforcement; EU Community Acquis; Collective institutions	*Unregistered, firm and cooperative farms; Water User Associations; Vertically integrated modes; NGOs; Codes of behavior; Eco-labels*	*Trade with formal brands, origins, organic products, and ecosystem services; Free (monopoly) agricultural water pricing; Insurance against natural disasters*	*EU eco-regulations and standards; EU Operational Programs; National programs for eco-management; National Plan for Agrarian and Rural Development (NPARD); Direct payments; Advisory Service; Eco-monitoring and assessment; Protected zones (NATURA); Compensations for natural disasters*

Fig. 6.1 Evolution of environmental management in Bulgarian agriculture

structures (organizations under privatization, liquidation or reorganization) with no interests in effective and sustainable exploitation. Short-lease of natural resources and material assets was a major form for farm extension.

Out-dated and sectoral system of public policing, regulations and control dominated until recently, which corresponded little to contemporary needs of eco-management. There was no modern system for monitoring the state of soil, water, and air quality, and credible information on the extent of environmental degradation. There was no awareness of "concept" of sustainable development and needs to include it in public policy and private and community agenda. Lack of culture of sustainability has impeded evolution of voluntary measures, and private and collective actions (and institutions) for effective eco-management.

Before EU accession, country's laws, standards and institutions were harmonized with Community Acquis. That introduced a modern framework for eco-governance including new rights (restrictions) on protection of environment, integrated territory, water and biodiversity management, preservation of traditional varieties and breeds, animal welfare, polluter pay principle, and corresponding control, monitoring, and assessment institutions (Executive Environmental, Hydro-melioration etc. Agencies).

EU accession introduces and enforces a "new order" – strict regulations and control; tough quality, environmental etc. standards; financial support for eco-conservation, market instability etc. Huge European markets are opened which enhances competition and lets local farms explore their comparative advantages (low costs, high quality, specificity and purity of produce) and give strong incentives for investments in farm modernization and conforming to high product, technology and eco-standards.

External demand, monitoring, pressure, and sanctions by EU lead to better enforcement of laws and standards. Internal collective actions and social demand for good governance have also got momentum leading to improvement of public management – e.g. success of eco-organizations putting a ban on GM crops, timely reaction against eco-violation in protected zones, revoking unlawful "exchanges" of valuable public lands.

Nevertheless, new "rules of the game" are not clearly understood by public authorities, private organizations and individuals. There is not readiness for effective implementation of new public order because of lack of information and experience or administrative capacity (lack of comprehension, deficient court system, corruption). Often, enforcement of eco-standards is difficult since costs for detection and penalizing of offenders are high, or there is no direct links between performance and eco-impact – e.g. banned fields burning is still widespread in the country [3]. Institutional modernization is also associated with new conflicts between diverse interests. However, results of public choices has not always been for the advantage of effective eco-management – e.g. strong lobbying efforts led to 20% reduction in numbers and 50% reduction in area of initially identified sites for pan-European network NATURA 2000. During much of transition, evolving market and private structures were inefficient to deal with economic and eco-issues. Most farming activities were carried in inefficient and unsustainable structures – public farms, part-time

Table 6.1 Evolution of farming structures in Bulgaria (Source: National Statistical Institute, Ministry of Agriculture and Food)

Indicators	Year	Public farms	Physical persons	Cooperatives	Agro-firms	Total
Share in total farms%	1989	0.13	99.9			1,602,101
	1995		99.7	0.1	0.1	1,777,000
	2007		98.6	0.3	1.1	465,084
Share in farmland%	1989	89.9	10.1			100
	1995	7.2	43.1	37.8	11.9	100
	2007		32.2	24.7	43.1	100
Average size, ha	1989	2423.1	0.4			3.6
	1995	338.3	1.3	800	300	2.8
	2007		2.2	613.3	364.4	6.8

and subsistence farms, production cooperatives, huge business farms based on provisional lease contracts (Table 6.1). Livestock holdings are mostly miniature "unprofessional" breading majority of animals [4]. Farms adjustments have been associated with a significant decrease in number of unregistered, cooperative and livestock holdings without adequate transfer of land, livestock, and eco-system services management to other structures.

Most farms had little incentives for long-term investment to enhance productivity and eco-performance. Cooperative's big membership makes control on management difficult focusing managerial efforts on current indicators and giving possibility for mismanagement. Most members are small shareholders, older in age, and non-permanent employees having no incentives for long-term investment for renovation and eco-conservation. Small farms possess insignificant capacity for investment and potential to explore economy of scale/scope (fragmentation, inadequate scale). There are no incentives for eco-spending due to lack of public control on informal sector. Primitive technologies and low compliance with modern agronomic, safety and eco-standards are widespread. Dairy farming is particularly vulnerable since only one third of holdings meet EU standards [4]. Larger farms operate mainly on leased land concentrating on high pay-off cereals and industrial crops. They are sensitive to market demand and institutional regulations since largely benefit/lose from timely adaptation to new standards and demand. They have higher capacity to fund and adapt to new requirements. Nevertheless, survivor tactics rather than a long-term strategy toward sustainability are common among commercial farms [2]. Smaller size, owner operating and extensive nature of majority of farms let avoid certain problems of old public enterprises (over-intensification, lost landscape and biodiversity, chemical contamination, livestock and manure concentration, erosion); revived local (sustainable) technologies, varieties, products; and avert Mad cow disease and Avian flu epidemic. Private mode has introduced incentives and possibilities for integral eco-management (revival of eco- and cultural heritage, anti-pollution, esthetic, comfort measures; investing in eco-system services, origins, labels) profiting from inter-dependent activities such farming, fishing, agro-tourism, processing, marketing. There are good examples for private introduction and enforcement of quality and

Table 6.2 Evolution of organic production in Bulgaria (Source: Ministry of Agriculture and Food, EUROSTAT)

Organic indicators	2003	2004	2005	2006	2007	2008
Farming area, ha	650	1,113	2,432	3,061	11,808	16,663
% in UAA[a]	0.01	0.02	0.05	0.06	0.23	0.33
Wild herbs, fruits, ha	–	–	–	110,143	397,835	397,835
Cattle	na	na	395	na	395	470
% in all cattle			0.11		0.11	0.14
Sheep	na	na	294	na	1,690	2,471
% in all sheep			0.02		0.14	0.21
Goats	na	na	32	na	1,058	na
% in all goats			0.01		0.12	
Bees colonies	na	na	23,508	na	35,747	na

[a]Utilized agricultural area

eco-standards by individual farms (voluntary and trade initiatives), vertical integrator (dairy and vine processor, retailer, exporter), foreign investor (cereals, oil crops). Private management shows improved eco-stewardship on owned and marketed resources, but less concern to manure and garbage management, over-exploitation of leased and common resources, air and water contamination. Since 2001, state irrigation assets were transferred to 70 newly-evolved Water Users Associations servicing 30% of total equipped for irrigation area. Expected "boom" in efficiency from collective management of irrigation has not materialized because of semi-monopoly situation (terms, pricing) of regional water suppliers, few incentives for water users to innovate facilities and expand irrigation, and uncompleted privatization [5]. Evolution of farmers and eco-associations is hampered by agents' big number and diversified interests (ownership, operation, preferences, age). Market-driven organic farming has emerged and registered a significant growth in recent years. Since 2003 there is 11 folds increase in number of organic operators while organic producers comprise the largest part (74%). There is enormous augmentation of organic areas and livestock but they are a tiny portion of farmland and livestock (Table 6.2). "Fully converted organic areas" accounts for 25.4% of total organic areas with Industrial crops, Pastures and meadows, and Permanent crops comprising the biggest shares [6]. There are few livestock farms and apiaries certified for bio-production.

Eco-labeling of farm and food products (relying on self-regulation) appeared but it is more a marketing strategy of certain companies rather than a genuine action for eco-improvement [2]. Organic form is introduced by business entrepreneurs who managed to organize new venture, arranging costly certification and marketing of highly specific output. Produced bio fruits, vegetables, essential oil plants, herbs, spices, and honey are predominately for export. Internal organic market is tiny due to higher prices and limited consumer confidence in authentic character of products. (Free) market management of semi-monopoly servicing companies comes with unfavorable pricing and terms for farmers, and only few purchase water or insurance against natural disasters (draughts, floods etc.). During transition public intervention in eco-management was not significant, comprehensive, sustainable, or

related [2]. Eco-policies were fragmented and reactive to urgent problems (floods, storms, drought) with different agencies responsible for individual aspects of eco-management. In past years, national programs were developed to deal with major eco-challenges (biodiversity and environment preservation; Sulphur Dioxide, VOC, Ammonia emissions limitation; waste management; development of water sector; combating climate change; organic agriculture; lands management and desertification fights; agrarian and rural development). National eco-monitoring system is set up and mandatory eco-assessment of public programs introduced. Nevertheless, bad coordination, gaps, and ineffective enforcement have been frequent for public management. During transition agrarian long-term credit market was blocked while evolving farming structures unsupported. Multifunctional role of farming was not recognized, and provision of "environmental service" funded by society. SAPARD actual eco-financing was little and reached few farms [1]. EU accession brought new opportunities for public support to private and collective eco-activities. NPARD 2007–2013 provides significant funding for area-based and agro-environmental payments (organic farming, management of agricultural lands with high natural value and handicaps, traditional livestock, protection of soils and water, preservation of landshaft); modernization of farms, processing, and marketing; diversification of activity; infrastructural development; keeping traditions; training etc. Specialized budget of NPARD directed for various eco-measures accounts for 27% of total. Funding for eco-projects is also available from EU Fund LIFE+and Operational Programs "Environment", "Fishery and Aquaculture", and "Regional Development". Cross-compliance (with safety, animal-welfare, eco-standards) for receiving public support is introduced. Area-based direct payments induce farming on abandoned lands and improve eco-situation. Mostly bigger farms participate in public programs because of superior entrepreneurial experience, available resources, capability for adaptation to requirements and wining projects. Due to restrictive criteria, lack of formal land management titles, complicated and costly procedures, and widespread mismanagement (corruption), new public support is not effectively utilized and benefits unevenly different farms. Overall support to agriculture rests low and a small proportion of farms benefits from public aid most of them large enterprises from regions with less socio-economic and eco-problems.

It was difficult to reform inefficient system of management of public programs and significant EU funding blocked (2008) and later lost. In 2007 no public payment was made for projects associated with NPARD measures but area-based payments for regions with handicaps. Progression in implementation of public support is slow and far behind targets (Table 6.3). While measures "Setting up of young farmers" and "Payments to farmers in regions with handicaps" are successful, number of approved and funded projects in other areas is insignificant. Bulk of public contracts and funding continues to go to a limited number of farms while many effective small-scale farms receive no or a tiny fraction of public support [2]. Minor amount of supported farms and agro-ecosystems, deficiency of clear criteria for eco-performance, and lack of effective control leads to little contribution of new public measures to improvement of eco-situation in the country.

Table 6.3 Progress in implementation of NPARD in Bulgaria (% of targets) (Source: Ministry of Agriculture and Food)

	December 31, 2008		December 31, 2009		August 23, 2010	
Measures	Projects	Funding	Projects	Funding	Projects	Funding
111	0	–	0	–	na	–
112	11.25	–	55.20	–	99.73	–
121	6.77	6.27	27.86	16.09	35.62	25.49
122	0	0	0	0	0	0
123	0	0	0	0	5.81	4.41
141	0	–	0	–	3.37	–
142	0	0	0	0	0	0
143	3.62	–	9.30	–	24.38	–
211	40.04	–	43.50	–	43.50	na
212	100.17	–	107.85	–	107.85	na
214	2.80	–	4.45	–	4.45	na
223	0.00	–	1.00		1.85	–
226	0.00	–	0.90	–	2.30	–
311	0	–	0	–	0.09	0
312	0	–	0	–	2.09	–
313	0	0	0	0	0	0
321	0.00	–	4.77	–	8.15	46.19
322	0.00	–	18.00	–	19.50	43.07
43-2	0	–	0	–	7.92	–

6.4 Agricultural Impact on Environment

Market and private governance has led to a decline in all (but sunflower and goat) productions from 33% (potatoes) to 94% (apples) [7]. Some traditional varieties and breeds are recovered. Considerable portion of agricultural lands has been left uncultivated for a long period – in some years abandoned land reached one third of total [4]. Now 10% of farmlands are unutilized while fallow land accounts for 9.5% of arable land. Average yields for major products shrunk to 40–80% of pre-reform level. Number of cattle has decreased from 53% (poultry) to 81% (sheep). Tractors and combines employed diminished by 64%, and now 5.6% of farms own tractors and 0.7% harvesters while 30–40% hire or use them in association [4]. All these have relaxed overall agricultural pressure on the environment. Improper practices caused erosion and uncontrolled development of some species and suppressing others. Some of valuable ecosystems (permanent grassland) have been severely damaged. Part of meadows has been left under-grazed or under-mowed, and intrusion of shrubs and trees took place. Some fertile semi-natural grasslands are converted to crops, vineyards or orchards. This has resulted in irreversible disappearance of plant species diversity. Many municipal and state pastures are degraded by unsustainable use (over-grazing) by private and domestic animals. Reckless collection of

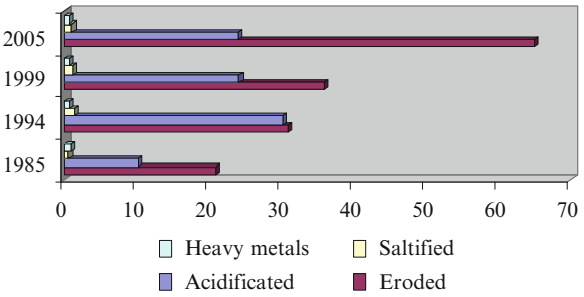

Fig. 6.2 Share of degraded agricultural lands in Bulgaria

valuable wild plants and animals led to destruction of some natural habitats. Degrading impacts of agriculture on biodiversity has been significant – all 37 typical animal breeds are endangered – 6 are irreversibly extinct, 12 almost extinct, 16 endangered and 3 potentially endangered [8]. Amount of fertilizers and pesticides used in agriculture has declined considerably, and their per hectare application is 22% and 31% of 1989 level. Now, N, P and K fertilizers are applied for 37.4%, 3.4% and 1.9% of UAA. This trend diminished drastically pressure on environment and risk of chemical contamination of soils, waters, farm produce. Part of production has got eco-character and reputation for high quality and safety.

Negative rate of fertilizer compensation of N, P and K intakes dominate being particularly low for P and K. Average of 23595.4 t N, 61033.3 t $P_2 0_5$ and 184392 t $K_2 0$ have been irreversibly removed annually from soils since 1990 [4]. Unbalance of nutrient components has been typical with application of 5.3 times less P and 6.7 times less K with appropriate N rate. Monoculture or simple rotation has been constantly practiced by large operators concentrating on few crops. All these practices further contributed to deterioration of soil quality and soil organic matter content.

Nitrate Vulnerable Zones cover 53% of country's territory and 68% of UAA. Lack of effective manure storage capacity and sewer systems contributes significantly to problem – e.g. 0.1% of livestock farms possess safe manure-pile sites, 81% use primitive dunghills, and 116,000 holdings have no facilities at all [4]. Decreasing amount of manure has been used for fertilization of 0.17% of utilized farmlands in recent years.

There has been a considerable increase in agricultural land affected by acidification (Fig. 6.2) as a result of a long-term application of specific N fertilizers and unbalanced fertilizer application. Share of acidified soil decreased after 1994, but in recent years there is a reverse tendency along with augmentation of N use. As much as 4.5% of acidified farmlands are with level harmful for crops. Fraction of salinized land doubled after 1989 but it is still insignificant part of total farmland. There has been taken no effective measures to normalize soil acidity and salinity during the period.

Erosion is a major factor for land degradation (Fig. 6.2) and its progressing level has been adversely affected by dominant agro-techniques, deficiency of anti-erosion

Table 6.4 Agricultural use of water resources in Bulgaria (Source: FAO, AQUASTAT)

Indicators	1988–1992	1993–1997	1998–2002	2003–2007
Agricultural water withdrawal $10^9/m^3$/year	3,058	0,141	0,144	0,143
Agriculture share in total withdrawal, %	21.78	–	1.66	–
Actual water resources withdrawn, %	14.6	0.66	0.68	0.67
Area equipped for irrigation, 000 ha	1,263	789	622	104,6
Cultivated area equipped for irrigation, %	29.7	17.55	17.36	3.18
Area equipped actually irrigated, %	na	5.42	4.96	51.29

measures, and uncontrolled deforestation. One third of arable lands are subjected to wind erosion and 70% to water erosion [3]. Since 1990, erosion affects 25–65% of farmland and losses varied from 0.2 to 40 t/ha in different years. Soil losses from water erosion depend on cultivation practices (8 t/year for permanent crops, 48 t/year for arable lands). Losses from wind erosion are around 30 t/year and depend on deforestation, uncontrolled pasture, ineffective crop rotation, plowing pastures etc. Serious eco-challenge has been posed by inadequate storage and disposal of expired and prohibited pesticides as 28% of country's polluted localities are associated with these chemicals [3]. Despite progression in management there are 333 abandoned storehouses in 324 locations for 2,050 t pesticides. Polluted with heavy metals and pesticides soils represents bellow 1% of farmlands. Re-cultivation of degradated farmlands is under way, but it is merely 200–250 ha per year [4]. Illegal garbage dumps in rural areas has noticeably increased reaching official figure 4,000, and farms contribute to waste "production" bringing air, soil and water pollution [3]. There is 21 folds decline in water used in agriculture comparing to pre-reform level and that contributed to reduction of water stress. In recent years, sector "Agriculture, hunting, forestry and fishery" comprises merely 3.17% of total water use and 0.34% of generated waste waters [7]. Restructuring of farms and production has been accompanied with sharp reduction in irrigated farmland and considerable distortion of irrigation facilities (Table 6.4). Negative impact of intensive irrigation on erosion and salinization diminished significantly after 1990. Primitive irrigation techniques are widespread and augment inefficiency of water use and local soil erosion. Decline in irrigation had direct harmful effect on crop yields and structure of rotation. Irrigation is not effectively used to counterbalance effect of global warming on farming and degradation of agricultural land.

There is considerable amelioration of surface and ground waters quality. Nitrate and P content in surface water decreases and only 0.7% of samples exceed Ecological Limit Value (ELV) for N [3]. In drinking water 5% of analyses show deviation of N up to five times above appropriate level. Later is mostly restricted to small residential locations but it is also typical for 9% of big water collection zones. Improper use of N fertilizers, crop and livestock practices, non-incompliance

with rules for farming in water supply zones is responsible. In 45% of samples of water for irrigation N concentrations exceed contamination limit 2–20 folds [4]. Nitrates are most common pollutants in ground water with N levels slightly exceeding eco-limit in recent years. Reduction in concentration of pesticides in ground water is reported with occasional cases of triasines over ELV since 2000. There is five times reduction of GHG emissions from agriculture since 1988 [9]. The N_2O emissions comprise 59% of agricultural emissions and it enlarged slightly since 2000. Agriculture has been major ammonia source accounting for two thirds of national emission. Majority of NO_2 emissions comes from agricultural soils (87%), manure management and burning of stubble fields (13%). Methane emission from agriculture represents about a quarter of national. Biggest portion of CH_4 comes from fermentation from domestic livestock (72%) and manure management (24%).

6.5 Policy Recommendations

First, better integrate eco- and water (including neglected ground water) policy in agrarian and development policies as effective design and enforcement of long-term eco-measures get priority. Most public efforts are put on addressing urgent socio-economic problems while improvement of eco-management is perceived as unimportant. Accordingly, no measures are taken to mitigate or prevent various eco-risks (e.g. impacts of climate change, constant monoculture, re-intensification). It is to be stability and certainty in eco-policy (long-term public commitment) in order to induce effective private and collective actions – e.g. major reason for low investments in green energy is uncertainty about the long-term policy.

Second, apply integral approach of soil, water and biodiversity management in planning, funding, management, monitoring, controlling and assessment at all levels with stakeholders involvement in decision-making. Eco-system services, life-cycle, eco (water) accounts, and other modern approaches are to be incorporated in programs design.

Third, improve coordination and efficiency of public and private agents involved in eco-management. Individual elements and responsibilities are usually divided between various agents with poor coordination, conflicting interests, and inconsistency, controversies, gaps and inefficiency of actions.

Fourth, further define, regulate and privatize property, user, management, trading, discharge etc. rights and assets related to eco-resources, eco-system services, renewable energy supply, (N, GHG) emissions, waste discharge.

Fifth, employ range of instruments including appropriate pricing, quotas, public funding and insurance, taxing, interlinking to improve eco-resources use efficiency and risk-sharing, prevent over-intensification and pressure on natural resources, and support farms adaptation to changing environment.

Sixth, organizationally and financially secure adequate eco-data collection, monitoring, and independent assessment including agricultural linkages with the state of environment – soil, water and air contamination; waste production and decomposition; total social costs, energy intensity, eco- (water) foot-print, benefits from farming; effect on eco-conservation and improvement; renewable energy production; impacts of climate change; existing and likely risks. Assure mechanisms for timely disclosure and effective communication to decision-makers, stakeholders and society.

Seventh, adapt CAP instruments to local conditions supporting farm modernization and adaptation, eco-innovations, and prospective business and non-for profit modes; relaxing EU criteria for semi-market and young farmers; directing funds to prospective (Farm modernization and adaptation, Young farmers), and unsupported (Organic livestock, restoration of abandoned farmland) measures and organizations (livestock, public academic centers); and better implementing planed eco- measures.

Eight, employ hybrid (public-private, public-collective) modes given coordination, incentives, and control advantages. Public organization and enforcement of most eco-standards is difficult (informal sectors, remote areas). Public support to voluntary initiatives of professional, community and non-governmental organizations (informing, training, assisting, funding, risk-sharing), and assistance in cooperation at grass-root, eco-system, watershed, trans-regional, trans-border levels is more efficient. Real participation of farmers and stakeholders in priority setting, management, and assessment of public programs and regulations is to be institutionalized.

Ninth, improve eco-education and training of farmers, administrators, and public modernizing Agricultural Education and Advisory Service. They are to reach all agents via effective methods of education, advice, and information (TV, radio, on line information; demonstration) suited to specific needs; set up system of continues training and sharing experiences; include eco, water, waste management, climate change, rural development issues; cooperate with other (public, private) organizations; involve farmers and stakeholders in programs management, implementation and assessment.

Tenth, improve overall institutional environment and public governance – property rights protection, laws and contracts enforcement, fight against mismanagement (corruption), removal of restrictions for private initiatives.

Eleventh, support multidisciplinary and interdisciplinary research on all aspects and impacts of eco-management, including factors and forms of eco-governance, and their impact on individual and collective eco-behavior and eco-security. Efforts of Ecologists, Technologists, Economists, Law, Sociologists, Behavioral and Political Scientists are rarely united; studies focus on individual aspect of sustainability, certain form(s), management level, location; governance of farming is separated from households and rural activities; “normative” (ideal, foreign model) rather than comparative (between feasible alternatives) approach employed; significant social

(third-party, recovery, transaction) costs ignored. Institutional, behavioral, economic, ecological, international factors of sustainability and security are not properly understood, spectrum of feasible governance modes identified, and efficiency, complementarities, and prospects of development assessed.

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Chapter 7
Environmental Security and Its Economical Aspect

Ganna Kharlamova

Abstract The purpose of this paper is twofold: firstly, to describe how environmental security issues grow rapidly in the convergence with other aspects of life in modern world, and, secondly, to furnish an example showing how the various elements of economy correlate with environmental security.

Keywords Security • Environment • Economical sustainability • Convergence

7.1 Introduction

> The whole notion of security as traditionally understood – in terms of political and military threats to national sovereignty – must be expanded to include the growing impact of environmental stress – locally, nationally, regionally, and globally.
>
> World Commission on Environment and Development

The environment is constantly changing. Environmental concerns have been growing extremely intensively in recent years. Despite the huge attention to world economic problems, like crisis, the attention to ecology and its safety is a hot point for international meetings, publishing editions [1]. Researchers identify problems and develop solutions to help the Earth tackle all environmental threats, and they all agree in one – the main background and source of ecological threats of the twenty-first century is in the population growth and STD. We have a challenge in the opposition: the growth of production – the ecological restrictions of the planet.

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7.2 Economic Sustainability and Environmental Security: Convergence of Scientific Approaches

Environmental Security – an important component of strategic defense analysis (Scheme 7.1).

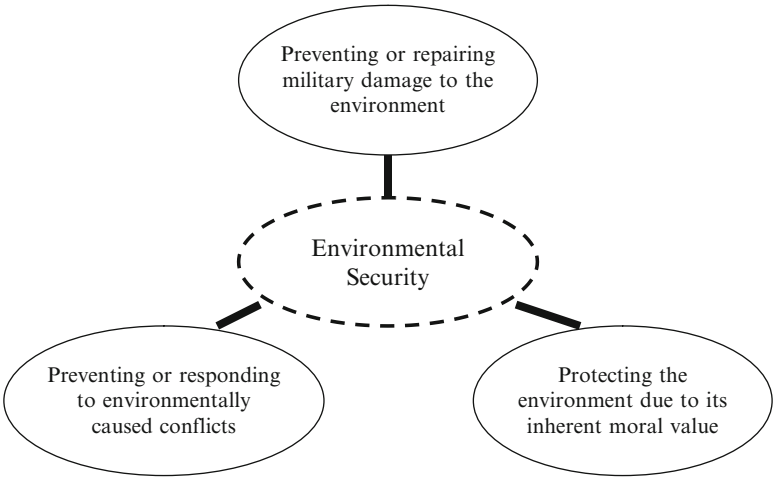
Environmental security is a process that effectively responds to changing environmental conditions that have the potential to reduce peace and stability in the world. Threats for the environment changed so the management of environment security also need be changed from traditional methods and ways to new interdisciplinary approaches.

Scientific convergence is rather new term and there is still no exact definition, so everyone understands it due own background. Merriam-Webster [2] dictionary gives such definition of convergence:

1. The act of converging and especially moving toward union or uniformity;
2. The state or property of being convergent;
3. Independent development of similar characters (as of bodily structure of unrelated organisms or cultural traits) often associated with similarity of habits or environment;
4. The merging of distinct technologies, industries, or devices into a unified whole.

We basically see a *convergence* as an integration and co-working of different science fields for giving the maximum synergetic effect in the limit point of best combining of techniques.

Recently in US the Institute for Advanced Sciences Convergence (IASC) has been created to analyze and evaluate basic science advances in nanotechnology, bioengineering, information management, and cognitive sciences (NBIC) and to



Scheme 7.1 Context sub-elements of environmental security

propose an investment roadmap for the Department of Defense (DoD). This is additional evidence in the basket of positive results that such approach can give.

There are an increasing per cent of papers appeared now in which we can find the science convergence approach, as an example:

- ***Economy and theory of terrorism*:** like works of Mancuso Anthony J., Dirienzo Cassandra E., Das Jayoti "Assessing terrorist risk and FDI using relative information measures" [3]; Enders Walter, Sachsida Adolfo, Sandler Todd "The impact of transnational terrorism on US foreign direct investment" [4]; Arno Tausch "Quantitative World System Studies Contradict Current Islamophobia: World Political Cycles, Global Terrorism, and World Development" [5] and others;
- ***Ecology and theory of terrorism*:** like Kharlamova Ganna "Ecoterrorism: an ecological-economic convergence" [6], Elhanani Doron "Environmental protection vs. eco & environmental terrorism: threats, impact and contingency plans" [7] and others;
- ***Economy and environmental theory*:** like works of Mathukumara Mani and Shreyasi Jha "Trade liberalization and the Environment in Vietnam" [8], Patricia Silva and others "Poverty and Environmental Impacts of Electricity Price Reforms in Montenegro" [9] and others.

Reasons for differences of opinion on the problem of connection between economic sustainable growth and environmental security to be found in different approaches to the interpretation of following key questions:

- Explanation of causes of the ecological crisis;
- Ways of ecological crisis overcoming;
- Compatibility of economic growth with environmental protection;
- Choice of mechanisms and instruments of environmental policy.

Items mentioned above can be considered as original criteria for grouping of existing environmental management theories (Table 7.1).

As it is impossible to create universal global mechanism of economic growth appropriate for any state without adaptations, it is same impossible to propose the universal strategy of the environment security under economic sustainable development.

7.3 Environmental Security in the Context of Sustainable Economic Development: Environmental Risk Analysis

One of the first apologists of the sustainable development conception Herman E. Daly defines the term of "sustainable development" as a link "equal to nature's ability to replenish – environmental equilibrium – steady state economy" [10, 11]. So it should be harmonic in sense of steady innovative intensive economic development where ecological factor is very crucial. Ecological limits should not rapidly convert "economic growth" into "uneconomic growth" [12]. So we have classic definition of economy as a science just supplemented by the goal of the environment protection.

Table 7.1 Grouping of existing environmental management theories

Criteria for grouping	Main head-note of the theory	Definition, sense of conception
Explanation of the main cause of the ecological crisis	Defects in the development of productive forces	Technological determinism
	Imperfection of institutions in society	Institutionalism
	Limited development of market relations	Neoclassicism
Attitude to the possibility of ecological crisis overcoming	Negative: enduring crisis	Ecological pessimism
	Positive: crisis may be overcoming	Technological optimism
Attitude to the idea of compatibility of economic growth with environmental protection	Negative: economic growth should be paused	Theories of "anti-growth"
	Positive: economic growth may be	Theories of "qualitative" and "modified" growth
Proposed mechanisms of ecological policy implementation	Methods of direct state regulation	Dirigisme
	Indirect (market) methods	Neoclassicism
	Mixed methods	"Neoclassical synthesis"

The logical development of these thoughts could be that environmental security has the basis in economical theory with its laws and principles but the goal in this variation is not a maximization of benefits and yield but sustainable economic growth in terms of preventing ecological hazards. Ecological economics, in such a way, is distinguishable from neoclassical economics primarily by its assertion that the economy is embedded within an environmental system. So ecological and economic sciences work together to identify values for society. Economics provides a decision-making framework within which maximization of the environmental policy benefits accomplishing the economically calculated fixed allocation of resources.

Like in the eternal dilemma – what was the initial – a chicken or an egg, we look at the economic system and the environmental security to range them like a source and a consequence. Here could be different points of view. But is it important to look for the only right one? It is fully obvious the existence of the contradiction between economic system and environmental state, and it becomes revealing in the process of contemporary socio-economic development. Such contradiction undoubtedly leads to the potential ecological threats and that causes the necessity to evaluate the probability of its occurrence, determination of possible economic losses and the price of the preventive measures implementation for the achievement optimum in the levels of economic and ecological risks, some saddle point.

It is necessary to expand the number of identified risk factors for the best management of potential risk situation for the environment as the increasing of new factors variety increases the depth of the term "risk". Then on the basis of factor analysis we need to develop indicators of real and potential risk for the environment. And then only on the basis of numerical indicators we manage to adjust famouse

Table 7.2 An example of indicators for creating a risk factor variable at the regional and industry levels

Regional level	Ecological risk indicators EcoR	EcoR1 – consumption of coal per area unit and per capita in region, in terms of E. Engel; EcoR2 – natural gas consumption per area unit and per capita in region, in terms of E. Engel; EcoR3 – material damage caused by fires, $ per one inhabitant of the region; EcoR4 – number of reported fires and ignition, % of the total amount in the country; EcoR5 – emissions of harmful substances in the atmosphere from stationary sources of pollution per area unit and per capita in region, in terms of E. Engel; EcoR6 – share of the turnover and consistently (re) used water in the total water usage in manufacture, %; EcoR7 – polluted waters return to the natural surface water, mln. m^3	Terms of E.Engel means calculating every statistic data in such a way: ENG = $\sqrt{T \cdot H}$, where T – the area km^2, N – the population of the region, thousands of people. This approach helps to remove the influence of the size of the region and the number of people living on its territory. $EcoR_n$ reflect the likelihood of occurrence and development of adverse natural and industrial processes that can be accompanied by significant environmental impacts and/or excessive costs of environmental compensation; energy dependence of the region
Industry sector level		$EcoR_1$ – emissions of harmful substances into the atmosphere from stationary sources of pollution, thous. tons; $EcoR_2$ – emissions of harmful substances in the atmosphere from stationary sources of pollution, % to the previous year level; $EcoR_3$ – emissions of harmful substances in the atmosphere from stationary sources of pollution average per 1 enterprise, tons; $EcoR_4$ – total volume of primary and secondary fuel, % of total	$EcoR_n$ reflect the level of environmental pollution from enterprises in industry sector, which can lead to significant environmental consequences and excessive costs for environmental compensation

math-methods of risk analysis or to apply new ones at the local, regional and national levels. As a variant of risk factor identification in terms of its numerical indicators we can propose our "environmental risk factor" based on Ukrainian statistic information available [13] (Table 7.2). Of course, the amount of $EcoR_n$ can be more than listed below, but this example shows the simple possibility to transfer from complex to integrally simple in the analysis of environment security. After the estimation of complex and integral ECOR – environmental risk level we can implement the

economic character of this class of the decision be a basic cost-loss model. As most risk avoidance decisions require some basic level of cost-benefit analysis to justify the imposition of any additional corporate or social burden.

– Method of economic analysis should start with a data base of stability:
– Conduct statistical correlations of groups of environmental parameters with stability index.
– Conduct correlations for threshold values of critical environmental parameters.

But effective decision-making and statistical analysis are only possible for further sober and realistic policy making when entering the field of real, "clear", objective information management and timely manner. Only such information that arrives is processed, interpreted and used in time when making decisions to optimize ecological management activities. However, for most Eastern European states and CIS states it's a weak part, the Achilles' heal of any management, economical and environmental as well.

The determined correlation between the evidence, utilization and management of the resources on one hand, and durable economic and social development and environment protection on the other hand will lead us to equilibrium, so to the science convergence equilibrium between ecology and economy, synergy of economic sustainability and environmental safety. Consequently, environmental risks need to be evaluated more carefully than in the past. The simplest example is building of new nuclear station, food security links. Currently, businesses are taking leading role in creating a sustainable future and the governments are only providing support for those initiatives that itself adds risks to the environmental security as in most issues touch moral and ethic aspects, level of ecological education of business management.

Consideration of some economic-ecological links:

– Increased production contributes to higher pollution through physical scale of production;
– Higher income per capita increases the demand for higher environmental quality;
– The relationship between income per capita and some types of pollution follows environmental Kuznets curve [14];
– Higher income means more consumption and consequently more consumption pollution [15].

So, we see one more prove of stable linkage between sustainable growth and ecological safety, on one hand, and on the other hand, there is very high sensitivity of correlation between indicators of this two fields.

The following measures necessary for environmental sustainability and security, in our view, include:

1. Energy and resource cost-effective use in industry and agriculture;
2. Reduce environmental emissions by introducing low-waste technologies;
3. Quality improvement of monitoring and equipment for pollution control, especially it is urgent for the CIS;

4. Stimulation and funding of the development of environmentally friendly technologies and products.

All this obvious items are possible only on the basis of scientific convergence between ecology, economics and mathematics, cybernetics, in priority:

1. Quantitative simulation modeling of land use in conditions of increasing anthropogenic loads;
2. Study of so called "industrial metabolism" (anthropogenic whirlpools of substances and energy) for integrated natural systems and changes of the chemical composition of the natural environment (including pollution, acidification, salinization, eutrophication, etc.) can be exemplified by the development of ecology Rhine basine;
3. Risk analysis and evaluation of stability limits in anthropogenic ecosystems;
4. Ground surveying system optimized with the specific features of the social and economic development of each country.

7.4 Conclusions

While during the last century economic and ecological goals were mostly conflicting with each other, nowadays those goals are getting more and more aligned. The target of future sustainable economical development assumes the point meeting in needs of human beings and the Environment (The Earth). However, there is still present misunderstanding, like a typical example of a logical fallacy "what was said in the collective sense is said in the sense of separating, and vice versa" – deliberately usage of resources by those who are destroying nature: to justify their actions they use a syllogism, a conclusion which presented in a statement "from the nature of my actions the nature will not perish".

Despite the variety of options are available to fit our collaborative research needs across the environment-related disciplines only multidisciplinary research addressing complex issues and civil society participation can give crucially positive results in enhancing of environmental security. Maximize the correlation between the summary environmental parameters and current and historical regional stability data (economic sustainability, particular) is the main task for now.

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Chapter 8
Sustainable Strategies of Phytoremediation of the Sites Polluted with Obsolete Pesticides

Lidiya Moklyachuk, Vitalina Petryshyna, Olena Slobodenyuk, and Yuliia Zatsarinna

Abstract The land around former warehouses has an increased likelihood of contaminant exposure for local population. For phytoremediation of phytotoxic soil, polluted with pesticides, around the former warehouses, we propose a method of phytoremediation using phytotoxicity tolerant plants. In a soil polluted with wide range of obsolete pesticides, changes in quantitative proportions of plant species, structure, productivity and floristic features are observed. In the structure of such phytocenosis, perennial plant species with vegetative reproduction prevail. Current study demonstrates that pesticide-tolerant wild plants decrease DDT concentration in soil by rhyzodegradation and rhyzostabilization of the pesticide. Wild plants with translocation factor, i.e. the ratio of concentration in the shoots over the root concentration, below 1 are suitable for phytostabilization of DDT and prevention of its migration in conditions of polycomponent pesticide pollution. Resistance to a high pesticide pollution of pesticide-tolerant plants is acquired during vegetation under the influence of persistent toxicants.

Keywords Dichlorodiphenyltrichloroethane (DDT) • Phytoremediation • Phytostabilization • Rhyzodegradation • Persistent organic pollutants (POPs)

8.1 Introduction

Management of semi-natural ecosystems, which have been exposed to different complex pollution, as well as remediation of former industrial sites contaminated with radionuclides, heavy metals or organic compounds, is of major concern in

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several European countries. For example, uranium mining and milling, phosphate processing or coal mining resulted in high accumulation of radionuclides and/or heavy metals in the soil surface layers of the surrounding environment. Contamination also could be caused by accidents at nuclear power plants, similar to the Cherobyl incident occurred in 1986.

Soil pollution is a serious environmental problem, not only in the highly industrialized western countries, but also in developing countries. Polluted areas are very dangerous for people and ecosystems. The extent of pollution and its characteristics vary widely. A substantial portion of polluted sites consists of large areas where the surface soil is more or less diffusely polluted with pesticides. These pesticides are often persistent organic pollutants (POPs), and their decomposition in nature is very slow.

Many republics of the former Soviet Union, including Ukraine [1], accumulated large reserves of persistent pesticides during the existence of the Soviet Union. Many of these reserves are now stored in bad conditions and serve as a source of POPs pollution of surrounding agricultural lands. There are substantial health threats to the population from poisoning of surface water from pesticide residues suspended in the air, and from groundwater pollution as a result of the vertical migration of pesticides [4].

Accordingly to the Ministry of Ecology and Natural Resources of Ukraine, at the end of 2009, the amount of obsolete pesticides and agricultural chemicals in Ukraine was about 21 t. The total number of warehouses is about 3,000. In addition, there are about 2,000 contaminated areas located on sites of destroyed warehouses.

Some of these warehouses are located in the areas affected by the Chernobyl accident. As known from scientific sources, the combined action of pesticides and radionuclides may have a synergistic negative impact on wildlife and human population. In 2010, the Ministry of Ecology and Natural Resources began implementation of a major program of cleaning of hazardous wastes in Ukraine. In particular, the Ministry planned to solve the problem of obsolete pesticides completely by the end of 2012. This is one of the priority programs of the Ministry. Given the lack of the pesticide destruction capacities in Ukraine, pesticides will be transported for safe disposal to specialized factories outside Ukraine.

Nevertheless, the problem of remediation of soil polluted with POPs remains unsolved. Two aspects of obsolete pesticides problem could be indicated. Firstly, there are obsolete pesticides that are stored in warehouses; secondly, there are pesticides in the soil. Polluted soil cannot be cleaned using known physical or chemical methods due to extremely high cost of transportation and processing of large soil quantities [3].

Our team examined soil samples taken from areas around pesticide warehouses in Kiev, Khmelnitsky, Vinnitsa, Zhitomir and Chernovtsy regions of Ukraine. Analysis of these samples conducted in the Institute of Agroecology revealed concentration of DDT and its metabolytes of 100–10,000 μg/kg and concentration of sim-triazine herbicides about 500 μg/kg. Pesticide residuals had been found at the distance of 500 m from the warehouse and at the depth of 6 m [16, 17]. Those studies confirmed the actuality of the problem of pesticide warehouses as sources of

environmental POPs pollution. An important aim of research in the field of neutralization of pesticide polluted objects is development of long-term strategy of soil and water phytoremediation.

8.2 Phytoremediation of Polluted Soil

Phytoremediation is a complex of "green" technology that uses plants for remediation of soil, sediments, and surface and ground water polluted with toxic metals, organic pollutants and radionuclides. Phytotechnologies do not require large investments. They are effective and inexpensive tools of soil remediation [18, 24]. Phytotechnologies are more profitable than any alternative mechanical or chemical method of soil pollution treatment. They are also ecologically safe. It was concluded that plants have genetic potential to remove or block a wide range of pollutants [14]. A large-scale research was conducted on phytoextraction of inorganic elements [13] revealing a number of plant species capable to hyperaccumulate metals [25]. Mechanisms of accumulation of chemical elements by plants were also investigated [2].

Phytoremediation of organic pollutants originally was developed for three classes of compounds: chlorine solvents, explosive materials and petroleum hydrocarbons. Recently, phytoremediation was also studied for soil remediation from POPs and polycyclic aromatic hydrocarbons (PAHs). POPs include industrial chemicals, byproducts of chlororganic synthesis and some chlorine-containing pesticides: DDT, hexachlorocyclohexane (HCH), chlordane, aldrin, dieldrin, and mirex. All these compounds have a long half-life in the environment and are able to bioaccumulate in trophic chains. They exert many negative effects on human and animal organisms, such as immunodepressive, cancer-causing, mutagenic, and teratogenic effects [15]. DDT and HCH were widely used in Ukraine in the past as insecticides for plant protection.

The goal of phytoextraction is to accumulate as much pollutants as possible in the vegetative part of the plant to clean the soil. In order for a plant to be classified as a hyperaccumulator of pollutants, the plant has to produce a large biomass, and the concentration in the ground part of the plant has to be considerably higher than concentration in the underground part [13]. The translocation factor represents the ratio of pesticide concentration in the shoots over the root concentration. If the translocation factor is greater than unit, processing of polluted plants is more efficient than processing of polluted soil [23].

Earlier studies and our research revealed great perspective in the use of phytotechnologies for remediation of soil polluted with POPs [22, 19]. However, soil polluted with a wide range of pesticides is often toxic for known remediator plants. Phytotoxicity of soil is an obstacle for use of phytotechnologies. For example, cultural plants, that are able to accumulate POPs, are dying after 30–35 days of vegetation. Plants intended for use in phytoremediation of soil contaminated with organochlorine insecticides and persistent herbicides should

be tolerant to these pesticides. Thus, there is an urgent need to find remediator plants, which are tolerant to soil phytotoxicity, and study their phytoremediation capabilities.

8.3 Materials and Methods

To characterize the residual amount of pesticides in soil near the pesticide warehouses, we studied organochlorine pesticide 4,4′-DDT and its metabolites (4,4′-DDE, 4,4′-DDD). Soil and plant sampling was conducted according to engineering specifications and state standards for Ukraine [7, 11]. Rhizosphere soil sampling was conducted according to methods of soil microbiology [26]. At each location, plant species' identity and coverage were determined within four 50 × 50 cm squares using Roshensky grids [5]. Common plant species were selected to estimate plant uptake of DDT metabolites. The sampled plants were carefully cleaned and separated into roots and shoots for analysis.

Organochlorine pesticides were quantified by gas chromatography (GC) using electronic-capture detector (ECD) according to accepted engineering specifications and state standards of Ukraine [8, 10, 12]. Vegetative studies were conducted in climatic chambers [6, 9]. Soil phytotoxicity was studied according to international and Ukrainian standards ISO 11269(1–2).

8.4 Plant Selection

The purpose of this study was to find plant species persistent to pesticides and capable either to accumulate large concentrations of pollutants or decompose organic xenobiotics in the rhizosphere.

During the process of natural plants settlement on the territories polluted with xenobiotics, changes in physical, chemical and biological properties of soil are observed. With development of secondary succession, persistent plant groups that include tolerant plant species are formed. Those tolerant species show potential for use in phytoremediation of such soil.

Our experiments were conducted to observe the adaptation processes of wild plants on a soil highly polluted with pesticides. The experiments were conducted in the climatic chamber. Gray sandy podzolized soil polluted with organochlorine pesticides and persistent herbicides was sampled near the pesticide warehouse in Kyiv region from the depth of 0–30 cm. The following plants of local phytocenosis were selected for the experiments: *Taraxacum officinalis* Wigg., *Oenothera biennis* L., *Erigeron canadensis*, *Xanthium strumarium* L., *Potentilla argentea* L., *Daucus carota* L., *Plantago lagopus* L., *Achillea millefolium* L. Each plant was vegetated separately in 51 plastic pots with 3 pots per species. The seeds of these plants were picked on unpolluted territory.

The observations of the adaptation processes of *Taraxacum officinalis* Wigg., *Oenothera biennis* L., *Potentilla argentea* L., and *Daucus carota* L. were conducted during 2 years (2 vegetation periods). Plants *Xanthium strumarium* L., *Plantago lagopus* L., *Erigeron canadensis* L. and *Achillea millefolium* L. were vegetated for one vegetation period.

For the plants growing on polycomponent polluted soil from seeds, visible toxicity symptoms were found for the following plant species: *Taraxacum officinalis* Wigg., *Plantago lagopus* L.i *Oenothera biennis* L. – point necrosis, yellowing and dying off of lover leaves; for *Potentilla argentea* L. and *Achillea millefolium* L. – decrease of growth processes and miniaturization of habitus; for *Xanthium strumarium* L. – dying off of leaves borders. Visible morphological changes were not observed on *Daucus carota* L. and *Erigeron canadensis* L.

The change of leave color, necrosis marks, chlorosis, yellowing and dying off of the lower leaves are non-specific reaction of plants to toxic influence of pesticides in soil. Such damages are classified as chematotraumosis [20]. Premature yellowing of leaves is followed by a major breach of metabolism. In chlorotic leaves, concentrations of the non-protein forms of nitrogen and ash elements increased, and their proportions changed. In addition, the amount of dry matter decreases, concentration of water increased, many ferments became less active, and osmotic pressure and suction force increased. Total concentration of organic acids in such leaves is always high [20]. In addition to mentioned symptoms of toxicity, the decrease of growth rate of all studied plants was detected. This phenomenon is caused by oppression of synthetic functions of plants, which are critical for the growth processes [20].

Weakened synthesis of organic compounds as the result of reduction of the leave area decreases reproductive ability of the studied plants. During the first year of study, only *Xanthium strumarium* L. had fruit (with recessed size). *Daucus carota* L. in its second year and *Erigeron canadensis* L. have formed seeds at the end of vegetation. *Taraxacum officinalis* Wigg., *Oenothera biennis* L. and *Potentilla argentea* L. did not form flowers and fruits even after 2 years of vegetation.

These studies showed difference in the reaction of plants on polycomponent pesticide pollution of soil, while growing from seeds, and in the field under the chronic influence of the pollution, where above phenomena did not take place. This difference can be explained by few reasons. Firstly, the compensation of the negative pesticide influence is much more effective on the group level in the field than on the species level in pots [20]. Secondly, the species with wide geographical distribution almost always form populations adapted to local conditions by physiological adaptation or producing new genetic types (with morphological differences or without them). Herewith, the cell adaptation is the most important feature of plants exposed to a long-term adverse toxicant influence [21]. There are biotypes of plants species formed in the field, which are persistent to local soil toxicants. These plants have seeds carrying genetic information for adaptation to local conditions, including pollutants.

The resistance of plants to pesticides in the field is revealed only when the population of persistent plants reaches at least 30% of total population.

Table 8.1 Distribution of DDT between root and shoot tissue for the pesticide-tolerant plants grown on contaminate soil (vegetative experiment)

Name	Soil or tissue	Total DDT, μg/kg dry matter	Translocation factor
Xanthium strumarium L.	Soil	398.0 ± 2.5	0.40
	Root	304.1 ± 11.1	
	Shoot	118.4 ± 3.3	
Achillea millefolium L.	Soil	757.9 ± 0.7	0.15
	Root	1,090.4 ± 18.3	
	Shoot	163.9 ± 3.9	
Achillea millefolium L.	Soil	1,567.0 ± 1.9	0.27
	Root	1,701.3 ± 18.0	
	Shoot	466.9 ± 5.1	
Plantago lagopus L.	Soil	543.9 ± 14.7	0.21
	Root	1,727.9 ± 41.1	
	Shoot	362.8 ± 12.3	

Development of this phenomenon in agriculture is prevented using different alternating herbicides and a crop rotation [21]. Hence, under the influence of negative factors, plants are able to expand the toxicity tolerance limits by changing the orientation of synthesis processes – decomposition of physiologically active compounds in plant cells or regain of normal growth rate by adapting on the cell or genetic level.

Beside phenological observation of growth and development of plants in climatic chamber, we conducted research of phytoextractional and phytodegradational ability of wild plants. We studied the following species, present in the phytocenosis formed in conditions of long-term polycomponent pesticides pollution: *Xanthium strumarium* L., *Achillea millefolium* L., *Plantago lagopus* L.

To evaluate the washout of toxicants during watering of plants, we used soil sample, polluted with DDT and its metabolytes, without plants. Experiments included determination of HCH residuals in soil of every pot before and after the experiment, and also in vegetative organs of plants.

The study of the accumulation and degradation of DDT in *Xanthium strumarium* L., *Achillea millefolium* L., and *Plantago lagopus* L. at various levels of DDT pollution, has shown that these plants mainly accumulate toxicants in the root system with low translocation factor (Table 8.1).

For all studied wild plants, a significant decrease of toxicants concentration in soil compared to the original levels occurs (Table 8.2). Depending on the species, total DDT concentration decreased by 15.2–30.7% (4.4–19.9% in the pot soil). Hence, wild plants cause a decrease of DDT and its metabolites in soil having long-term polycomponent pesticide pollution. Notably, concentration of HCH had decreased by 3.6% due to watering.

Table 8.2 Decrease of DDT concentration in soil as the result of growing persistent to phytotixicants plant species (vegetative experiment)

	Before the experiment	After the experiment			
Plant species	Total DDT, μg/kg dry soil, in pot soil	Total DDT, μg/kg dry soil, in pot soil	Total DDT, μg/kg dry soil, in rhyzospheric soil	Decrease in rhyzospheric soil, %	Total decrease, %
Xanthium strumarium L.	398.0±2.5	359.9±1.0	276.0±3.9	30.6	19.9
Achillea millefolium L.	757.9±0.7	659.6±64.6	564.7±1.7	25.5	13.0
Achillea millefolium L.	1,567.0±1.9	1,445.4±15.8	1,328.7±2.4	15.2	7.8
Plantago lagopus L.	543.9±14.7	520.1±50.8	445.4±1.7	18.1	4.4
Soil only	1,253.3±37.5	1,208.0±1.9	–	–	3.6

8.5 Conclusions

The persistence of plant species *Taraxacum officinalis* Wigg., *Oenothera biennis* L., *Erigeron canadensis*, *Xanthium strumarium L.*, *Potentilla argentea L.*, *Daucus carota L.*, *Plantago lagopus L.*, *Achillea millefolium L.* tolerant to high pollution level was studied. It was shown that tolerance to the pesticide pollution is gained during the vegetation process in conditions of chronic toxicant influence.

Wild plant species *Xanthium strumarium L.*, *Achillea millefolium L.*, *Achillea millefolium L.*, *Plantago lagopus L.* have low translocation coefficients, but are able to grow on phytotoxic soil and decrease DDT concentration by phytostabilization and rhyzodegradation.

Local citizens are advised to assist in planting of wild plants on polluted sites. Fencing of these sites and monitoring of the soil and plant pollution by pesticides and their metabolites is essential.

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Chapter 9
Innovative Method for Utilization of Wastewater for Security and Safety in Rural Areas

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Abstract In the rural areas building of sewerage systems and central wastewater treatment plants (WWTPs) often brings too high costs, due to high distances from one farm to another and terrain configuration. Individual treatment systems for each farm are a good solution. The treatment wetlands for nine individual farms were constructed in Kaszubian Lake District in summer and autumn 2009. Three configurations of reed beds were chosen. In the paper design criteria and results from 2 years of operation are presented. The average BOD removal varied from 64% to 92% and N removal from 44% to 77%. Individual construction of the treatment facilities by farmers under the supervision of technical personnel makes the framers aware of the significance of each element of the treatment process and guarantees proper future operation of the system.

Keywords Surface water quality • Single-family TWs • Vertical flow treatment wetlands • Wastewater

9.1 Introduction

High quality food production requires good quality of water. During last decades we observed more and more degradation of water bodies. The latest report of Environmental Protection Inspection about the condition of Polish waters indicates that the protection of waters against eutrophication is one of the most significant issues. In order to solve

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the problem of the eutrophication of waters in Poland, strategies not only for big agglomerations but, first of all, for rural areas with dispersed farms and buildings have to be developed. Surface waters are especially exposed to eutrophication due to the way land is used in rural areas. One of the most important reasons of this is discharging of untreated or only partly (mechanically) treated wastewater from rural areas. Also sanitation of rural areas is the most curtail task according to the newest EU Water Directive. These areas are characterized with a huge disproportion between number of people are supplied with water system and those who are using wastewater system equipped with wastewater treatment plant. Therefore to resolve the eutrophication and sanitation problem, there is a demand for working out the strategy for less compact settlement. According to the Polish Water Management Board, at present there are 1,636 agglomerations, with 23 million of people connected to sewerage systems. It means that the other 15 million of people in Poland do not use sewerage systems. According to the new administration regulations, building of sewerage systems is not profitable if there are less than 120 inhabitants per 1 km of newly constructed system. This means that in a major part of rural Poland no sewerage systems will be built. Thus, simple, reliable and cost-saving solutions in the field of sewage management in rural areas are needed. Treatment wetlands (TWs) can be an alternative solution to sewage treatment in rural communities, schools, at campsites and in individual houses. These systems are simple in operation, cheap and effective and they can have landscape and educational values. Although TWs in Europe are usually designed to serve up to 500 inhabitants, most of the existing facilities receive sewage from fewer than 50 inhabitants or even from individual farms. TWs for more than 1,000 inhabitants are scarce [1, 2]. Within the research project *Innovative Solutions for Wastewater Management in Rural Areas* (financed by Polish Ministry of Science and Higher Education E033/P01/2008/02 and EOG Financial Mechanism and Norwegian Financial Mechanism PL0271) the conception of sewage treatment and sewage sludge utilization at the TWs for individual households in a rural area was created. The project has been launched in the catchment area of the river Borucinka in the Municipality of Stezyca, Pommerania Region. The idea was to prepare a ready-to-implement solution for community in a rural area through using TWs serving individual households. Local terrain configuration and dispersed development in the selected area do not justify building a sewerage system. Domestic sewage is collected at cesspools, which often leak to groundwater. After the review of existing TWs in Poland and in Europe, three configurations of hydrophyte beds are proposed. These facilities were constructed in summer 2009.

In the paper design criteria and treatment results of single-family TWs from 2 years of operation are presented.

9.2 Materials and Methods

The proposal of an innovative sanitary system is basing on an idea of a closed cycle of matter in the environment. The nutrient substances: N, P, K compounds present in sewage should be used as soil fertilizers. Sewage from individual

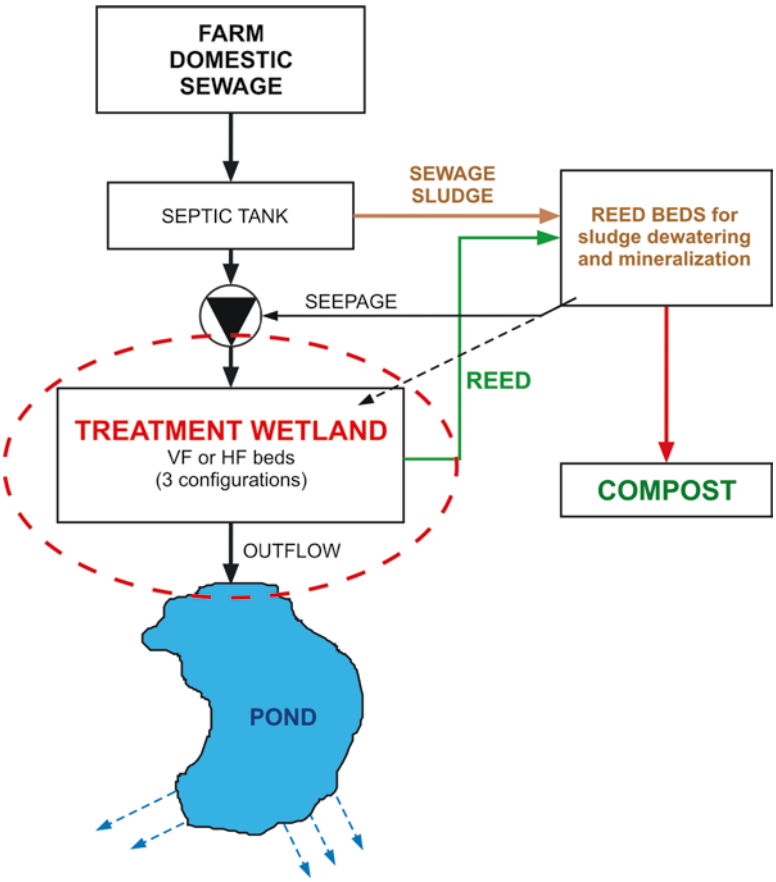

Fig. 9.1 The concept of sewage and sludge management for an individual household

households is treated at individual treatment plants for a single household. The benefit of applying treatment wetlands (TWs) is luck of excessive sludge generation during the wastewater treatment thus. Primary treatment of sewage takes place in septic tanks. Then the wastewater is discharged to treatment wetlands working with three configurations. The sludge gathered in septic tanks is periodically removed and performed in reed draying beds, where intensified natural processes take place. The seepage generated in the dewatering process are reticulated to treatment wetlands and treated together with wastewater (Fig. 9.1). The dewatered and stabilized sewage sludge becomes a valuable humus substance that can be used as soil fertilizer at the farm lands. In this way the matter cycle is closed. The sewage treatment technology as well as sludge processing is simple in operation. Described method of sewage and sludge utilization has positive social, economic and environmental aspects. The strategy of sustainable development of the municipality is fulfilled.

Another significant aspect of the undertaken research is the selection of the optimal configuration of hydrophyte beds, which will depend on the local conditions. Within the project, nine individual farms were selected in the analysed catchment area. The farms selected to take part in the project were already equipped in the septic tank. The investors (farmers) accepted the project conditions. Within the Project the formal questions were settled up at the local administration level and the necessary materials for TWs construction were purchased. The construction of TWs was carried out by the farmers. Three configurations were proposed: two with vertical subsurface flow (VSSF) beds and the third one with a horizontal subsurface flow (HSSF) bed preceded by a pre-filter:

– Configuration I: primary sedimentation tank with elongated detention time (5–6 days), followed by a single VSSF bed (the unit area of 4 m^2/PE) and a pond,
– Configuration II: existing primary sedimentation tank (with short retention time up to 2 days), then two sequential VSSF beds followed by a pond,
– Configuration III: primary sedimentation tank, pre-filter (pre-treatment), HSSF bed.

In all TWs additional pumps were used. A timer, overrun by a float switch, controls the dosing pump. The depth of vertical flow beds was 0.7 m. The bottom was laid with HDPE foil (1 mm). Common reed was planted on the beds surface with the density of 4 plants per m^2. Each of the investors individually arranged the aesthetical appearance of his TW. The investors were allowed to extend the area of the polishing pond. The only "strong" recommendations were to keep the gravitational outflow of sewage from the bed to the pond and to isolate of the significant part of the pond's bottom from the subsoil with the 1 mm PE foil. The role of the pond is removal of nitrates V (denitrification), further removal or the organics, fine suspended solids and colloids. Additionally, the pond will provide retention during the winter period. In the vegetation season the treated sewage will feed the plants growing on the pond's edges. In order to promote biodiversity, it was recommended to plant the pond with possibly large number of aquatic plant species and to systematically remove the extensive vegetation to avoid recontamination of the treated sewage. The TWs were built in summer and autumn 2009.

9.3 Laboratory Analyses

The samples of sewage were collected from August to October 2009 (three series) and in spring (May–June 2010) (three series). In the grab samples of sewage the pH and concentrations of BOD_5, COD, TSS, organic TSS (VSS) total nitrogen (TN), ammonia nitrogen, organic nitrogen, nitrates V and total phosphorus (TP) were determined. The analytical procedure recommended by Hach Chemical Company and Dr. Lange GmbH was used. The analyses were performed according to Polish norms and guidelines given in Polish Environmental Ministry Regulation of 24th July 2006 [3].

9.4 Results and Discussion

9.4.1 Pollutants Concentration

Although the concentrations of pollutants in discharged sewage varied significantly among the analyzed TWs, they were much higher than reported by Vymazal [4], Heistad et al. [5], Steer et al. [6] and Jenssen et al. [7]. The average concentration of COD varied from 537.3 to 1140.9 mg O_2/L and for TN from 107.9 to 134.1 mg N/L (Table 9.1). These concentrations are two to three times higher in comparison to the values reported by Vymazal [4] and Heistad et al. [5] (36.3–77.5 mg/L). Similar high concentration of nitrogen was present in the septic tank effluent in Podlasie region of Poland [8]. Enormous concentrations of organics COD and BOD_5 were discharged to TWs – at four out of nine analyzed farms. COD was exceeding 1,000 mg/L and at another one was 970 mg/L. The inflow BOD_5 concentrations were also high – the highest amounting 1,200 mg/L. So, high inflow concentrations could be caused either by improper maintenance and operation of septic tanks or the inflow of high strength wastewater (manure, run-off from the fields or leakages from farmyard).

Although the concentration of pollutants decreased, however the sewage samples collected from the last stage of treatment (the pond) in many cases not fulfil the requirements of the Regulation of Environmental Ministry from 24th July 2006. Relatively low quality of the treated effluent is likely be a result of short period of operation (low development of roots and rizhomes as well as biofilms) and short period of sewage retention in the pond. Further monitoring of the TWs is necessary in order to explain the sewage treatment mechanisms as well the role of the purification pond in the treatment process.

9.4.2 Removal Efficiency

Despite so high inflow pollutants concentrations, quite effective removal of pollutants was observed at most TWs. The effectiveness of TN removal at the analyzed configurations varied from 49% to 79% and the highest was observed in two sequential VSSF beds (configuration II). Good removal efficiency of TP was observed (from 29% to 68%). The best efficiency was achieved in configuration II while for configuration I and III the mean efficiency of TP removal was almost similar. The achieved high efficiency of pollutants removal, especially TP, could result from filtration and sorption processes. The effectiveness of TSS removal varied from 43% to 89% what could be concluded as a fail at all TWs. Such low effectiveness in TSS removal could be due to some leakages from farmyards. Among organics, BOD was removed with slightly higher efficiency than COD, which is characteristic for both conventional and natural wastewater treatment technologies. The highest effectiveness of BOD removal (88.1%) was observed for configuration II. On the contrary,

Table 9.1 Average concentrations of pollutants at individual household TWs, mg/L

	Configuration					
	I (VSSF + pond)		II (VSSF I + VSSF + pond)		III (pre-filter + HSSF + pond)	
Parameter	Influent	Effluent	Influent	Effluent	Influent	Effluent
TSS	289.7	69.9	178.3	37.2	269.4	100.3
	186.7–441.4	47.9–100.2	161.2–202.7	29.3–44.6	179.7–413.9	83.3–116.2
VSS	236.9	52.7	137.5	30.5	221.5	91.4
	157.0–348.1	32.8–77.4	113.1–173.2	25.7–39.7	143.0–346	73.3–123
BOD_5	236.0	46.8	328.3	36.0	558.1	69.5
	166.1–330.5	37.5–64.2	201.9–415.6	33.4–40.6	466.6–723.9	50.6–80.8
COD	537.3	170.3	745.3	165.9	1140.9	204.5
	292.5–679.1	101.7–271.8	568.1–836.6	140.4–179.1	940.0–1306.4	189.0–206.4
TN	107.9	39.9	134.1	42.4	115.7	48.0
	59.4–159.5	26.4–46.9	111.2–158.5	26.9–72.4	80.5–138.6	35.6–69.7
$N\text{-}NH_4^+$	76.6	17.7	78.5	15.4	61.4	16.8
	38.2–118.2	8.15–23.6	44.8–97.7	7.4–30.1	53.8–69.4	8.4–29.0
TP	11.2	4.3	15.5	5.5	14.3	6.6
	4.8–17.9	3.4–5.7	14.6–17.2	4.7–6.2	13.2–15.9	6.0–7.2

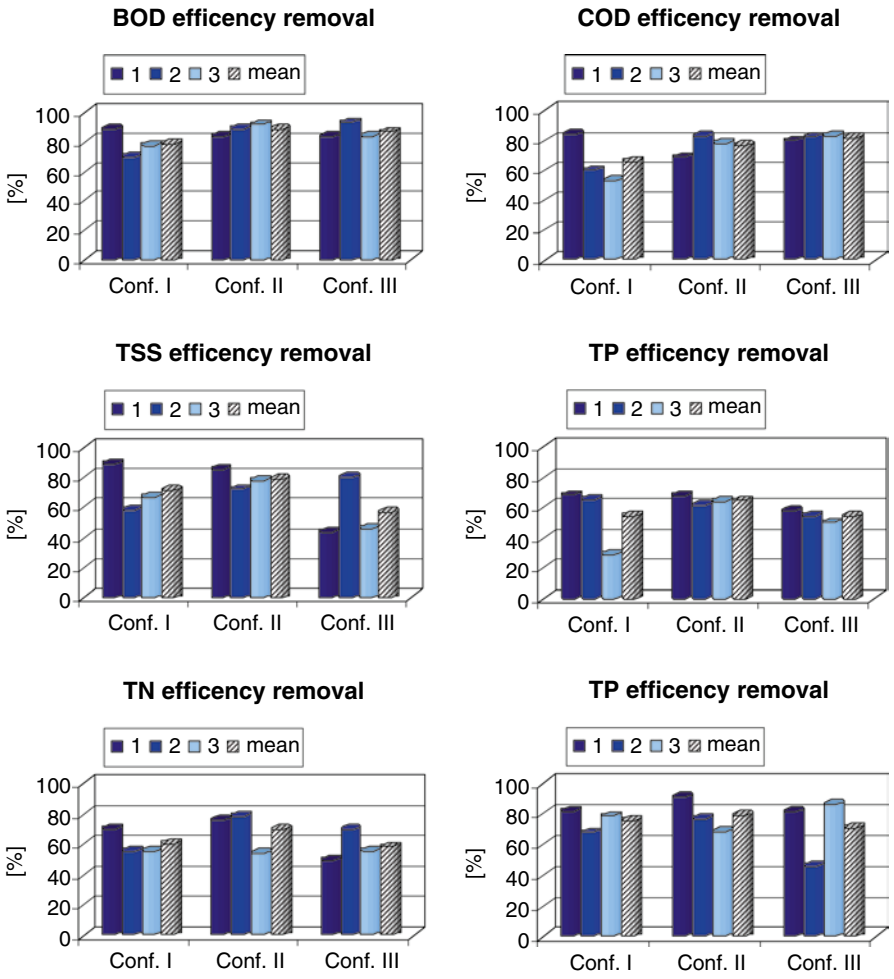

Fig. 9.2 The mean treatment efficiency for single family TWs, (1, 2, 3 – facility working in configuration)

long retention time in HSSF bed, applied in configuration III, favored the decomposition of COD. The pollutants removal efficiency in TWs in Kaszuby Lake District is similar to the monitoring results of the individual household TWs in Poland. It was indicated that the HSSF facilities working at the second stage of sewage treatment provided effective removal of BOD_5 and COD as well as TSS. The effectiveness of BOD_5 removal varied from 25.6% to 99.1% (average 62.4%) for the loadings from 11.2 to 115 kg/ha/day. However, the removal effectiveness of the total nitrogen was lower and varied from 22.4 to 84.2% (average 44.5%), for the loadings from 8.5 to 34.0 kg/ha/day [9] (Fig. 9.2).

The analysis performed in Germany showed that 24 VSSF facilities achieved significantly better treatment effects than 83 HF-CW facilities [10]. The average concentrations of COD and ammonia nitrogen in the effluent of the VSSF facility were equal to 68.2 mg O_2/L and 9.5 mg/L, respectively, and were lower than the corresponding values for the HSSF facilities (102.5 mg O_2/L and 36.0 mg/L, respectively). According to Langergraber [11], the effluents of one-stage VSSF beds in a unit area equal to 4 m^2 PE^{-1} and organic matter load equal to 20 g/m^2/day can meet rigorous Austrian outflow standards (below 90 mg/L COD and 25 mg/L BOD_5), regardless of the season of a year and air temperature. According to Molle et al. [12], two sequential VSSF beds, periodically supplied with raw sewage, provide effective treatment. This configuration of VSSF beds allows for reducing pollutant concentrations to the following level: COD – 60 mg/L, TSS – 15 mg/L, Kjeldahl nitrogen – 8.0 mg/L. In France there are over 200 TWs constructed according to this scheme. More than 60 of these facilities were built in 2003. In 2005 the analysis of the operation of 81 treatment wetlands (53 with sequential VSSF I and VSSF II beds) was performed. The treatment effectiveness of the analyzed facilities was very high: over 91.0% for COD, 95.0% for TSS and 85.0% for Kjeldahl nitrogen. Due to high treatment effectiveness, the concentrations of pollutants in treated sewage were very low: 66.0 mg/L COD, 15 mg/L TSS and 13.0 mg/L $N_{Kjeldahl}$. The removal effectiveness of Kjeldahl nitrogen in the first stage of treatment was equal to 50.0%. The one-stage vertical flow systems had not been used until 2004, when they were implemented as a solution to sewage problem in the rural area in Podlasie region (east part of Poland).The VSSF TWs in Podlasie are being monitored by several research institutes, however, the monitoring results are not obvious. The analyses performed by the authors of the article indicated that the analysed facilities were very effective in pollutant removal. The removal effectiveness of BOD_5 varied from 86% to 98%, and of COD – from 79% to 94%. The results of the analyses confirmed the low effectiveness of total phosphorus removal (from 13.4% to 41.3%) [8]. Additionally, it was found that the effluent concentrations of TSS exceeded the admissible value of 50 mg/L (The Regulation of Environmental Ministry from 24th July 2006). The share of organic suspended solids in the total suspended solids at the effluent varied from 49% to 95%. Very good conditions for the nitrification process existed in the treatment facilities. This is confirmed by the very low concentrations of ammonia nitrogen at the effluents of the three analyzed facilities. However, the removal of total nitrogen was substantially lower in comparison to that of ammonia nitrogen. As a result, nitrates V were the dominant form of nitrogen at the effluents, which indicates that the denitrification pond failed to play its role [8]. Comparison of all three configurations so far indicated the best preferment for configuration II consisted of two sequential VSSF beds followed by a pond. Since the contact time in sequentially working beds is twice as long as in a single bed, it can be concluded that elongation of the contact time has more positive impact on treatment results than application of larger unit area of a single VSSF bed.

9.5 Conclusions

1. The TWs operated in Poland receive much higher concentration of pollutants in comparison to the TWs operated in Europe and USA.
2. Good treatment effectiveness (BOD 69–93%, TN 49–79%, TP 29–69%) was observed, however the sewage samples collected from the last stage of treatment (the pond) in many cases did not fulfill the requirements of the Regulation of Environmental Ministry from 24th July 2006.
3. Comparison of all three configurations so far indicated the best preferment for configuration II consisted of two sequential VSSF beds followed by a pond.
4. The application of treatment wetlands for single-family effluent is a good solution for sewage treatment in the rural areas.
5. Individual construction of the treatment facilities by farmers under the supervision of technical personnel makes the framers aware of the significance of each element of the treatment process and guarantees proper future operation of the system.

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Chapter 10
Three Stage Biotechnology for the Rehabilitation of Soils Polluted with Explosives

Giorgi Kvesitadze

Abstract Screening of plants according to their potential to assimilate TNT and RDX was carried out. As a result, 3 plant species (soybean, alfalfa and ryegrass), 41 bacterial cultures, 14 strains of microscopic fungi, 3 strains of yeast were selected. Two bacterial consortia for bioconversion of TNT were composed. Selected cultures of microorganisms and consortia were tested in conditions of submerge cultivation on the areas containing 0.5 mM TNT. Optimum conditions of submerge cultivation (pH, temperature, duration of incubation) for selected active strains has been established. As a result of the experiments performed for water cleaning, it has been established that 12-days-old soybean seedlings effectively absorb [1-^{14}C] TNT from the polluted water and after 8 days approximately 80% of clearing effect is reached. It has been shown that that during destruction of biomass of plants by microscopic fungi *Aspergillus niger* J 3-5 the release of part of labeled TNT and its metabolites from plant biomass and the release of radioactive CO_2 takes place.

Keywords Explosives • Polluted soil • Soil rehabilitation

10.1 First-Value Heading

Widespread contamination of the environment by explosives due to the manufacture, disposal and testing of munitions has become a major international concern connected with the increasing scale of soil and ground water contamination.

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Most explosives are considered to be a major hazard to the biological system due to their high toxicity and mutagenic effects. Research on biotransformation of explosives has clearly shown that some plants and several families of aerobic microorganisms, representing different taxonomic groups of microorganisms, have the potential to transform TNT (2,4,6-trinitrotoluene), RDX (hexogen – hexahydro-1,3,5-trinitro-1,3,5-triazine) and other nitro organic explosives to non toxic products [1–5]. Despite the existence of such extensive data, there is no reliable information dealing with the affordable clean up technology of explosives. To evaluate the disappearance of TNT and its intermediate products, in the last decade, great attention has been paid to microorganisms (both eukaryotes and prokaryotes) and plants. Microorganisms being well established detoxifiers, of structurally varying contaminants, are promising natural tools that allows to sustain the ecological balance. The ability of plants to clean up the environment has still attracted relatively little attention, having no long story. Although plants play an important role in sustaining and restoring all niches of the environment due to their ability to absorb and metabolize quite a wide spectrum of various contaminants of organic nature, plants for cleanup the environment has attracted relatively little attention having no long story. Research carried out for more than 30 years, with annual and perennial plants and various families of different taxonomic groups of microorganisms have revealed their potential to absorb and metabolize organic contaminants of different structure. Three laboratories, at Durmishidze Institute of Biochemistry and Biotechnology (Georgia) have carried out research aimed at the creation of a new biotechnological approach for rehabilitation of soils polluted with explosives based on joint, symbiotic action of microorganisms and plants.

10.2 Materials and Methods

10.2.1 Effect of Explosives on Seed Germination and Plant Growth

Experiments were carried out with the following annual mono and dicotyledonous plants: ryegrass (*Lolium multiflorum*), maize (*Zea mays*), chickling vetch (*Lathyrus sativum*), chickpea (*Cicer arietinum*), alfalfa (*Medicago sativa*), china bean (*Vigna sinensis*), mung bean (*Vigna radiata*), and soybean (*Glycine max*). Plant seeds were soaked in running water or solutions containing different concentrations of TNT (0.1, 0.5 and 1.0 mM) and RDX (0.10, 0.25, 0.50, 1,0 and 2.5 mM), at temperature 22–25°C. After 4 days of seeds germination, i.e. the correlation between the number of germinated and sowed seeds was estimated. The germinated seedlings were exposed to different concentrations of TNT or RDX solutions in tap water and cultivated hydroponically at ambient illumination and temperature (22–25°C). Plant growth parameters: plant biomass, height of stems, length of roots and chlorophyll content have been determined daily, during 10 days.

10.2.2 Microorganisms Growth

To screen active strains of microorganisms on their capability to degrade TNT and RDX, strains of bacteria, fungi, yeasts and actinobacteria from the collections of microorganisms (kept at the Durmishidze Institute of Biochemistry and Biotechnology) have been tested. The diversity of the strains is stipulated due to the fact that microorganisms have been isolated from different soil zones of the country, including polluted soils from military proving grounds [6]. The capability of microorganisms to absorb and degrade organic contaminants was revealed by strains growth on solid agar or on liquid media containing explosives in a shaker at 180 rpm and 28–30°C. Microorganisms were cultivated in modified Czapek's medium, containing (g/l): $NaNO_3$ – 0.91; KH_2PO_4 – 0.1: $MgSO_4 \cdot 7H_2O$ – 0.05; KCl – 0.05; $FeSO_4 \cdot 7H_2O$ – 0.02. In some special cases, glucose (60 g/l), or TNT (0.1, 0.2 or 0.5 mM), or RDX (0.25 or 0.5 mM) were used as a sole source of carbon. Microorganisms grown up to the exponential phase were used as inoculums. The nutrient media were inoculated with suspension (10% of total broth volume) of microorganisms. The intensity of growth was estimated visually according to the following point scale: – no growth, + weak growth, ++ normal growth, +++ intensive growth, ++++ highly intensive growth.

10.2.3 Preparation of Soil Polluted with [1-^{14}C] TNT

Diethyl ether solution (0.5 L) containing 1.05 g of [1-^{14}C] TNT (specific radioactivity of 500 Bq/mg) was added to air-dried soil (7 kg) and then the soil was thoroughly mixed. The ether evaporated during 2 days. The suspension of microorganisms was added to the soil samples. Thereafter the soil was placed in special boxes for experiments and the plant seeds were sowed in samples (80 seeds on 7 kg of soil). To determine the radioactivity of soil samples, residual TNT and its metabolites were extracted by methanol. Methanol extracts were evaporated and dry residue was dissolved in 5 ml of benzene. One ml of benzene solution was taken for measurement, on the scintillation spectrometer SL-30 Rackbeta, with an efficiency of 95%.

10.2.4 Model Experiments for Cleaning Water Polluted with [1-^{14}C] TNT

At the beginning of experiments the water (volume 2 L) containing 0.1 mM ***[1-^{14}C] TNT*** (specific radioactivity – 500 Bq/mg) was equally distributed in 20 flasks, in which bacterial strains *Pseudomonas* sp. TNT-44 and *Rhodococcus* sp. TNT-74 were cultivated. Incubation of polluted water with bacterial consortium was carried out for 3 days in shaken flasks, at shaking speed of 180 rpm and temperature of 28–30°C. After incubation the contents of flasks were united, heated up to 100°C

and filtered. The received solution was filled up with tap water to a 6 L volume and the container subsequently was filled with this solution. Plate with seedlings of soybean was placed in the container. The container with the polluted water and the plants were placed in the hermetic chamber, with a magnetic mixer. Experiments were carried out at temperature of 20–25°C, in the dark, for prevention of re-fixation of the released radioactive CO_2 by plants. For fixation of released $^{14}CO_2$ in the chamber, a solution with 30% KOH was placed in the chamber. Periodically, the radioactivity of polluted water, alkaline solution, and residual amount of TNT in polluted water were measured. At the end of experiments the roots of plants were carefully and repeatedly washed, and the biomass was dried for further application.

In 1,500 ml of Czapek's medium, ((g/l): $NaNO_3$ – 0.91; KH_2PO_4 – 0.1; $MgSO_4 \cdot 7H_2O$ – 0.05; KCl – 0.05; $FeSO_4 \cdot 7H_2O$ – 0.02) 36.5 g of soybean biomass was added with radioactivity 178,000 Bq. The cultivation of microscopic fungi with soybean biomass was carried out in a hermetic chamber, on a magnetic mixer at ambient temperature of 20–25°C, in the dark, to prevent reification of released radioactive CO_2 by plant. For the fixation of released $^{14}CO_2$, a glass with 30% solution of KOH was placed in the chamber.

10.2.5 Quantitative Determination of TNT and RDX

To determine the amount of explosive left in TNT-containing media (water solution), 1 ml of a solution was added to 1 ml of 1 M of KOH. TNT content was determined according to the difference of extinction at 447 nm [6]. In case of RDX, the analyses were carried out by reverse phase on HPLC [7]. Thin-layer chromatographic (TLC) analysis was carried out on methanol extracts from polluted [1-^{14}C] TNT soil samples. TCL-plates were developed with a mixture of benzene:dioxane:acetic acid (90:10:1). The chromatograms were exposed on X-ray film for 45 days to identify radioactive spots. After exposition the radioactive spots were extracted from chromatograms by benzene and their radioactivity was measured.

10.2.6 Determination of Enzymes Activities

Nitroreductase activity was determined according to the rate of TNT reduction by measuring untransformed TNT in an incubation mixture [6]. In highly alkaline solution, TNT has absorption at 447 nm, whereas its major metabolites: 2-amino-4,6-dinitrotoluene, 4-amino-2,6-dinitrotoluene, and 2,6-diamino-4-nitrotoluene, has no absorbance at this wavelength [8]. Peroxidase activity was determined according to the intensity of H_2O_2-dependent guaiacol oxidation at 450 nm [9]. Phenoloxidase activity was determined by the intensity of catechol oxidation at 420 nm [10]. Glutathion S-transferase activity was measured according to rate of oxidation of reduced glutathione [11]. The activity of Cytochrome P450, containing monooxygenase was determined polarographically by determining the oxygen

consumption rate of NADPH-dependent oxidation of N,N-dimethylaniline [12]. Protein concentration was measured according to Bradford [13]. Specific activities of enzymes were calculated as mmole transfered substrate in min per mg protein.

10.3 Results and Discussion

TNT exposed negative influence on germination of the tested plants seeds: 0.1 mM of TNT on average decreased the number of germinated seeds by 10–15%; at 0.5 mM TNT concentration, the lagging seeds germination was up to 15–25%; at 1 mM TNT concentration, the difference between the germ ability of test and control group variants was equal to 30–40%. As for development of germinated seedlings, much slower growth of the plants was detected when the roots of seedlings were submerged in 0.5 mM solution of TNT (corresponding to 100 ppm pollution level, which exceeds ecological harmless concentration 50 times [9]). Parts of seedlings above the ground lag in growing two to four times, as compared with control plants. Accordingly, the plant biomass decreased. The soybean seedlings germinated from the seeds soaked in 0.5 mM solution of TNT, adapt comparatively better to coexistence with explosive. At higher concentration of TNT, equal to 1 mM, all plant seedlings have shorter stems; their roots become shorter and brown. This process was accompanied by the significantly decreasing chlorophyll content (25–30%). High tolerance of soybean to TNT among the tested plants should be mentioned. Growth parameters of this plant decrease only by 5–10% on saturated solution of the explosive (1.0 mM). To summarize the data of plants tolerance toward explosives, it can be concluded that the tolerance of eight tested plants to TNT decreases according in the following order:

Soybean > Mung been > Ryegrass > Chickpea > Chickling vetch > Alfalfa > China bean > Maize.

Statistically valid difference between growth parameters of control and test plants hasn't been obtained in analogous experiments with another explosive – RDX. All the tested plants have practically identical tolerance to increased concentrations of RDX. Moreover, the highest concentration of this explosive (2.5 mM), increases formed biomass (by 20%) of 10-days old seedlings. Presumably, these plants are able not only to detoxify RDX, but to use this compound as nitrogen and/or carbon source. Parallel to plant growth, the residual content of TNT in nutrient medium was determined. The results indicated that TNT uptake of tested legumes decreases in the following order:

Alfalfa > Soybean > Chickpea > Chikling vetch > Mung bean

It should also be noted that germination of soybean seeds on solution of TNT promotes the assimilation of this explosive by seedlings. Analogous investigations with RDX show that all tested plants completely uptake the explosive from water solutions during 5–7 days. The seedlings of soybean are characterized with the highest rate of RDX uptake.

Table 10.1 Effect of nicotinamide coenzyme and ferricyanide on TNT-nitroreductase activity in the homogenate in 14-days alfalfa seedling roots, grown on medium containing TNT (0.5 mM) and RDX (1.0 mM)

	Rate of TNT-reduction, nmole/min per mg of protein		
Incubation medium	Control	Induced by TNT	Induced by RDX
Homogenate + 50 mM TNT	6.30 ± 0.32	10.77 ± 0.54	9.14 ± 0.46
Homogenate + 50 mM TNT + 2 mM NADH	17.07 ± 0.85	28.33 ± 1.42	23.89 ± 1.19
Homogenate + 50 mM TNT + 2 mM NADPH	21.01 ± 1.05	36.56 ± 1.83	29.84 ± 1.49
Homogenate + 50 mM TNT + 1 mM NADH + 1 mM NADPH	18.10 ± 0.91	30.60 ± 1.53	26.79 ± 1.34
Homogenate + 50 mM TNT + 2 mM NADH + 0.1 mM $K_3[Fe(CN)_6]$	0.42 ± 0.02	0.75 ± 0.04	0.59 ± 0.03
Homogenate + 50 mM TNT + 2 mM NADPH + 0.1 mM $K_3[Fe(CN)_6]$	0.48 ± 0.02	0.86 ± 0.04	0.72 ± 0.04
Homogenate + 50 mM TNT + 1 mM NADH + 1 mM NADPH + 0.1 mM $K_3[Fe(CN)_6]$	0.45 ± 0.02	0.79 ± 0.04	0.66 ± 0.03

TNT-nitroreductase activity in plants grown on TNT and RDX containing media has been studied. The results are given in Table 10.1.

According to the data presented in Table 10.1, in plant roots, TNT-nitroreductase is actively functioning. The enzyme has clearly expressed inducible nature by both substrates: TNT (induction degree 1.7) and RDX (induction degree 1.45). Activity of the enzyme is significantly enhanced by the presence of electron donors NADH and NADPH and does not reveal its specificity towards a concrete nicotinamide coenzyme. It seems that this enzyme corresponds to nonspecific NAD(P)H dependent nitroreductase [2]. Potassium ferricyanide acts as a electron acceptor that (this reagent is used for shunting of reducing equivalents of NAD(P)H Cytochrome P450 reductase) causes inhibition of TNT transformation.

10.3.1 Microorganisms Degrading TNT and RDX

As a result of screening, from 205 bacterial strains: 34 – *Pseudomonas*, 1 – Bacillus, 8 – *Rhodococcus* and 5 – *Mycobacterium* strains were selected that had the best growth on solid nutrient medium containing 1 mM TNT. According to the growth intensity on media containing RDX, eight strains of *Pseudomonas* and three strains of *Bacillus* were selected. It is interesting to note that some selected bacterial strains, particularly, *Bacillus sphaericus* 9 J123, Pseudomonas sp.3JL3, *Pseudomonas* sp.R59 and *Pseudomonas* sp.6Ru57, grew better at high concentration of RDX (1 mM) than at low concentrations. From the 240 strains collection of microscopic fungi, 14 cultures representing genera *Aspergillus, Mortiella, Mucor, Chaetomium,*

Fusarium, Penicillium, Trichoderma, and *Trichotecium* actively growing on media containing 0.5 mM TNT and RDX have been selected. As a result of screening, among 45 yeasts cultures, 8 representatives of genus *Saccharomyces,* 6 – *Torulopsis and* 1 – *Hansenula*, characterized by accumulation of biomass while growing on at high concentrations of TNT and RDX have been selected. Growth in other cultures was significantly inhibited or stopped when concentration of explosives in nutrient medium was increased. It should be noted that in such cases rugosity of the surface of colonies and secondary growth was observed. Actinomycetes reveal much lower TNT assimilation potential. Only 3 strains (*Streptomyces griseus* 138A, *Streptomyces griseus* 241 H and *Streptomyces griseus* 242 H) among 90 tested strains exposed average growth on the medium containing 0.1 mM TNT. It should be noted that indicated strains were growing more intensively during the presence of 0.5% starch as cosubstrate. In the same way actinomycetes exposed low RDX assimilation feature. 37 cultures among 90 tested strains were grown on the medium containing 0.1 mM RDX; at 0.5 mM RDX concentration growth ability was exposed by 15 strains of genus Streptomyces (representatives of groups: *Ruber, Violaceus, Griseus u Fradia*); and at 1.0 mM of RDX concentration neither of cultures of tested actinomycetes survived. Selected cultures of microorganisms and artificially created consortia composed by some of them were tested in conditions of submerged cultivation on the media containing 0.5 mM TNT. The cultivation was conducted for 3–7 days on shaker (180–200 rpm, at temperature 28–30°C) in modified Czapek's medium, containing 0.5 mM TNT. Glucose was added as the additional carbon source for the activation of strains metabolism. According to the results obtained strains: *Mucor* sp. D1-1, *Trichoderma viridae* X 1-3 and *Trichoderma viridae* M 3-3 are themost effective degraders of TNT, decreasing the concentration of the explosive by more than 90% in 3 days. From the active bacterial strains, two consortia were composed, which assimilate TNT and at the same time effectively accumulate the biomass (Consortium 1: *Pseudomonas* sp. 211 + *Pseudomonas* sp. R67 + *Rhodococcus* sp. TNT124; Consortium 2: *Pseudomonas* sp. 6Ru56 + *Pseudomonas* sp. GN32 + *Rhodococcus* sp.TNT74 + *Pseudomonas* sp. 211 + *Pseudomonas* sp. R67 + *Rhodococcus* sp. TNT124). The dynamics of revelation of TNT-nitroreductase activity in cultures of microorganisms, selected according to the results of targeted screening have been studied. The results show that all tested strains form nitroreductase. Almost in all cases this enzyme is induced by the presence of TNT in the cultivation medium, with the exception of *Chaetomium* J 1-4. The maximum level of nitroreductase activity for this strain was achieved on the second day of cultivation (on third day for *Mucor* D 1-1). The nitroreductase activity of tested microscopic fungi strains decreases according to the following order:

***Aspergillus niger* D 35 > *Penicillium* D T-1 > *Aspergillus niger* J 3-4 > *Mucor* D 1-1 > *Chaetomium* J 1-4 > *Trichoderma* D 1-1**

Comparison nitroreductase activity of two cultures from the genus *Bacillus* showed that strain *Bacillus* sp. 95 has good ability to grow on TNT containing media and has fourfold higher nitroreductase activity than *Bacillus* sp. 92, which is weakly adapted to growth on the explosive. In addition, is observed for *Bacillus* sp. 95 that

incubation on medium containing TNT increased nitroreductase activity fivefold, whereas for *Bacillus* sp. 92 the induction is only expressed by burst of enzymatic activity on the second day of cultivation. Strains *Saccharomyces vini* 38 and *Saccharomyces vini* 41 were grown on TNT-containing medium in aerobic and anaerobic conditions. It has been established that for both cultures the nitroreductase was induced more intensively in the medium without oxygen. The study of nitroreductase activity accumulation dynamics in strains of Pseudomonas has revealed that the presence of glucose (at 2% concentration) stimulates induction of enzyme catalyzing reductive transformation of nitro groups during the degradation of TNT. The TNT-nitroreductase activity was revealed by fungi strains growing on RDX-containing medium. This indicates that fungi nitroreductase is capable of reducing not only nitro groups of TNT that are substituted in the aromatic ring but also nitro groups that are bound to non aromatic hexahydrotriazine heterocycle in RDX. The maximum of nitroreductase activity was achieved on the second and third days of cultivation on RDX-containing medium (on fourth day for *Aspergillus niger* J 3-4). The nitroreductase activity of tested microscopic fungi decreases, according to following order:

***Mucor* D 1-1 > *Aspergillus terreus* J 2-3 > *Trichoderma viride* J 3-1 > *Aspergillus niger* J 3-4 > *Chaetomium* J 1-4 > *Penicillium* sp. G 1**

Similar to microscopic fungi, bacterial strains of genera *Pseudomonas, Bacillus, Rhodococcus,* as well as yeast cultures grown on RDX-containing medium, produce enzymes that reduces TNT in the presence of NADPH. However, while the cultivation/grown of these microorganisms the effect of induction for bacterial strains was expressed to a lower extent for microscopic fungi. It must be also noted that the tested microorganisms grow weakly in conditions when RDX is used as the sole carbon source, whereas in the presence of small amount of glucose the majority of tasted strains reveal good growth. The growth potential for these microorganisms decreases according to following order while growing on RDX-containing medium:

Rhodococcus* > *Pseudomonas* > *Bacillus

According to the obtained results, soybean and alfalfa are best phytoremediators for cleaning of soils contaminated with explosives. Among the fungi: *Aspergillus niger* D 35, *Mucor* sp. D 1-1 expose the best RDX assimilating potential. The bacterial consortium of genera Rhodococcus and Pseudomonas are serviceable for initiating and effectively leading the remediation process.

10.3.2 Model Experiment for Remediation of Soils Polluted with TNT

The model experiments for testing the remediation potential of selected microorganisms and plants for soil remediation and water cleaning were carried out.

Table 10.2 Residual content of TNT in samples of polluted soil (Model experiment #1) after 30 days of incubation. Initial pollution – 62 mg/kg

Test variant	Residual TNT, mg/kg	Remediation degree (decrease of TNT from initial pollution), %
Control (without inoculation of microorganisms)	44.7	28.0
Pseudomonas sp. TNT-44	31.0	50.0
Rhodococcus sp. TNT-74	29.8	52.0
Aspergillus niger J 3-4	39.7	36.0
Mucor sp. D 1-1	33.5	46.0
Consortium (*Pseudomonas* sp. TNT-44 + *Rhodococcus* sp. TNT-74)	25.4	59.0

Experiment #1

Cleaning object: Red soil (1 kg) artificially polluted with TNT, equal to 62 mg/kg.

Tools for cleaning:

1. Bacterial strain *Rhodococcus* sp. TNT-74
2. Bacterial strain *Pseudomonas* sp. TNT-44
3. Fungi *Aspergillus niger* J 3-4
4. Fungi *Mucor* sp. D 1-1
5. Consortium composed of bacterial strains *Rhodococcus* sp. TNT-74 and *Pseudomonas* sp. TNT-44.

The incubation was carried out at ambient temperature of 20–25°C and illumination. After 30 days of incubation the residual content of TNT was determined in soil samples. The results are presented in Table 10.2.

Experiment #2

Cleaning object: Red soil (mass 1 kg) artificially polluted with TNT equal to 62 mg/kg.

Tools of cleaning:

1. Soybean (25 seedlings on 1 kg soil);
2. Alfalfa (100 seedlings on 1 kg soil);
3. Consortium composed with bacterial strains *Rhodococcus* sp. TNT-74 and *Pseudomonas* sp. TNT-44;
4. Soybean (25 seedlings on 1 kg soil) and Consortium;
5. Alfalfa (100 seedlings on 1 kg soil) and Consortium.

The incubation was carried out at ambient temperature of 20–25°C and illumination. After 15 and 30 days of incubation, residual content of TNT was determined in soil samples. The thin-layer chromatographic (TLC) analysis of methanol extracts from polluted soil samples was carried out. The chromatographic plates were developed with mixture of: benzene:dioxane:acetic acid (90:10:1). The results are presented in Tables 10.3 and 10.4.

Table 10.3 Residual content of TNT in samples of polluted Red soil in Model experiment #2. Initial pollution – 62 mg/kg

Test variant	Residual TNT, mg/kg		Remediation degree (decrease of TNT), % of initial pollution	
	Exposure time 15 days	Exposure time 30 days	Exposure time 15 days	Exposure time 30 days
Control (without plants and microorganisms)	50.1	43.7	19.2	29.5
Soybean (without microorganisms)	37.1	27.5	40.2	55.6
Alfalfa (without microorganisms)	38	30.6	38.7	50.6
Consortium[a] (without plants)	43.4	27.2	30.0	56.1
Soybean + Consortium[a]	27.9	17.7	55.0	71.5
Alfalfa + Consortium[a]	31.5	20.4	49.2	67.1

[a]*Pseudomonas* sp. TNT-44 + *Rhodococcus* sp. TNT-74

Table 10.4 TLC analysis of TNT metabolites extracted from samples of polluted Red soil, after 30 days of remediation process (Model experiment #2)

Test variant	Metabolites, developed on chromatogram				
	$R_f = 0.34$	$R_f = 046$	$R_f = 0.61$	$R_f = 0.74$	$R_f = 0.87$
TNT (control)	–	–	–	–	+
Soybean (without microorganisms)	+	+	–	–	+
Consortium[a] (without plants)	+	+	+	+	+
Soybean + Consortium[a]	–	+	–	–	+

[a]*Pseudomonas* sp. TNT-44 + *Rhodococcus* sp. TNT-74

Experiment #3

Cleaning object: Red soil (mass 7 kg) artificially polluted with [1-^{14}C] TNT.
Initial pollution – 142 mg/kg.
Tools of cleaning: Bacterial consortium composed with bacterial strains *Rhodococcus* sp. TNT-74 and *Pseudomonas* sp. TNT-44 and soybean (80 seedlings on 7 kg soil).

The incubation was carried out at ambient temperature (20–25°C) and illumination. The results of experiments are presented in Figs. 10.1 and 10.2.

The results of TNT degradation indicate that the aboriginal microflora of soils assimilated up to 30% of explosive, and the introduced microbial inoculation composed by TNT-degrading cultures raises the intensity of bioremediation process additionally by 25–30%. Using plants allows reaching the same result almost twice as faster than in case of applying only microorganisms. Soybean and Ryegrass revealed the highest phytoremediation ability (remediation degree up to 45–50%). The highest remediation effects have been detected after sowing soybean in soil treated by consortium containing bacterial strains *Pseudomonas* sp. TNT-44 and *Rhodococcus* sp. TNT-74. In such case the remediation degree is increased up to

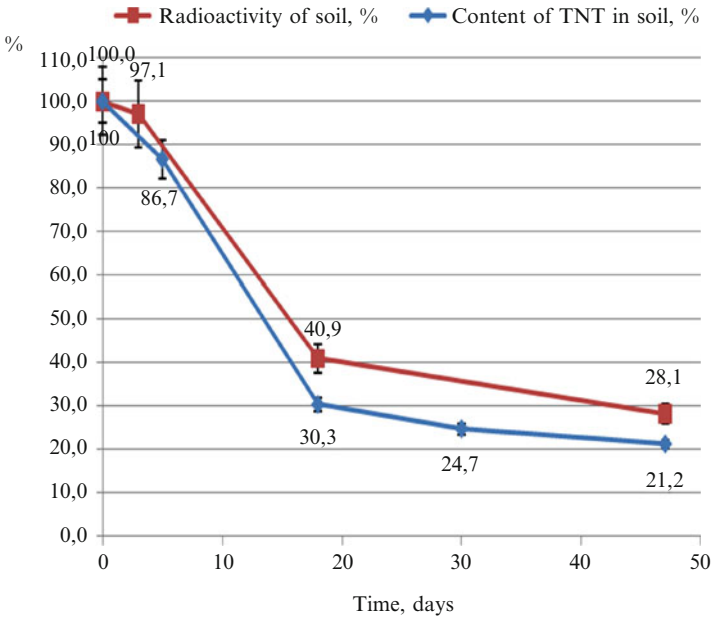

Fig. 10.1 The dynamics of changing of TNT content in Red soil contaminated with [1-^{14}C] TNT during cleaning by soybean and bacterial consortium of *Pseudomonas* sp. TNT-44 and *Rhodococcus* sp. TNT-74

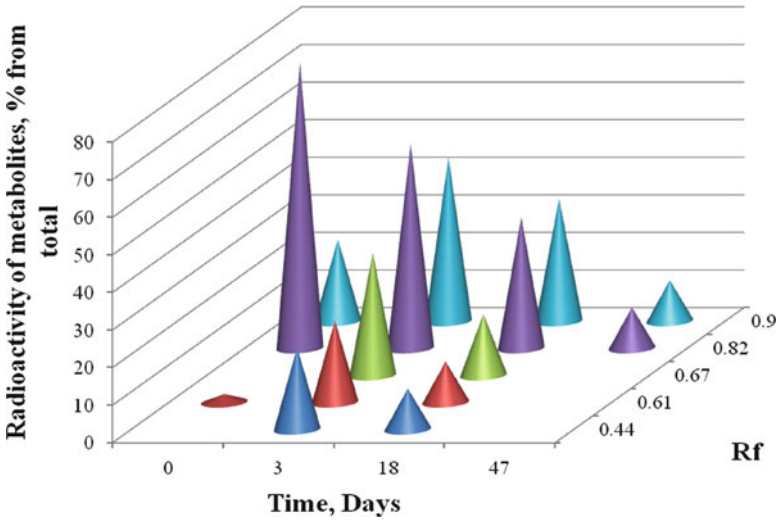

Fig. 10.2 TLC analyses of [1-^{14}C] TNT metabolites extracted from samples of polluted Red soil on 3rd, 18th and 47th days during the remediation process

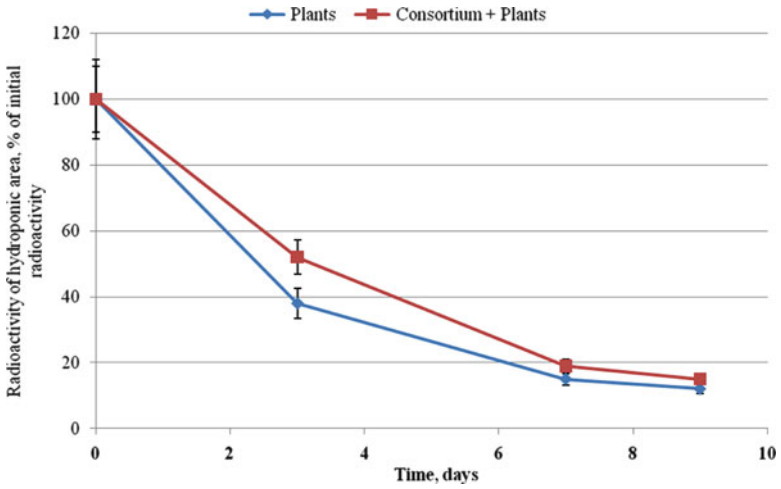

Fig. 10.3 The dynamics of changing of TNT content in water contaminated with [1-^{14}C] TNT during cleaning by soybean and bacterial consortium of strains *Pseudomonas* sp. TNT-44 and *Rhodococcus* sp. TNT-74

70–75% and amount of TNT metabolites in soil is decreased to 20%. The chromatographic analysis of soils shows that in both cases similar products of TNT transformation are formed. The radioactive metabolites formed as a result of bacterial transformation of [1-^{14}C] TNT are effectively up taken from soil by the plants.

Experiment # 4

Cleaning object: Tap water (volume 6 L) artificially polluted with radioactive [1-^{14}C] TNT (specific radioactivity – 500 Bq/mg). Concentration of TNT– 0.1 mM, total initial radioactivity of polluted water – 210 400 Bq.
Tools of cleaning: Bacterial consortium composed by *Pseudomonas* sp. TNT-44 и *Rhodococcus* sp. TNT-74 and 12-days-old seedlings of soybean, preliminarily cultivated on pure tap water.

The results presented in Fig. 10.3 show that in both cases (using plants without and with bacteria) a significant decrease of water pollution level takes place. Intensity of these processes is almost identical. This fact indicates that plants are capable to completely extract almost all metabolites from water, formed as a result of biotransformation [1-^{14}C] by bacterial strains.

10.3.3 Bioutilization of Plants After Phytoremediation

Research to establish the optimal conditions for bioutilization of plants used in phytoremediation of soils polluted with explosives was carried out. For this task, TNT-degrading cultures of microscopic fungi *Trichoderma viride* X 1-3,

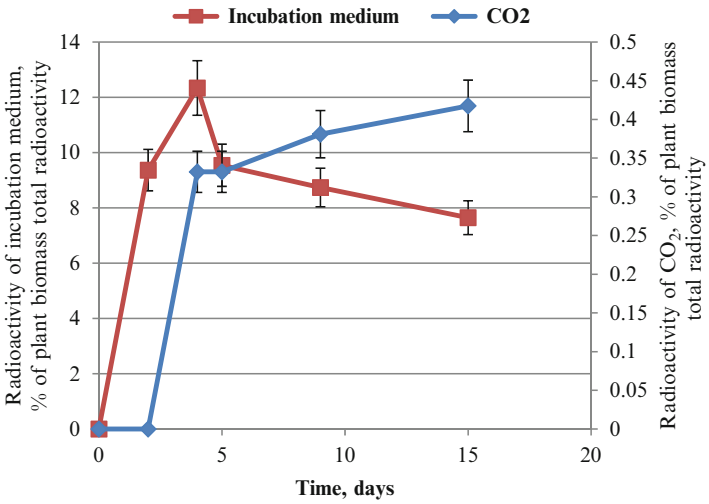

Fig. 10.4 The dynamics of the radioactivity of incubation medium changing and release of $^{14}CO2$ during the utilization of soybean biomass by microscopic fungi *Aspergillus niger* J 3-5

Aspergillus niger J 3-5 and *Mucor* sp. D 1-1 were cultivated on medium with dried and grinded biomass of aboveground parts of soybean seedlings as a sole carbon source. The results show that the highest destruction of plant biomass is achieved when 5 g of soybean biomass was added to 100 ml suspension of microscopic fungi. Research to determine the optimal conditions for bioutilization of plants previously used in phytoremediation of water polluted with [1-^{14}C] TNT was carried out. For these aims TNT-degrading cultures of microscopic fungi *Aspergillus niger* J 3-5 were cultivated on medium with dried and grinded biomass of soybean seedlings (from Experiment #4) as a sole carbon source. The results are presented in Fig. 10.4.

Content of incubation medium (g/l): $NaNO_3$ – 0.91; KH_2PO_4 – 0.1; $MgSO_4 \cdot 7H_2O$ – 0.05; KCl – 0.05; $FeSO_4 \cdot 7H_2O$ – 0.02; and 36.5 g of soybean biomass with total radioactivity of 178,000 Bq total. The obtained data indicate that during the destruction of biomass of plants by microscopic fungi *Aspergillus niger* J 3-5 the release of part of labeled TNT and its metabolites from plant biomass takes place. The occurrence of radioactivity in alkaline solution specifies that extracellular enzymes of fungi oxidized TNT and its metabolites to carbon dioxide. TCL analysis of incubation medium shows that in cultural liquid six compounds with different R_f values are presented. Only two metabolites (with R_f=0.12 and R_f=0.15) from them are radioactive i.e. they are formed from [1-^{14}C] TNT. Apparently, in parallel with the release of intermediates of [1-^{14}C] TNT its transformation and further degradation occurs. Analysis of [1-^{14}C] TNT metabolites shows that the basic part of TNT is metabolized to fumaric acid as a result of the action of *Aspergillus niger* J 3-5 enzymes.

10.4 Conclusion

Screening of plants according to their potential to assimilate TNT and RDX was carried out. As a result, 3 plant species (soybean, alfalfa and ryegrass), 41 bacterial cultures, 14 strains of microscopic fungi, 3 strains of yeast were selected. Two bacterial consortia for bioconversion of TNT were composed. Selected cultures of microorganisms and consortia were tested in conditions of submerge cultivation on the areas containing 0.5 mM TNT. Optimum conditions of submerge cultivation (pH, temperature, duration of incubation) for selected active strains has been established. Model experiments were carried out for testing selected microorganisms and plants for soil remediation and water cleaning. As a result of the experiments performed for water cleaning, it has been established that 12-days-old soybean seedlings effectively absorb [1-^{14}C] TNT from the polluted water and after 8 days approximately 80% of clearing effect is reached. It has been shown that that during destruction of biomass of plants by microscopic fungi *Aspergillus niger* J 3-5 the release of part of labeled TNT and its metabolites from plant biomass and the release of radioactive CO_2 takes place.

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Chapter 11
Transfer of Radionuclides to Crops, Foodstuff Contamination and Application of Countermeasures

Gerassimos Arapis

Abstract The behavior of radionuclides into the soils and their uptake by plants is a competitive physiological process. For radiocaesium and radiostrontium, the main competing elements are potassium and calcium, respectively. Migration velocity into the soil is lower for radiocaesium, compared to radiostrontium, and also it depends on soil type. The migration velocity of radionuclides into the soil is discussed, based on experimental works in northern Greece (a region seriously affected by the Chernobyl accident). Concerning ^{137}Cs, the vertical velocity was found to range from 0.1 to 0.3 cm/year. The major processes influencing radionuclide-transport processes within the rooting zone varies mainly with the soil type.

Radionuclides transfer from soil to plants is commonly quantified using the Transfer Factor (TF). The highest radiocaesium uptake by roots from soil to plants occurs in peaty, boggy soils, and is one to two orders of magnitude higher than in sandy soils; this uptake often exceeds that of crops grown on fertile agricultural soils by more than three orders of magnitude. The high radiocaesium uptake from peaty soil became important because such soils are used for the production of animal feeding crops. In long term, the amount of radiocaesium in agricultural products depends not only on soil type, but also on the density of contamination, soil moisture regime and texture, agrochemical properties and, finally, the species of plants.

Currently, due to natural processes and the applied agricultural countermeasures, the radiocaesium-activity concentrations in agricultural foodstuff produced in areas affected by the Chernobyl accident, are generally below local (national), regional (EU) and international action levels. However, in some limited areas, with high

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radionuclide contamination (i.e., the region of Gomel in Belarus), or with poor organic soils, the radiocaesium-activity concentrations in food products, especially milk, may exceed the higher permissible levels. Therefore, the application of additional specific measures might be still necessary.

The countermeasures used in crop production, in order to minimize the contamination of foodstuff, can be classified in two main groups: organizational and agrotechnical. The organizational countermeasures are mainly based on the principle of change of the use of land in order to: (a) increase the area of crops characterized by low accumulation of radionuclide, (b) abandon any land of very high contamination, and (c) substitute the existing crop by others, less critical (i.e., hay, grain, potatoes). The agrotechnical countermeasures include: (a) change of crops, (b) mechanical soil treatment, and (c) application of agrochemical measures. A short description of the above countermeasures and a comparison of their efficacy are presented in this paper.

Keywords Radionuclides • Food contamination • Countermeasures

11.1 Introduction

Nuclear accidents in many cases can have long-term environmental contamination consequences for agro-ecosystems and the use of rural areas. The accident of Chernobyl, in 1986, contaminated not only Ukrainian, Belorussian and Russian territories, but also large areas of Europe [1] and, thus, affected a wide range of extensive and intensive agricultural systems. The radionuclides of most concern in Europe where caesium-137 and strontium-90, both with a half-life of approximately 30 years.

The behavior of radionuclides into the soils and their uptake by plants is a competitive physiological process. For radiocaesium and radiostrontium, the main competing elements are potassium and calcium, respectively. In this paper the migration velocity of radionuclides into the soil will be firstly discussed, based on our experimental works in northern Greece (a region seriously affected by the Chernobyl accident). The transfer of radiunuclides to the crops and the contamination of foodstuffs will be also discussed using scientific data from the affected areas in the regions around Chernobyl. Finally the countermeasures applied in crop production and a comparison of their efficacy is presented in this paper.

11.2 Migration Velocity of Radionuclides into the Soil

Migration velocity into the soil is lower for radiocaesium, compared to radiostrontium, and it depends on the soil type.

Following the accident in the nuclear power reactor at Chernobyl on 26 April 1986, the released radionuclides were also detected in northern Greece on May 1st [2]. Among the deposited radioactive elements, ^{137}Cs was considered of great

importance due to its long residence half time and its low mobility in the majority of soil types. The regions of Greece, where heavy ^{137}Cs deposition from the Chernobyl accident occurred, were those of Northwest Thessalia and West Macedonia [3]. Radiocaesium's slow rates of migration in the soil, both horizontally (on surface) and vertically (downward migration), are due to its irreversible sorption onto various soil particles, its fixation by mineral and organic components, and to the low content of ^{137}Cs mobile forms, i.e., forms that are ionic, exchangeable and water-soluble [4–9].

Radiocaesium's horizontal migration depends mainly on water runoff. This leads to the displacement of radioactive material in its dissolved state, and in the solid state that is adsorbed on migrating micro-particles. Surface displacement depends on the quantity of radionuclides in the upper soil's surface layer, and their water solubility [10–12]. As a result of the radionuclide's mechanical transportation by water runoff, it was hypothesized that accumulation occurred at local geochemical barriers, and in meadow soils at the lower borders of slopes. A weak indication of such accumulation was reported by [13]. However, under the conditions of our study in Greece, accumulation was not detected approximately 15 years after the accident. On the contrary, the lowest level of each site contained less ^{137}Cs than the higher ones. These data obtained in Greek semi-natural environments, seem to be in agreement with observations obtained abroad, concerning alpine meadow sites [14].

11.2.1 Vertical Migration

Vertical migration depends on many factors such as the physicochemical forms of radionuclides, the type of soils, the hydrological regime and the ecological conditions of the radio-polluted area [13]. In order to study the vertical migration, a good approach is the evaluation of the effective vertical velocity, which is the shift, with time, of the center of the distribution in the radionuclide's concentration along the soil profile [15].

The purpose of was to explore the vertical migration of ^{137}Cs in the soils of sloping semi-natural ecosystems in Greece. It should be pointed out that there have been very few previous studies of radionuclide migration on sloping semi-natural ecosystems, especially in the Mediterranean region.

The data of ^{137}Cs concentrations (as% of the total) at the various depths, at our experimental plots in northern Greece (location of Karpero), are summarized in Fig. 11.1. The vertical velocity was found to range from 0.1 to 0.3 cm/year [15].

11.3 Transfer of Radionuclides to Crops

The major processes influencing radionuclide-transport processes within the rooting zone varies mainly with the soil type.

Radionuclides transfer from soil to plants is commonly quantified using the Transfer Factor (TF), equal to plant activity concentration (Bq kg^{-1}), divided by soil

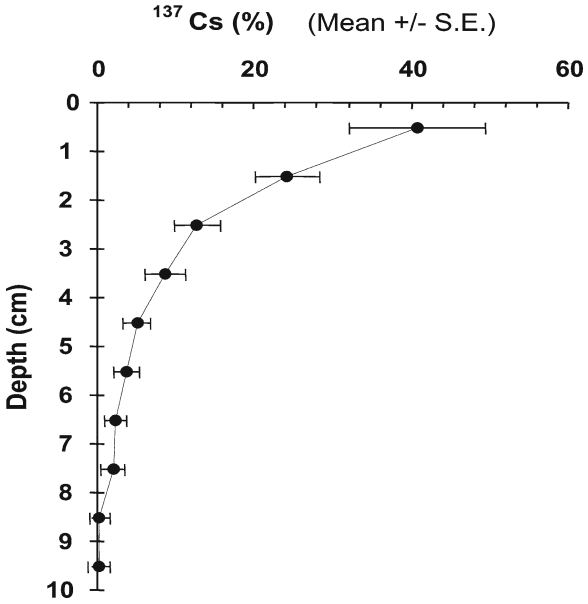

Fig. 11.1 Percentage of total ^{137}Cs concentration as a function of depth (Northern Greece – Karpero) (Source: [15])

activity concentration (Bq kg^{-1}). The highest radiocaesium uptake by roots from soil to plants occurs in peaty, boggy soils, and is one to two orders of magnitude higher than in sandy soils; this uptake often exceeds that of crops grown on fertile agricultural soils by more than three orders of magnitude. The high radiocaesium uptake from peaty soil became important after the Chernobyl accident, because in many European countries such soils are used for the production of animal feeding crops.

In the long term, the amount of radiocaesium in agricultural products depends not only on the soil type, but also on the density of contamination, the soil moisture regime, the particular soil texture, the agrochemical properties and, finally, the plant species. Among the different species of crops, the variations in transfer between soil and plant may exceed one or more magnitude for radiocaesium. The effect of other factors known to influence the plant root uptake of radionuclides (like for example soil moisture) is less clear or can be explained by basic mechanisms. For example, the accumulation of radiocaesium into crops and pastures is related to soil texture. Regarding sandy soils, the radiocaesium is approximately twice the value as on loam, but this effect is caused mostly due to the lower concentrations of its main competing element, potassium, in the sand. The main process controlling plant-root uptake of radiocaesium, is the interaction between soil matrix and solution which depends primarily on the cation-exchange capacity of the soil. For the mineral soils, this is influenced by the concentrations and types of clay minerals and the concentrations of competitive major cations, especially potassium and ammonium. Thus, differences in radioecological sensitivities of soils explain why in some areas of low deposition high concentrations of radiocaesium are found in plants and mushrooms

harvested from semi-natural ecosystems and conversely why areas of high deposition can show only low to moderate concentrations of radiocaesium in plants.

For soil-to-plant transfer of radiocaesium, a decrease with time is likely to reflect:

- Physical decay,
- The downward migration of the radionuclide out of the rooting zone and
- Physicochemical interactions with the soil matrix that result in decreasing bioavailability.

The potential of the decrease of ^{137}Cs availability in the soil-plant system is greatly determined by the properties of the soil, and the rates of decreasing ^{137}Cs uptake by plants can differ by a factor of 3–5, depending on the soil characteristics [16]. Compared to radiocaesium, plant uptake of ^{90}Sr often has not shown such a marked decrease with time. In the areas close to the Chernobyl NPP gradual dissolution of fuel particles has enhanced the bioavailability of ^{90}Sr, and, therefore, there was an increase with time in ^{90}Sr uptake by plants [17]. The difference in ^{90}Sr/^{137}Cs ratios was affected by plant species, rooting depth, soil pH, and calcium content in soil. In remote areas, where strontium radionuclides were predominantly deposited in condensed form, and in lesser amounts as fine dispersed fuel particles, the dynamics of long-term transfer of ^{90}Sr to plants was similar to that of radiocaesium, however, with different ecological half-lives of plant-root uptake and their contributions. This difference reflected various mechanisms of soil transfer of these two elements. Regarding radiostrontium, its fixation by soil components less depends on clay content of soil, which is not the case for radiocaesium. More generally, values of ^{90}Sr-transfer parameters from soil to plants depend less on soil properties than for radiocaesium.

11.4 Foodstuff Contamination

Currently, due to natural processes and the applied agricultural countermeasures, the radiocaesium-activity concentrations in agricultural foodstuff produced in areas affected by the Chernobyl accident, are generally below local (national), regional (EU) and international action levels. However, in some limited areas, with high radionuclide contamination (i.e., the region of Gomel in Belarus), or with poor organic soils, the radiocaesium-activity concentrations in food products, especially milk, may exceed the higher permissible levels. Therefore, the application of additional specific measures might be still necessary. The ingestion of radio-contaminated food is one of the pathways leading to internal retention and contributes to human exposure from natural and man-made sources. Excessive contamination of agricultural land, such as may occur in a severe accident, can lead to unacceptable levels of radionuclides in the aliments. The most important radionuclide contaminants in agriculture are those which are relatively highly taken up by the crops, have high rates of transfer to animal products such as milk and meat, and have relatively long radiological half-lives. However, the ecological pathways leading to crop

contamination and the radioecological behavior of the radionuclides are complex and are affected not only by the physical and chemical properties of the radionuclides but also by factors which include the soil type, the cropping system (including tillage), the climate, the season and, where relevant, the biological half-life within animals. The major radionuclides of long term concern in agriculture after a large reactor accident are ^{137}Cs and ^{90}Sr. direct deposition on plants is the major source of contamination of agricultural produce during the first year after the accident, the following years is the root uptake.

11.5 Application of Countermeasures

The countermeasures used in crop production, in order to minimize the contamination of foodstuff, can be classified in two main groups: organizational and agro-technical.

The **organizational** countermeasures are mainly based on the principle of change the use of land in order to:

- Increase the area of crops characterized by low accumulation of radionuclide,
- Abandon any land of very high contamination, and
- Substitute the existing crop by others, less critical (i.e., hay, grain, potatoes).

The **agro-technical** countermeasures include:

- Change of crops,
- Mechanical soil treatment, and
- Application of agrochemical measures.

The countermeasure of crop change of existing crops can be used for both medium and long term periods, but only for long life radionuclides, when other countermeasures are not appropriated.

Always under the same perspective another countermeasure is the selection of crop species that accumulate low levels of radionuclide, in respect to their rate of uptake of radionuclide from soil and radionuclide distribution between edible and non-edible parts.

As far as mechanical soil treatment and cultivation is concerned, a major advantage of this countermeasure is that, the majority of the deposed radionuclides can be removed successfully by skimming off a relatively shallow layer of the topsoil, because of their low mobility. The removal of the soil upper layer (0–10 cm) of course cannot be applied on soils that present a thin humus horizon.

Agrochemical measures can include: liming of acid soils; application of increased doses of K and P-K fertilizers; supplementing soils with natural sorbents (different kinds of clay minerals) and the use of organic fertilizers. The agrochemical countermeasure is based on reducing the biological availability of radionuclides for cultivated crops. The radionuclides present in the topsoil can be made potentially available for uptake by plant roots, even if radionuclide plant uptake of radionuclides is regulated by a certain variety of factors.

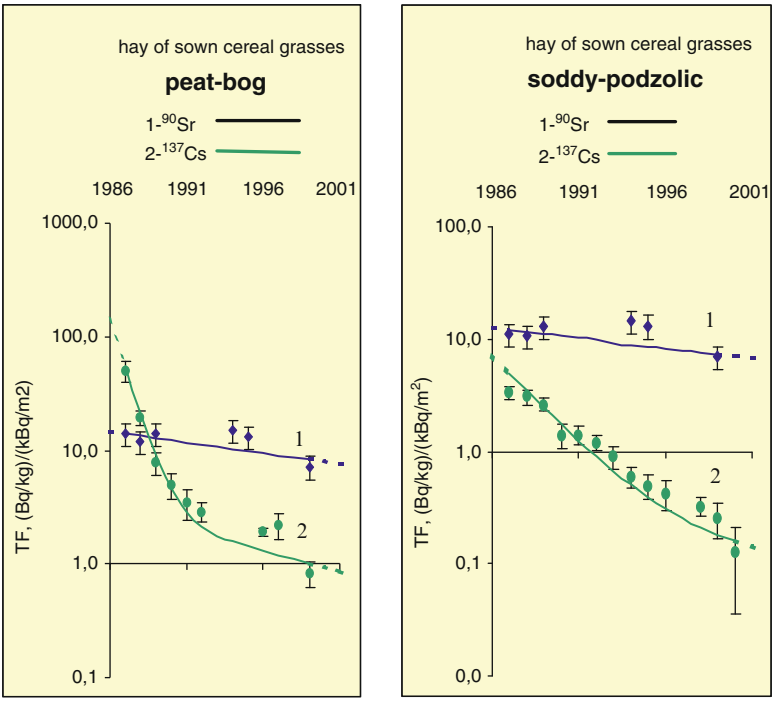

Fig. 11.2 Dynamics of ^{137}Cs and ^{90}Sr TF for hay of sown cereal grasses

11.5.1 Efficacy of Countermeasures in Crop Production

The main factors influencing countermeasures applicability and effectiveness are the following:

- Radionuclides composition (half-life, type of fallout, mobility, physico-chemical form);
- Post-accidental time;
- Environmental conditions (soils, landscape);
- Food practices and import/export ratio;
- Financial applicability;
- Ecological acceptability;
- Social perception;

The diagrams below (Fig. 11.2) show for perennial grasses the mostly constant content of ^{90}Sr and a rather regular decreasing for ^{137}Cs. Keeping the same relative efficiency, absolute effectiveness of a countermeasure shall be in this case greater for strontium that for caesium because applied to a higher content.

In the above example (Fig. 11.2), the influence of soil type appears clearly for ^{137}Cs. Peat-bog soil causes a faster decreasing in Transfer Factor (TF) than soddy-podzolic. For ^{90}Sr no significant difference appears between the two soil types [18].

11.5.2 Evaluation of Agrotechnical Countermeasures

An evaluation of the most important countermeasures used in crop production in order to rehabilitate the radio contaminated areas affected by the Chernobyl accident is presented below.

11.5.2.1 Selection or Change of Crops

Medium or long term, changes in the type of land use, including crop selection, can prove quite effective but not with out the cost of long term consequences both social and economical. Usually rigorous changes present a higher probability of potential consequences. This is why these countermeasures are suited for the treatment of long life radionuclides only when other measures are not possible.

11.5.2.2 Ploughing or Other Mechanical Soil Treatment

The countermeasure involves the removal of the upper soil layer up to 10 cm. This measure is an effective technique of soil decontamination if its application does not diminish the existing soil fertility or water quality. The effectiveness can be very high (about 80%) based to the fact that most of the contamination, right after the accident, is concentrated in the upper soil layer. The disadvantages of this method are its high costs and also the difficulty in the disposal and burial of the radioactive soils.

The efficiency of this countermeasure depends on the type of the soil, its depth, the type of crops, and mostly on the root depth.

Deep ploughing can reduce quite effectively radionuclide uptake and it is recommended especially for those types of soils with a highly productive layer.

11.5.2.3 Agrochemical Measures (Fertilization or Liming)

The effectiveness of liming is quite high but varies in function with the pH. The average reduction factor for ^{90}Sr is 2 but could be higher (up to 10), for ^{137}Cs average 1.8 (up to 3). Liming can reduce radiocaesium and radiostrontium uptake from crops and plants by increasing soil cation exchange capacity. It is mostly of low cost and of easy application.

As far as fertilizers are concerned, potassium and organic fertilizers are quite inexpensive and relatively easy to carry out. In case of the application of potassium fertilizer, ^{137}Cs uptake by crops in various soils is reduced on an average of 2 or even up to 3–5 times. Clearly the effectiveness of potassium fertilizer is higher for those soils that significantly lack the nutrient. The average reduction of accumulation of radionuclides for crop production countermeasures is presented below in Table 11.1.

Table 11.1 Average efficiency of crop production countermeasures (reduction factor) (Source: [18])

	^{90}Sr	^{137}Cs
Type of countermeasure	Reduction factor	
Use of mineral fertilizing	1.6	2.0
Liming	2.0	1.8
Ploughing (or other mechanical treatments)	1.4	1.4
Crop selection	4	4

11.6 Conclusions and Discussion

The vertical migration velocity of ^{137}Cs in northern Greece was found to vary, from place to place by a factor of three, being the most important from all the investigated locations at the site of Carpero. This variability is high, especially if we take into account the fact that the results come from soils of one region, of a relatively small area and of a rather similar texture. However, it is of importance to note that the behavior of any radionuclide in the soil, as well as its migration velocity, are rather complex phenomena and depend on the physical, chemical and biological properties of the soil, which are strongly related to soils characteristics such as acidity, humic or mineral component content, surface-horizons structure, granulometric composition, adsorption ability, microorganism's abundance and overall water content. Also, the migration behavior of radionuclides (controlled by different migration parameters) depends on the solubility of its various chemical forms (movement by solution into soil water) and also on the adsorptive ability of the soil. In the long term, the quantity of radiocaesium in agricultural alimentary goods depends on the type of soil, on the density of the contamination, on the soil moisture regime, by the particular soil texture, by the agrochemical properties and, finally, by the plant species. In some limited areas around Chernobyl, with high radionuclide contamination (i.e., the region of Gomel in Belarus), or with poor organic soils, the radiocaesium-activity concentrations in food products, especially milk, may exceed the higher permissible levels. Therefore, the application of additional measures might be still necessary. Regarding countermeasures, the most efficient for ^{137}Cs is the selection of crop species with a mean value about 4, which represents a very high level of efficiency. The less efficient methods are probably application of clay minerals and biological active matter. For ^{90}Sr, the efficiency of the agricultural countermeasures is generally lower than for ^{137}Cs or at least at the same level, except for liming because of competitive action of calcium. The most efficient method seams also to be the selection of crop selection with the same efficacy as for ^{137}Cs (reduction factor of 4). The remaining ones (plowing, fertilization and liming) are in the same order ranging from 1.4 to 2. Summarizing available information about countermeasures and taking into account the experience in the elimination of the nuclear accident consequences, it is demonstrated that the most widespread countermeasures after the Chernobyl accident were soil ameliorations [19]. These methods

are simple, inexpensive and effective in reducing radionuclide transfer from the soil to agricultural crops. Among the above mentioned methods, agrotechnical countermeasures were the most applicable, decreasing the contamination of plant products many times [20, 21].

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Chapter 12
Sustainability of Mining Waste in Basin Bor, Serbia

Zoran S. Markovic

Abstract Mining and Metallurgical Basin Bor exists over 100 years. During that period copper ore has been mined and processed by pyro metallurgical process, mainly for the rich copper ore. Flotation concentration process was introduced due to the decreasing copper grades in ore and started in 1930s. Since that time, mining production grew up generating large amounts of waste materials in form of solid, liquid and gas. They are all clearly visible all around of mining area. This paper deals with flotation testing results preceded by an attrition step, on copper minerals and pyrite obtained under laboratory condition. Also, the main problems with other type of wastes were discussed and some recommendation for its further treatment was given in the paper. All proposed concept are based on sustainable development in this area and wider.

Keywords Overburden • Flotation tailings • Flotation • Copper minerals • Sustainability

12.1 Introduction

Mining of copper ore in Bor, Serbia, has over 100 years long history. During this period 650 Mt solid materials (mining and flotation tailings) were generated which, by geology estimation contains about 750,000 t of metal copper. Compared to the area affected by mining, of about 1.22 million m^2, areas affected by mining wastes are over 2.84 million m^2. Some data on areas of degraded terrain are given in Table 12.1 [1].

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DOI 10.1007/978-94-007-2953-7_12,

Table 12.1 Data on terrain surfaces occupied by the open pit mines and overburden disposals and flotation tailings

Mine	Open pit mine	Overburden disposals	Flotation tailings	Sub total
Bor	1,220,950	2,840,800	733,300	4,795,050
Veliki Krivelj	2,239,000	660,000	2,465,700	5,364,700
Total:	**3,459,950**	**3,500,800**	**3,199,000**	**10,159,750**

In addition areas that are directly affected by mining operations, the area of damaged and degraded agricultural land is estimated to over 25,000 ha. This represents about 60% of agricultural land in the municipality of Bor [2]. Smelting of copper ore with sulfur dioxide emissions have led to soil acidification, destruction of vegetation and erosion. The pollution of waters and water courses are the most significant consequences of mining on the Bor's river, which is biologically almost completely destroyed, with significant concentrations of heavy metals. Also, the big problem is decades of consumption of drinking water for industrial production, reducing the abundance of existing natural water resources. All these wastes have a damaging environment impact, but utmost has flotation tailings. The tailings consists of very fine dust and contaminated water that is leaking out from tailing pond in nearby rivers as well as valuable quantities of copper, gold and silver. Old Bor flotation tailing is located near to Bor city center and therefore the main objective is providing recycling and delocation process of flotation tailing to the location where will be no environment impact. By reprocessing of flotation tailings, metal copper and other valuable compounds could be produced which could create possibility for self financing of Bor's creek cleaning. The flotation tailings reprocessing is consisted of following main steps: excavation, repulping, attrition, bulk sulfide and precious metals flotation, bulk concentrate treatment by selective flotation and newly obtained tailings disposed to the closed old open pit mine. The extensive laboratory work was undertaken in order to obtain bulk flotation concentrate with high recoveries of above mention compounds. For example, copper recovery in bulk concentrate of up to 98% was achieved. Current activities are concentrated on copper minerals separation from pyrite from the bulk. Attrition technique is applied, rather than regrinding, in mineral liberation and cleaning of their surfaces prior to flotation. Attrition is much cheaper than grinding, and provides better results in flotation.

12.2 Old Flotation Tailings

The are many ways of old mining wastes treatment depending on waste type, place and type of valuable components. The simplest way to solve those problems is reclamation of tailings and overburden surfaces by planting trees and grass without prior surface preparation [3]. Some authors propose desulphurization of tailings surface by froth flotation to the deep of 1–2 m, prior reclamation [4, 5]. Those

Fig. 12.1 Location of old flotation tailings and closed open pit Bor

mentioned achievements have a primary goal to remediate waste surfaces and not take in account extraction of valuable compounds from the waste materials. Some investigation were undertaken at UB-Technical faculty Bor in order to recover useful compounds from old flotation tailings by froth flotation [6, 7]. Figure 12.1 presents situation plan of old flotation tailings and closed open pit Bor and industrial zone with old flotation plant between them.

Old flotation tailings has most negative environmental impact such as surface degradation due to change of original land topography and damage to fertile soils, pollution of water and soil with heavy metals. Moreover underground and surface waters in the vicinity of tailings pond can be contaminated by heavy metals such as lead, zinc, copper and arsenic, which have already been found in soil and plants in Bor region. In the case of a tailings dam failure there is the potential for a significant portion of the toxic material to run directly to the Bor's River and onward to the Danube River, with enormous environmental consequences to the entire region.

The main concept of our investigation is completely reprocessing of old flotation tailings by flotation and the residue material reject to old closed open pit mine with economical effects. Overburden materials from open pit mine "Veliki Krivelj" is disposed into the space of closed open pit mine "Bor." This form of waste disposal is a permanent and sustainable solution for mining waste management, at present and in the future. Also, this paper provides a new solution for disposal of tailing from the old flotation tailings in space of closed open pit "Bor," after its reprocessing.

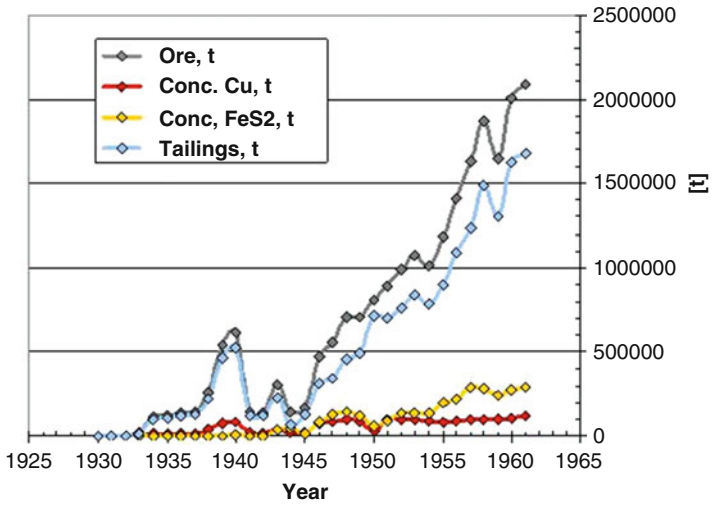

Fig. 12.2 Production of copper and pyrite concentrates and tailings

12.2.1 Characteristics of Flotation Tailings Material

During the period of exploitation of copper ore from Bor open pit mine, the ore grade was gradually decreased. In order to maintain the same level of concentrate production the more ore to be mined. Figure 12.2, show the ore, copper concentrate, pyrite concentrate and final tailings production over the years. Increased ore production was followed by increasing tailings, but the masses of concentrates, with the little variations, remained on the constant levels [8].

12.2.1.1 Mineralogy of Tailings Materials

Mineralogical analysis was carried out by polarized ore microscopy on prepared sample, in reflected light. The principal sulphide mineral is pyrite. Copper minerals are present as: covelite, chalcocite, chalcopyrite, enargite, bornite and cuprite. Gangue minerals are present mainly as quartz, alumosilicates and feldspars. Maximal grain size of pyrite and copper minerals are up to 200 μm, but the gangue minerals are up to 500 μm. They mainly occur in the form of middling rather than liberated particles.

12.2.1.2 Chemical Composition

According to chemical analyses of tailings sample, given in Table 12.2, one can see significant copper contents. Copper is found mainly in sulphide form, (97.80%) in total copper contents of 0.401%. The pH of the sample was about 3.8 but the lower

Table 12.2 Chemical analyses of tailings sample

Element/compound	Contents (%)	Copper distribution R_{Cu}(%)
Cu_{total}	0.41	100
$Cu_{sulphide}$	0.401	97.80
Cu_{oxide}	0.009	2.20
S	14.51	–
Fe	13.91	–
SiO_2	52.60	–
Al_2O_3	14.83	–
CaO	0.87	–

values can be found in dipper zone of tailings materials with values down to pH = 2. This is a typical characteristic for spontaneous chemical processes into tailing materials caused by sulphide oxidation. The average content of gold is 0.8 g/t and silver 2.4 g/t.

12.2.1.3 Flotation

Extensive investigations on bulk sulphide flotation, were undertaken in Laboratory for mineral processing at the Technical faculty in Bor. Tests were carried out at various conditions: pH, type of collectors and their concentration and pulp preparation prior flotation. Preparation of pulp prior flotation was done by regrinding in a steel ball mill, by attrition and in original condition without any mechanical pretreatment. The best results were achieved by applying attrition prior flotation in pulp preparation, flotation at pH of 10 and collector PIBX (potassium isobutyl xanthate). In that case flotation recovery of copper was over 97% and pyrite recovery was over 87%. Copper and pyrite grades in bulk concentrate were 1.34% and 42.74% respectively. Recoveries of precious metals such as Au and Ag have not been analyzed in the initial phase of investigations, but it is believed that they follow sulphide minerals. Present investigations on these metals are in progress, as well as the further treatment of bulk concentrate.

12.3 Other Wastes

During the long period of exploitation of copper ore in the Mining basin Bor, has generated large amounts of waste. Mining and Metallurgy complex in Bor consists of three open pit copper mines (Bor, Veliki Krivelj and Cerovo) and a smelting plant for metallurgical treatment of copper concentrate. Of the three mines, two were closed (Bor and Cerovo) and only "Veliki Krivelj" is in operation. There are significant quantities of ore wastes around these mines that occupy large areas and have a negative impact on the environment.

12.3.1 Open Pit Mine Bor

Open pit mine Bor is the oldest copper mine in Serbia. The mining has ended and the mine was closed. This mine has left behind two stockpiles with overburden material and a flotation tailing pond. Overburden has been re-cultivated and the soil forested. Recultivation of these areas was designed to prevent erosion thereof and at the same time reduce the emphasis on acid solutions from them. Reclamation for the flotation tailing was planned in beginning by means of planting trees of various types as a short-term rehabilitation of its surface. In the future, the proposed processing of tailings and its relocation in the area of the former open pit Bor are planned. This process would be cost effective and the whole venture implemented in a profitable way.

12.3.2 Open Pit Mine Cerovo

Open pit mine Cerovo was closed as well as. This mine was opened in 1993 and was shut down after 10 years of operation. In the vicinity of the mine, crushing and milling plant for treatment of copper ore was built. Milled copper was hydrotransported to the flotation plant in Bor and processed there. At this mine there are overburdens waste ore. These overburdens have a large impact on water pollution because they contain copper oxide minerals that are easily dissolved under the atmospheric influence. There is a reception pond with the dam for acceptance of these solutions that are leaking from overburden heap. In addition to build the dam, this problem has not been completely solved. In numerous occasion leakage of this solution into nearby waterways has occurred.

12.3.3 Open Pit Mine Veliki Krivelj

This mine was open in 1982 and it is still operational. There are two areas with overburden and a flotation tailing in its vicinity. Overburden was deposited in two areas near the open pit. Today, this overburden is deposited into area of closed pit of mine Bor, as a permanent and sustainable solution. Flotation tailings from this mine are deposited in a special place, but a lot of problems with leaking of waste water solution occurred, due to the tunnel positioned underneath the tailings through which passes nearby river. At the moment tunnel is under the reconstruction.

12.3.4 Copper Smelting Plant

Metallurgical plant for processing copper concentrate has as long history as the Bor mine. From this plant two types of solid and gaseous wastes are produced. The solid waste is smelter slag and gaseous wastes are smoke and gases that carry large

amounts of sulfur dioxide and other harmful components and aerosol. Smelter slag does not pose a grave danger to the environment because it dissolves very slowly under the atmospheric conditions. Emission of gasses from the smelter has a great impact on the environment because it is disseminated at long distances. As it is planned to build a new smelter and the introduction of modern metallurgical technology, it is expected to permanently eliminate this problem.

12.4 Conclusions

According to above presented, the following can be concluded:

1. Wastes in mining and metallurgy have a major impact on the environment because they occupy large areas.
2. In Mining and Metallurgy Basin Bor there are many problems associated with mining and metallurgical wastes, some of these are successfully resolved, and some are in preparation for solving.
3. There is a real possibility to obtain copper and other valuable compounds from tailings materials and at the same time obtain desulphured new tailings which is less hazardous than the primary one.
4. There are possibilities for long term solutions of ecological problems by removing old tailing material and its relocation in the space of old open pit, after reprocessing.
5. This offer opportunity to clean existing tailings pond and downstream waterways.
6. Prevention of acid mine draining from mine Cerovo overburden materials is the primary task for the near future.
7. According to recommendations from sustainable development principles, subsequent steps include long-term repair of all degraded areas especially revitalization of devastated river beds.
8. The river valleys should again return to farming vegetables and other crops, which is a traditional form of agricultural production in Serbia.
9. Solving problems of protection and rehabilitation of the environment opens up opportunities for employment of various labor profiles.

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Chapter 13
Electrolyzer for Production of Environmentally Safe Functional Aqueous Solutions: Analysis of Mass Transfer in Annulus

Elena N. Gnatko, Vasilij I. Kravets, Elena V. Leschenko, Volodymyr K. Rybalachenko, Alexander Omelchenko, and Mohammed Aider

Abstract The first attempts to apply electrolyzed aqueous solutions for water disinfection were performed in Russia in the late XIX century. However, the rapid development in the technology of electrolytic water oxidation and application of electrolyzed aqueous solutions have commenced in the USSR only in 1960s. At present, this technology is being developed in numerous countries around the world. It is reflected in an increased number of the publications in peer-reviewed journals and other informational sources. Moreover, a number of companies were established to pursue commercialization of the technology. The possibility of use of electrolyzed aqueous solutions as power disinfectants of potable water and swimming pools, bactericidal agent for disinfection and sterilization of living tissues, materials, medical and food processing equipment, etc. have been demonstrated in various trials. Nevertheless, the broader application of the technology is hindered by a lack of profound theoretical and experimental studies of production of electroactivated aqueous solutions and the mechanisms of their activity. Particularly, the characteristics of mass transport inside the electrolyzer and their relation to the constructional features of the apparatus and

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functional properties of produced solutions are poorly understood. We described the transport phenomena inside the permanently flowing electrochemical reactor and investigated the current–voltage relationships to gain understanding of the nature of the electrode reactions.

Keywords Functional aqueous solutions • Electrolysis • Mass transfer • Disinfection • Sterilization • Theoretical analysis • Limited current • Current–voltage relationship

13.1 Introduction

Despite the wide application of the electrolytically produced functional aqueous solutions as environmentally safe agents [1–6], the basic principals governing the performance of the apparatus are poorly understood. Accurate determination of the mass transfer coefficient, K_m, and evaluation of the factors influencing its magnitude are of paramount importance for designing the most efficient electrochemical reactors. The values of K_m calculated through the magnitudes of the limited current obtained from the current–voltage relationships have empirical character, and pertains only to the specific electrolyzer. Hence, they have only limited predictive capability and cannot be used on early stages of designing. On the other hand, juxtaposition of quantitative and qualitative parameters of the current–voltage relations, such as their shape and the magnitude of current inside the electrolyzer, with calculated values of K_m allows making reasonable suggestions regarding the mechanisms of the electrode reactions. The evaluation of the mass transfer coefficient in electrolyzers did not get appropriate attention notwithstanding the existence of developed theoretical basis for description of mass transport phenomena [7, 8]. Existing publications sometimes contain inaccurate formulas, e.g., Eq. 13.6 in [9]. On the other hand, it is very important to consider the geometry of the electrolyzer and the type of the diaphragm used to separate its compartments. Some previous studies suggested the use of neutral membrane, whereas other researchers preferred ion exchanges membranes to allow differential separation of the targeted ionic spices. The last approach seems to be more promising for practical purposes. For theoretical studies, the use of ion exchange membranes is favorable for the differentiation of charged ionic species mass transfer and migration.

Here, we present theoretical analysis of the mass transfer coefficient in annulus of the coaxial cylindrical flow-through electrolyzer similar to those employed in a variety of electrochemical processes [10–14]. The principal scheme of the electrolyzer comprising the anode and cathode chambers separated by the porous diaphragm was presented in [13]. We also provide the comparison of theoretical and experimental results.

13.2 Theoretical and Experimental Study of the Electrolyzer

Initially, whether the flow of electrolyte inside the electrolyzer is laminar or turbulent should be evaluated. Transition from laminar to turbulent flow occurs near Re = 2,000, where Re is the Reynolds number given for the liquid flowing through the annulus of the anode chamber by

$$\mathrm{Re} = \frac{d_h U_{avg} \rho}{\mu} = \frac{2 r_{DA} (1 - a_{AD}) U_{avg} \rho}{\mu} \tag{13.1}$$

where d_h, $d_h = 2r_{DA}$ $(1\text{-}a_{AD})$, is the hydraulic equivalent diameter, r_{DA} is the inner radius of the diaphragm, $a_{AD} = r_{AD}/r_{DA}$ is the aspect ratio of the anode chamber, r_{AD} is the outer radius of anode, U_{avg} is the average velocity of the electrolyte flow through the annulus, ρ is the density of electrolyte, and μ is the dynamic viscosity of electrolyte. Here, we will apply our analysis to the anode chamber only, since, in the cathode chamber, the discharge of abundant H_2O molecules takes place, which is not limited by diffusion. For $r_{AD} = 0.4$ cm, $r_{DA} = 0.52$ cm and maximal practically used value of the volumetric velocity of the electrolyte flow through the annulus, Q, of about 30 mL/s, the Reynolds number is ~2000, meaning that the flow through the electrolyzer is laminar in most practical cases.

The convective diffusion in electrolyte representing incompressible liquid flowing between concentric cylindrical electrodes for the stationary and laminar profile of the linear velocities is described by [8]:

$$U_x \frac{\partial c}{\partial x} = D \frac{\partial^2 c}{\partial r^2} \tag{13.2}$$

where U_x is the linear velocity of the electrolyte flow in axial direction, x is the distance in axial direction, r is the distance in radial direction, D is the diffusion coefficient, c is the bulk concentration of electrolyte.

The coefficient of mass transfer across the border electrolyte-solid electrode could be found by solution of the Eq. 13.2 using the approximation of Leveque [15] that the gradient of U_x is linearly dependent on r at very small distances from the electrode surface. This assumption is connected to the fact that the value of kinematic viscosity of liquids, ν, is usually larger than the diffusion coefficient, i.e., the Schmidt number, Sc = ν/D, is always large. In this case, the thickness of diffusion layer is small compared to the electrode radius. The solution of the Eq. 13.2 provides the expression for the mass transfer coefficient between electrolyte and the electrode surface:

$$K_m = 0.807 D \left(\frac{\beta}{DL} \right)^{1/3} \tag{13.3}$$

where $\beta = \left(\frac{\partial U_x}{\partial r}\right)_{r=r_{AD}}$ is the gradient of the linear velocity of the electrolyte flow in axial direction at the very small distances near the electrode surface, and L is the length of the annulus. Thus, to use Eq. 13.3 for estimation of K_m the distribution of the linear velocities of the flow in the radial direction needs to be found.

In the anode chamber, the distribution of the linear velocities of the flow in the radial direction is described by the equation derived basing on the formulas given in [8]:

$$U_x = \frac{\Delta p r_{DA}^2}{4\mu L}\left[1-\left(\frac{r}{r_{DA}}\right)^2 - \frac{1-a_{AD}^2}{\ln\left(1/a_{AD}\right)}\ln\left(\frac{r_{DA}}{r}\right)\right] \tag{13.4}$$

where Δp is the difference in hydraulic pressures at the enter into and exit from the annulus, including the pressure due to gravitation.

At the aspect ratio reaching zero, $a_{AD} \to 0$, Eq. 13.4 is transformed into known formula, describing the radial distribution of the linear velocities of the liquid flow inside the hollow circular tube [8]:

$$U_x = \frac{\Delta p R^2}{4\mu L}\left[1-\left(\frac{r}{r_{DA}}\right)^2\right] \tag{13.5}$$

where R is the radius of the hollow tube. In this case, the velocity of the flux would be maximal at the axis of the tube:

$$U_{max} = (U_x)_{r=0} = \frac{\Delta p R^2}{4\mu L}$$

and would become zero at the inner surface of the tube, at $r = r_{DA}$. Thereat, the parabolic velocity distribution symmetric with respect to the axis of the tube will occur.

It is seen from the Eq. 13.4 that, in contrast with the hollow circular tube, the linear velocity of the electrolyte flow inside the annulus equals zero at $r = r_{AD}$ and $r = r_{DA}$, i.e., at the outer surface of the anode and at the inner surface of the diaphragm. The radial position of the maximal linear velocity of the electrolyte flow in the annulus of the anode chamber, r_{max}, is given by formula:

$$r_{max} = \lambda r_{DA} \tag{13.6}$$

where λ is exclusively defined by the geometry of the annulus, namely, by its aspect ratio:

$$2\lambda^2 = \frac{1-a_{AD}^2}{\ln(1/a_{AD})} \tag{13.7}$$

In our specific case, $a_{AD} = 0.4/0.52 = 0.769$, $\lambda = 0.882$ and $r_{max} = 0.459$ cm, meaning that the radial position of the maximal linear velocity of the electrolyte flow in

the annulus of the anode chamber practically coincides with the middle distance between the outer surface of the anode and the inner surface of the diaphragm, which is:

$$\frac{r_{AD} + r_{DA}}{2} = 0.46\,\text{cm}$$

Using Eqs. 13.4, 13.6 and 13.7, one can obtain the expression for the maximal velocity of the electrolyte flow inside the annulus:

$$U_{max} = (U_x)_{r=\lambda r_{DA}} = \frac{\Delta p r_{DA}^2}{4\mu L}[1 - \lambda^2(1 - \ln\lambda^2)]$$

The equation for the volumetric velocity of the electrolyte flow through the annulus, Q, could be obtained by integration of the Eq. 13.4:

$$Q = \frac{\pi\Delta p r_{DA}^4}{8\mu L}[(1 - a_{AD}^4) - \frac{(1 - a_{AD}^2)^2}{\ln(1/a_{AD})}]$$

From this, the average velocity of the electrolyte flow through the annulus having the cross-sectional area A is given by:

$$U_{avg} = \frac{Q}{A} = \frac{\Delta p r_{DA}^2}{8\mu L}\left[1 + a_{AD}^2 - \frac{1 - a_{AD}^2}{\ln\left(1/a_{AD}\right)}\right] \tag{13.8}$$

By substitution of the Eq. 13.8 into Eq. 13.4, one can obtain the formula for the radial distribution of the linear velocities in the annulus of the anode chamber expressed through the average velocity of the electrolyte flow:

$$U_x = 2U_{avg}\left[\frac{1 - \left(\frac{r}{r_{DA}}\right)^2 - \frac{1 - a_{AD}^2}{\ln\left(1/a_{AD}\right)}\ln\frac{r_{DA}}{r}}{1 + a_{AD}^2 - \frac{1 - a_{AD}^2}{\ln\left(1/a_{AD}\right)}}\right] \tag{13.9}$$

The radial distribution of the linear velocities of the electrolyte flow in the annulus of the anode chamber calculated using the Eq. 13.9 for $r_{AD} = 0.4$ cm and $r_{DA} = 0.52$ cm is shown in Fig. 13.1.

Now, the expression for β could be obtained by differentiation of Eq. 13.9 by r:

$$\beta = \frac{8U_{avg}\psi_a}{d_h}, \quad \text{where} \quad \psi_a = \frac{1 - a_{AD}}{a_{AD}}\left[\frac{0.5 - \frac{a_{AD}^2}{1 - a_{AD}^2}\ln\left(1/a_{AD}\right)}{\frac{1 + a_{AD}^2}{1 - a_{AD}^2}\ln\left(1/a_{AD}\right) - 1}\right] \tag{13.10}$$

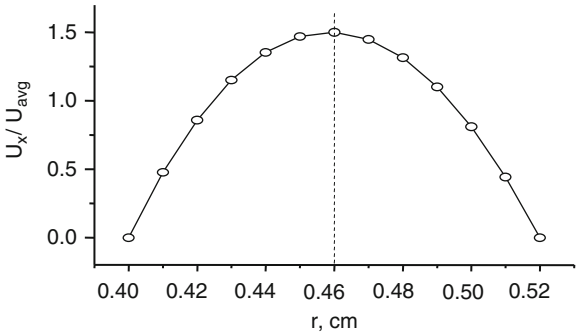

Fig. 13.1 The radial distribution of the linear velocities in the annulus for $r_{AD} = 0.4$ cm and $r_{DA} = 0.52$ cm. The linear velocities were normalized with respect to the average linear velocity. *Dash line* corresponds to the middle of the annulus

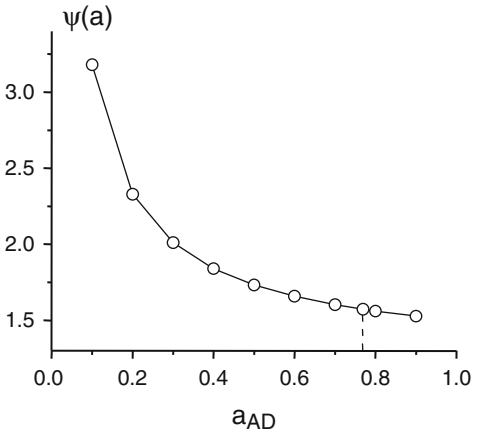

Fig. 13.2 Dependence of $\psi(a)$ on the aspect ratio a_{AD}. *Dash line* shows the value corresponding to $a_{AD} = 0.769$

It is seen from the Eq. 13.10 that ψ_a depends only on the aspect ratio a_{AD}, and is a decreasing function having discontinuities at $a_{AD} = 0$ and 1.

The dependence of ψ_a on the aspect ratio a_{AD} calculated by the Eq. 13.10 is presented in Fig. 13.2.

Using numerical values L = 21 cm, $D = 2 \times 10^{-5}$ cm^2/s, and $d_h = 0.24$ cm, we calculated the values of K_m for the anode reaction depending on the volumetric velocity of the electrolyte flow (Fig. 13.3).

The theoretical limited diffusion anodic current, I_L, could be calculated using:

$$I_L = zFK_mA_ac,$$

where z is the electric charge of the ions participating in the anode reaction (z = 1 for chloride ions), F is the Faraday number, A_a is the anode surface area. The value of K_m calculated using Eq. 13.3 equal about 2×10^{-3} cm/s for the volumetric velocity of 300 mL/min. The anode surface area $A_a = 2\pi r_{AD}L = 52.8$ cm^2. Therefore, for the bulk concentration of 4 g/L or 7×10^{-5} mol/mL, the value of I_L is 0.71 A.

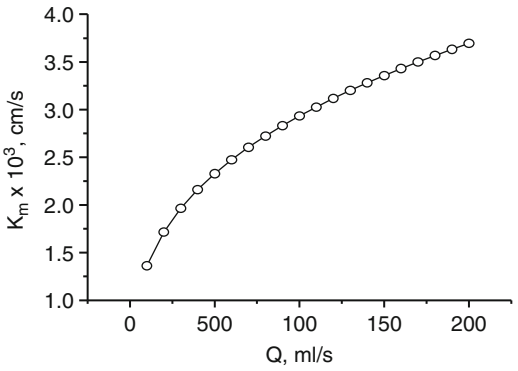

Fig. 13.3 Dependence of the mass transfer coefficient on the volumetric velocity in the annulus of the anode chamber

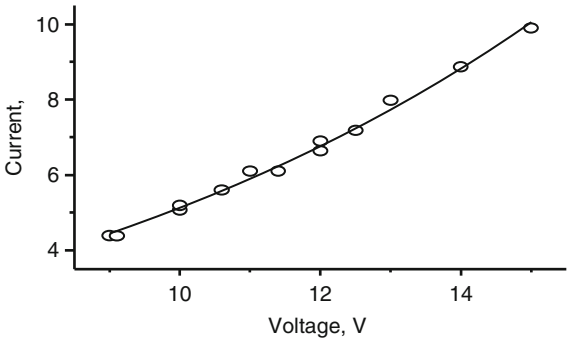

Fig. 13.4 Current–voltage curve of the electrolyzer. The electrolyte flow rate 300 mL/min. Bulk concentration 4 g/L NaCl. Room temperature

To evaluate the nature of the limiting step of the process we studied current–voltage relationships using voltage step protocol. Experimentally obtained current–voltage relationship is presented in Fig. 13.4. It is seen that current at practically used voltages (8–16 V) exceeds the theoretically estimated maximal diffusion-limited current.

13.3 Concluding Remarks

The chemical nature of the electroactivated aqueous solutions and the mechanism of their action are still controversial. For example, the skeptics claim that the formed solutions are not more than solutions of hypochlorite, a derivative of chlorine-gas evolved on the anode. At the same time, it is thought that some highly reactive agents of radical and non-radical nature could be formed during the electrolysis. This notion is consistent with the observation that chemically prepared solutions, e.g., aqueous solutions of hypochlorite, do not manifest the same activity as electro-activated solutions having the same concentrations and pH. The existing controversy

hinders the broader application and commercialization of the technology. The study undertaken here tends to clarify this issue using theoretical description of the mass transfer inside the electrolyzer and experimental investigation of the current–voltage relationships of the electrolyzer. The current–voltage relationships of the reactor, demonstrating maximal, diffusion-limited current independent from voltage, would strongly suggest that the anodic process represents mainly a discharge of chloride-ions. Experimental current–voltage relationships, obtained in this study, do not manifest limited current attributable to the electrolytic diffusion-controlled processes. Moreover, the magnitude of the working current significantly exceeds the value of the theoretical limited current. This allows suggesting that the process in the electrolyzer is not limited by the evolution of chlorine-gas at the anode, but it is accompanied by the production of other active species.

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Chapter 14
Electrochemical Incineration of Some Phenolic Compounds and MTBE

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Abstract Electrochemistry along with the microbial and photochemical approaches is a well established method for the degradation of wastes. It has been frequently stressed, however, that these methods often cannot bring about complete mineralization of several compounds, and to this end various methods broadly classified as AOPs (Advanced Oxidation Processes) provide complementary and alternative means of environment remediation, as outlined in comprehensive recent surveys. These AO systems include ozone, hydrogen peroxide as well as a mixture of them called "Peroxone" which can be activated by Fenton reactions leading to formation of a large amount of OH radicals and, consequently, to a highly oxidizing environment. In this work we investigate incineration of some phenolic compounds (4-chlorophenol, 4-nitrophenol, 2,4-dichlorophenoxyacetic acid, trans-3,4-dihydroxycinnamic acid) and methyl tert-butyl ether (MTBE) using different electrochemical systems involving ozone formation at PbO_2 based anodes: (1) direct electrolysis at constant current; (2) ex-situ use of O_3 and (3) combined use of anodically generated O_3 and H_2O_2 produced at a graphite cathode. According to the results described in this work, what we called *direct electrolysis* actually turns out to be a mediated process by oxygenated radical intermediates. Subsequently, the active oxygen intermediates can react with the organic pollutants. The homogeneous

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chemical decomposition by ozone is the basis of the ex-situ methods. It involves the direct reaction of O_3 with the organic substrate and/or a radical chain reaction initiated by radicals formed in the reaction of O_3 with OH^- or with HO_2^-. The latter pathway is favoured by an increase of the pH. The examination of the results shows that the combined use of anodically generated O_3 and H_2O_2 produced at cathode is the most efficient approach.

Keywords Advance oxidation process • Electrochemical incineration • MTBE • Phenolic compounds

14.1 Introduction

Electrochemistry along with the microbial and photochemical approaches is a well established method for the degradation of wastes. It has been frequently stressed, however, that often these methods cannot bring about complete mineralization of several compounds, and to this end various methods broadly classified as AOPs (Advanced Oxidation Processes) provide complementary and alternative means of environment remediation, as outlined in comprehensive recent surveys. These AO systems include ozone, hydrogen peroxide as well as a mixture of them called "Peroxone" which can be activated by Fenton reactions leading to formation of a large amount of OH radicals and, consequently, to a highly oxidizing environment.

In our previous work [2, 4], H_2O_2 was electrogenerated at the cathode which was fed by a gaseous mixture of O_2 and O_3 that are, in turn, electrogenerated at the PbO_2 anode of the same electrochemical cell; the cathode also contained the target organic species to be degraded. It is now well recognized that, in the direct electrolysis process, the oxidation of a large number of organic and inorganic compounds on different electrode materials, including PbO_2, proceeds simultaneously with the evolution of oxygen. Lead dioxide is a promising electrocatalyst widely used in different applications [1–4, 6]. Electrodeposited pure lead dioxide was demonstrated to exhibit a moderate electrocatalytic activity toward various anodic reactions in acidic media. However, this activity can often be enhanced greatly by incorporation of some ions, for example Bi^{3+}, As^{3+}, Fe^{3+}, Cl^-, F^- [8, 9, 11–13]. There is much less information on effects of polyelectrolyte and surfactant additives on the process of oxide electrodeposition and the physicochemical properties of the resulting materials. It was shown [13] that both polyelectrolytes and anionic surfactants are adsorbed on PbO_2 and that the composite materials prepared from plating solutions containing those additives have new physicochemical properties. Composite materials based on lead dioxide and containing various oxides have been reported in the literature, in particular: $A1_2O_3$, Co_3O_4, RuO_2, TiO_2 and ZrO_2 [5, 14, 15].

At the high anodic potentials involved, the same oxygen species may react to form O_3 in addition to O_2, as illustrated by the pathway below [3, 7]:

$$H_2O \rightarrow H^+ + (OH^{\cdot})_{ads} + e^- \quad (14.1)$$

$$(OH^{\cdot})_{ads} \rightarrow (O)_{ads} + H^+ + e^- \quad (14.2)$$

$$2(OH^{\cdot})_{ads} \rightarrow O_2 + 2H^+ + 2e^- \quad (14.3)$$

$$2(O)_{ads} \rightarrow O_2 \quad (14.4)$$

$$(O)_{ads} + O_2 \rightarrow O_3 \quad (14.5)$$

We show that the direct electrooxidation of an organic substrates is kinetically controlled and competes with O_2 evolution and O_3 formation. Moreover, concerning cathodically activated reactions, we show that an O_2 cathode with a graphite electrode, in weakly alkaline solutions, forms HO_2^- which reacts with O_3 when the cathode is fed by O_2/O_3, confirming the mechanism that we proposed earlier [2, 4] where the active oxygen species are intermediates in the reaction of O_3 with co-electrogenerated H_2O_2.

14.2 Experimental

Ultrapure sulfuric acid was obtained from Merck; all other chemicals were Fluka reagents and were used as received. PbO_2 electrodes were prepared by electrodeposition at constant current from $Pb(NO_3)_2$ acid solutions onto Ti substrates previously etched in hot oxalic acid and then platinized. The electrodeposition of Pt was carried out from a solution containing 32.5 $g\,L^{-1}$ K_2PtCl_6 in 30 $g\,L^{-1}$ KOH at 75°C, using a constant current of 8 mA cm^{-2} for 2.5 min. In the present work, however, the oxide electrodeposition was carried out as described before[33] from a solution containing 0.1 M HNO_3 and 0.1 M $Pb(NO_3)_2$, which contains in some case ions, surfactants and polyelectrolytes as additives, at room temperature and at a constant current of 5 mA cm^{-2}. The PbO_2–TiO_2 composites were deposited in the galvanostatic mode onto pretreated platinum-plated titanium electrodes of area 4 cm^2. The deposition electrolyte contained 0.1 M $Pb(NO_3)_2$ and 0.1 M HNO_3. Additionally, TiO_2 (35 nm) powder was added into the electrolyte.

Analyses of the reaction products were conducted by HPLC using a TSP instrument equipped with a Spectro Monitor detector 5,000 and a 25 cm Econosphere C 18 5U column. Ozone analysis was carried out mostly by iodometric titration. In some cases the results so obtained were checked by the spectrophotometric method. The chemical oxygen demand (COD) was evaluated as described elsewhere. In one experiment ozone was generated by the arc discharge method using a Fischer Ozone-Generator Instrument.

A CHROM 5 gas chromatograph equipped with a SE-30 column (polyethylene glycol saturated by ferrochromium) with a length of 2.5 m and an ID of 3 mm and

a flame ionization detector was used for solution analyses in case of MTBE degradation. The oven temperature was 70°C. Carrier gas was argon at a flow rate of 25 mL min^{-1}. Sampling was performed at 0, 30, 60, 90, 120, 150, 180, 210, 240, 270 and 300 min (each kinetic curve consists in 11 points). The experiments have been performed 3 times in order to verify the reproducibility of the results. The constant rates were calculated from the experimental kinetic curves ($\ln C = f(t)$ for the first order reaction) using linear regression procedure. Correlation coefficient was in range 0.990–0.998 and maximum standard error was 5×10^{-4}.

The formation of colored compounds during electrolysis was followed by UV-visible spectroscopy using a Kontron Uvikon 940 spectrometer, and the analysis of Pb^{2+} released into the solution was carried out by atomic absorption spectroscopy using a Perkin-Elmer 1,100 spectrometer.

14.3 Results and Discussion

14.3.1 Electrooxidation of MTBE

Methyl tert-butyl ether (MTBE) is one of the most widely used motor fuel additives. Despite its success in the improvement of fuel combustion efficiency and air quality, numerous corrosion failures have caused it to leak from fuel storage tanks and now MTBE has been widely found in both groundwater and surface waters [10, 11, 16]. We will examine the performance of PbO_2–TiO_2 anodes in the EC degradation of MTBE. It is also relevant to mention that only very little information concerning EC oxidation of MTBE is available in the literature. Gas chromatographic measurements showed that tert-butanol (TBA), acetone, acetic acid and CO_2 were main by-products of electrochemical degradation of MTBE at PbO_2 electrodes. The concentration of TBA reached a maximum after 1 h of electrolysis, while the concentrations of acetone and acetic acid increased for treatment times of up to 3 h. After 6 h of electrolysis, only impurity level of acetic acid was found.

According to our calculation performed on kinetic data (evolution of MTBE concentration vs time) electrochemical oxidation of MTBE is a pseudo first order reaction with main kinetic parameters presented in Table 14.1. It is important to note that increasing TiO_2 content in composite electrodes leads to increase the rate of MTBE electrooxidation in two times (Table 14.1) with decreasing half-life of reaction from 126 to 69 min. The stronger electrocatalytic activity of composite PbO_2–TiO_2 electrodes to some oxygen-transfer reaction may be linked to a larger amount of strongly-bonded oxygen-containing particles on the electrode surface.

Another interesting effect is the increase of the rate of MTBE electrooxidation under UV irradiation (Table 14.1). In case of the composite containing 6 wt.% of TiO_2 the constant rate of MTBE electrooxidation increases in two times under UV irradiation with decreasing of half-life of reaction from 99 to 58 min. We suggest that this effect is related to the additional generation of OH-radicals due to photocatalysis

Table 14.1 Kinetic parameters (constant rate, k, and half-life, $t_{1/2}$) of electrochemical MTBE degradation at different PbO_2 anodes

Electrode material	k/min^{-1}	$t_{1/2}$/min
PbO_2	5.5×10^{-3}	126
PbO_2–TiO_2 (6 wt.%)	7.0×10^{-3}	99
PbO_2–TiO_2 (9 wt.%)	1.0×10^{-2}	69
PbO_2–TiO_2 (6 wt.%) Under UV irradiation	1.2×10^{-2}	58
PbO_2–TiO_2 (9 wt.%) Under UV irradiation	1.4×10^{-2}	49

as it was clearly shown in [9, 10]. Synergistic effects of irradiation in oxidation reactions at PbO_2–TiO_2 electrodes have been previously observed [5]. Some of these studies have dealt with the degradation of dyes using UV illumination at 254 nm where, admittedly, the organic substrate absorbs light.

14.3.2 Oxidation of Some Phenolic Compounds

Direct Electrolysis Experiments. In these experiments the organic substrate is added to the anodic compartment. Lead dioxide on Pt-Ti substrates was used as anode with Pt-Ti sheets as counter electrode. Since under some conditions ozone formation can occur, the gas evolved at the anode was collected by an argon stream and analyzed for O_3.

Since the formation of O_3 appears to provide a possible measure of efficient pollutants degradation at PbO_2, we carried out some experiments under conditions in which formation and detection of O_3 is improved, i.e., in buffer phosphate electrolyte at low temperatures and addition of fluoride. The oxidation of CPh was then followed at room temperature and, for comparison, at 0°C and in the presence of NaF too (Fig. 14.1). From the experimental data, evaluation of the initial rate of CPh disappearance gives 0.016 mol L^{-1} min^{-1} at 0°C and 0.01 mol L^{-1} min^{-1} at 25°C; the slightly higher value at low temperature is likely due of a decreases of the OH radicals condensations reaction that leads to the parallel O_2 evolution process. Since, however, one effect of decreasing the temperature is also the enhancement of the efficiency of O_3 formation, one could possibly explain the above results on the basis of a direct reaction of the organic substrates with electrogenerated O_3 at the electrode surface. Actually, all literature data report that the reaction rates of organic species with OH radicals are at least some orders of magnitude higher than those with ozone.

It is important to note that the conversion rate of chlorophenol strongly depends from nature of micro-doped and composite PbO_2-based electrodes. Activity of PbO_2-based anode to chlorophenol oxidation changes in sequence: Ni-PbO_2 > F-PbO_2 > Fe-PbO_2 > Co-PbO_2 > PbO_2 > porous-PbO_2. This sequence does

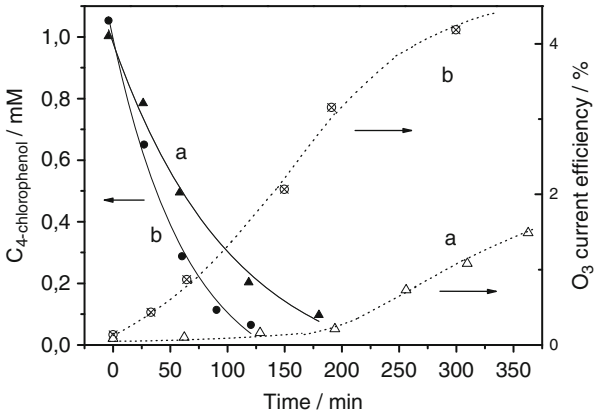

Fig. 14.1 Oxidation of 4-chlorophenol (*full symbols*) and O_3 formation (*open symbols*) on a β-PbO_2 electrode in buffer phosphate at 25 °C (**a**) and buffer phosphate with 0.01 M NaF at 0°C (**b**). Applied constant current: 50 mA cm^{-2}

not have direct correspondence for ozone evolution efficiency. An investigation on the electrodeposition of nickel-doped lead dioxide has been carried out, as part of a study ultimately aimed at producing anodes with improved electrocatalytic activity and stability. Experiments showed that Ni^{2+} is weakly adsorbed on PbO_2, and that the adsorption follows Langmuir conditions. Surface analysis by SEM and XRD of the Ni-doped PbO_2 films does not reveal major changes compared to undoped samples. At the same time X-ray photoelectron pectroscopy (XPS) measurements show a marked increase in the signal due to adsorbed oxygen species, water and hydroxyl groups at the Ni-doped PbO_2.

Another interesting effect was observed for composite PbO_2 anodes with surfactants and polyelectrolytes. According to our data, some materials seems to be very promising for their application in organic pollutants conversion processes, for instance, Nafion®-PbO_2.

Reactions with Ex-situ Generated Ozone. This AOP approach using ozone for the abatement of pollutants is probably the most extensively investigated and the object of a large number of publications and reviews. In the present work, the gas evolved at the PbO_2 anode (O_3+O_2) is collected and passed through an external vessel containing the phenolic compounds in H_2O. As O_3 is present at low concentrations in the mixture, the rate of ozone consumption will be also limited by mass transfer of O_3 from the gas phase to the liquid (aqueous) phase. Under these conditions the rate of O_3 formation will depend also on the apparent volumetric mass transfer coefficient (k_La) of the investigated system. For this reason the results reported in this section are compared at a constant gas flux.

It is finally important to note that disappearance of the main intermediate initially observed (BQ) is fast and little dependent on pH (Fig. 14.2). It is, however, converted into secondary intermediates which cause the solution to become intensely brown colored. We have not investigated on the chemical nature of these intermediates

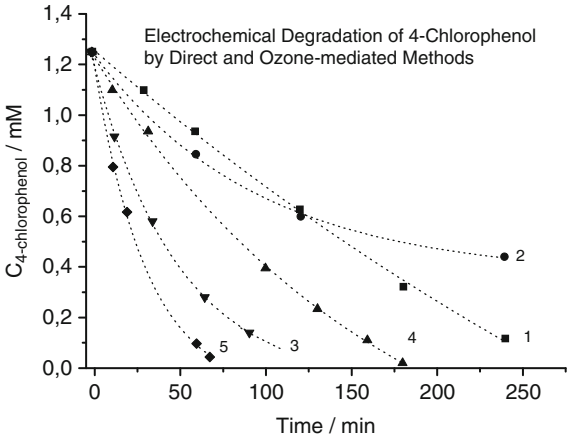

Fig. 14.2 Comparison of different methods for the oxidation of 1.25 mM 4-chlorophenol using β-PbO_2 electrodes in neutral or weakly alkaline media at 25°C: (1) direct electrolysis at pH 7.2 (phosphate buffer); (2) ozone-mediated ex-situ method, in water at pH 6.5; (3) ozone-mediated ex-situ method, in water at pH 9 (borate buffer); (4) cathodic O_2/O_3 approach at pH 7.2 (phosphate buffer); (5) cathodic O_2/O_3 approach at pH 9 (borate buffer). See text for full explanation

but they are likely products of condensation of 1,4-benzoquinone as reported previously. Particularly in the case of CPh, in contrast to a relatively fast conversion, the color disappears slowly. Thus, while the conversion of CPh is essentially complete after 120 min, it takes about 300 min for a COD value of 10 to be measured.

Indirect oxidation with simultaneously electrogenerated O_3 and H_2O_2. In the approach described here, the organic substrate is present in the cathodic compartment and hydrogen peroxide is conveniently generated in-situ, at a graphite cathode, when a O_2/O_3 mixture produced at the PbO_2 anode is collected by a stream of an inert gas and swept at a constant flux through the catholyte. The attention attracting term "*cathodic oxidation*" has also been used in this connection.

We limited experiments to the case of CPh as a test substrate since NPh could undergo reduction reactions at the cathode, making a comparison with other methods difficult. Experiments were conducted in buffer phosphate (pH 7.2) and buffer borate + 1 M NH_4PF_6 (pH 9). The choice was dictated by the necessity to maximize the amount of O_3 formation and for this purpose the solution in the anode compartment was also added with NaF and kept at a temperature of 0°C. Current efficiencies for ozone formation at a current of 50 mA cm^{-2} were 8–10% in buffer phosphate and 10–12% in buffer borate.

The results for the advanced oxidation of CPh by cathodically activated O_2/O_3 are shown in Fig. 14.2 (curves 4, 5) and compared, in the same figure, with the data discussed in the previous sections obtained with the external cell O_3 approach (curves 2, 3) and direct electrolysis (curve 1). The comparison is done in the pH range from 6.5 to 9 where the stability of H_2O_2 in the presence of O_3 decreases with increasing pH and even a relatively low concentration of

hydrogen peroxide can cause formation of a high concentration of OH radicals through reaction with ozone.

It is clear that the O_2/O_3 cathodic activation AO method always offers the best results. We observed that the methods based on the use of O_3 generally feature an improved degradation of the organic substrate as pH increases from neutral to weakly alkaline media. As discussed above, this is due to reaction of the phenolate XRO^-directly with O_3 and/or indirectly with OH radicals. Additionally, we have proposed earlier that in the neutral or weakly alkaline pH range reaction of O_3 with hydrogen peroxide:

$$O_3 + HO_2^- \rightarrow {}^{\bullet}OH + O_2^- + O_2 \tag{14.6}$$

Reaction (12) contributes significantly to creating a highly oxidizing environment leading to degradation of the organic pollutants. It can occur in addition to or instead of the above mentioned reactions; the rate constant for Reaction 6 ($2.8 \times 10^6\ M^{-1}\,s^{-1}$) is several orders of magnitude higher than those of reactions.

In our experiments, the potential of the graphite cathode reached values between −0.9 to −1.0 V when the PbO_2 anode worked under conditions of O_3 generation, i.e., typically at 2.0–2.1 V, and thus reduction of O_2 needs to be considered and discussed. Indeed, an ample literature has much to recommend consideration of the role played by O_2 reduction in the system described herein (Reaction 6). In particular, it has long been established that on some electrode materials, including graphite, O_2 reduction follows a two-electron route yielding hydrogen peroxide. However, in order to collect additional data that can buttress conclusions on the reduction processes involved, we performed the experiments described in the following.

Ozone is a rather small fraction of the total gas evolved at the anode, i.e., typically 18 mg L^{-1} or ~ 1% v/v (STP) in the present case. Then with pure O_2 bubbling, under otherwise identical operative conditions as with O_2/O_3, analysis of the catholite during 20 min showed that hydrogen peroxide is produced at an average rate of 4 mg L^{-1} min^{-1}. For a comparison, in the experiment with O_2/O_3 and 18 mg L^{-1} O_3 in the gas phase, the amount of dissolved ozone was about 4 mg L^{-1} at 20°C in agreement with the value calculated according to literature. As a consequence, at steady state conditions, ozone can react with an excess HO_2^- according to fast Reaction 6 giving rise to a highly oxidizing environment.

We verified that the reaction of O_3 with peroxide is indeed fast in an experiment in which a stream of O_2/O_3 generated, this time, by arc discharge (80 mg L^{-1} O_3 at 70 mL min^{-1}) was fluxed for 2 min through a 3 mL of 8 mM H_2O_2 at pH 9. We observed a decrease of the peroxide concentration by over an order of magnitude, and no dissolved ozone was measured. For prolonged experiments ($t > 4$ min), the concentration of ozone dissolved in water increased again and reached 17 mg L^{-1}, i.e., the value measured in an analogous experiment with no added H_2O_2.

On the basis of our data we cannot support the key role of O_3^- from electroreduction of O_3 proposed by some authors. Assuming parallel one-electron and two-electron reductions of O_3, the subsequent reaction of O_3^- should be that with water to give OH radicals. On the other hand, according to the cited authors, the main

source of hydroperoxy species is the reaction of O_3 with OH^-. Our results actually show efficient formation of hydrogen peroxide, then both the above cited processes cannot compete with Reaction 6 which is several orders of magnitude faster.

We think that a final comment is due on this complex system: it concerns the role of H_2O_2 itself, in the absence of ozone, in the degradation of the target phenol. It has, in fact, been recently shown that H_2O_2 electrogenerated at carbon cathodes can bring about an efficient degradation of chlorophenols present in the catholyte. We then examined the abatement of 4-chlorophenol at a O_2-reducing cathode under the same conditions described above but in the absence of O_3 and found that, in experiments carried out on the same time scale as those with O_3, no significant decrease of the COD was observed despite a fast disappearance of the initial phenol. We only observed accumulation of intermediate products, among which we detected 2-hydroxy-1,4-benzoquinone and 2,5-dihydroxy-1,4-benzoquinone. The results are at variance with those of the above cited authors and one possible reason is the difference in current density: 39 mA cm^{-2} in their work and 4–5 mA cm^{-2} in the present one. We can therefore claim that the use of an O_3/O_2 cathode is a better choice in that it allows efficient removal of the pollutant under milder experimental conditions.

14.4 Conclusions

The present work is a comparative investigation of the oxidation of phenolic compounds using electrochemical methods. Three different methods were employed having in common the formation of ozone as a potential oxidant: (1) direct electrolysis (2) ex-situ use of electrogenerated O_3 and (3) a "cathodic oxidation" in which an O_2/O_3 mixture is fed into the cathodic compartment, where the H_2O_2 produced reacts with ozone to yield radicals that bring about the demolition of the organic compounds. According to the results described in this work, what we called *direct electrolysis* actually turns out to be a process mediated by oxygenated radical intermediates. Subsequently, the active oxygen intermediates can react with the phenolic compounds.

The homogeneous decomposition of the organic species, which is the basis of the ex-situ method, can compete with direct electrolysis on weakly alkaline solutions since degradation of the phenols is initiated by radicals formed in the reaction of O_3 with OH^-, with HO_2^- or with phenates. On the basis of the data collected for CPh, the so called "cathodic oxidation" approach offers the best advantages, and results can be further improved since there are promising developments in the study of efficient systems for the electrochemical ozone production. In a perspective development of the research it would be interesting to examine the oxidation of organic species with O_2/O_3 as a function of the applied cathodic potential and of the amount of O_3 in the gas phase so as to create conditions for O_3 reduction to be the preferred process or, in other words, establish the role of active oxygen intermediates.

The electrocatalytic activity of the micro-doped and composite anodes strongly depends on the amount and nature of doping elements, surfactants, polyelectrolytes

and foreign oxides. It was shown that rate of chlorophenol and MTBE direct electroxidation increase dramatically at Ni-doped PbO_2, Nafion®-PbO_2 and TiO_2–PbO_2electrodes. According to presented data, all these materials are interesting for industrial applications as electrocatalysts with good mechanical properties and long service life.

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Chapter 15
The Status of Research on Constructed Wetlands

Peter Kuschk, Arndt Wiessner, Eva M. Seeger, Matthias Kästner, Uwe Kappelmeyer, Diego Paredes, and Natalia I. Shtemenko

Abstract Constructed (treatment) wetlands have been state of the art in wastewater treatment technology for decades. Design and operation are determined mainly on the basis of the results of long-term experience. Knowledge of the role of specific removal processes like anaerobic ammonium oxidation, predatory activity of protozoa, bdellovibrio, bacteriophages etc. in the rhizosphere is still insufficient. Basic research is necessary to investigate the transformations involved and to understand the interrelations of element cycles. New findings from research and practice will identify new and expanded applications for highly efficient treatment, particularly of industrial wastewaters. New technologies, such as the combination of wastewater treatment and energy production using "energy" plants, should be the focus of future research. Basic research on element cycle dynamics will help to understand the fundamental processes of greenhouse gas generation in wetlands.

Keywords Contaminant and pathogen removal • Greenhouse gases • Industrial wastewater • Treatment wetland • Wastewater treatment

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15.1 Constructed Wetlands – Established Water Treatment Technologies

Over the last three decades, constructed wetlands have gone from being a "green idea" of a simple nature-oriented process for treating contaminated waters to become a state-of-the-art technology [57, 92]. Constructed wetlands got widespread in their application, due to their particular advantages like low external energy consumption, no need of complex technical apparatuses etc. [57]. Depending on the hydraulic conditions, three main types of constructed wetlands can be identified:

1. Vertical flow wetlands
2. Horizontal subsurface flow wetlands, and
3. Free water surface constructed wetlands.

Different specific redox dynamics inside the rhizosphere of the various systems determine different removal, transformation and/or immobilization efficiencies for wastewater contaminants [7, 120]. Aerobic conditions are dominant in the pore water of vertical flow systems, due to the usual interval-loading of the wastewater. The helophytes in the systems are of secondary importance for the removal efficiency [56, 57]. In contrast, in horizontal subsurface flow constructed wetlands oxygen supply is affected by diffusion from the atmosphere into the near-surface unsaturated soil layer and by oxygen release from the roots of the helophytes [19, 103]. Oxygen supply to these subsurface flow systems is limited and anaerobic conditions are found to be dominant. Only close to root surfaces and in the upper layer of the unsaturated soil body is oxygen present for chemical and/or biological consumption [7, 125]. However, in the biofilms generated at oxic/anoxic interfaces, aerobic and anaerobic microbiological processes simultaneously achieve highly efficient removal under the dominant micro-gradient redox conditions [82, 121]. The total efficiency of such systems is decisively influenced by these redox – micro-gradients and superimposed by flow-determined macro-gradients related to concentrations, temperatures, pH, redox and other parameters [7]. The resulting process correlations and the usually low flow rates in the rhizosphere lead to a high biodiversity, which is advantageous for enhanced removal efficacy and special applications [103, 122]. Subsurface flow wetlands are particularly of interest for removing contaminants such as trace elements, pharmaceuticals, toxic organic compounds, heavy metals and others. Concerning the importance of the micro-gradient processes only a few studies have been carried out to characterize redox dynamics in near-root environments [7, 25]. More information (regarding redox dynamics) is necessary for broader and more specific application of constructed wetlands. In planted ponds or surface flow systems with water levels more or less over the surface of the soil body, specific redox-conditions result in the water body and in the pore water of the soil, depending on the flow rate and the rate of oxygen consumption [56, 57]. In these systems, the diffusive exchange of water from the water body and the pore volume of the soil is strongly limited. Transport of contaminated water from the water body into the rhizosphere for redox/removal processes is mainly

realized by substitution of pore water being transpired by the helophytes. Accordingly, mean annual specific transpiration of 1,600–2,000 mm were found for reed in Central Europe, for example [44, 63]. This means a specific annual water transfer of 1.6–2 m^3 per m^2 of soil. The diffusion of atmospheric oxygen into the water body of surface flow systems is small and the redox conditions in the rhizosphere of the soil body are comparable to conditions in paddy soils and contaminated sediments of freshwater lakes [47, 101, 129]. In the whole soil, strong anaerobic conditions are dominant with the exception of the micro-gradient zones close to parts of the root surfaces. In constructed wetlands, the removal of water contaminants is mainly realized by microbiological transformations [103]. Non-biological processes, such as sorption, volatilization, filtration and autoxidation, are usually of secondary importance. Depending on redox conditions and availability of nutrients, specific microbial consortia are established in the system with different spatial distributions. In general, the knowledge of microbiological dynamics and the correlation of biological and non-biological processes in constructed wetlands is still insufficient. In the following, the state of the art in constructed (treatment) wetlands, future potential of ecological wastewater treatment, and research needs will be presented from the viewpoints of both scientists and engineers.

15.2 Removal of Carbon, Nitrogen and Phosphorus Compounds

The removal of organic carbon pollutants in vertical flow constructed wetlands with intermittent loading is known to be dominated by aerobic microbiological mineralization. In contrast, the importance and efficiency of different involved microbiological processes in horizontal subsurface flow systems is insufficiently investigated. In general, oxygen, nitrite, nitrate, iron(III), manganese(IV), inorganic sulphur compounds with oxidation values equal or higher than zero may act as electron acceptors for the microbiological mineralization of organic compounds. Even more toxic compounds, such as chlorate, perchlorate, chromium(VI), uranium(VI), arsenic(V), found in industrial effluents and in contaminated groundwater, are also suitable as electron acceptors. Otherwise, anaerobic fermentation of organic carbon, including methanogenesis, may be the main processes in case of a limitation of electron acceptors for mineralization. Methane emission from wetlands has mainly been investigated for paddy soils [60, 67, 126]. Information for constructed wetlands is increasing especially within the last 5 years [27, 49, 71]. Calculated a small share of less than 1% in the global trace gas emission if constructed wetlands were used for treating the entire world's domestic wastewater. In contrast, Ström et al. [104] give support to the finding that constructed wetlands can be a significant source of this greenhouse gas to the atmosphere. Knowledge of the generation of trace gases like methane and nitrous oxide in the rhizosphere of helophytes and interrelations of the carbon, nitrogen and sulphur cycles is essential for understanding the dynamics of greenhouse gas emission from wetlands [50, 117, 121].

Laboratory and small-scale experimental wetlands represent useful model systems for the investigation of geo-biochemical processes in planted water-logged soils [49, 104, 121]. The correlation of sulphur dynamics and the carbon cycle (microbial sulphate reduction/methanogenesis, reoxidation of reduced sulphur/methane) is of special interest here [61, 119, 108]. For the efficient treatment of industrial wastewater and contaminated groundwater, the fate and behavior of chlorinated hydrocarbons and other xenobiotics (methyl-tertiary-butylether (MTBE), BTEX aromatics, explosives, pesticides, pharmaceuticals and etc.) in the rhizosphere of constructed wetlands are increasingly the focus of research [48, 111]. The initial microbial degradation step should be aerobic but may also be anaerobic, depending on the degree of halogenation [8]. These compounds often are highly volatile; uptake into, transport through, and release out of the plants into the phyllosphere should be taken into account [48, 103]. However, the distinctive removal processes for distinctive chemicals mainly realized by the microorganisms and plants is still a wide field of the wetland research. In particular, quantitative data from field tests considering the water balance (precipitation and evapotranspiration) are still insufficient. Nitrogen is removed from wastewater mainly by microbial denitrification [57, 65]. In intermittently loaded vertical flow wetlands, in particular, the availability of organic compounds, essential for this microbial process, is mostly deficient. Recirculation of the nitrate-rich outflow, as known from conventional wastewater treatment technologies, may be helpful for improving nitrogen removal. This means that nitrate-rich outflow is added to the inflowing wastewater with a hydraulic retention time that is sufficient for a successful denitrification. Hybrids of vertical and horizontal systems in series are also known to provide a successful application [95, 110]. Nitrification affects the generation of nitrite from ammonium via a first oxidation step. Particularly in usually oxygen-deficient horizontal subsurface flow wetlands, the subsequent transformations are unknown. In theory, nitrite may be further oxidized to nitrate, or direct denitrification of the generated nitrite may be the dominant process. Different reductants may be efficient for reducing nitrite, such as organic compounds from the wastewater, dead plant matter, plant root exudates, hydrogen sulphide, iron(II) or even ammonium itself (anaerobic ammonium oxidation – so-called "Anammox") [113]. The "Anammox" process could be particularly efficient in wetlands loaded with ammonium-rich and wastewater poor in organic carbon. Basic investigation of the "Anammox" process is necessary. Long-term nitrogen removal on a high level could be observed in a laboratory-scale planted soil bed treating a model wastewater by using a highly concentrated inoculation of "Anammox"-bacteria [87]. Because of extremely low growth rates of "Anammox"–bacteria, the self-selection of these bacteria to a useful high cell density within the biofilms of newly constructed large-scale wetlands would take several years [87]. Possibility and efficacy of large-scale inoculation are still unknown. A lot of work has been done over the last years to evaluate the potential of wetlands to release greenhouse gases. The highly reactive greenhouse gas N_2O was found to be released from treatment wetlands but emission data differ extremely [71, 104, 124]. Information on the generation of N_2O and on interrelations in the rhizosphere is still insufficient. Better knowledge of dynamics in wetlands is necessary because of the

high potential of nitrous oxide to contribute to global warming. Investigations using laboratory-scale model treatment wetlands would be helpful in gaining fundamental information on the element dynamics in wetlands [121]. Information on the microbial reduction of phosphate to phosphine (PH_3) is rare and so far it has not been possible to provide evidence for this process [17, 23, 32, 53]. Generally, microbial processes and plant uptake of phosphorus compounds are of subordinate importance and it is mainly physico-chemical processes, such as precipitation and adsorption, which are responsible for phosphate removal from wastewater. The use of special soil material such as alumn sludge [128], the addition of iron or increasing the amount of adsorbent surface by using special soil material can improve removal efficiency. Phosphate from domestic sewage may also be used as fertilizer in agriculture. The wastewater used for such purposes has to be low in organic carbon, pre-treated for hygienization, and wastewater from industrial sources has to be separated from the domestic sewer system to avoid accumulation of contaminants in agricultural soils.

15.3 Removal of Trace Elements and Treatment of Industrial Wastewaters

Trace compounds from pharmaceuticals, health care products and chemicals from other industries, are detected in significant amounts in effluents from wastewater plants treating domestic sewage. This is increasingly discussed as a risk for human health [86, 96, 105]. Post-treatment using several physico-chemical methods is known to be expensive [38]. Treatment wetlands have a higher potential for removing trace compounds from wastewater than conventional treatment system due to the comparably higher microbial biodiversity and longer hydraulic retention times, particularly in horizontal subsurface flow wetlands [56, 57, 103]. The fate and behavior of pharmaceuticals inside the rhizosphere of treatment wetlands is insufficiently investigated [68, 73, 74]. Expensive trace analytics and the development of new or adapted analytical methods are necessary for the qualitative and quantitative determination of the substances and their possible metabolites in water, soil, and biological matter (plants, microorganisms) [39, 46]. The potential of constructed wetlands for removing industrial chemicals from wastewaters was already described many years ago [93, 94]. Today, the treatment of industrial wastewater with treatment wetlands is state of the art [57]. Different constructed wetland systems are used to treat agricultural wastewater, contaminated groundwater, landfill leachate, and wastewater from tannery, mining and many other industries [57, 111]. All chemicals which can be degraded by microorganisms are potentially metabolized in wetlands and the efficacy of the plants can increase the total removal potential. The plants are of subordinate importance for high concentrations of contaminants but significant in case of low concentrations and long hydraulic retention times. Therefore, wetlands can be used as post-treatment systems to remove chemicals from effluents of conventional wastewater treatment plants [4].

15.4 Removal of Sulphur Compounds, Metals and Metalloids (Arsenics)

Redox processes of the sulphur cycle in the rhizosphere of treatment wetlands were found to influence toxicities and removal processes [18, 81, 119]. Knowledge of the interrelation with the processes of the carbon and nitrogen cycles bears still open questions [119]. For example, microbial reduction of 100 mg/l sulphate in wastewater can decrease the chemical oxygen demand caused by organic compounds by about 65.5 mg/l and theoretically generates nearly 35 mg/l toxic sulphide. Furthermore, simultaneous methanogenesis was observed in natural and technical ecosystems, despite a comparably higher thermodynamic and kinetic potential for sulphidogenesis [66, 76]. Different retention of the bacteria species in biofilms, flocs and micro-niches is postulated as the reason for this observation. The dynamics and correlation of sulphide generation and methanogenesis as well as the efficacy of reoxidation of sulphide and methane in the rhizosphere and in the unsaturated soil zone in subsurface flow wetlands are important subjects of future research. Moreover, response of biological systems (microorganisms, plants) to sulphide toxicity and the formation and stability of deposited sulphur pools are of special interest [18, 28, 81]. High concentrations of sulphate are found to be a main problem in wastewater from industries, such as mining, paper production, leather production, and landfills. The treatment of acid mine drainage (AMD) focuses on increasing the pH, for example. The removal of metals from wastewater is mainly based on precipitation as stable metal sulphides, hydroxides and carbonates. Accordingly, one of the most often used conventional technologies is limestone treatment and the promotion of the microbial dissimilatory sulphate reduction [12]. The biological process promotes mineralization of organic carbon and the generation of bicarbonate, which leads to an increase of the pH value. These chemical and biological processes can be stimulated in so-called “passive technologies” for AMD treatment, such as constructed wetlands [12, 58]. Constructed wetlands are increasingly used for this field of application [40, 54, 75]. One of the main problems for efficient processing here is the limitation of biologically available organic carbon. The supply can be realized by root exudates and/or dead plant matter. The qualitative and quantitative determination of organic compounds originating from plants and the efficacy for beneficial microbial processes should be important aims of further basic research. Additional organic matter and lime stone can be added to the soil body of the wetland to enhance efficiency of the neutralization. The selection and efficient dosage of suitable electron donors (mainly organic waste materials), facilitation of sulphidogenesis and efficiency of simultaneous metal precipitation, prevention of methanogenesis, and obtaining an improved understanding of nature of the extremophile microbial communities are aspects of future research [24, 75, 83]. Arsenic is a ubiquitous trace metalloid and is found in the environment through natural and anthropogenic processes [1]. The situation becomes particularly problematic in many regions worldwide and several millions of humans are exposed to drinking, ground and surface water contaminated with arsenic [59, 70]. Wetland systems were

found to be advantageous for the treatment of wastewater contaminated with arsenic [98]. The fate and behavior of arsenic compounds in treatment wetlands is very complex mainly because of the potentially high chemical and biological process dynamics of arsenic and the dynamic redox conditions in the rhizosphere [90, 91, 15]. On the one hand, As(V) can easily be adsorbed by $Fe(OH)_3$ under aerobic conditions. On the other hand, anoxic conditions favor bacterial reduction of Fe^{3+} to Fe^{2+} and dissolution of $Fe(OH)_3$ in the same system. Accordingly, generated As(V) should be metabolized by bacteria to As(III), methylated arsenic compounds with a low potential for adsorption to solid Fe(III) and undissolved As_2S_3 [85, 97]. The rooted zone of the wetland was found to offer specific macro- and micro-gradients of redox conditions, which enable the development of highly diverse still not well characterized microbial consortia capable of different redox reactions of the arsenic cycle. In many countries, especially in the tropics, domestic sewage treatment is often realized by an anaerobic fermentation step, such as a septic tank, an anaerobic filter, etc. [55, 118]. The biochemical oxygen demand (BOD) of the wastewater is considerably reduced by such a treatment step, but it still contains a relatively high residual BOD, and ammonia and sulphide formed by the bacterial dissimilatory sulphate reduction. Because of the toxicity of sulphide and the bad odor of H_2S, such anaerobically pre-treated wastewater needs further post-treatment steps. Several methods are available for sulphide oxidation/detoxification in wastewater, such as chemical oxidation [123], adsorption [11] and biological oxidation [43]. Due to restrict financial budgets, simple methods, like ponds or wetlands, are often preferred [3]. Treatment wetlands offer a potential method to tackle the sulphide problem of anaerobic digester wastewater [33, 119]. Oxidation of reduced sulphur by oxygen released by the helophytes into their rhizosphere is not well understood but is beneficial, particularly for generating immobilized elemental sulphur from the sulphide of the wastewater, as realized already in bioreactors [2, 51, 52].

15.5 Hygienization

The reuse of treated wastewater, for example for irrigation, demands removal of pathogenic germs. Health risks rise sharply with the ingestion of unsafe water: diseases related to water sanitation are estimated to account for 4.0% of all deaths and 5.7% of the total disease burden occurring worldwide [88]. Therefore, research into sewage treatment is needed in order to reduce the risks associated with improper sanitation. Potential physico-chemical methods for hygienization, such as ozonization, chlorination, UV illumination, and filtration, are useful but expensive in terms of equipment [78]. Another way is to apply constructed wetlands as an easy-to-handle technique that is relatively inexpensive and particularly applicable in developing countries [72]. To remove bacteria, constructed wetlands are generally considered to feature a combination of biological, chemical and physical factors, including mechanical filtration and sedimentation [57, 89]. The physico-chemical factors are oxidation, exposure to UV irradiation of sunshine, aggregation and sorption

on organic matter/particles. The biological removal mechanisms may be antimicrobial activity of root exudates [84], predation by nematodes and protists [20, 21], activity of lytic bacteria or viruses [5], retention in biofilms [14], and natural die-off [29, 30]. Even though possible mechanisms of bacterial removal have been discussed in many articles and books [22, 57, 79], no systematic analyses on the removal processes and the fate of potential pathogenic bacteria in constructed wetlands are yet known [106]. In particular, the structure of microbial populations in response to vertical or horizontal subsurface flow systems, soil type, plant presence, and BOD load, etc. is still not well understood [24, 106]. The removal efficiency of bacterial pollution and pathogenic protozoa like *Cryptosporidium* and *Girardia* in constructed wetlands has been widely reported ([45, 99, 109]; Thursten et al. 2001). Mostly, orders of magnitude for decreasing the pathogens are in a range of 1–3 [31, 80] and a high order of magnitude of 5 could be determined for removing 10^7 ml^{-1} *Escherichia coli* cells in a combination of horizontal subsurface flow and vertical flow constructed wetland.

The removal efficiency depends strongly on technological aspects. While for a subsurface-flow constructed wetland Graczyk et al. [37] reported a removal of *Cryptosporidium* (97.4%) and *Giardia* (95.4%) they observed in case of a surface-flow constructed wetland even an increase of the number by 84.0%. The authors discussed this phenomenon in relation to wildlife contribution. The processes responsible for hygienization in the planted soil beds are not yet fully understood and basic research is necessary for optimization and management. Flocculation and sedimentation should be main removal processes and filtration of single germs seems to be of subordinate importance because of usually long hydraulic retention times and comparably coarse-grained size bed material. Furthermore, cell lysis by nutrient limitation and predatory activities of protozoa, bdellovibrios, and bacteriophages are found to be of importance, as investigated in model-scale constructed wetlands [102, 115, 116]. Accordingly, bdellovibrios of up to 10^4 g^{-1} sand were estimated in laboratory-scale planted columns [115]. Knowledge of the influence of the plants on hygienization is still insufficient. A biocide effect of root exudates [93, 94] seems to be unrealistic because of dilution by the streaming pore water in the saturated soil filter. No concentrations of physiological effectiveness are realistic at usual rates of exudations. Besides pathogenic bacteria, enteric viruses propose a real danger of infection [64]. Viruses are able to survive under extreme environmental conditions and infectious dosages are comparably low [114]. The pathogenic potential of enteric viruses is not reflected by existing laws and regulations [77]. In consideration of possible global epidemics a change of the situation is urgently necessary. Related research is mainly focused on establishing necessary new indicator systems to detect contaminations with fecal viruses [36]. Bacteriophages associated with intestine microorganisms are being studied [35, 64]. The $\log_{10}$ removal of viruses in constructed wetlands was reported in the range of −0.2–4.5 [57]. The intrinsic removal mechanisms still are not well characterized. In future, these aspects of pathogen removal mechanisms will be increasingly investigated. By this, a better understanding of the processes and in consequence probably even better removal efficiency can be realized.

15.6 Technical Innovations

Technical progress and expansive use results in development of numerous variations and modifications of wetland designs, creating hybrid systems and combinations with other water treatment technologies [57]. For example, classical design principles of an intermittently loaded vertical system and a continuous-flow horizontal system were combined to create a new intermittent outflow horizontal subsurface flow wetland to guarantee optimum oxygen support with minimum technical expenditure [112]. Floating plant mats also termed floating-raft hydroponic systems with emergent water plants like *Phragmites*, *Typha* etc. were reported for the treatment of polluted river water and wastewaters [9, 127]. Valipour et al. [107] described using instead of sand/gravel for soil matrix a rack system constructed by perforated PVC pipes in a hydroponic/wetland system for wastewater treatment. These technical examples lead to the general question about the role of soil matrix in constructed wetlands. Beside anchorage the plants and surface for microbes attachment (biofilms), the adsorption capacity plays a significant role mainly for phosphate. Nevertheless, this adsorption capacity is usually very limited. First, still unpublished results about the treatment of a groundwater contaminated by benzene and ammonia show that a non-floated root mat system of *Phragmites* without any soil matter or rack system for anchorage the plants (this is realized in the dense root mat by itself) realized similar results like a gravel bed planted with *Phragmites*. By this it can be assumed that such a non-floated plant mat the root/rhizome system serves also enough surface for attached bacterial growth like a planted soil filter (wetland). Constructed wetlands on roofs are a further example for new solutions and design variability [26]. The selection of plant species for further maximization of pollutant removal is still in discussion [13]. In general, especially the use of genetically modified superplants is difficult because of auto-selection of helophytes in the wetlands and also due to problems of acceptance and legal restrictions in many countries. Ecologically well-adapted plants, such as reed, rushes, and cattail, are dominant in uninfluenced ecosystems and specially designed plants probably would be displaced by the wildlife species in treatment wetlands. The implementation of constructed wetlands in urban planning for wastewater treatment and for landscape architecture respectively urban ecology is still at the beginning of its development [100].

15.7 Production of Energy Plants

The use of plant biomass of constructed wetlands for different purposes such as ornamental flowers [130], food [34], construction material [69] etc. has a long history.

At present there is an increasing interest in simultaneous wastewater treatment and production of biomass for energy generation and this interest is stimulated by:

- Climate protection
- Increasing costs for wastewater treatment because of continually increasing prices for energy and higher standards of water quality

– Increasing aridity of many areas and an increasing water demand in agriculture
– Increasing costs for the production of fertilizer because of higher prices for energy (nitrogen fertilizer) and limitation of resources in nature (phosphate)

The use of wastewater to fertilize areas in agriculture is well known and wastewater is already used as a fertilizer for the production of energy plants, such as willow trees or poplars [10, 16, 41, 42]. The harvested biomass is usually burned in local power stations to generate heat or electrical energy [6]. Apart from the benefit, this simple kind of irrigation may cause new soil contaminations by enrichment of heavy metals and stable organic trace compounds from the wastewater. New solutions for the design and operation of treatment wetlands in this field of application are necessary. Numerical modeling of constructed wetlands gained increasing interest during the last years, especially for engineers. At present, there are intense activities to develop appropriate models [62]. Further research on constructed wetlands will result in a better understanding of the complex purification processes and this will result in more reliable models for design and running of constructed wetlands.

15.8 Conclusion (Summary)

Treatment wetlands are planted soil bodies flooded by wastewater. Microorganisms and plants in the soil filter have the potential to transform, mineralize and/or immobilize contaminants from the wastewater. The efficiency of biotic and non-biotic transformations is determined by the dynamics of the redox conditions, temperature, flow conditions, etc. The redox dynamics are influenced by oxygen consumption, plant physiology with regard to uptake, transport and release of oxygen by the plants into their rhizosphere, as well as the design of the wetland and the operation. Redox micro-gradients in near-root environments of the rhizosphere enable simultaneous anaerobic and aerobic processes, particularly in horizontal flow systems. Still there are gaps of knowledge, especially on anaerobic microbiological processes in horizontal flow systems. The potential of alternative pathways of microbial transformations (e.g., bacterial denitrification via anaerobic ammonium oxidation) to increase efficiency of constructed wetlands has to be investigated.

Moreover, the influence of redox dynamics on the emission of greenhouse gases, such as methane, nitrous gases, and carbon dioxide, and the interrelation with other processes in the rhizosphere of wetlands is not well understood. The understanding of the microbiological correlation of sulphur dynamics and methanogenesis is of special interest for balancing methane emission and for determining both the efficiency of sulfidogenesis for removal of heavy metals and the bioavailability of organic carbon for different beneficial heterotrophic transformations. Limitation of available organic carbon is one of the main problems for the efficiency of removal processes and further research in this field should focus on:

– Identification and quantification of plant exudates
– Effect of plant exudates on microbial activities

- Selection of additional electron donor materials to support microbial dissimilatory sulphate reduction
- Evaluation of optimum amounts of organic carbon to support sulfidogenesis and repress methanogenesis
- Toxicity of generated sulphide on plants and microorganisms
- Investigations to guarantee optimum hydraulic conductivity of the soil bodies
- Evaluation of biological and non-biological transformation of metalloids, such as arsenic.

Horizontal flow subsurface treatment wetlands are particularly useful for the removal of trace chemicals, such as pharmaceuticals, because of the well-known high biodiversity and the usually long hydraulic retention times in the rhizosphere. The knowledge of fundamental removal processes in this field of application is still insufficient, and design and operation of efficient systems is a challenge for scientists and engineers. The necessary interdisciplinary basic research is lacking mainly because of high costs for the trace analytics and the high number of potential substances. Constructed wetlands are highly suitable for the successful removal of pathogenic germs from wastewater because of the heterogeneous efficacy of the planted soil filter. Predatory activities by protozoa, bdellovibrios, and bacteriophages are supposed to be of importance here. However, removal processes have not been investigated sufficiently and basic research is necessary to develop efficient systems. The development of new technological solutions by intensified basic research, in particular for the treatment of industrial wastewater, has to include the simultaneous production of valuable materials, such as energy plants for power stations. Fundamental research on redox processes in the planted soil bed is necessary to understand the heterogeneous correlations and efficacies of carbon, sulphur, nitrogen, phosphorous cycles in biofilms on root surfaces, in the near-root and root-free environments under different hydraulic conditions. Suitable methods of investigation can be developed by gradually scaling up the experimental systems under laboratory and field conditions.

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Chapter 16
Effects of Hurricane Katrina on Land Cover Within the Grand Bay National Estuarine Research Reserve in Mississippi, USA

Eric D. Evans, Yerramilli Anjaneyulu, and Paul B. Tchnouwou

Abstract Hurricane Katrina hit the Mississippi Gulf Coast on August 29, 2005 as a Category 3 hurricane at the mouth of the Pearl River, on the Mississippi/Louisiana border. Katrina is considered one of the costliest natural disasters in United States history. Grand Bay National Wildlife Refuge (GBNWR) is located in the coastal zone of Jackson County in Mississippi, and Mobile County in Alabama. The Mississippi portion of GBNWR is part of the 18,400-acre Grand Bay National Estuarine Research Reserve (NERR), which was designated in 1999. The objectives of this study were to map changes to wetland and forest habitats resulting from hurricane Katrina and to discuss the implications of changes in these habitats on biodiversity within the Grand Bay NERR. Pre- and post-Katrina subsets of the Grand Bay NERR were derived from Landsat images downloaded from The Coastal Change Analysis Program's (C-CAP) website. Unsupervised classification and change detection analysis were applied to each Landsat-derived, 3-band datasets.

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The land cover change analysis revealed that hurricane Katrina caused a decrease in evergreen forest, and the conversion of evergreen forest into grassland. The major land cover changes were due to the expansion of open water. The increase in open water caused the decrease in estuarine emergent wetlands (salt marsh habitats) and the conversion of one type of land cover into another. These land cover changes could have a profound effect on the flora and fauna located within the reserve. Remote sensing technology appears to be a valuable tool for monitoring and implementing restoration and conservation strategies by the Grand Bay NERR managers.

Keywords Hurricane Katrina • Grand Bay NERR • Remote sensing • Landsat imagery • Land cover change • Biodiversity • Ecosystems alterations

16.1 Introduction

Hurricane Katrina made landfall on the U.S. Gulf Coast on twice August 29, 2005; first in southeast Louisiana as a high level Category 3 storm with sustained wind speeds of about 112–124 mph (180–200 km h^{-1}) and second on the Mississippi coastline near the mouth of the Pearl River. On 28 August 2005 it was centered over the Gulf of Mexico where it was considered a Category 5 storm with estimated maximum sustained winds of 174 mph (280 km h^{-1}) [16]. On the basis of its barometric pressure, Katrina was the third-most intense hurricane to hit the United States; exceeding only the "Labor Day Hurricane" that hit the Florida Keys in 1935 and Hurricane Camille that hit the Gulf Coast in 1969. In addition to strong winds, the disaster was characterized by heavy rains and a devastating storm surge up to 10 meters (m) high. Katrina's intensity took a heavy toll on coastal landforms. Wide-spread tidal damage to coastal areas of all Gulf Coast states resulted in the destruction of many areas, especially in Mississippi and Louisiana. It also induced severe flooding in many coastal cities including New Orleans (Louisiana), and Gulfport and Biloxi (Mississippi) [5, 10, 22, 28]. Other devastating effects included heavy stress on populations who found themselves entrapped with no food, drinking water, and/or electricity [2, 5, 6]. The immediate impact and related socio-economic issues included over 1,800 deaths in all affected areas with about 1,600 in Louisiana and 240 in Mississippi. Also, a significant increase in the incidence of infectious diseases has been reported in Katrina-affected areas [4, 5, 22, 24]. Moreover, Katrina is considered one of the costliest natural disasters in United States history. The total economic loss from Katrina was over 125 billion US dollars [5]. Hurricane Katrina hit the Mississippi Gulf Coast as a Category 3 storm on the Mississippi/Louisiana border, with an estimated wind speed of 190 km/h (118 mph). The Mississippi Gulf Coast was particularly vulnerable to Katrina's storm surge because of its low lying, coastal plain topography and lack of effective barriers [10]. Grand Bay National Wildlife Refuge is located in the coastal zone of Jackson County, Mississippi and Mobile County, Alabama, approximately 10 miles east of

Pascagoula, Mississippi and about 20 miles west of Mobile, Alabama [21]. The Mississippi portion of Grand Bay National Wildlife Refuge is part of the 18,400-acre Grand Bay National Estuarine Research Reserve (NERR), which was designated in 1999 [8, 21]. The Grand Bay NERR's climate is characterized by having long springs, long, hot and humid summers, and mild winters. January, which is the coldest month of the year, has an average temperature of 50°F, with the average maximum temperature of 61°F and an average minimum temperature of 42°F. With an average maximum temperature of 90°F, July and August are the hottest months of the year in the Grand Bay NERR. The reserve receives substantial amounts of rainfall during the year (annual average >64 in.), with most of it occurring during the summer and July being the wettest month [8]. The land within the Grand Bay NERR is comprised of a core area consisting of approximately 12,800 acres of estuarine tidal marsh, tidal creeks or bayous, shallow, open-water habitats, oyster reefs, seagrass beds, maritime forests, salt flats, sandy beaches and shell middens. In addition, there is an adjacent buffer zone consisting of about 5,600 acres of tidal marsh, scrub shrub, pine flatwood and wet pine savanna habitats that provide additional protection for estuarine-dependent species [20]. The Grand Bay NERR is characterized by a flat topography and a low elevation just a few feet above mean sea level. The soils are utilisols that tend to be acidic and infertile, and associated with clay-like horizons that frequently prevent groundwater percolation. The dominant soil types include loamy sands, very fine sandy loams, loams, silt-loams, and an undefined series that support swamps and tidal marsh. The soils are further classified as (a) hydric- soils that are poorly drained, with long periods of saturation (days to weeks), and moist surfaces and poor oxygenation, or (b) nonhydric soils- non-saturated, well oxygenated soils [8]. The Grand Bay NERR is located in the southeastern region of the State of Mississippi, an area that receives a substantial amount of rainfall (>64 in.). There are three groundwater sources that supply the savannas and flatwoods located within the NERR. Where loamy sands occur, the hydrology is driven by water arising from below from an apparent water table. In the case of loams, the hydrology is driven by a perched water table consisting of saturated soils resting on top of impenetrable subsurface. Episodic rainfall events create temporary perching (water tables) or ponding (saturation) without the benefit of having an impenetrable subsurface. This occurs in very fine sandy loams [8]. Freshwater flow from the Escatawpa River also drives the hydrology of various habitats located within the Grand Bay NERR. This river, which has its source in Washington County, Alabama, flows south into Mississippi and empties into a series of water bodies that form the mouth of the Pascagoula River. The Grand Bay NERR and the Escatawpa River drainage are a part of the Southeast Conifer Forest ecoregion, which is a stretch of land covering the coastal areas of eastern Louisiana to coastal Georgia. Some of the distinct terrestrial communities found in coastal Mississippi include: pitcher plant bogs, longleaf pine savannas, and bayhead swamps, all of which are found on the refuge. These areas, present within this system, are critical because they provide a variety of habitats to many migratory bird species [8]. The Grand Bay Refuge includes the following regions: the Southern Pine Hills predominantly north of Interstate 10; the Gulf Coast Flatwoods just south of Interstate 10; and the

Marsh regions in the southern portions of Jackson County. Flatwoods are characterized by various species of pine, including slash, loblolly, and longleaf. Commonly encountered hardwoods and shrubs include *Quercus nigra* (water oak), *Quercus virginiana* (live oak), *Magnolia* spp. (magnolias and bay trees), *Myrica cerifera* (wax myrtle), *Ilex* spp. (hollies), and *Cyrilla racemiflora* (titi). The flatwoods ecosystems provide important habitat for neotropical birds and wood ducks [8]. As the flatwoods transition to the marsh, a gradient effect occurs. This is characterized by the coastal area widening into floodplain swamps dominated by *Taxodium distichum* (southern bald cypress), *Nysssa aquatica* (black gum), *Carya* spp. (hickories), and *Acer rubrum,* (red maple). These bottomland hardwood swamps provide feeding and resting habitat for a variety of waterfowl, such as mallards, green-winged teal, and blue-winged teal, as well as other species. In the northernmost marshes there are isolated pockets and of freshwater marsh dominated by freshwater herbaceous plant species, such as *Pontederia* spp. (pickerel weed), *Typha* spp. (cattail), and *Sagittaria* spp. (arrowhead). Located further to the south, brackish marshes exist where tidal influence is constant. Saline marsh vegetation found along the coastal area includes *Juncus roemerianus (*black needlerush*),* and *Spartina* spp. (cordgrasses). This area supports a number of open water ducks, including canvasback, American wigeon, gadwalls, and shovelers [21]. These habitats support many important species of fish, such as speckled trout, Atlantic croaker, spot, menhaden, spotted sea trout, flounder, red drum, oysters, and several species of shrimp. Also, sea turtles, bottlenose dolphins, and manatees, on occasion, can be found in the deeper waters of the reserve. Wildlife that is present in the reserve include, wading birds, shorebirds, waterfowl, bald eagles, peregrine falcons, wild turkey, white-tailed deer, Gulf salt marsh water snakes, Mississippi diamondback terrapins, alligators, and a colony of gopher tortoises. Many species of carnivorous plants, such as pitcher plants, and orchids can be found in the higher savanna habitats [8, 21].

The objective of this study was to map changes to wetland and forest habitats within the Grand Bay NERR that have been affected by hurricane Katrina and to discuss the implications of these changes on the NERR biodiversity. We hypothesized that hurricane Katrina will induce land cover changes leading to ecosystem alterations and adverse effect on biodiversity.

16.2 Experimental Design and Methods

Two previously geo-referenced images of the Northern Gulf of Mexico including a pre-Katrina map AND post-Katrina map were downloaded from The Coastal Change Analysis Program's (C-CAP) website [6]. This is a nationally standardized database of land cover and land change information, developed using remotely sensed imagery, for the coastal regions of the U.S. C-CAP's goal is to monitor these habitats by updating the land cover maps every 5 years [22]. Landsat 5 TM Scenes used for map classification pre-and post-Katrina are presented in

Table 16.1 Landsat 5 TM Scenes used in the classification of the maps of the Northern Gulf of Mexico, pre- and post-Katrina

Landsat 5 TM Scenes			
Pre-Katrina		Post-Katrina	
Scene	Date	Scene	Date
P019r038	04/27/2005	P021r038	02/07/2006
P019r039	04/27/2005	P021r039	02/07/2006
P020r038	030/1/2005	P021r040	02/07/2006
P020r039	03/01/2005	P022r038	03/02/2006
P021r038	03/08/2005	P022r039	03/02/2006
P021r039	03/24/2005	P022r040	03/02/2006
P021r040	05/11/2005	P023r038	02/05/2006
P022r038	02/11/2005	P023r039	02/05/2006
P022r039	02/11/2005	P023r040	020/5/2006
P022r040	04/16/2005	P024r039	02/12/2006
P023r038	01/17/2005	P025r039	01/18/2006
P023r039	01/17/2005	P025r040	01/18/2005
P023r040	01/17/2005	P026r038	11/22/2005
P024r038	11/05/2005	P026r039	11/22/2005
P024r039	11/05/2005	P026r040	11/22/2005
P025r038	12/14/2004		
P025r039	12/14/2004		
P025r040	12/14/2004		
P026r038	03/11/2005		
P026r039	03/11/2005		

Table 16.1. Pre- and post-Katrina data subsets of the Grand Bay NERR were derived from the Landsat images provided by the C-CAP website using the ENVI 4.1 remote sensing software.

Unsupervised classification was done by applying the IOSDATA algorithm to each Landsat-derived, 3 band datasets. The classes produced were labeled and IOSDATA maps were changed to match original datasets using a class color map provided by the C-CAP website. Classification accuracy was determined by the ENVI 4.1 software confusion matrix. Post-classification statistics were also done with the ENVI 4.1 software. Change Detection analysis was performed using 2003 Microsoft Excel software.

16.3 Results and Discussion

Unsupervised classification and post-classification statistics of the pre-Katrina and post-Katrina Grand Bay NERR Landsat images yielded 16 land cover classes for each image and included point totals (total number of pixels), percentage of area covered, and total area covered represented in square meter (Fig. 16.1; Tables 16.2, 16.3).

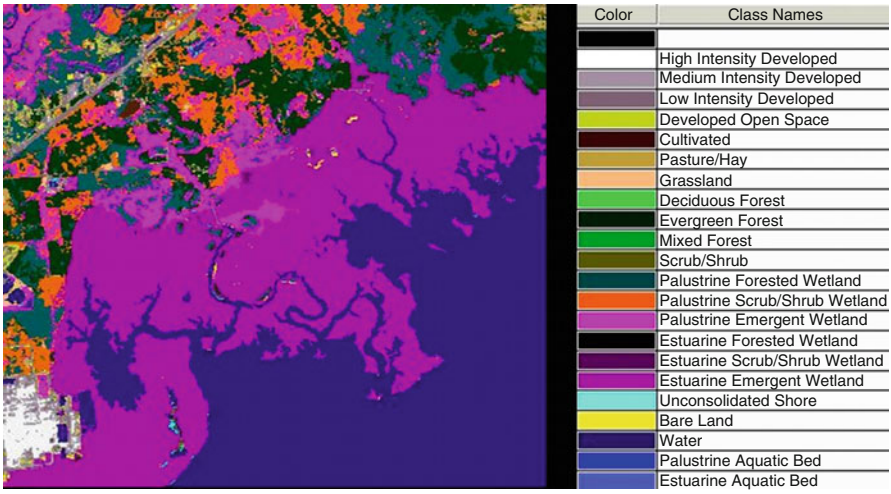

Fig. 16.1 Map of Grand Bay National Estuarine Research Reserve subset from the Landsat TM Map of the Northern Gulf of Mexico (Pre-Katrina December, 2004–April, 2005) downloaded from The Coastal Change Analysis Program website (http://www.csc.noaa.gov/crs/lca/ccap.html)

Table 16.2 Pre-Katrina class distribution summary for Grand Bay National Estuarine Research Reserve

Class distribution	Post-classification statistics[a]		
Summary	Points	Percent (%)	Area (m²)
Evergreen forest	14,195.00	9.007	12,775,500.00
Water	50,418.00	31.989	45,376,200.00
Cultivated	132.00	0.084	118,800.00
Palustrine forested wetland	13,771.00	8.738	12,393,900.00
Deciduous forest	2.00	0.001	1,800.00
Mixed forest	51.00	0.032	45,900.00
Scrub/shrub	547.00	0.347	492,300.00
Palustrine aquatic bed	3.00	0.002	2,700.00
Estuarine scrub/shrub wetland	194.00	0.123	174,600.00
Medium intensity developed	4,067.00	2.58	3,660,300.00
Estuarine emergent wetland	49,551.00	31.439	44,595,900.00
Unconsolidated shore	130.00	0.082	117,000.00
Palustrine scrub/shrub wetland	9,033.00	5.731	8,129,700.00
Bare land	1,839.00	1.167	1,655,100.00
High intensity developed	2,134.00	1.354	1,920,600.00
Palustrine emergent wetland	11,541.00	7.323	10,386,900.00

[a]The data were obtained using ENVI 4.1 remote sensing software and applying unsupervised classification to Landsat satellite images (November 2005–March 2006)

Table 16.3 Post-Katrina class distribution summary for Grand Bay National Estuarine Research Reserve

Class distribution	Post-classification statistics[a]		
Summary	Points	Percent (%)	Area (m²)
Evergreen forest	14,150.00	8.978	12,735,000.00
Water	51,086.00	32.413	45,977,400.00
Cultivated	132.00	0.084	118,800.00
Palustrine forested wetland	13,623.00	8.644	12,260,700.00
Deciduous forest	2.00	0.001	1,800.00
Mixed forest	51.00	0.032	45,900.00
Scrub/shrub	553.00	0.351	497,700.00
Estuarine scrub/shrub wetland	193.00	0.122	173,700.00
Low intensity developed	2,830.00	1.796	2,547,000.00
Estuarine emergent wetland	48,932.00	31.047	44,038,800.00
Class 11	–	0	–
Palustrine scrub/shrub wetland	8,910.00	5.653	8,019,000.00
Medium intensity developed	1,879.00	1.192	1,691,100.00
Palustrine emergent wetland	11,728.00	7.441	10,555,200.00
Unconsolidated shore	123.00	0.078	110,700.00
High intensity developed	1,595.00	1.012	1,435,500.00

[a]The data were obtained using ENVI 4.1 remote sensing software and applying unsupervised classification to Landsat satellite images (November 2005–March 2006)

The results of this study show that the Grand Bay NERR sustained increases in open water, and decreases in evergreen forest, palustrine scrub/shrub wetland, and bare land. The reserve also sustained a loss of estuarine emergent wetland, and a total loss of palustrine aquatic bed land cover types. Grassland was not identified in the Grand Bay NERR class distribution summary. There was a decrease in estuarine scrub/shrub wetland as well as in unconsolidated shore.

Land cover change analysis of the Grand Bay NERR (Tables 16.4, 16.5) indicate that between April 2005 and March 2006, the land cover changes included the expansion of open water (601,200 m^2, 0.424%); the decrease in evergreen forest (−40,500 m^2,−0.029%), palustrine forested wetland (−133,200 m^2,−0.094%), palustrine scrub/shrub wetland (−10,700 m^2,−0.078%) estuarine scrub/shrub wetland (−900 m^2,−0.001%), estuarine emergent wetland (−557,100 m^2,−0.392%), unconsolidated shore (−6,300 m^2,−0.004%); and the total loss of palustrine aquatic bed (−2,700 m^2) and bare land (−1,655,100 m^2). There were also increases in scrub/shrub (5,400 m^2, 0.004%), and palustrine emergent wetland (168,300 m^2, 0.118%). The classification accuracy for the Grand Bay NERR was 98.939% with a Kappa Coefficient = 0.9859 (Table 16.6).

Stanturf et al. [30] stated that damage to coastal habitats comes from three primary features of the hurricane including rainfall, storm surge, and winds. The combination of storm surge, a rise in sea level, and high tide can increase the amount of damage to coastal forest habitats. The decrease in evergreen forest can be attributed to the strong winds of hurricane Katrina. This decrease could be affected by

Table 16.4 Grand Bay Ecosystem Change Detection. The results contained in this table were obtained by using the post-classified pre-Katrina (December 2004–April 2005) and post-Katrina (November 2005–March 2006) Grand Bay NERR images. 2003 Microsoft Office Excel software was used to do the change detection analysis. Pre-Katrina, post-Katrina and change detection data are expressed square meter of surface area (Numbers in parenthesis denote a decrease)

	Pre-Katrina	Post-Katrina	Change detection
Class	Area (m²)	Area (m²)	Area (m²)
Evergreen forest	12,775,500.00	12,735,000.00	(40,500.00)
Water	45,376,200.00	45,977,400.00	601,200.00
Cultivated	118,800.00	118,800.00	–
Palustrine forested wetland	12,393,900.00	12,260,700.00	(133,200.00)
Deciduous forest	1,800.00	1,800.00	–
Mixed forest	45,900.00	45,900.00	–
Scrub/shrub	492,300.00	497,700.00	5,400.00
Palustine aquatic bed	2,700.00		(2,700.00)
Estuarine scrub/shrub wetland	174,600.00	173,700.00	(900.00)
Medium intensity developed	3,660,300.00	1,691,100.00	(1,969,200.00)
Estuarine emergent wetland	44,595,900.00	44,038,800.00	(557,100.00)
Unconsolidated shore	117,000.00	110,700.00	(6,300.00)
Palustrine scrub/shrub wetland	8,129,700.00	8,019,000.00	(110,700.00)
Bare land	1,655,100.00		(1,655,100.00)
High intensity developed	1,920,600.00	1,435,500.00	(485,100.00)
Palustrine emergent wetland	10,386,900.00	10,555,200.00	168,300.00
Low intensity developed		2,547,000.00	2,547,000.00

factors such as tree height and diameter, topography, insect and disease infestation, drought, and prior site history [23, 30]. The removal of trees by hurricane Katrina was also responsible for the conversion of forest to scrub/shrub and grassland.

The major factor affecting land cover change in the Grand Bay NERR as a result of hurricane Katrina was the expansion of open water. Hurricanes can add to the natural progression of sea level rise due to global warming and can likely enhance saltwater intrusion into coastal habitats. The expansion of open water was responsible for the loss of bare land and grassland (inundation), estuarine emergent wetlands (inundation, erosion, land subsidence) [12], palustrine aquatic beds (increased rainfall, salt water intrusion), and forest habitats (inundation, increased salinity, erosion, land subsidence) [30]. It also induced conversion of forest stands into palustrine forested wetlands (inundation) and of palustrine emergent wetlands, palustrine scrub/shrub, palustrine forested wetlands into estuarine emergent wetlands (inundation, increased salinity).

Hilbert [12] studied land cover change at the Grand Bay NERR and reported a 5% increase in open water and a 6% decrease in herbaceous wetland that during the years 1974–2001. Between the years of 1974 and 1991 there was a 3% increase in open water and a 4% decrease in herbaceous wetland, and between the years 1991 and 2001 there was a 2% increase in open water and 2% decrease in herbaceous wetland. Data from our study are in support Hilbert's findings, showing the same

Table 16.5 Grand Bay Ecosystem Change Detection. The results contained in this table were obtained by using the post-classified pre-Katrina (December 2004–April 2005) and post-Katrina (November 2005–March 2006) Grand Bay National Research Reserve images. 2003 Microsoft Office Excel software was used to do the change detection analysis. Pre-Katrina, post-Katrina and change detection data are represented in percentages (%)

	Pre-Katrina	Post-Katrina	Change detection
Class	Percent (%)	Percent (%)	Percent (%)
Evergreen forest	9.007	8.978	−0.029
Water	31.989	32.413	0.424
Cultivated	0.084	0.084	0
Palustrine forested wetland	8.738	8.644	−0.094
Deciduous forest	0.001	0.001	0
Mixed forest	0.032	0.032	0
Scrub/shrub	0.347	0.351	0.004
Palustine aquatic bed	0.002		−0.002
Estuarine scrub/shrub wetland	0.123	0.122	−0.001
Medium intensity developed	2.580	1.192	−1.388
Estuarine emergent wetland	31.439	31.047	−0.392
Unconsolidated shore	0.082	0.078	−0.004
Palustrine scrub/shrub wetland	5.731	5.653	−0.078
Bare land	1.167		−1.167
High intensity developed	1.354	1.012	−0.342
Palustrine emergent wetland	7.323	7.441	0.118
Low intensity developed		1.796	1.796

Table 16.6 Grand Bay National Estuarine Research Reserve Confusion Matrix. ENVI 4.1 remote sensing software was used to assess producer and user accuracy in classifying land cover within the Grand Bay NERR. Overall Accuracy = 98.9309% (151298/152933 pixels identified) with a Kappa Coefficient = 0.9859

	Prod. Acc.	User Acc.	Prod. Acc.	User Acc.
Class	(Percent)	(Percent)	(Pixels)	(Pixels)
Evergreen forest	99.87	99.56	14132/14150	14132/14195
Water	98.73	100	50415/51062	50415/50415
Cultivated	100	100	132/132	132/132
Palustrine forest	99.38	98.32	13539/13623	13539/13770
Deciduous forest	100	100	2//2	2//2
Mixed forest	100	100	51/51	51/51
Scrub/shrub	96.75	97.81	535/553	535/547
Estuarine scrub/shrub	100	99.48	193/193	193/194
Medium intensity developed	64.77	97.59	1217/1247	1217/1247
Estuarine emergent wetland	100	98.75	48931/48932	48931/49551
Unconsolidated shore	100	94.62	123/123	123/130
Palustrine scrub/shrub	99.98	98.62	8908/8910	8908/9033
High intensity dev.	99.18	74.75	1582/1595	1582/2125
Palustrine emergent wetland	98.38	99.97	11538/11728	11538/11541

trend in the expansion of open water coinciding with a loss of herbaceous wetland habitats or a conversion from one type of habitat to another. These losses or conversions of habitats can have a profound effect on the local/resident flora and fauna.

A study done by Hoeppner et al. [13] in Lake Maurepas, Louisiana reported that coastal swamps that are subject to saltwater intrusion and affected by drought, had unprecedented soil salinities that resulted in cumulative tree mortalities of up to 85%. They also pointed out that most of the Lake Pontchartrain coastal swamps were deteriorating and would eventually be lost to the increase in open water without restoration efforts. The Grand Bay NERR supports a wealth of wildlife that includes wild turkey, white-tailed deer, wading birds, waterfowl, shorebirds, bald eagles, peregrine falcons, Gulf salt marsh water snakes, Mississippi diamondback terrapins, alligators, and a colony of endangered gopher tortoises. Also found in the reserve are many species of carnivorous plants, such as pitcher plants, and orchids can be found in the higher savanna habitats [8, 20]. Various species of pine, including longleaf, slash, loblolly, and makeup the flatwood habitats found in the reserve. Live oak, water oak, magnolias and bay trees, wax myrtle, and hollies are the most commonly encountered hardwoods and shrubs. This ecosystem also provides very important habitats for neotropical birds and wood ducks [21, 30]. A gradient effect occurs as the flatwoods transition to the marsh. This area is associated with floodplain swamps dominated by red maple, black gum, hickories, tupelo, and southern bald cypress [21, 30]. These hardwood swamps provide feeding and resting habitat for various species of waterfowl, such as, green-winged teal, blue-winged teal, and mallards as well as other species. Pockets and of freshwater marsh dominated by freshwater plant species, such as cattail, pickerel weed, and arrowhead are found in the northernmost marshes. Salt marsh vegetation found along the coastal area includes black needlerush, and cordgrasses. A number of open water ducks, including canvasback, American wigeon, gadwalls, and shovelers make use of this area as feeding and nesting grounds [21]. With the loss or conversion of important coastal habitats and the plant species such as mash grasses, shrubs and deciduous and pines trees, comes a loss in animal species that use these habitats as food sources, breeding and nesting grounds, and places of refuge. These land cover changes could trigger a bottom-up trophic cascade that could have huge impacts on the wildlife and the commercial and recreational fisheries enterprises that operate within the reserve. Piazza and Peyre [25] found that vegetative loss in freshwater marshes in the Brenton Sound, Louisiana, due to hurricane Katrina was extensive, and elevated salinity persisted for almost 6 months. Their results also showed an increase in nekton density and biomass in these areas increased significantly, and the nekton community shifted from one of tidal freshwater/resident species to one containing brackish/migrant species. However, the nekton community had shifted back to freshwater/resident by spring 2007. Another study by Mazaris et al. [18] showed that the loss of breeding habitat for loggerhead sea turtles (*Caretta caretta*) due to land cover conversions such as, vegetative encroachment onto beaches and sea level rise, could result in density dependent factors (increased predation, disease) that may affect hatchling viability. It has also been projected that the conversion of grassland to forest land will cause the increase in one forest species and the decrease in two open

habitat species in parts of Western Europe [3]. A Post-Katrina study of Nile tilapia, an invasive species to the Mississippi Gulf Coast revealed that Nile tilapia, due to damage and flooding associated with hurricane Katrina, expanded its range into an aquaculture facility and a nearby bayou [28]. Rodgers and Murrah [27] investigated the impact of Hurricane Katrina on coastal vegetation of the Weeks Bay Reserve in Alabama and reported that this hurricane negatively affected the Reserve's vegetation due to changes in estuary salinity and exposure to high intensity winds. In a similar study, Faulkner et al. [7] reported a catastrophic destruction of the floodplain forests of the Pearl River as a consequence of Hurricane Katrina; with a significant impact on the ecology of local plant species and migratory birds. Melloul and Collin [20] linked Hurricane's frequency and intensity to global warming, which will eventually lead to higher and potentially more damaging waves. Also due to the complete inundation and the enormous amount of soil erosion sustained to the barrier islands off the coast of Mississippi, there is less coastal protection for these coastal habitats [10]. Therefore, assessing the damage caused by hurricane Katrina and its effects on these coastal environments, will better serve managers of the Grand Bay NERR as they implement conservation and restoration programs within the reserve.

Remote sensing has proved to be a valuable tool in the monitoring of land cover and land use changes in sensitive or protected areas of the world. Oswalt and Oswalt [23] used remotely sensed data to compare and contrast hurricane related damage in Mississippi 2 years following hurricane Katrina. Their study addressed four questions concerning damage caused by the hurricane: (1) do inventory data substantiate damage zone estimates made using remotely sensed and climate data following Hurricane Katrina? (2) are softwoods or hardwoods more susceptible to hurricane damage and does that susceptibility change as distance from landfall increases? (3) what are the primary stand-level factors influencing vulnerability to damage, based on observed damage and measured stand characteristics? and, (4) is tree-level damage related to tree species, and do damage types (bole, branch, lean, or windthrow) differ by species? Through their study, the investigators found that: (a) hurricane damage differed between the different developed zones; (b) they were not able to accept the hypothesis that hardwoods experienced more damage than softwoods and that the likelihood of hardwood damage increased with increasing distance from the zone of impact; (c) the data suggested that tree characteristics, such as tree height and diameter consistently influenced damage probabilities; and (d) that wind events combined with landscape-level attributes, insect and disease infestation, drought, prior site history, that affect stand and tree condition and contribute to an element of uncertainty that makes damage prediction highly variable and very difficult. A study by Bayarsaikhan et al. [1] used remote sensing to monitor land cover change in Hustai National Park, Mongolia, an ecological hotspot containing rare species. Land cover types were classified and their temporal changes were evaluated using Landsat MSS TM/ETM data between 1994 and 2000. The classification data revealed several changes between the 1994 and 2000 maps: (1) There was a 166.5 km^2 increase in mountain steepe and a 12 km^2 increase in sand dune; (2) Agricultural areas and degraded areas affected by human activities decreased by

46.1 and 194.8 km^2, respectively, during this period. These areas were replaced by mountain steepes; (3) Forest area during this period was fragmented and included a loss of about 400 ha. Other studies have been conducted that have involved the use of remote sensing to monitor land cover change in sensitive or protected areas. In 2000 a Panel on the Ecological Integrity of Canada's National Parks concluded that basically all of Canada's national parks are under some kind of internal or external stress. In response to the Panel's conclusion, Fraser et al. [9], along with the Parks Canada Agency set out to use Earth Observation technology to be the basis for a Park Ecological Integrity Observing System (Park-EIOS), a national parks ecological integrity (EI) monitoring program. The Park-EIOS incorporates coarse filter EI indicators that correspond to landscape pattern, succession and retrogression, net primary productivity, and focal species distributions within parks and their surrounding park ecosystems. The investigators used a change detection design developed for Park-EIOS, identified as Automated Multi-temporal Updating through Signature Extension (AMUSE). AMUSE was used on six pilot parks using time series of Landsat TM/ETM+imagery from 1985 to 2005. These investigators concluded that the AMUSE method was effective in capturing land surface changes where reflectance changed sufficiently to alter land cover class. Changes to vegetation that were less dramatic, such as those caused by insect defoliation and drought, were usually not detectable due to the design requirement that the method produce low levels of commission error. The Park-EIOS started operational use in 2008. Data related to changes in the amount of old-growth forests was derived from the 1990 to 2005 land cover time series and will serve as one of the first inputs into the 2008 State of the Park Report. Liu et al. [17] used remotely-sensed land cover data, along with a biochemical model to estimate modern and future ecosystem carbon trends. The investigators applied the General Ensemble Biogeochemical Modeling System (GEMS) for the Laurentian Plains and Hills eco-region in the northeastern United States for the period of 1975–2025. The land cover changes were detected on 30 randomly located 10 km by 10 km sample plots, forest stand-replacing events being of particular interest, and were assimilated by GEMS for biogeochemical simulations. Forest type, forest age, forest biomass, and soil C, based on the Forest Inventory and Analysis (FIA) data and the U.S. General Soil Map (STATSGO) data was determined using the Monte Carlo process, a forest simulation unit. The results of this study showed that on average, forests of the Laurentian Plains and Hills eco-region have been sequestrating 4.2 teragram (Tg) of carbon per year, including 1.9 Tg of carbon removed from the ecosystem as the consequences of land cover change. Wang et al. [32] implemented a multiscaled protocol to detect and monitor land cover change and in and around National Parks and ten segments of the Appalachian National Scenic Trail (AT) in the northeastern United States. Landsat imagery from 1970 to 2002 was used, along with recent ground based photographs to evaluate changes within the parks and buffer areas within 0.5, 1, and 5 km of the parks. Through their studies the investigators concluded that all of the parks studied, except for one park in AT (Maine), showed an increase in urban land (172% and 181% within 0.5 and 1 km, respectively, of the park boundary or trail centerline) and conversely, a decrease in forested area (5% and 6% within 0.5 and 1 km, respectively,

of the park boundary or trail centerline). More loss of forest occurred near the parks (18%) than the trail segments (2%). Some investigators have been using Landsat data to monitor changes in U. S. National Forests (NFs). Huang et al. [14] used time series stacks of Landsat images (LTSS) spanning from 1984 to 2006 to evaluate the dynamics of seven NFs in the eastern United States, which included the De Soto NF, the Talladega NF, the Francis Marion NF, and the Uwharrie NF in southeastern U.S., and the Chequamegon NF, the Hiawatha NF, and the Superior NF in northern U.S. A vegetation change tracker (VCT) algorithm was used to analyze each LTSS to map forest disturbance. The disturbance year maps that were generated from the study revealed that although each of the seven NFs consisted of 90% or more forest land, significant portions of the forests have been disturbed since 1984. The disturbances that were mapped accounted for about 30–45% of total land area in the four NFs in southeastern U.S. and about 10–20% in the three NFs in northern U.S. Buffer zones surrounding the NFs usually had higher rates of disturbance, which varied considerably over time. MacAlister and Mahaxay [19] used remote sensing to map wetland changes in the Mekong River Basin, which is considered the second most species rich river basins in the world. In response to the need for updated maps of this river basin, the Mekong River Commission (MRC) took methods necessary to produce new coverage maps. Field surveys were conducted at five pilot sites covering a range of typical wetland habitats to supply data for a supervised classification of Landsat ETM images from the existing MRC archive. These images were analyzed using ERDAS IMAGINE and applying Maximum Likelihood Classification. Field data were reserved to apply formal accuracy assessment to the final wetland habitat maps, with resulting accuracy ranging from 77% to 94%. These new maps are now being used at a Provincial and National level in three countries for resource and conservation planning and management applications, that also include the designation of a Ramsar wetland site of international importance. Huang et al. [15] used Landsat images acquired since the 1970s to map land cover and land cover change between 1989 and 2000, and to estimate the forest area of Paraguay's Atlantic Forest Eco-region (PAFE). The investigators found that in 1973 the PAFE was 73.4% covered by forest. As of 1989, only 40.7% of the PAFE was covered by forest and by 2000 only 24.9% of this area was covered by forest. This study also revealed that the major causes of deforestation were private land owners who were responsible for 80% of forest removal, and settlers who were responsible for the remaining 20% of the deforestation that occurred during this time period. Another study that involved the use of remote sensing to detect land cover change was done by Zhoua et al. [33] to assess the Grain for Green (GfG) program's effect on the mean annual vegetation cover in the Shaanxi province, China. The objective of the GfG program, which was funded by the Chinese government, was to increase the forest cover on steep slopes in the landscape by planting trees and sowing grasses on former cropland. SPOT VEGETATION imagery from 1998 till 2005 was used to calculate NDVI-values in four counties with different rainfall regimes. The results showed a significant increase of the vegetation cover on farmland in the northern part of Shaanxi province, while in the southern part of the province, which has a more humid climate and large areas of irrigation

farmland, no significant increase of the vegetation cover could be detected. Gibbes et al. [11] used remote sensing and habitat fragmentation analysis to investigate land cover change in two protected wetlands, Caroni and Nariva, in Trinidad. The results of the study revealed that Caroni showed a shift toward anthropogenic land cover types (urbanization, developed space) within the protected park. Further findings showed a decrease in mean NDVI and greenness values which suggest a decrease in healthy vegetation. Classification data from Nariva indicated a return of natural land cover, but the continuous measures of land cover change indicated the opposite.

16.4 Conclusion

The major aim of this study was to detect land cover changes that occurred within the Grand Bay NERR, as a result of hurricane Katrina. The land cover change analysis revealed that the winds from hurricane Katrina cause the decrease in evergreen forest, and the conversion of evergreen forest into grassland. However, the major land cover changes were due to the expansion of open water. The increase in open water caused the decrease in estuarine emergent wetlands (salt marsh habitats) and the conversion of one type of land cover into another. These land cover changes could have a profound effect on the flora and fauna located within the reserve. This study shows that remote sensing is a valuable tool for reserve managers in monitoring and implementing restoration and conservation strategies within the Grand Bay NERR.

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Chapter 17
Protecting and Improving Water Quality in Vojvodina

Vesela Radovic

Abstract Ratification of the Stabilization and Association Agreement of the European Union by the European Parliament have encouraged Serbia to be more proactive in achieving the remaining claims in this process. In the area of water management Serbia recognized the most demanding investment directives which are to be implemented. Numerous studies show that the major problem of Serbia is the way to ensure adequate water management. Problems related to water management are more obvious in everyday life. The Province of Vojvodina is in worse situation considering assessment that 40% of population drinks poor quality water which contains some toxic substance (like arsenic). The author presents the results of the research of implemented water quality in Vojvodina, which is related to the city of Novi Sad, and in other parts of the Province. Analysis results confirmed a legitimate concern for the quality of drinking water, excessive pumping of underground water and a small percentage of pollutants which have wastewater treatment systems, insufficient development of the sewerage network in the settlements and so on. In some towns, water supply problems have a long history due to lack of financial resources to solve them. In the light of the global economic crisis, we could not expect significant improvement in the nearest future. The aim of the paper is to show that unless there are urgent changes in the legislative framework of Serbia, at the time when all indicators of social development are rather problematic, expectation of any improvement in the nearest future represent a big issue. Vojvodina and Serbia have also numerous "hot spots" which need more help from international community in implementing positive practices and the provision of financial resources. Only an integral approach will ensure that Serbia does not continue

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endangering water resources and public health that may in certain circumstances constitute a threat to neighboring countries.

Keywords Water management • Drinking water • Waste water treatment plant • Sewage

17.1 Introduction

The Autonomous Province of Vojvodina (APV) has 27% of total population of the Republic of Serbia, according to the 2002 Census. It is located in the northern part of the country. Its capital and largest city is Novi Sad, with over 300,000 inhabitants. Vojvodina has six official languages, and there are more than 26 ethnic groups in the region. The Province of Vojvodina cover an area of 21,506 km^2. Vojvodina has its own Government. It is executive organ of the Province, accountable to the Assembly of the APV. In accordance with the Constitution of the Republic of Serbia environmental protection and sustainable development are guaranteed [1]. Article 183 of the Constitution defines the competences of autonomous province, empowers the province to organize and provide for environmental protection on its territory in accordance with the Law. Article 190, describes the competences of the municipality as a local self-government unit, empowers the municipalities to organize and provide for environmental protection, through its bodies. In the past years the Government the APV has undertaken a wide range of intensive activities aimed at regaining the competencies of the Province. Certain jurisdictions in the field of environmental protection were transferred to Vojvodina in 2002 [2]. Through the Provincial Secretariat for Environmental Protection and Sustainable Development, APV is responsible for affairs related to: development of programs for environmental protection and sustainable development on the territory of the province and establishing measures for its implementation, monitoring of the current state and information subsystem, forests and waters, inspection control in all aspects of environmental protection and other matters relevant for the province. The Province is also responsible for strategic assessment of plans and programs and for issuing integrated permit for facilities and activities on the territory of the province. Provincial Secretariat for Agriculture, Water Management and Forestry [3] has the jurisdiction over water management on the level of APV. Water management activities are performed by Public Water Management Company (PWMC) "Vojvodina vode." The "in situ" tasks related to water management are performed by water management companies and the activities related to municipal water supply and sewerage are performed by a great number of public utility companies. In addition to mentioned agency, special organizations within the state administration and local governments and institutions and public companies that operate outside of environment and water sector also perform some tasks in this field. Among special organization Republic Hydro meteorological Service of Serbia should be noted as well as a network of Public Health Institutes. Despite many different water management activities

situation are so far to be evaluated as satisfactory, the quality of drinking water in many towns is inappropriate, as well as quality of surface waters and it is proved by many scientific research, documents and independent reports. The Environmental Protection Agency (Agency) functions as an administrative body of Ministry of Environment and Spatial Planning (MESP) and is obliged to prepare the Annual Report about environmental state. In the Report for 2009 one of the conclusion is "the quality of surface water in Vojvodina is bad. The Region of Great Backa Canal is European environmental hot spot" [4]. Many of the most polluted flows, old and navigable Begej River and Canal Vrbas-Becej are situated in Vojvodina. The research performed during 2001–2006 about Vojvodina water quality, analysis the data provided by the Hydrometeorological service of Republic of Serbia in final result showed a general deterioration of quality expressed using Water Quality Index (WQI) for the observed period. River Tisa and Tamis as well as Hydro system Danube Tisa Danube (HS DTD) decreased in quality. The quality of water during 2001–2002 was classified as good (74–73 WQI), since in 2003–2006 the quality start to be in group bad (65–71 WQI) [5]. According to many official reports and research supplying of drinking water is the worst in Vojvodina, as well as its quality [6]. Vojvodina has great water resources in flows of river Danube, Sava, Tisa and developed canal system Danube-Tisa-Danube. These resources are important factor in the goal of generally accepted National Sustainable Development Strategy [7]. Despite the quantity of water Vojvodina has severe problems in providing adequate quality of drinking water. It does not meet physicochemical and bacteriological drinking water standards; 67% of water samples do not meet the requirements. Reserves of underground water in the Autonomous Province of Vojvodina are polluted with heavy metals. School children appear to be particularly at risk, since 90 schools in the Province of Vojvodina have no water supply facilities, and in 508 schools the bacteriological quality of water was found to be unsatisfactory [8].

17.2 Water Quality in Vojvodina

The amount of water available for use depends on its quality and the availability of water dictates where we can live, build cities, and create industry. It is well known that water quality can be damaged by any number of pollutants in the air, on land, or from other water supplies [9]. The greatest problem detected in Vojvodina is to ensure safe drinkable water for the inhabitants. There are 465 settlements in Vojvodina, out of which 69 do not have piped water. Organized water supply is present in 372 settlements. Water supply through pipe lines is present in 307. There are 157 pipe lines under the supervision of public utility companies which supply 222 settlements. 150 pipelines are supervised by local authorities. There are problems both in rural and urban areas of the Province. People living in rural areas get their drinking water from three different sources: official piped water systems owned and operated by the municipality; private piped systems built and operated by the communities themselves, and private wells.

17.2.1 Quality of Drinking Water in Some Vojvodina Districts

Drinking water can be contaminated from a variety of sources and by variety of contaminants. Since very early times, people have created ways to remove debris and other contaminants from drinking water to make it look and taste better [9]. The quality of drinking water in Vojvodina is the most important factor considering current requirements and need of population in this region. In Vojvodina region slow gradual improvement in water quality is not matched by an improvement of the microbiological quality of water. That means that water still contains coli form bacteria, but also those which cause intestinal infections and Escherichia coli. Apart from the poor quality of drinking water, a separate problem in Vojvodina is the deficiency of quality fresh water wells. More than 75% of wells in Vojvodina used for public supply systems are underground waters [10]. Groundwater protection from pollution is especially important since groundwater in Vojvodina is a major source of drinking water. The row water too often does not meet hygiene-sanitary regulation, and need to be purified, and adequately treated before use. Only one third of taken underground water is treated in drinking water plants. The treatments applied for this purpose are mainly projected for decreasing the concentration level of iron, manganese and ammonia. The major part of these facilities was not designed to remove highly toxic arsenic from underground water, and for that reason the concentration of arsenic in drinking water at the great part of Vojvodina is higher than allowed (above 10 μg/l) according to legislative [11]. Only in Subotica there are drinking water plants which use the proper treatment for removing arsenic from underground water. The astonishing fact is that treatment facilities in other municipalities do not perform any additional and necessary water treatment except the basic chlorine disinfection. From that point of view it is obvious why drinking water is such of bad quality. This problem is particularly acute in the region of Banat, where waters are rich humid substances, ammonia, iron, manganese, sodium and the extremely toxic arsenic.

At the average, around 270 l/day is taken daily out of underground waters in Vojvodina, and through public water pipes, around 245 l/person/day. There is a problem of loss in the water supply system ranging from 30% to 50% [12]. More precise data are not available because of the lack of measuring in the system. The most part of the data is based on measuring water consumption, and on the estimation of the quantity of repressed water. According to the World Health Organization (WHO) water and health safety of drinking water ranked among the 12 basic indicators of health of a population. It could be presume that insufficient quantity and quality of waters joint with others environmental indicators could be a cause of permanent migration in some parts of Vojvodina. That fact could be presumed from analyses of health status of population perfomed in Middle Banat District. The population decreased since 1971 according to the data provided from Statistical Office of the Republic of Serbia (SORS). In 2009 decreasing was approximately about 7, 47%, total number of inhabitants who left was 15,000. The researches which were implemented proved that drinking water does not meet sanitary standards. The health

Table 17.1 Results of research of sanitarian accuracy (physical-chemical parameters) of row water in Vojvodina districts, data 2006 (Source: B.Dalmacija and others, part of official report for 2010)

	Analysis of physical-chemical parameters		
District	The total number of samples	% cause of inaccuracy	The cause of inaccuracy
South Backa	790	77	Color, $KMnO_4$ consumption, electrical conductivity, ammonia, arsenic, chloroform, nitrites, iron, manganese, turbidity, odor, Mg, pH, chloride, trihalomethanes, Na, phosphate, Ni, fluor, suspended solid particles
West Backa	132	92	Color, turbidity, iron, $KMnO_4$ consumption, manganese, ammonia, arsenic, chloride, evaporation rest
North Backa	493	94	Color, odor, turbidity, ammonia, iron, arsenic, manganese, nitrates, kalium, Al, mineral oils
North Banat	412	98	Color, turbidity, $KMnO_4$ consumption, electrical conductivity, arsenic, odor, chloride
Middle Banat	624	100	Color odor, turbidity, electrical conductivity, $KMnO_4$ consumption, ammonia, phosphate, nitrites, chloride, arsenic
South Banat	43	88	Color, turbidity, ammonia, iron, $KMnO_4$ consumption, arsenic, electrical conductivity, chloride, odor
Srem District	360	25	Manganese, ammonia, color, nitrites, iron, arsenic, turbidity

status is unsatisfied due to microbiological; neither physicochemical requirements (samples did not meet any regulation for basic A volume) [13]. The quality of underground water used for water supply of population is the best in the Srem area and South East Banat, and is the worst in the area of Middle Banat, North Banat and West Backa. The evidence for this statement is high percentage of incorrectness of samples in meeting hygiene-sanitary standards in settlements which use underground water (high concentration of natural organic matters). In Kikinda and Zrenjanin municipalities the percentage of incorrectness sample reaches whole 100% [14] (Table 17.1).

Serbia is situated in the region where the impact of global climate change is visible. The future projections for climate change suggest a negative influence on the water balance of Serbia [15]. During the last year, Serbia was exposed to great losses due to extreme weather conditions. The loss was four times more than in the previous year. Due to heavy rain population in some parts of Vojvodina was faced with floods and with increased level of underground water. It caused pollution of wells in the region after waters from septic tanks and waters from wells were mixed. The State declared emergency state in Kikinda municipality and banned use of

waters. The chlorine disinfection was necessary but emergency services could not do it because of long period of heavy rain. They had to wait for appropriate weather conditions. The problem was obvious during the summer 2010 and after that in winter months population was again faced with the same situations [16].

In small town Odzaci, district of West Backa water was banned for use 13 months because of threadworms, nematodes [17]. Vojvodina's Fund for capital investment provided financial means for settled two eco-tap for supplying population and daily water supply from water tank from neighboring town Sombor was organized. After 13 months Republic Sanitary Inspections allowed the use of drinking water [18]. There were and still are many controversial issues about this problem. Many experts on first place put neglected technological solutions and political will just to build water factors without considering local circumstances and scientific facts which kind of water treatment is needed. From that point even after cleaning from nematodes, the troubles are still present (high hardness and high concentrations of toxic bromates). There are same rumors about increasing rate of malignant disease among population, but it is not followed by official statistic.

The Zrenjanin municipality is perhaps a unique case in whole Europe. The provincial health inspectorate has banned the use of Zrenjanin water for drinking and food preparation because of increases in the arsenic levels of underground springs [19]. Despite those fact, the City Council in Zrenjanin allows the PU "Water and Sewage" to increase price of water since 1. October 2010. There are 295 employees in the Company and they work without any profits (the loss of this utility was more than half million euros). The banishment of using water lasts more than 6 years and for that banishment citizens became "immune" and government parties only announced the building of drinking water treatment plant during the period of elections. The state claims that there are not sufficient financial means for final solution of the problem. If we take into consideration that Serbia is one of the most corrupted state, and only in process of public procurement disappears about million of euros there is a reasonable doubt for that claim.

Management of health safety of drinking water in our country is governed by the legal basis based on the recommendations of the World Health Organization, European Union directives and international standards of quality and many national regulations. Sampling and analysis of drinking water can be performed only by health institutions that are authorized by Ministry of Health. These institutions are in the system of Public Health Institute network that is distributed across the Republic of Serbia according to territorial principle. At the territory of APV it is a competence of Institute of public Health of Vojvodina.

17.2.2 Quality of Drinking Water in Novi Sad

Public company Water Works Novi Sad has a task to ensure safe drinking water in Novi Sad and its settlements. Public water system must provide water treatment, monitor drinking water to ensure proper quality in treatment plant, and provide

public information of contaminant problems and reasons for interruption in supplying. It would be interesting in short to highlight a history of pollution of drinking water source in Novi Sad. In October 1999 UNEP presented its findings in the report entitled: "The Kosovo Conflict-Consequences for the Environment and Human Settlements." This drew a number of important conclusions on the post–conflict situation in the region and in particular singled out four heavily polluted environmental "hot spots" and among them the city Novi Sad for immediate humanitarian assistance [20]. The spillage of large quantities of oil caused by the military intervention in 1999 caused that oil refinery became a source of longer-term and ongoing pollution. As the refinery is constructed on back-filled sand, spilled oil easily reaches the shallow groundwater table. Because the Ratno Ostrvo wells are located downstream, in the vicinity of the refinery, immediate measures for protecting the wells were seen as Novi Sad's highest priority. Working in close cooperation with Novi Sad Water Works, UNEP applied a precautionary approach and immediately initiated construction of a hydraulic barrier to prevent the migration of contaminated groundwater from the refinery area towards the drinking-water wells. The Ratno Ostrvo drinking water wells is in the area between Novi Sad oil refinery and the Danube River. The wells constitute approximately 40% of the water supply intake for the city of Novi Sad which does not have alternative water intake sources for equivalent volumes. Since November 2000, monitoring of groundwater quality has been carried out by a partnership between UNEP, Novi Sad Water Works, the oil refinery, the University of Novi Sad/Institute of Chemistry and etc. After many activities in April 2000 UNEP Feasibility Study showed that Novi Sad is no longer considered as an "environmental hot spot" [21]. There is a question: Does all above mentioned mean that groundwater is not now jeopardized by pollution, the answer would be: Not at all. As city of Novi Sad uses groundwater as drinking water source from Danube alluvium from abstracted at three sites (Strand, Petrovaradinska ada and Ratno ostrvo) pollution is present significantly, now in peaceful time [22]. The City Administration for Municipal Affairs of Novi Sad is aware of the importance of public health care. Health care control of drinking water available to the final consumer in the city of Novi Sad and Novi Sad areas connected to the water, and in accordance with the legal bases [23]. The Institute for public health of Vojvodina has done the assessment and monitoring of food safety and drinking water from public water system in the city of Novi Sad in 2010. During 2010 skilled personnel of the Institute for public health of Vojvodina controlled water samples of drinking water from public water wells on the territory of the town of Novi Sad and surrounding settlements, as well as the quality of waters of natural baths. Sanitary inspection plays a very important role in surveillance of health status of water for bathing [24]. In some areas of Serbia, the Danubian endemic familial nephropathy (also known as Balkan endemic nephropathy) occurs and seems to be linked with drinking-water quality. Under the health safety of drinking water there are included microbial and physicochemical quality of drinking water with a secured source protection, health and safe supply of drinking water handling. These include: daily sampling, receiving samples, microbiological and physical-chemical analysis, and preparation of reports on sampling and testing and production of specialist opinion on the health

Table 17.2 The sanitary accuracy of water samples during the period 2007–2010 (Source www.izjzv.org.rs)

Year	% of microbiological accuracy	Percentage of physical chemical accuracy
2010	98	76
2009	97	92
2008	99	97
2007	99	96

safety of drinking water samples from 18 measurement sites from the water plant and water system PUC "Water Works" in Novi Sad and settlements: Novi Sad, Petrovaradin Stepanovićevo, Futog, Kovilj, Sremski Karlovci, Sremska Kamenica, Veternik, Čenej, Bukovac, Kisač, Rumenka, Kac and Budisava.

The Institute for public health of Vojvodina carried out an analysis of samples of drinking water of different volumes (samples of primary "A" scale, samples of primary "A" scale with the determination of the concentration of degradation products of disinfection and a periodic pattern of "B" scale). The results clearly showed an increased concentration of residues of desinfectant used (residual chlor) and also the fact that the change of sensory characteristics compromises the usage of water for drinking. The occurence of degradating products (trihalomethane and other halogenized compounds) represents health hazards. The comparison of results obtained for 2010 with the results obtained for previous years is exceptionally important and is shown in Table 17.2.

The fact is that inhabitants are reconciled to the evident increase of the percentage of inaccurate samples, which is the consequence of global economic crises. Inhabitants have no possibilities to influence the work of monopolists, the so called public companies, such as PUC "Water Works" in Novi Sad. They pay their bills under the threat of being disconnected, or dispossesing their property without having a right to demand a quality service. A significant absence of reaction of the community is present for many objective and subjective reasons. The findings of the presence of nematodes in drinking water during summer 2010 speak in favor of the above statement. The Institute for public health of Vojvodina performed biological analysis of drinking water and confirmed the presence of nematodes. The presence of nematodes points out the existence of technical problems from water source to water tanks. World Heath Organization does not give any norms for the presence of nematodes in drinking water recording to the present regulations in Serbia, the presence of nematodes is not allowed [25]. Great panic was caused among the citizens. Having in mind the presence of significant number of cases of viral meningitis (55 within only 1 month) panic increased [26] . Unlike developed countries, there are no experts in Serbia for risk communication. Citizen are very often exposed to different rumors. From a sociological perspective, rumors are not pathological. If officials do not release information to the public quickly enough, the public might conjecture as it tries to figure out what s happening. This is a important reason for emergency managers to be integrated into these channels of communication so they are provide

accurate information as qickly as possible preventing the start of rumors [27]. One among given explanations to the public is that the analysis is used in "order to attack the managing structure" of the company by the opposition. "The attack" was seen as incorrect.

The irony is that the company, only a few months later totally renewed rolling stock by buying a greater number of vehicles, with the permission of the City Assembly, explaining this act as safing activity. As a consequence of this event the usage of bottled water became massive. PUC "Water Works and sewage" started flushing the water network and injecting chlorine in greater concentration than usual, the sediment from the distributive system arose and it caused additional disturbances among the citizens. After a period of time media attention ceased, (the freedom of media is topic of numerous discussions and are given different opinions). Months later, the citizens are still dissatisfied with the price and with the quality of drinking water in Novi Sad (the most expensive in Serbia) and with political compromises which are legally carried out by govern political parties in public companies, whose founder is the town. It is expected that the situation will be changed by passing a law on communal activities and through realization of the first public partnership which has been announced in the upcoming proces of ownership transformation [28] and in in the field of supplying the citizens with drinking water, in favor of citizens.

17.3 Existing Problems Which Significantly Influence Water Quality in Vojvodina

The most important problems referring to the water quality in Vojvodina are the pollution from settlements, from industry and agriculture. Quality of water in Vojvodina is seen from the aspect of surface waters, underground waters and bathing water. Especially important is the quality of drinking water. Jeopardize of surface waters is especially expressed in the vicinity of big towns which have no waste water treatment plants. It is important to know whether a certain water quality problem pertains only to local community or weather is a national problem. Generally speaking, the quality of underground waters is not at satisfactory level because the most part of water which is used for water supply of the citizens is naturally polluted with natural organic substances, arsenic, sodium, iron, manganese, ammonium etc.

On the territory of Vojvodina there are 503 registered water pollutants. They consist of: industry (326 polluters), settlements (44 polluters), agriculture (113) and others (20). Total emission of canal waste water (communal and industrial) in Vojvodina is about 5,250,000 equilent per person, out of which 40% of pollution originates from citizens. Data reffer to canal waste water, excluding households waste water which go to septic tanks. Out of 447 industrial polluters, 293 polluters do not have waste water treatment, 71 polluters treat their waste water together with communal waste water, and 83 polluters primarily treat their waste water. Out of total amount of industrial waste water which originate from the territory of

Vojvodina, 10% is covered by secondary treatment. It is very important to point out accidents which jeopardize water quality. The cause for most of the accidents is not known, except when they are recorded in a neighboring country and in the region, as was the case with red sludge from Hungary and some accidents from Romania. In Serbia there does not exist a system of early announcement of accidents in monitoring surface water. There is no register of polluters, and already established system of protection and rescuing within the activities of MUP in RS is not operative yet. Within the activities in accidents there are certain obligations which are still obligatory and within the authorities of Water Directorate of the RS, which only partially implement them. Restrictions on the provision of sufficient quantities of safe drinking water accessible to everyone are: insufficient number of water sources, insufficient yield of springs, limited and insufficient financial resources, technological problems (lack of training of persons employed in the process of producing safe drinking water, lack of equipment and acquisition of equipment) and insufficient education of the population and improper handling of drinking water. The state of public drainage of water in Vojvodina is one of the most important issues referring water quality. Out of 463 settlements in Vojvodina, only 44 have some kind of sewerage system. Only 30% of population is connected to any form of sewerage system. Waste water treatment plants include only 11% of waste water produced by citizens. Vojvodina is the area of intensive agricultural production. Serbia has carried out initial steps in the implementation of the EU Nitrate Directive in order to protect waters identified as sensitive to pollution from agricultural sources [29]. Nitrate concentrations in Vojvodina waters are decreasing. Evaluation the status of river quality is shown using the nitrate indicator. The analysis included 6,497 water samples from 52 measurement sites for the control [30]. There is an assessment that 40% of total burden of nitrogen in Danube originates from agriculture. Summarizing all above is quote obvious that the main problems of water quality in Vojvodina are:

- Significant health risks due to contamination of surface and underground water and almost no effective sanitary protection zones at water intakes;
- No effective water pollution charges or water conservation mechanisms;
- Problems associated with an absence of clear responsibilities, with the overlapping of institutional boundaries, duplication of work and a lack of coordination; and
- An insufficient number of specialists and inspectors trained in the water management area and connected activities in environmental health.

There is obvious need for analise the complexicity of water quality problems as a complex of economic impact, human health impact, impact on ecosystem, geographical extent of impact, duration of impact etc. The best way to describe the state in water management in Vojvodina under current circumstances in accordance of implement many EU Directives, national laws and sub-lawes could be the old proverb “Easier sad than done”, but in future it should be better if Vojvodina, Serbia want to be equal part of European Family.

17.4 Future Steps in Improving Water Quality

There are many specific features in Vojvodina in the process of adequate water protection in order to obtain satisfactory quality of raw water, water management and protection, water treatment applying different technologies and passing necessary law regulations for such activities. No matter many positive changes, there is a need for additional harmonization of strategic documents, and adoptation, in accordance with European regulations. One of such urgent documents should regulate emission standards after a long period of time. In the RS the history of water management is full of inadequate solutions which were often forced due to the situation of that moment, sometimes without a vision and without respection skilled personnel. In order to solve the problem of water supply, in Vojvodina there was made a Strategy of water supply and water protection [31]. The Strategy defined priorities which are necessary to be solved in the next period. The priorities include the water supply to the population (settlements) and priorities in water protection. It is expected that the most part of issues will be solved in the next two decades and that more than a billion euros is needed. In accordance with the Law on control and prevention of pollution it is necessary to establish the basis for integral management of water quality in the sense of »a polluter pays« system. In all stated above, it is necessary to follow the Directives (first of all Water Framework Directive and Directive on integrated prevention pollution and the control) [32] with recommendation to take care about the specific features of Vojvodina and local distribution of industry and protected zones. Making canals within settlements and building of waste water treatment plant will be a priority task within communal economy. Apart from acute problem of discharging waste waters and the quality of recipients, it is necessary to discuss the problem of quality of sediments. In this country, there are still no such regulations for this matter. Improvement of quality in the Backa region, demands, first of all, a solution regarding waste water and the conditions in the Hydro system Danude-Tisa-Danube. In the region of Banat it is necessary to achieve better international cooperation in order to improve quality. Water resources, for instance, are subject to negative influences from Romania. As mentioned in the beginning, Serbia derives part of its raw water from several trans boundary rivers. On the other hand, Serbian wastewater management is still too little developed to ensure that major impacts on its neighboring countries can be avoided. In the context with the need to rehabilitate water infrastructure, it is not surprising that Serbia has a strong interest in working together with other countries in the Danube Region – and Sava region [33] . In the future it is necessary to improve, first of all, the quality of drinking water. In order to ensure health safety it is necessary to renew wter supply network, and to introduce treatment technologies which will lead to quality demanded. Speaking about water quality it is inevitable to mention ditributive network. In Vojvodina there is a satisfactory density of distributive network, but its quality is bad because of it is built of asbestos cement pipes. Pipes of bigger dimensions are made of steel, and because of damage anticorosive protection they cause much more problems in the system. In water supply in Vovodina it is necessary to

pay significant attention to small systems and to the local sources of water which may have huge importance in emergency situations. Investigation planned in order to ensure sufficient quantity of qualitative underground drinking waters for the needs of population, should be as followed:

- Investigation of potential new local water sources,
- Widening of already existing water sources, and
- Investigation of potential new micro regional and regional water sources.

There are four fundamental strategies to combat water quality degradation that can form the basis of solutions for improving water quality, prevention of pollution, safe reuse of wastewater treatment of polluted water restoration and protection of ecosystems. Mainstream relevant policies should not be implementing only in the water sector but also in others. Serbia is very grateful for significant international help in water management area. Implementation some of the project was extremely useful in the area of water management like project titled: Strengthening capacities of the Water Directorate of the Republic of Serbia, CARDS project with result: Analysis and proposal for harmonization of our legislation with the EU legislation. Construction of central communal waste water treatment plant in Vrbas municipality, expanding of sewage network and connection of Kula to central plant (EU grant International pre asistance help 2008). Construction started at the 2010 completion should be in 2013. Within the cooperation of RWD and Government of Netherlands two projects have been planned: Project on WFD (Water Framework Directive) implementation on pilot sub-basin in the competences of PWMC "Vode Vojvodine." The project for comparative and strategic analysis of future water supply in AP Vojvodina is the most important for Vojvodina populations. The future plans are to intensify (in cooperation with local government) preparation of investment technical documentation for water supply facilities, and facilities for communal waste water collection, transport and treatment, and introduction of feasible water price. In addition, it is planned to commit significant amounts of money for fulfillment of obligation to apply directives related to water management and other recommendations.

17.5 Conclusion

Degradated waters have significant implications on the environment and human health, impacting education, poverty and development and economic growth. Safe handling of drinking water means to prevent secondary pollution of water sources, sanitary protection and purified drinking water from the distribution system or from a local source of water supply. Responsibility in the chain of use belongs to all participants, or a manufacturer, distributor and consumer. Realization of safe handling of drinking water can only be achieved by permanent education of all age groups of the population with the help of various teaching forms of education and support of all forms of mass media.The wakest part of the water protection system in Serbia is

the application of legal regulation for wastewater treatment. The main question for all stakeholders is still same like years and years before: "How can the resources be quantified and protected for future use in the most sustainable manner." Hence, taking in account impacts of global climate change there is additional task of improving the effectivness of existing policy instruments with regard to disaster prevention in the area of water management. Presenting many data about water protection in Vojvodina region is obvious that problem of how to improve it is a subject at national level and also issue of bilateral and multilateral cooperation. Some of positive examples are presented in a short, as some negative, but still there are a lot of opened question and room for improvement. In the expected approaching to EU and ongoing transition Vojvodina Government is aware that water management will be complicated and expensive taking into accout present circumstances.

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Chapter 18
Public Health Risks and Economic Impact of Counterfeit Medicines

Bram Ramjiawan, Angela Ramjiawan, Paramjit S. Tappia, and Grant N. Pierce

Abstract According to the World Health Organization, counterfeit medicines are medicines that are fraudulently mislabeled, contain wrong ingredients, absent of active ingredients, contain insufficient active or over-active ingredients or have fake packaging. The production and distribution of counterfeit medicines has become a significant problem in both affluent and developing nations. It is now widely believed that approximately 15% of drugs worldwide could be counterfeit, although in some developing countries it may be closer to 50% and rising. Due to consumer-initiated importation of prescription drugs through internet sites and other means of cross border purchasing, the potential risks of exposure to counterfeit products has increased dramatically. In addition, counterfeit medicines have become more difficult to detect as counterfeiters have become more creative and bold in the manufacturing of fake drugs. This poses an important threat to global public health and patient safety. In addition, the financial loss and irreparable damage to the reputation of pharmaceutical companies, health care centers and dispensing pharmacies is substantial. This paper briefly highlights the incidence and global impact of counterfeit medicines from a public health and economic perspective and addresses some of the strategies including identifying the risks and threat from counterfeit medicines as well as the analytical procedures for combating the growth, availability and trafficking of counterfeit drugs.

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Keywords Counterfeit medicines • Illegal on-line pharmacies • Global health risks • Economic impact of fake drugs

18.1 Introduction

The pharmaceutical industry is a multi-billion dollar industry that is tightly regulated by laws and regulations to ensure the safety and efficacy of drugs available on the market. However, it is estimated that 15% of all drugs sold globally are fake [1]. Counterfeit pharmaceuticals are medicines, both brand name and generic, which are deliberately and fraudulently mislabeled with respect to identity and/or source. This is not a new problem. Throughout history, poor quality medicines have been a persistent concern with periodical crises in the supply of medicines. The reporting of fake cinchona bark in the 1600s and fake quinine in the 1800s are good examples of this practice [2]. Unfortunately, this problem seems to have grown in the last decade, especially afflicting unsuspecting patients and those seeking medicines via on-line pharmacies [2]. Most of the counterfeit medicines are manufactured in uncontrolled or street laboratories that do not conform to good manufacturing practices [3]. Counterfeits may be produced with the correct medicinal ingredients in insufficient quantities or may be absent altogether or may contain toxic or poisonous chemicals [4]. It is likely that the chemical composition and purity of raw materials may change during production each time because of the inconsistencies in the processes involved in manufacturing fake medicines [4]. The appearance of the medication and its packaging may also be visibly different (or inferior) from the genuine product [5]. This may result in an unwanted or unknown acceleration of drug decomposition and changes in shelf life. Thus it has been suggested that the public health problem of counterfeit drugs is largely due to the qualitative and quantitative variability in formulation and purity profiles [3].

18.2 Widespread Availability of Counterfeit Medications

Although these medications are mostly manufactured and distributed within many developing nations, counterfeit medicines are emerging in industrialized countries at an alarming rate [6–8]. In the 2008 report from the Pharmaceutical Security Institute, the production of fake drugs was surprisingly high with over 100 countries reporting incidents of fake drugs [9]. Counterfeit medicines encompass all types of therapeutic classes and involve a complex global supply chain network to enable this illegal activity [10]. Drugs at risk for being counterfeited are often the most widely prescribed medications. Some of the more common counterfeited medications include classes of drugs that are used to lower circulating levels of cholesterol, or treat hypertension, AIDS, depression and a variety of haematological conditions. With the growth of the Internet and the advent of on-line pharmacies,

illegal Internet pharmacies have appeared and have become engaged in selling a variety of medications that can pose health and safety risks. This includes unapproved drugs, legal prescription drugs dispensed without a valid prescription, products that are marketed with fraudulent health claims, or counterfeit or grey market pharmaceuticals [11–13]. Medication trafficked by criminal groups or sold by illegal internet pharmacies are more likely to be counterfeit than medication received from licensed sources. In addition, information technologies available throughout the Internet and sales via on-line pharmacies have allowed the criminal element to thrive in an unregulated environment of anonymity, deception and lack of adequate enforcement [10]. Most on-line pharmacies do not require a medical prescription from the consumer's physician and some do not even declare any side-effects on the medications available [14]. This creates an environment in which the counterfeit process will thrive. Moreover, on-line pharmacies advertise their products in order to increase aspects of the on-line trade that consumers might find appealing and convenient [14]. It has been suggested that one in five Europeans, representing over 77 million people, is currently putting themselves at risk and admit purchasing prescription-only medicines without a prescription [15].

The import of prescription drugs has also played a significant role in the counterfeit sales market. The import of medicines into the US was designed to allow patients to have access to drugs that were not available to them in the US. This could occur when continuing therapy that was begun in another country or when all other US Food and Drug Administration (FDA)-approved drug options for their condition had been exhausted. However, this system presented challenges to both the US regulatory system and pharmaceutical companies. The potential risks of exposure to counterfeit products have increased as more and more consumers have opted to import drugs from other countries through Internet sites and other means presumably because of the lower cost of purchase [16].

Despite the recent global enforcement efforts, the incidence of drug counterfeiting shows no evidence of declining. The number of counterfeit medicines on the market has been suggested to have increased as much as 25% each year over the last several years [9]. In a 9 month investigation of counterfeit prescription drugs by 60 min (a US news program aired on the CBS Network), it was revealed how the dangerous imitation drugs get into the nation's drug pipeline in the US [17]. In this investigation lead by John Clark, Vice President and Chief Security Officer, Pfizer Global Security, an underground illegal operation to produce counterfeit medication was uncovered in Peru. The report brought to light that in countries such as Canada, the United States, and many of the European Union, counterfeit Pfizer medicines had entered the legitimate supply chain. This included imitations of well-known drugs like Lipitor®, Norvasc®, Viagra®, Zithromax®, and Celebrex®. The infiltration of counterfeit Pfizer medicines into the legal market was so large that in 2010, authorities from 53 countries seized almost 8.4 million tablets, capsules and vials of counterfeit Pfizer medicines [17]. Counterfeited drugs are not just limited to Pfizer company, for example, in 2001, it was reported that three injectable counterfeit prescription drugs were discovered in American pharmacies, some vials of which were found to contain no active ingredient. The medications were filgrastim

(Neupogen®, Amgen Inc.) an anticancer drug, and human growth hormones, Serostim® (Merk Serono) and Nutropin® (Roche) used in the treatment of AIDS related weight loss [18].

According to a report by the International Policy Network, a detailed study of medicines in Africa and South East Asia revealed that between 30% and 60% of medicines were "substandard" [19]. An estimated 25% of the medicines consumed in developing countries are believed to be counterfeit [20]. In some countries, this figure is thought to be as high as 50% [20]. It should be noted that the WHO conducted a survey of counterfeit medicines in 20 countries from 1999 to 2000 and found that 60% of counterfeit medicine cases occurred in poor countries and 40% in industrialized countries [20]. Thus, it is clear that counterfeit medicines have become readily available and have penetrated the legal prescription drug markets across the world irrespective of a country's economic standing. Of late, with advancing technologies counterfeiters have been able to very closely reproduce the packaging and labeling of legitimate prescription drugs. In fact, the labeling of counterfeit medications is duplicated with such accuracy that the subtle differences from the legitimate product can only be detected and identified through extremely close inspection by experts [21].

18.3 Economic and Health Consequences of Counterfeit Medicines

In 2010, it was estimated that the global business of counterfeit medicines was worth $75 billion [20, 22]. This represented an increase of more than 90% from 2005 [20, 22]. Not only is this a loss in revenue and profit for the legal pharmaceutical companies but, without these additional finances, it also limits their ability to carry out research that would ultimately allow them to bring novel drugs into the market in the future. These fraudulent medications not only have an economic impact, but more importantly, affect the health of the patients consuming these fraudulent medications. The counterfeit medications contribute to morbidity, mortality, and drug resistance [6, 23]. Counterfeit medicines can result in unexpected side effects, incorrect dosages, dangerous drug interactions, allergic reactions or the worsening of medical conditions. Substandard antibiotics that do not effectively treat bacterial infections result in antibiotic resistance creating infections that are difficult to treat and are more virulent. Counterfeiting may also result in a loss of confidence in the public health system and pharmaceutical industry by patients who unknowingly take counterfeit drugs that are ineffective. Ultimately, this can threaten the reputation of pharmaceutical companies [6]. There are many examples of counterfeit medications that have penetrated the legal supply line and consumer markets that have negatively impacted health and resulted in economic losses. According to the FDA, there are a number of instances of fraudulent activity used for manufacturing counterfeit medicines. In this regard, the recently counterfeited Procrit, an important drug for cancer and AIDS patients was found to contain non-sterile

components, which posed an increased risk of infections in these groups of patients [24–26]. Anti-depressant drugs have been sold as anti-retrovirals in DR Congo which resulted in a major setback in the treatment of and fight against AIDS in sub-Saharan Africa [27, 28]. Another recent example involved counterfeiters emptying bottles of Zyprexa, a drug used for schizophrenia and acute bipolar mania, and replacing them with white tablets imprinted with the word "aspirin" [29] that represented an increase in the risk of an adverse outcome for this particular patient population group. In North America, several adverse side effects of bitter tasting Lipitor® (atorvastatin) pills were also reported that resulted in the discovery of counterfeit tables [30]. It has been estimated that 30–50% of artesunate tablets, an anti-malarial drug, bought in Southeast Asia contained no active ingredient that led to deaths from untreated malaria, as well as financial repercussions to legitimate manufacturers [31–33]. A total of 192,000 Chinese patients have been reported to have died in 2001 from fake drugs that subsequently led the Chinese authorities to close 1,300 factories while investigating 480,000 cases of counterfeit drugs reported to be worth $57 million [34]. In 2004, in highly publicized cases, Chinese authorities arrested 22 manufacturers of substandard infant milk powder and closed three factories after the death of over 50 infants [35]. In Haiti, Nigeria, Bangladesh, India, and Argentina, more than 500 patients, predominantly children, died from exposure to diethylene glycol (a compound found in printing ink and glue) that counterfeiters had used in the manufacture of fake paracetamol syrup [36–38]. During the 1995 meningitis epidemic in Niger, authorities received a donation of 88,000 Pasteur Merieux and SmithKline Beecham vaccines from neighboring Nigeria. After almost 60,000 people were inoculated with the vaccines, the entire stock was later found to be counterfeit and contained no active ingredient [39]. It is pointed out that counterfeiters are always seeking opportunities to capitalize on market demands with their illegal trade, which was demonstrated by a rapid production of fake vaccines and antiviral medicines during the peak of the H1N1 flu pandemic [40].

18.4 The Origin of Counterfeit Drugs

The largest counterfeit market with proximity to the EU free trade zone is Russia, where the generally accepted estimate is that 10–12% of drugs are counterfeit [41, 42]. Now that the Baltic nations of Latvia, Lithuania, and Estonia have joined the European Union, the WHO has warned that the risk of counterfeits entering the EU supply chain will increase [1]. According to a report by the Organization for Economic Co-operation and Development, approximately 75% of counterfeit drugs produced and supplied around the world have some origins in India, while 7% has been estimated to have origins in Egypt and 6% from China [43]. Nigeria recently threatened to ban the import of all drugs from India, a major supplier of legal pharmaceuticals to Nigeria, because of the high prevalence of fake anti-malarial drugs with "Made in India" label that were detected among the imported drugs [44], however, it was later found that the medicines were in fact produced in China [45].

A less than optimal adherence to patent regulations not only in the pharmaceutical industry, but in a variety of product fields is a well known problem in China. The counterfeit drug industry is, at the very least, indirectly or implicitly supported by the governments of the countries in which the drugs originate when they do not devote the resources needed to detect and/or do not take the legal action necessary to stop the production and distribution of counterfeit drugs.

18.5 Strategies to Combat Counterfeit Medications

Strong and urgent action is required to stop the proliferation of illegal pharmacies. However, despite the global enforcement to combat fake medicines, the supply of illegal drugs has not been diminished. A global policy framework needs to be established that would use public-private partnerships with centralized surveillance reporting to fight against counterfeit medications in a synergistic and coordinated manner [10]. The American Academy of Family Physicians has posted a "VIPPS" seal on approved, on-line pharmacies that links to the National Association Boards of Pharmacy. Sites without the VIPPS seal may be selling counterfeit products [46]. One important strategy in the campaign against counterfeit medicines would be to use health professionals. Pharmacists and doctors should stay vigilant and report any suspicious products [47]. They should consider counterfeit medicines as a possible cause of adverse reactions or therapeutic failure [47]. In addition, the patients should also take the initiative and inform pharmacists and doctors if they experience side effects or a decrease in a beneficial effect [47]. Interestingly, a recent study in California was undertaken to examine their pharmacists' knowledge of counterfeit medicines, the impact of technology and barriers to pharmacist involvement and the potential role of the pharmacist in combating counterfeit medicines. A lack of knowledge and resources were identified as barriers to detecting fake medications.

An obstacle in the fight against counterfeit medicines is the lack of unequivocal evidence and information on the prevalence of this problem. Although it is generally accepted that about 10% of drugs worldwide could be counterfeit, it is also well known that this number covers different situations depending on the country, the site of purchase of the drugs and the definition of counterfeit drugs [48]. Accordingly, the chemical analysis of counterfeit medicines is now considered as a crucial step needed to distinguish between genuine, substandard, degraded and counterfeit medicines [2, 48]. Several analytical tools including colorimetry, thin layer chromatography, mass spectrometry, nuclear magnetic resonance and vibrational spectroscopies have been reviewed for their relative performance and selection as the most appropriate analytical approach in the fight against poor quality or counterfeit drugs [2, 48–51]. Another approach is to use Radio frequency identification technology (RFID) that can help track and authenticate medicines. The RFID system has been designed to operate with electronic product codes that are item-specific coding [52]. With a global mass-serialized RFID system, such as the Worldwide Track and Trace Bank, it will be possible to automatically track products from raw materials to

post-consumer waste [52]. Clearly, such a scheme will add to the fight against the manufacture and trade of fake medicines. In this regard, the California Board of Pharmacy has planned to use RFID technology to identify authentic drugs and differentiate them from counterfeit drugs [53]. However, while about half of the pharmacists that responded to a questionnaire on the topic were aware of RFID technology, they did not believe that RFID would be effective [53].

With respect to parliamentary action, international organizations, governments of developed and developing nations and the pharmaceutical industry created the IMPACT initiative (International Medical Products Anti-Counterfeiting Taskforce created by the WHO) to tackle the fake drugs trade [54]. In addition the European Union (EU) approved EU-wide legislation to prevent fake medicines from entering the legal supply chain and becoming available to the general public [55]. The law will cover internet sales, will introduce new measures to improve safety and traceability as well as provide for penalties for any violations [55]. The FDA in conjunction with the U.S. Bureau of Customs and Border Patrol has been working to identify packages suspected of containing counterfeit drugs that cross the border into the US from other countries and enter into the US distribution structure. A recently developed voluntary program has been initiated to encourage drug manufacturers to notify the FDA within 5 working days if a product is believed to be counterfeited. The program also extends to counterfeits discovered in countries outside the US if there is clear evidence to believe that they are intended for distribution within the US. The program went into effect in early 2011 and it has already been used to provide several reports of counterfeit drugs to FDA, including those involving Procrit, and Lipitor, a widely prescribed cholesterol-lowering drug. The FDA's new Counterfeit Drug Initiative is designed to better identify the risks and threats from counterfeit drugs, to coordinate public and private efforts to fight counterfeiters, and to identify technologies and tools to aid in identifying, deterring, and combating counterfeiting. A new internal Counterfeit Drug Task Force recently announced by the FDA will be assigned to determine measures to prevent patients from being exposed to counterfeit drugs. This includes higher penalties, public education and engaging other government agencies and the private sector. Taken together, these strategies represent a viable initial response for international authorities tasked with combating the global trade of counterfeit medicines.

18.6 Conclusion

The production and distribution of counterfeit medicines has become a significant issue for public health and economic concerns. Despite improved deterrence and detection of counterfeit medicines, the increased sophistication of the counterfeiters and increasing opportunities for drug counterfeiting has presented new challenges in the global fight against fake medicines. Stronger national and international cooperation and coordination in the surveillance, reporting, public education and implementation of stiffer penalties would certainly help to lessen the global health risks

and economic consequences of counterfeit medicines. Each country should introduce measures to monitor and identify the type and amounts of medicines arriving from other countries and determine the extent of counterfeit drugs among such imports. Health professionals including physicians and pharmacists should be made aware of the prevalence of counterfeit medicines such that they can be included in and cooperate with strategies to combat the distribution of fake medicines. Simple and affordable technologies should be developed with widespread applicability for the detection of counterfeit drugs that could include analyses of the tablet and/or the packaging. It is evident that both financial and human resources need to be dedicated immediately in order to decrease the production and use of counterfeit medicines.

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Chapter 19
Patients Practice and Knowledge of Pharmaceuticals Disposal: Example from Some Rural Areas of Croatia

Ksenija Vitale, Magdalena Palian, Danijela Jonjic, Marko Milic, Slavica Sovic, and Aleksandar Dzakula

Abstract Consumption of the pharmaceutical products for both human and veterinary use is constantly increasing. As the consequence considerable amount of pharmaceuticals and its metabolites is released into the environment at therapeutic concentrations. The aim of this article is to describe patient practice and knowledge of unused drugs disposal in some rural areas of Croatia. Results showed that mostly older population live in these areas with considerable co morbidity of various chronic diseases, using considerable number of pharmaceuticals. Educational level and therapy compliance is low, as well as knowledge on how to dispose unused pharmaceuticals. On the other hand, due to the relative geographical isolation there is tendency to stock various pharmaceuticals as well as self-medication. Unused drugs end up improperly disposed in the ecologically delicate environment rich in drinking water supply. Strong support from primary health care is needed as well from pharmacies for returning of unused pharmaceutical when possible or clear advice how to properly dispose them if they cannot be returned.

Keywords Pharmaceuticals disposal • Patient practice • Rural environment • Croatia

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19.1 Introduction

Consumption of the pharmaceutical products for both human and veterinary use is constantly increasing, even more as population grows old and more chronic diseases are present. As the consequence considerable amount of pharmaceuticals and its metabolites is released in the environment at therapeutic concentrations causing adverse effects on flora, fauna and environment as a whole [1]. Presence of the pharmaceuticals in the environment was first published in the late 70s and the beginning of 80s [2] in England, USA and Canada. Today in Croatia a number of studies have confirmed presence of pharmaceuticals in waste waters and fresh water sediments. Out of 44 analyzed compounds 31 was above limit detection and concentrations were in range of 1–10 ng/L. The most abundant were analgesics and antiimflamatories followed by sulfonamides. These findings reflect the patterns of production as well as principles of drug prescription, along with patients' knowledge, awareness and practice of disposal [3–5]. Unused pharmaceuticals are considered as pharmaceutical waste, out of which some is in the category of hazardous waste. In Croatia pharmaceutical waste is regulated with law on waste and low on pharmaceuticals [6, 7]. While pharmaceuticals waste from the technology processes are well regulated, waste from households is often underestimated as a problem. Currently the model of disposal of unused pharmaceuticals through pharmacies is available, but there are few downsides that are disabling the functioning of the model. The distribution of pharmacies is not equal in the whole territory of Croatia, pharmacies are not well recognized as the places to dispose unused drugs, and pharmacies itself do not have any incentives for collection of unused drugs. On the contrary they are charged for the collected quantity of the waste from the households. So in the most cases unused pharmaceuticals from the households are disposed together with the communal waste ending on the landfills, most of which are unsanitary. The aim of this article is to describe patient practice and knowledge of unused pharmaceuticals disposal in some rural areas of Croatia that are under national protection as national parks or nature parks.

19.2 Materials and Methods

Sample consisted of inhabitants of five villages in the environmentally delicate region bordering to National Park in the southwest Croatia who were 65+. Questionnaire analyzed socio-demographic characteristics of population, presence of chronic diseases, pharmaceutical use, use of health care services and knowledge and practice regarding disposal of unused pharmaceuticals. Data for continuous variables were expressed as mean (±) standard deviation (SD), Pearson's χ^2 test was used for comparison of frequencies. As statistically significant was considered $P < 0.05$.

19.3 Results

Results showed that mostly older population live in these areas with considerable co morbidity of various chronic diseases, using considerable number of pharmaceuticals. Final sample consisted of 163 persons 98 females and 65 males. All inhabitants who were 65 or older were approached except persons who were not capable of answering or who were terminally ill. Secluded hamlets and isolated houses were not approached. Response rate was 84.46%, around 16% of inhabitants refused to answer for unknown reasons. Average age of examinees was 74.01 (73.83 for males and 74.12 for females). Unfinished elementary school had 58.28% examinees, elementary school finished 21.47% examinees, and 19.63% examinees finished high school. Only female examinees had college level education (0.61%). More than 60% had chronic disease and those patients get prescription drugs at each visit to the doctor's office. Around 50% of examinees take 1–5 medications, and 4.08% women take more than 10 medications. Educational level and therapy compliance is low, as well as knowledge on how to dispose unused pharmaceuticals. Compliance is statistically significant higher in those who go to the pharmacy more often ($\chi^2 = 8.66$ $p = 0.034$). Unused drugs more than 66% throw in the communal garbage, around 30% collect and swap drugs and nobody returns them to the pharmacy. Only 16.5% got some advice on drug disposal, and those advices were received from pharmacists and family and friends. Doctors or nurses did not advice anybody. Patients with higher education got significantly more advice ($\chi^2 = 56.54$ $p < 0.001$) than those wilt lower education, but there is no statistically significant difference in the disposal practice regarding education. Regarding gender, women take more drugs and have better compliance, while man had higher prevalence of chronic disease but take fewer drugs and tend to stock them. On the other hand, due to the relative geographical isolation tendency to stock various pharmaceuticals as well as self-medication is somehow understandable. The problem is that in the end they are improperly disposed in the ecologically delicate environment rich in drinking water supply.

19.4 Discussion

This study revealed high percentage of inappropriate drug disposal in the examined villages, but we can speculate that those results reflect the practice in the whole community. According to the 2001 census there were 2,623 persons living in the 25 villages and hamlets, more than 60% being 65 years and older. The 66.25% of unused pharmaceuticals end up in the communal waste which is higher than in some other countries such as America, New Zealand, England or most of the east Europe countries where around 51% of patients have same practice [8, 9]. However, this number has decreased between 2005 and 2008 and due to the public health campaigns number of patients who return drugs to the pharmacies has risen to 12.25%.

According to the European Environment Agency Croatia is among the countries with lowest return rate of unused pharmaceuticals with only 0.19 t per million of inhabitants, as oppose to the Switzerland with 237 t per million of inhabitants, followed by Ireland, Luxemburg, Sweden and France. Most of the states reported return rate between 10 and 100 t per million of inhabitants [10]. These results depend on medical practice in each country, but at the same time they reflect knowledge, awareness, practice of the patients, availability of health care and legislative of each country. In city of Zagreb, Craotia in 2007 campaign 1,580 t of pharmaceuticals were collected in 2 months. More than 93% were prescription drugs with financial value around 100,000 Euros [11]. Such campaigns are uniquely important but they are sporadic due to the scarce financial means, as example, this one was financed by pharmaceutical industry. The practice of unused drug disposal along with communal waste is particularly dangerous in the karst areas due to the high porosity of such geological formations. In examined community communal waste is deposited in karst cave only 2 km away from the last inhabited house in the area, due to the non existence of sanitary landfill. Periodically cave is buried with layers of soil. In this way hazardous components from the pharmaceutical waste are concentrated with high possibility of leakage in the nearby drinking water supplies for the broader area including two towns. It is important to stress that in Croatia available systems for drinking water production and waste water purification are not capable of such a compounds removal. Some other studies demonstrated leakage of pharmaceutical compounds from biggest landfill in Zagreb in nearby aquifers, regardless the fact that this landfill is in its major part sanitary and impermeable. The most prominent was propyphenazone compound of large number of analgesics produced in local industry, which is resistant to the microbiological degradation and highly mobile through vertical flow [5]. This study also reveals a problem of education and communication between health care users and providers. The role of the doctor is very important particularly in small communities because in many cases this is the point of communication with the broader area, point of information and point of health education and health promotion. Unfortunately some other studies in Croatia have shown that doctors in primary health care do not dedicate enough time to advising and primary prevention [12]. According to the literature, there are three possible ways to reduce the entry of medicines in the environment and aquatic life. The first implies the improving of methods for waste water treatment, which requires appropriate technical and financial capabilities [1], which makes its questionable and probably unsustainable for many countries. The second involves the collection and return of medicines through pharmacies, and strongly highlights the need for education of doctors and pharmacists who would educate the population. A good example is which Stockholm County Council, in close co-operation with pharmacies gave some of the best guidelines for drug disposal. They put the emphasis on the communication between doctors, patients and pharmacists, with strict legal regulations focussed on pharmaceutical companies [1]. Systems for the return of outdated drugs are effective in several countries, and should be in the accordance with EU regulations. The third way highlights the rational design of new drugs which are "benign" for environment [1]. This design requires not only the pharmaceutical properties of new drugs, but also the environmental aspects such as improving the

degradation or removal from waste water by conventional means. Good example is praxis in America where clear guidelines for disposal of unused pharmaceuticals are given via printed material widely available for the public. Food and Drug Administration [13] gives instruction when drugs could be disposed into the sewage system, when disposed along with communal waste and what to do in other cases. They even give practical guidelines such as taking drugs out of the packages, mixing them with unpalatable substance such as used coffee, or kitty litter, placing the mixture in the plastic container and as such in communal waste. In Croatia such information is unavailable and there is no information on drug disposal on the drug package, which is in the contrast with the law because it states such information as obligatory. Also according to the law all pharmacies should accept unused drugs in the appropriate green box, but they tend to avoid that practice due to the financial loss. This is certainly lost opportunity for the society, and we strongly believe that pharmacies should have some incentive for unused pharmaceutical collection. At the moment they feel as if they have been punished for doing well for the community. As conclusion we emphases that, strong support from primary health care is needed as well from pharmacies for returning of unused pharmaceutical when possible or clear advice how properly dispose pharmaceuticals if they cannot be returned to health care facility.

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Chapter 20
Endemic (Balkan) Nephropathy: A Disease Caused by Aristolochic Acid

Bojan Jelaković, Živka Dika, and Arthur P. Grollman

Abstract Endemic (Balkan) nephropathy (EN) is a devastating chronic renal disease, affecting residents of rural villages situated near tributaries of the Danube river in Croatia, Bosnia and Herzegovina, Serbia, Bulgaria and Romania. The disease affects at least 25,000 men and women, while another 75,000 are estimated to be at risk. The prevalence of confirmed EN cases in the endemic regions of Croatia has fluctuated between 2% and 10% of the village population. Significant epidemiologic features of EN include its presence only in certain villages; a familial pattern of disease; occurrence in adults and never in children under 15 years of age; restriction to rural farming populations; and strong association (~50%) with transitional urothelial cell carcinoma of the upper urinary tract. This epidemiologic evidence strongly suggests that an environmental toxin is involved in the etiology of EN and its associated cancers.

Keywords Endemic nephropathy • Aristolochic acid • DNA adducts • Environmental p53 mutagens • Urothelial cancer

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20.1 Introduction

Over the past 50 years many environmental factors have been investigated as causative agents of EN. We have pursued an earlier, but largely ignored and untested hypothesis that seeds of *Aristolochia clematitis* contaminate wheat grain during the annual harvesting procedure and that a toxic constituent of this plant, aristolochic acid (AA), is introduced into home-made bread, a major source of food for farming families in the endemic region. This hypothesis is supported by striking similarities between the pathophysiology of EN and aristolochic acid nephropathy, a recently recognized syndrome traced to the ingestion of *Aristolochia fangchi* by women undergoing a slimming regimen in Belgium. We estimate that bread prepared from grain contaminated by a few seeds of *Aristolochia clematitis* would provide, over a period of years, dietary exposure to AA equivalent to that documented for the Belgian women. The nephrotoxic effects of AA in human and animal species are mediated by reactive metabolites that react with DNA to form covalent deoxyadenosine-aristolactam (dA-AL) and deoxyguanosine-aristolactam (dG-AL) adducts. These adducts persist for an extended period of time, facilitating their detection in target tissues. Our hypothesis that dietary exposure to AA is responsible for EN and its associated UUC is strongly supported by a pilot molecular epidemiologic study conducted in Croatia; (a) the detection of aristolactam-DNA adducts in the renal cortex of EN patients and in tumor tissues of affected individuals, and (b) the demonstration that the mutational spectra of the p53 suppressor gene in upper urothelial cell cancers is dominated by A:T → T:A transversions, now recognized as a mutational "signature" for human exposure to AA. This toxicogenomics and translational research is a collaborative project of scientists from the University of Zagreb School of Medicine University and from Stony Brook University, led by Drs Bojan Jelakovic and Arthur P. Grollman. Our aim is to provide definitive evidence associating exposure to an environmental carcinogen (aristolochic acid) with a disease (chronic interstitial nephritis and/or upper urothelial cancer) characterized by serious disability and death. Results of the proposed investigations are expected to lead promptly to the adoption of public health measures, including education and modified agricultural practices, which would ultimately result in the eradication of upper urothelial cancer in countries harboring the endemic disease.

Our primary goals are to identify the environmental toxin responsible for the carcinogenic process and the genes underlying susceptibility to the disease. Our research program pursues both aspects in a unique population. Additionally, confirmation and extension of our research on upper urinary tract urothelial cancers points the way to public health measures that can eradicate a debilitating and often fatal disease.

20.2 Basic Epidemiological Data

Endemic (Balkan) nephropathy (EN), a chronic tubulointerstitial nephritis affecting farmers living in rural areas of Bosnia and Herzegovina, Bulgaria, Croatia, Romania, and Serbia along the Danube river basin, is characterized by slow and

insidious clinical course and strong association with upper urinary tract urothelial cancer (UUC) [1–5]. In the literature, EN was first described in the late 1950s, although according to church reports and anecdotal observations it was likely present in this area many decades before. The first well documented case of EN was the autopsy case report of the men from the village of Slavonski Kobaš who died in uremia caused by endemic nephropathy at the University Hospital Centre Zagreb in 1948 (data on hospital record). Interestingly, over the past 50 years the geographical distribution of EN remained unchanged. In Croatia, the focus of the disease is strictly situated west from the city of Slavonski Brod in Jelas valley near the Sava river and it comprises 14 villages with the population of around 10,000 inhabitants based on the census from 2001 [6]. The prevalence of the confirmed EN cases in this area varies between 2% and 10% of the village population.

Of note is that the EN shows mosaic distribution affecting certain farming villages leaving the nearby ones spared. Remarkably, this distribution also is present within the endemic villages where several members of the same household, not necessarily blood related, are often affected. Some households are not affected at all, showing the clustering of EN cases within households. This mosaic phenomenon and not inherited pattern of the disease was confirmed by a natural experiment with Ukrainian immigrants who moved to this Croatian region at the end nineteenth and the beginning of the twentieth century from the then Austro-Hungarian province of Galicia (the neighborhood of Lvov and Ternopil) [6]. Both autochthonous Croats and Ukrainian immigrants and their descendants that settled in endemic villages were equally affected by EN and the associated UUC, while Ukrainians who settled nonendemic Croatian villages as well as Ukrainian relatives that stayed in Ukraine did not develop symptoms of EN. Moreover, the presence of EN has never been confirmed by nephrologists of the former Soviet Union, nor described in the literature, suggesting that individuals affected by the disease had been exposed to a nephrogenic and carcinogenic substance(s) present in the local environment. Thus, epidemiologic findings and striking geographical correlation of two, otherwise, rare diseases (chronic tubulointerstitial nephritis and upper urothelial cancers) points to a common etiologic agent.

20.3 Diagnosis

In Croatia, for the last four decades the modified WHO criteria have been used to diagnose EN [7, 8]. The modified WHO criteria classify subjects into four groups: “diseased”, “suspected” of having EN, “at risk” of having EN and “others”, based on the following diagnostic data: (a) positive family/household history of EN; (b) low molecular weight proteinuria (alpha-1 microglobulin > 10 mg/l or alpha-1 microglobulin/creatinine > 14 mg/g); (c) serum creatinine > 132.6 μmol/l; (d) anemia (hemoglobin < 120 g/l if male, Hb < 113 g/l if female); (e) exclusion of renal disease due to other causes (including diabetes). Subjects are considered “diseased” if they are positive for “a + b + c + d + e”, or “b + c + d + e”, or “a + b + d + e”;

"suspected" if they have combination of "a + b" or "b + d"; "at risk" if they are from family/household with EN and "others" if they do not fulfill the aforementionated criteria.

20.4 Pathology and Clinical Course

Extensive fibrosis is one of the hallmarks of the advanced phase of endemic nephropathy and kidneys of these patients were reported as the smallest among patients with any form of chronic renal disease. Histopathological findings in EN reveal that the pathological process starts from superficial cortex and gradually moves centrally, with the proximal tubules being the primary sites of injury [9, 10]. Accordingly, the biomarkers of proximal tubule damage (i.e. beta2 microglobulin, or alpha1 microglobulin) are the most reliable diagnostic tools in making an early diagnosis of the disease. In advanced stages of EN extensive fibrosis is present with paucicellular infiltrate [9]. EN has insidious onset slowly progressing to the end-stage renal disease. It was reported that anemia is more severe than the stage of chronic kidney disease (CKD). Hypertension is not a characteristic of early phases, and appeared only when accompanying advanced stages of CKD [1, 3, 11]. However, our last report showed that prevalence of hypertension in EN villages does not differ from other rural parts of Croatia, very probably reflecting the changes in lifestyle that have occurred in the past several decades [12]. In last few decades shift to older ages was observed [13]. As mentioned earlier, UUC are significantly frequently present in EN patients and specific mortality of UUC is several folds higher in endemic area than in other parts of Croatia [5, 14].

20.5 Etiology and Aristolochic Acid Research

Many environmental factors have been investigated as causative agents over the past 50 years with ochratoxin A (OTA) and, more recently, aristolochic acids (AA) being the focus of such research [15–17]. According to the recent report of the European committee on Food Safety there is no convincing evidence from human epidemiology to confirm the association between OTA, a ubiquitous micotoxin in common foodstuff (present in high concentration in residents of EN villages as well as certain otherwise healthy individuals throughout the world), exposure and the prevalence of EN or urothelial cancer [18, 19].

AAs found in all *Aristolochia* species, are well established nephrotoxic and carcinogenic agents in animals and humans (IARC Group 1 and Group 2A) [20, 21]. *Aristolochia* herbs are regularly used in traditional Chinese medicine, Japanese kampo and Ayurvedic medicine [20, 21]. This plant also has been observed as a weed growing in meadows and wheat fields in Bosnia, Croatia and Serbia [19, 22]. The first Croatian reports of AA nephrotoxicity is from veterinary medicine [23, 24]. Clinical course and histological findings were recorded in horses that ingested

hay contaminated with *Aristolochia clematitis*. Examination of renal tissue revealed histopathological findings similar to those associated with EN in humans. These findings showed marked renal tubular damage, interstitial fibrosis primarily in the cortex with few or no signs of inflammation or glomerular damage [23, 24]. In 1969, Ivić observed that, during harvesting, seeds from *Aristolochia clematitis* often co-mingled with wheat grain resulting in contamination of the flour used for baking bread. Being aware of the toxicity of AA, he speculated that the bread ingested by these farmers must be poisoned. Ivić performed toxicological studies on rabbits treated with the contaminated flour and demonstrated nephrotoxic effects similar to those observed in horses [19]. Furthermore, in rats, he showed that an aqueous extract of *Aristolochia* seeds induced sarcomas at the site of injection [25]. Unfortunately, no attempt was made to confirm Ivic's prescient observations. Then, in 1993, a cluster of renal failure cases were observed in a group of healthy Belgian women who had accidentally ingested *Aristolochia fangchi*, a Chinese herb, as a part of slimming regime [26]. Furthermore, those patients with chronic renal failure exhibited a high prevalence (>40%) of upper urothelial cancer [26]. In addition, renal and urothelial tissues of these patients contained AL-DNA adducts, a definitive marker of AA exposure. Since then, several hundred cases of Chinese herb nephropathy (CHN), as it was called at that time, have been reported worldwide [27]. After detection of AA DNA adducts in kidney tissue of those patients CHN was renamed aristolochic acid nephropathy (AAN). Cosyns first called attention to similarities in patohistological features of AAN and EN and their strong association with UUC [28].

In 2003 we started our collaborative research project to test the hypothesis that AA is a causative agent of EN and associated UUC. First, we explored the hypothesis that flour contaminated with AA is the route of ingestion of this potent nephrotoxin and carcinogen in EN [22]. A detailed questionnaire was designed to collect information on demographics, exposure to potentially toxic substances, diet, agricultural practices, and other factors relevant to residents of EN and non-EN villages. Interestingly, we did not find evidence to support a hypothesis implicating drinking water as a causative factor for EN previously cited as the most plausible risk factor for chronic tubulointerstitial nephritis in EN [29]. In our study, the majority of subjects clearly recalled seeing the plant of *Aristolochia clematitis* in their fields and meadows 20–30 years ago. Subjects with EN observed more frequently seeds of *Aristolochia clematitis* among wheat seeds than farmers who were not affected by EN. Obviously, flour used for preparing home baked bread was contaminated with AA, thereby poisoned exactly as Ivić suggested. At that time most residents of the endemic villages in Croatia grew their own wheat. In last 10 years, there have been changes in agricultural practices (increased use of fertilizers and herbicides), lifestyle (residents now rarely bake their own bread and some no longer farm) as well as changes in the microenvironment (installation of pumps and drainage ditches near the wheat fields). To test the predicted decrease in the prevalence of EN prevalence, we conducted several large epidemiologic surveys, enrolling residents of nine endemic villages. Indeed, we observed that the prevalence of EN shows a decreasing trend in the majority of EN villages included in this survey, in agreement with

other reports [30, 31]. Thirty years ago the average age of onset of EN was 44.5 year, but, in last 15 years, a shift to older ages was observed (average 61.9) [8, 32]. These developments indicate that the environmental agent is less active than in the past. We conclude that exposure to this powerful environmental nephrotoxin and carcinogen has decreased due to these facts, and to significantly improved farming and milling practices preventing contamination of flour. Harvesting, threshing and milling have evolved in the past 35 years. Modern combines and large mills were introduced in early 1980s containing multiple sieves with smaller holes enabling separation of wheat from weed seeds [33]. In addition, large mills contained storage space which allowed farmers to bring their seeds and immediately receive flour rather than wait for their own flour to be ground, as in the past when smaller mills and even mill boats were used. We have conducted molecular studies on kidney and tumor tissue of EN patients designed to confirm that AA is the causative agent of EN and associated UUC. Using ^{32}P postlabeling/PAGE we identified dA-aristolactam (AL) and dG-AL DNA adducts in renal cortex of patients with EN, but not in patients with other chronic kidney diseases [34]. Moreover, the same DNA adducts were detected in tumor tissue obtained from residents living in EN villages more than 15 years. Using the AmpliChip p53 microarray, 19 base substitutions in the sequence exons 2–11 of the p53 gene were identified. Mutations at A:T pairs accounted for 89% of all p53 mutations, with 78% of these being A:T → T:A transversions [34]. The significance of these p53 mutational spectra is best appreciated by comparing the EN data with that reported for sporadic transitional cell carcinomas collected in the IRAC p53 mutational database [35]. In that database, AT → TA transversions occurred in only 5% of all transitional cell carcinomas while in EN and UUC, these mutations were found in 78% of patients examined. This dominant AT → TA transversion is now considered a mutational signature for human exposure to AA. Of particular note is that these signature mutations were not only detected in Croatian but also Bosnian and Serbian patients with UUC, residents of endemic villages. Our data confirm that AA from *Aristolochia clematitis* is the causative agent of endemic (Balkan) nephropathy which may now be considered as an environmental form of the aristolochic acid nephropathy described firstly in Belgium and, more recently, worldwide. Our results indicate that consumption of bread was the sole route of AA ingestion in EN in contrast to other forms of AAN where AA was ingested inadvertently or intentionally, as a part of traditional medicine remedies. However, the question still remains whether AA might have been also ingested in EN by alternative routes mainly as a part of traditional medicine. Thus, we analyzed whether farmers from EN villages have also ingested AA in the form of herbal teas i.e. whether their habits in drinking herbal teas differed from inhabitants of non-endemic villages. Apart from the medical history, physical exam, blood drawing and urine sampling, extensive questionnaire was applied to 2,152 farmers from nine endemic and three non-endemic villages and we obtained data on agricultural practices, tobacco and alcohol use, diet and exposure to different environmental toxic substances. All participants were asked about drinking different herbal teas, and we particularly asked question about usage of parts of *Aristolochia clematitis* as herbal tea. Farmers from endemic villages do not differ in habits of drinking herbal teas

from inhabitants of non-endemic villages. Even more, in endemic villages we failed to observe differences in herbal tea consumption between diseased and suspect of having EN comparing to those at risk and others, non-affected inhabitants of endemic villages [36]. Those results additionally confirmed our previous observation that in EN, aristolochic acid was not ingested via herbal teas leading to the conclusion that EN is not an iatrogenic, but environmental form of AAN where bread is the principal route of AA ingestion.

20.6 Conclusion

Endemic nephropathy results from chronic poisoning with aristolochic acid, an environmental toxin found in an herbaceous plant that has been used in traditional medicine for hundreds of years. It is now appreciated that endemic (Balkan) nephropathy is the same entity as Chinese herb nephropathy and aristolochic acid nephropathy [17]. These entities are the same disease, or better the same syndrome, caused by the same very potent carcinogen and nephrotoxin [37, 38]. In Chinese herb nephropathy AA has been used as a medicinal agent, in Belgian women with AAN it was inadvertently used as part of a slimming regime, and in EN AA was unintentionally ingested at low doses for many years via contaminated bread. Of note is that in 2001 Food and Drug Administration (FDA) warned against the use of products containing AA after seeing an increase in kidney disease among users (BioSlim Doctor's Natural Weight Loss System Slim Tone Formula and Rheumixx for arthritis and gout). Additionally, the most recent official National Toxicology Program report on aristolochic acid carcinogenicity released in June 2011 by the Secretary of Health and Human Services listed AA as a human carcinogen and in the report emphasized the potential worldwide exposure risk from food via wheat flour primarily based on the results of our research group [39].

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Chapter 21
Catastrophe Medicine and Environmental Security: The Dietary Grape Polyphenol Concentrate Enoant as Functional Food in Prevention and Treatment

Vladimir I. Mizin and Yuriy A. Ogay

Abstract Modern medicine pays a great attention to environmental security, i.e. to elimination of adverse health effects due to environmental factors. From the end of twentieth century, the environment became a source of urban and industry induced threats, such as: air and water microbe and chemical pollution, increased levels of ionizing radiation, and high risk of traumas. Essentially, the food, as very important environmental factor, has also changed in the modern era due to new agriculture technologies, refining, synthetic food additives, the influences of ultra-high and ultra-low temperatures, gene engineering, etc. On the other hand, one of the important achievements of the modern medicine is an introduction of functional foods for maintenance of homeostasis of human metabolism under the current environmental conditions. Imbalance between free radicals in organism induced by a variety of stresses and dietary antioxidants is one of the factors damaging human health. Bioantioxidants, i.e. antioxidants naturally engaged in metabolism, are of special value for medical purposes of prevention, treatment and rehabilitation. The adverse effects related to the bioantioxidant deficiency are generated by the accumulation of products arising from oxidation induced by free radicals. An increased need for bioantioxidants may emerge under specific conditions: (1) stress, (2) exposure to ionizing radiation, xenobiotics and another harmful man-made factors of the environment, (3) too much or too little physical activity due to traumas and rescue efforts, (4) administration of pharmaceuticals activating processes of oxidation induced by free radicals, such as antibiotics, narcotic and non-narcotic analgesics, and non-steroid anti-inflammatory preparations. The alcohol-free dietary concentrate Enoant developed by the National Institute for Vine and Wine "Magarach" represents a practical approach to use the Cabernet Sauvignon grape for prevention and treatment of various maladies. Experimental and clinical studies have shown

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curative and preventive benefits of Enoant as a part of methodology used in catastrophe medicine. These may contribute to the achievement, with better effectiveness, of clinical goals in complex treatment and rehabilitation of humans, possessing impaired functions of the cardio-respiratory system, red blood cells, the antioxidant system and lipid metabolism by improving the immunity and lessening of toxic side effects of radiation and pharmacotherapy. Data from experiments and clinical studies on effects exerted by Enoant indicate that the criteria for optimization of medical technologies should include both a sufficient daily dose of the concentrate and the absence of individual negative reactions or grape-induced allergy.

Keywords Catastrophe medicine • Functional food • Grape polyphenol • Dietary concentrate Enoant • Beneficial health effects

21.1 Introduction

Modern medicine pays a great attention to environmental security, i.e. to elimination of damages in health due to environmental factors. From the end of twentieth century, the environment has changed dramatically. It became a source of urban and industrial induced threats, such as air and water microbial and chemical pollution, increased levels of ionizing radiation, and high risk of traumas. Environmental pollutants become a significant source of chronic stress and various diseases including arterial hypertension, chronic cardiac ischemia and chronic bronchitis [1, 2]. Essentially, the food, as a very important environmental factor, has also changed in the modern era due to new industrial and agriculture technologies, refining, synthetic food additives, the influences of ultra-high and ultra-low temperatures, genetic engineering, etc. On the other hand, one of the important achievements of modern medicine is an introduction of functional foods for maintenance of homeostasis of human metabolism under the recent abnormal environmental conditions.

Imbalance between the reactive oxygen substances (ROS) in organism induced by a variety of stresses and dietary bioantioxidants is one of the factors damaging human health due to environmental threats.

Recently, bioantioxidants had been appreciated as an important vital resource for human organism. Bioantioxidants reduce the ROS generation and, sequentially, enzyme oxidation. It creates optimal conditions for metabolism, cell and tissue growth and prevention of various diseases. The bioantioxidant deficiency usually attenuates the organism tolerance to the factors activating the ROS generation, such as stress, ionizing radiation, trauma and pollution. There is a functional antioxidant system (AS) in human organism, which consists of enzyme (including cytochrome P-450) and non-enzyme components (including polyphenols). The ROS generation and AS activity ratio indicate the antioxidant status of the human organism.

To correct the antioxidant status, the dietary risk factors causing the antioxidant deficiency have firstly to be determined. Among them: (1) low bioantioxidant levels

in the diet, (2) oxidants, including alcohol and substances impairing the functions of the human antioxidant system (such as nitrites, products of thermal oxidation of lipids, toxic chemicals, heavy metals), and (3) imbalance between oxidative nutrients and bioantioxidants in the diet due to increased caloric value and excessive intake of fat, especially, refined vegetable oil and products rich in cholesterol and vitamins A and D exerting pro-oxidant effects. An increased bioantioxidant need may also emerge under specific conditions: (1) stress, (2) exposure to ionizing radiation, xenobiotics and another harmful man-made factors of the environment, (3) too much or too little physical activity due to traumas and rescue efforts, (4) administration of pharmaceuticals activating the processes of oxidation, such as antibiotics, narcotic and non-narcotic analgesics, anti-inflammatory preparations, etc.

Polyphenols are the most active natural bioantioxidants, which may be protective against the adverse effects of the oxidative stress. Polyphenols are not synthesized by humans and may only be administrated via vegetable foods. Grapes are the richest source of vegetable polyphenols and flavonoids. Unfortunately, water solubility of polyphenols is small, which entails poor biological availability of these substances. Very small amounts of polyphenols are assimilated by the human organism via direct consumption of grape berries. When subjected to modern processing technologies, they can be made biologically available and administrated at substantially higher doses as a part of grape wines or nutritional concentrates [3].

It is well known that grape polyphenols have reliable curative and preventive effects consisting of reduced oxidation of low density lipids, decreased platelet aggregation, slower development of increased tonus of arterial smooth muscles, reduced coronary blood flow, and reduced frequency of heart attacks, myocardial infarction, oncology and bacterial infections of the gastrointestinal tract [4–9]. Polyphenols are bound by collagen and elastin in arteries improving their resistance to high blood pressure and restoring normal NO synthesis in the epithelia, which, in turn, regulates vascular relaxation [10]. Grape polyphenols have been found to prevent expression of viruses by reducing the levels of peroxide radicals in cultured blood cells [11]. In fact, every polyphenolic and flavonoid substance exerts antioxidant effects, although the best results are provided by the total polyphenolic extract from the grape skins and seeds.

The alcohol-free dietary concentrate from the Cabernet Sauvignon grape Enoant, developed by the National Institute for Vine and Wine “Magarach”, has shown be successfully used for prevention and treatment of adverse environmental effects. It contains 18–20 g/L of total polyphenols, which is about ten times the polyphenol content of red wines. Various polyphenolic substances were identified in Enoant by HPLC (Table 21.1) [12].

Antioxidant activity of Enoant was assessed analytically from data on the air oxidation kinetics of the reduced form of 2,6-dichlorophenol indophenol found in the concentrate. The antioxidant activity index was found to be $2.2 \cdot 10^{-1}$ min/l/ml, which is considerably high, being 15 times more than that for ascorbic acid and three orders of magnitude higher than normal antioxidant activity of human plasma.

Table 21.1 Comparison of polyphenol content (mg/l) of Enoant and Cabernet Sauvignon dry wine

Substances	Food concentrate Enoant	Cabernet Sauvignon dry (table) wine
1	2	3
Antocyans		
Delphinidin-3-O-glycoside	27.1	21.3
Cyanidin-3-O- glycoside	12.3	8.2
Peonidin-3-O- glycoside	13.0	12.6
Petunidin-3-O- glycoside	1.2	0.8
Malvidin-3-O- glycoside	167.5	135.7
Delphinidin-3-O-(6′acetyl –glycoside)	12.4	9.4
Cyanidin-3-O-(6′acetyl –glycoside)	3.6	2.2
Peonidin-3-O-(6′acetyl –glycoside)	6.0	3.2
Petunidin-3-O-(6′acetyl –glycoside)	2.3	1.2
Malvidin-3-O-(6′acetyl –glycoside)	6.2	1.5
Delphinidin-3-O-(6′-n-coumaroil-glycoside)	2.8	1.0
Petunidin-3-O-(6′-n-coumaroil –glycoside)	6.5	1.6
Malvidin-3-O-(6′-n-coumaroil –glycoside)	1.9	0.5
Flavons		
Quercetin	121.2	1.6
Quercetin-3-O- glycoside	46.0	3.9
Flavan-3-ols		
(+)-D-catehin	879.0	64.2
(−)-Epycatechin	567.0	32.1
(−)-Epycatechin-gallate	101.7	11.4
Acids		
n-Coumaric acid	25.3	9.1
Caffeic acid	29.0	5.6
Trans-cautaric acid (n-coumaroil-vinous)	42.6	4.2
Gallic acid	928.4	135.7
Stilbens		
Trans-resveratrol	5.6	0.5
Olygomeric procyanidins		
Procyanidin B1	1,857.0	267.0
Procyanidin B2	1,325.0	155.0
Procyanidin B3	421.0	56.0
Procyanidin B5	269.0	45.0
Procyanidin B7	128.0	15.0
Condensed tannins and polymeric procyanidins		
Total amount of polymeric polyphenols	16,370.0	2,210.0

21.2 Health Effects of Enoant

Enoant delivers to the human organism a complex of biologically important grape polyphenols and flavonoids in water-soluble form. The National Institute for Vine and Wine "Magarach" together with the National Crimean Medical University performed experiments and clinical trials of the biological and health effects exerted by Enoant.

The influence of alcohol and polyphenols on activity of cytochrome P-450 was studied in 70 persons during 24 days. About 70% of them regularly consume alcoholic beverages. Daily doses of ethanol were of 2–8 ml, mean daily dose was ~ 3.9 ml and total dose was ~ 70 ml. Forty persons consume Enoant in daily dose of ~0.45 ml/kg; thus, the mean daily dose of polyphenols was 0.27 g and total dose was 4.9 g. Alcohol and polyphenols caused the opposite effects on the cytochrome P-450 enzyme activity. Statistically significant ($p < 0.05$) increase of Phenazone Cl was observed in persons consumed Enoant.

The influence of Enoant is manifested in optimizing the energy efficiency of physiologic functions. Although, both alcohol and Enoant produce a reduction of psychological stress, however, an increasing of the stress value in composition of blood cells under the influence of ethanol (demonstrated by the Garkavi test) indicates its negative effect. Thus, the absence of alcohol in Enoant could be considered as advantageous.

The cytochrome P-450 enzymes control metabolism of substances entering the organism from the environment. The enzymes reduce biological activity of xenobiotics and increase their removal from organism. For example, the cytochrome P-450 enzymes usually inactivate pharmacologic preparations at about 80% by oxidation and at about 50% by removal. Induction of the cytochrome P-450 enzyme activity suggests the possibility to reduce negative effects of xenobiotics entering the organism due to environmental incidents.

The in vitro experimental study of Enoant has demonstrated the antibacterial effect on *S. aureus* at a minimum concentration of 1.25 g/l. The experimental studies of Enoant in rats have demonstrated the cell protective, liver protective, kidney protective, anti-ulcerous and antitoxic effects [13–17].

One of the important tasks for catastrophe medicine is prevention of side effects of non-steroid, anti-inflammatory medicines on stomach and intestine mucous surface. For example, a single administration of indometacin in dose of 20 mg/kg leads to ulcerous damage in the rat intestines. At the same time, an uptake of Enoant in daily dose of 0.45 ml/kg during a week prior to administration of indometacin diminished ulcerous index from 100% to about 52%. Enoant also diminished rat mortality; the maximum effect was caused by daily doses from 0.15 to 0.55 ml/kg [13].

High functional activity in single intact kidney after post-traumatic operative removal of another one is accompanied by changes in oxidative processes in the organ tissues. Alcohol in daily dose of 15 ml/kg generates disturbances in enzyme activity and microcirculation, dystrophy in epithelial cells and diminishes body

weight and adoptive hypertrophy of single kidney. Administration of Enoant in daily dose of 0.5 ml/kg improves microcirculation and increases the above mentioned adoptive hypertrophy. In connection with an optimizing effect of Enoant on reparation of kidney tissues, its application within the complex postoperative rehabilitation is practical [16]. Enoant consumption (0.25 ml/kg during 21 days) diminished morphologic changes of liver tissues, such as inflammatory, alterative and blood circulatory failures, in rats. Thus, Enoant is a highly effective liver-protecting agent [15]. The grape polyphenols have been specifically accumulated in the heart tissues in concentrations ranged 0.1–0. 5 mg/g, and the concentration remained that high for 3 years. Thus, Enoant could serve as a highly effective heart-protecting agent [17].

Experiments in rats had shown that Enoant reduces frequency of chromosome aberrations in the course of radiation-induced and post-stress thyroxin-induced mutagenesis [18, 19].

The modern environment becomes more and more polluted with mutagens of physical, chemical and biological origin. A great part of population is also subjected to a psychological stress due to various causes. During the environmental catastrophes, the air, water and food pollution usually increase up to dangerous levels. It evokes the stress and hormonal disorders and their consequence – chromosomal aberrations. Many natural bioantioxidants, including grape polyphenols, have the anti-mutagenic characteristics.

An ability of Enoant to modify the spontaneous (due to aging) and induced (due to radiation and thyroid hormone T4) mutagenesis was studied in nonlinear 3–5 months old rats. The control and experimental groups were kept at the same conditions. The liver was used as a test-object, since the liver cells are not divided in the adult rats. However, after partial (2/3) hepatoectomy liver actively regenerated, so the observation of the cells division and the state of the chromosomes became possible. Administration of 0.52–1.04 ml/kg of Enoant daily during 30 days reduced the frequency of hepatocytes with chromosome aberrations up to 1.8 times. The final value was even lower than in intact rats at the beginning of the experiment. The effect of Enoant was dose- and time-dependent. Enoant in daily dose of 0.52 ml/kg had induced effect only after the 30th day of administration. The dose of 0.78 ml/kg increased cytogenetic effect of Enoant, and the effects at the doses of 0.78 and 1.04 ml/kg were similar. Age and sex of animals did not modulate the cytogenetic effect.

Administration of Enoant in a dose of 0.78 ml/kg for 14 days after injections of thyroid hormone T4 in a daily dose of 10 mkg/100 g decreased the frequency of cells with chromosome aberration by 4.4 times comparing to animals with thyroid hormone only, and by 1.3 times comparing to intact animals at the beginning of the experiment. These results allow offering Enoant for prevention of the cytogenetic disorders due to post-stress thyroid gland pathology.

Administration of Enoant in a daily dose of 0.78 ml/kg for 30 days after exposure to a single total ionizing radiation of 250 cGy reduced the frequency of hepatocytes with chromosome aberrations by 1.6–3 times vs. control irradiated animals. Thus, Enoant can be offered for preventing the cytogenetic disorders due to post-radiation pathology [18, 19].

The assessment of the effects of Enoant on ischemia/reperfusion injury was done in bilateral common carotid artery occlusive/reperfusion traumatic ischemic model using Wistar albino rats. The brain is rich in polyunsaturated lipids and pro-oxidative metals and has very low antioxidant capacity. Thus, it is vulnerable to ischemia/reperfusion-induced ROS promoting damage to lipids, DNA, carbohydrates, and proteins and inducing production of several inflammatory proteins, which contribute to neuronal demise. Experiments showed significant changes in electroencephalogram (EEG) activity in rats treated with Enoant both before and after ischemia when compared to their basal EEG values. The glutation levels were significantly increased and the concentration of thiobarbituric acid reactive substance, the most frequently studied marker of oxidative tissue damage, was decreased in animals treated with Enoant in both pre- and post-ischemic periods. These results support the notion that both pre- and post ischemic administration of Enoant might produce protective action against cerebral ischemia. The neuron-protective action of Enoant could be mediated through the following mechanisms: (1) reduction of release of pro-inflammatory cytokines by inhibiting activation of NF-kB, which is a common regulatory element in the promoter region of many pro-inflammatory cytokines; (2) scavenging of ROS; (3) improving the glutathion level; (4) reduction of generation of excitatory amino acids, e.g. glutamic and aspartic [20].

The effects of Enoant in daily concentration of 0.52 mg/kg were studied in children with biocenosis disorders of the intestines as an antibiotics side-effect. Due to its strong antioxidant properties, Enoant considerably reduced concentration of reactive oxygen species, enhanced the activity of the antioxidant system and improved energy-exchange processes. Manifestation of biocenosis disorders of the intestines were considerably diminished, especially of those induced by antibiotic therapy [21, 22].

Putative effectiveness of Enoant was studied in the complex treatment of post-traumatic lower jaw fractures, which are often complicated with periodontitis and osteomyelitis. Administration of Enoant was performed orally in daily dose of 0.5 ml/kg and in 10–15 ml mouthwash three times a day. In 2–3 days, a microbial index was normalized, the immunity improved (including increased levels of T-lymphocytes, immunoglobulin A and lysozyme in saliva and decreased levels of immunoglobulin A and G in blood), the hygienic conditions of the oral cavity also considerably improved, the gingivitis severity decreased and the positive dynamics of main clinical symptoms was accelerated [23, 24].

The effects of Enoant on the dynamics of the condition were studied in operated patients. The concentrate was administered at 30 ml per day during 4 days before the operation and at 15 ml per day during 4 days after the operation for cholelithiasis. By the date of discharge, the conditions of the Enoant-treated patients were better relative to the control group. It was indicated by a considerably enhanced feeling of well-being, improved blood biochemical indices (reduced cholesterol, AST- and ALT-transaminases and lower atherogenic index), faster normalization of bilerubin level and functional status of the cardiovascular system. Administration of Enoant resulted in a considerable improvement of functional status of liver and cardiovascular system in patients with cholelithiasis accompanied by B and C hepatitis, which, in turn, led to a smooth recovery after the operation [25].

The effects of Enoant during treatment and prevention of the drug and alcohol dependence syndromes due to analgesia treatment were studied at the Center of Clinical and Experimental Narcology of National Academy of Ukraine and at the Center of Narcology and Psychosomatic Medicine "Medissa" (Simferopol). Increased alarm level and depression after the consumption of alcohol was stopped constitute the major difficulty in treatment of this pathology. The alarm level and depression were decreased in Enoant-treated patients by 12% (on the HAM-A scale) and 15% (on the BDI scale), respectively, which in turn increased the effectiveness of the therapy [26, 27].

Clinical effects of Enoant also were studied during complex treatment of patients with stress-induced diseases: adenomatous hyperplasia (AH), chronic cardiac ischemia, and chronic bronchitis.

The concentrate had pronounced positive effects on patients with AH in relation to parameters of the cardio-respiratory system and other systems of the organism, including improvement of the auscultative respiration pattern, increase in respiratory volume, decrease in minute blood volume and heart capacity, reduction in the heart rate frequency and respiration rate, decrease in β-lipoprotein content and increase in blood serum catalase activity and color index.

Parameters of the cardio-respiratory system and other systems of the organism were also beneficially affected in patients with chronic cardiac ischemia, including reduced complaints of fatigue, a reduction in labored respiration, reduction in heart pain, and, in general, in all kinds of complains. Positive effects of the concentrate were also indicated by improvement in auscultative respiration pattern, lower diastolic arterial pressure, reduction in heart rate frequency, lower β-lipoprotein content and decreased total blood bilerubin. Increase in blood serum catalase activity (as a part of AS), color index and tolerance to physical exercise were also registered as a reliable positive effect [28].

Many effects of Enoant, such as activation of the antioxidant system of the organism and reduction of the arterial pressure, are common for patients with chronic bronchitis and chronic cardiac ischemia. The benefits of Enoant in patients with chronic bronchitis include a reduction in labored respiration and cough, an increased value of the Hench test, an increased erythrocyte amount in parallel with increased red blood capacity, a decreased number of leucocytes and reduced stress level shown by the Garkavi test, a normalized minute respiratory volume, a reduction in the heart rate frequency, an increase in the maximum volumetric expiratory flow rate at a level of 75% and 50% of forced vital volume, and a lower indexes of Robinson and Cerdo [28–30].

Complex therapy received by Enoant-treated patients with AH, chronic cardiac ischemia and chronic bronchitis has entailed a desire to reduce consumption of alcohol beverages, which is often accompanying the stress. By the end of the course, a reduction in willingness to consume wine was registered in 57% of patients with hypertensive disease, in 37% of patients with chronic cardiac ischemia and in 16% of patients with chronic bronchitis.

The above data show that effects of Enoant, as a part of medical technologies, are related to successful achievement of a number of catastrophe medicine clinical

goals, such as (1) reduction in action of risk factors, (2) reduction in stress and elimination of distress, (3) correction of function of the vegetative nervous system, (4) reduction in the intensity of inflammatory processes, (5) improvement in patency and evacuation function of the bronchi, (6) normalization of function of external respiration, (7) normalization of parameters of oxygen-transport function of blood, (8) normalization of arterial pressure and blood circulation, (9) improvement of function of the cardio-respiratory system and increase in its functional reserves, (10) normalization of lipid exchange, (11) increase in detoxification reserves and antioxidant potential, (12) increase in tolerance to physical exercise, and (13) enhancement of well-being feeling in patients [28–30].

21.3 Conclusions

Experimental and clinical studies have shown curative and preventive benefits of the dietary grape polyphenol concentrate Enoant as a part of preventing, therapeutic and surgical technologies used in catastrophe medicine. These may contribute to the achievement, with better effectiveness, of clinical goals in complex treatment and rehabilitation of humans, including reduction in risk factors, clinical symptoms and negative consequences for cardio-respiratory system, red blood cells, antioxidant system and lipids exchange, and lessening of toxic side effects of radiation, environmental pollutants and pharmacotherapy.

Data from clinical studies and experiments on effects exerted by Enoant indicates that the criterion for optimization of medical technologies of catastrophe medicine should be based on both a sufficient daily dose of the Enoant concentrate (0.25–0.5 ml/kg body weight) and the absence of individual negative reactions or grape-induced allergy in the patients.

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Chapter 22
Thinner Exposure and Memory and Learning Deficits

Victor Nedzvetskii, Svetlana Kyrychenko, and Giyasettin Baydas

Abstract Neural functions are very sensitive to different kind treatment from pharmacology to environmental pollutants substances. Neurospecific proteins are involved in learning and memory. Thinner is a neurotoxic mixture which is widely used as an aromatic industrial solvent. This product has been shown to cause functional and structural changes in the central nervous system. We investigated the effect of exposure to high concentrations of thinner for 45 days on cognitive functions and the levels of neural cell adhesion molecules (NCAM) and lipid peroxidation products (LPO) in the hippocampus, cortex and cerebellum of rats. The actions of melatonin on the effects produced by thinner exposure were also tested. Thinner exposure caused a dramatic increase in LPO in all brain regions. Melatonin administration significantly reduced LPO in these brain regions. NCAM (180 kDa) was significantly decreased in hippocampus and cortex of thinner-exposed rats. Furthermore, thinner-exposed rats showed cognitive deficits in passive avoidance and Morris water maze tasks, whereas in the rats chronically treated with melatonin these effects were reversed. This study indicates that treatment with melatonin prevents learning and memory deficits caused by thinner exposure possibly by reducing oxidative stress and regulating neural plasticity.

Keywords Lipid peroxidation • Melatonin • Neural cell adhesion molecules thinner

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22.1 Introduction

Thinner is a compound containing a mixture of toluene, benzene, acetone, methanol, hexane, and other substances and is a widely used aromatic solvent in textile, paints and solvent-based cleaning fluids. The major component is the neurotoxin toluene (60–70%). Toluene is an abused substance and a well-known neurotoxic agent [1]. The chronic abuse of solvents results in structural and functional impairment to a variety of organs. Thinner fume inhalation is an important cause of encephalopathy and may lead to irreversible brain damage. Particularly, toluene abuse has been shown to cause permanent changes in brain structures which correlate with neural dysfunction [2]. Exposure to toluene is known to cause hearing impairment and reduce birth weight in rats [3]. Furthermore, Mattia et al. [3] demonstrated that an intraperitoneal injection of toluene caused a significant elevation in the rate of reactive oxygen species (ROS) generation and a reduction in glutathione (GSH) levels in the brain. ROS, in turn, damage lipids, proteins and nucleic acids. This leads to neurodegenerative disorders which mediate behavioral changes. Acute and chronic effects of toluene on neurons have been well documented [3]. Toluene can cause CNS depression, loss of memory and progressive brain and nerve damage [4]. Exposure to toluene has been shown to deplete spatial learning, as measured by the Morris water maze [5]. There are limited data to explain the mechanism of the neurotoxic effects of solvents such as thinner and toluene on behavioral and functional structures of the CNS. Neural cell adhesion molecules (NCAM) play important developmental and structural roles in the nervous system and are involved in learning and memorizing processes [6]. Melatonin, pineal-derived product, is a potent free radical scavenger [7] which is known to reduce oxidation-based neurotoxicity [8]. In addition to this effects, melatonin's ability to influence cognitive functions has also been studied. It has been demonstrated that melatonin modulates specific forms of plasticity in hippocampal pyramidal neurons [9]. Recently, we showed that melatonin modulates the expression of NCAM in brain areas concerned with cognitive function [10]. In the present study, we investigated the effects and possible mechanism of melatonin on learning and memory impairment in rats induced by thinner exposure.

22.2 Material and Methods. Animals and Treatments

Male Wistar rats (weighing 200–250 g) were kept in a temperature- and light-controlled room with free access to food pellets and tap water. The animals were randomly divided into three groups each consisting of 40 animals. Two groups were exposed to inhalation of 3,000 p.p.m. thinner 1 h/day between 17:00 and 18:00 h for 45 days. The exposure to thinner was performed in a whole-body inhalation chamber with glass walls. The control group was exposed only to fresh air. One of the thinner-exposed groups was given melatonin intraperitoneally in a dose of 10 mg/kg body weight once daily before thinner exposure over a period of 45 days.

All protocols described were reviewed and approved by the Local Institutional Committee for the Ethical Use of Animals. A one-trial step-down type passive avoidance task was used to evaluate memory retention deficits in rats as previously described [11]. The Morris water maze [12] was selected as a test of spatial learning and memory. A probe trial was performed wherein the extent of memory consolidation was assessed. To test possible deficits in sensor motor processes, rats were tested in the water maze with a visible platform on a new location on the final day of training [13]. For the visual test, the black target platform was placed inside the pool 1 cm above the water line. Latency times to reach the platform were recorded for each trial.

22.2.1 Immunoblotting

Fresh or frozen tissue samples were homogenized 1:10 (w/V) in buffer 10 mM Tris–HCl (pH 7.4), 0,1 mM phenylmethylsulphonyl fluoride (PMSF), 5 μM soybean trypsin inhibitor (soluble powder; Sigma, St, Louis, MO). Homogenates were centrifuged at 80,000 g for 60 min. Pellets were washed and resuspended in homogenizing buffer and re-centrifuged at 80,000 g for 60 min. The resulting pellets were washed and resuspended in buffer (25 mM Tris–HCl (pH 7.4), 0,1 mM PMSF and 2% Triton X-100). SDS-polyacrylamide gel electrophoresis (SDS-PAGE) sample buffer conltaining 2% β-mercaptoethanol was added to the supernatant. Samples were boiled and submitted to SDS-PAGE using 7,5-17,5 gradient gel. Separated proteins were transferred to nitrocellulose filters (Schleich & Schuell Inc., USA) using an electroblotter. Equal amounts of total protein were applied on each lane as described previously [10, 14]. Non-specific binding was blocked by incubation with 1% bovine serum albumin in 100 mM NaCl, 20 mM Na_2PO_4, 20 mM NaH_2PO_4 at pH 7.2. Primary antibody (rabbit anti-rat NCAM antibody) was diluted in the same buffer containing 0.05% Tween-20. Blots were visualized using 0.02–0.05% diaminobenzidine and peroxidase-conjugated goat anti-rabbit immunoglobulin. The relative amount of immunoreactive NCAM isoforms on Western blots was quantified in arbitrary units by scanning blots using a computerized software program (LabWorks 4.0; UVP, Inc. Cambridge, UK).

22.2.2 Protein, Lipid Peroxidation, and GSH Assays

Total protein levels were measured according to Lowry et al. [15]. Tissue lipid peroxidation (malondialdehyde + 4-hydroxyalkenals: MDA + 4-HDA) was determined using an LPO-586 kit (Oxis International, Inc., Corvallis, OR, USA); the method is based on a reaction of N-methyl-2-phenylindole with MDA + 4-HDA at 45°C. GSH levels were determined according to the method of Ellman [16].

22.2.3 Statistical Analysis

The results are expressed as means ± SE. Data were analyzed by ANOVA. Significance was accepted at $P < 0.05$.

22.3 Results and Discussion

Thinner exposure significantly increased the level of LPO, i.e. MDA and 4-HDA, in hippocampus, cerebellum ($P < 0.001$) and in cortex ($P < 0.01$) as compared with those in the control. Melatonin administration to thinner exposed rats significantly reduced the levels of LPO in brain tissues compared with thinner-exposed group. There was no significant effect of thinner inhalation on the levels of GSH in any brain region. Melatonin treatment significantly increased GSH levels in thinner-exposed rats compared with the thinner group.

The ANOVA test indicated that before the acquisition trial, there were no significant differences in the step through latency between groups. Retention of passive avoidance response was different from the control group, the mean retention latency in thinner-exposed group was significantly less ($P < 0.01$) as compared with that of control rats. A reduction in retention latency indicates impairment in memory retention of the passive-avoidance task in thinner exposed rats. Chronic administration of melatonin to thinner-exposed rats ameliorated significantly the impairment of passive avoidance memory in rats exposed to thinner ($P < 0.05$; Fig. 22.1).

All rats showed a gradual reduction in the time taken to find the escape platform as training proceeded. The mean latencies in thinner-exposed and control rats were

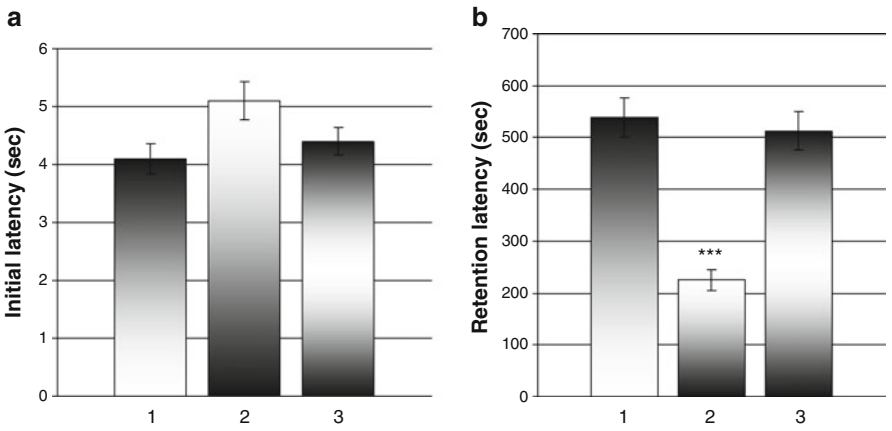

Fig. 22.1 Effects of thinner inhalation on the initial latency (**a**) and retention latency (**b**) in the passive avoidance test. 1 – control; 2 – thinner-exposed group; 3 – thinner + melatonin treated group. (***$P < 0.001$ vs. control)

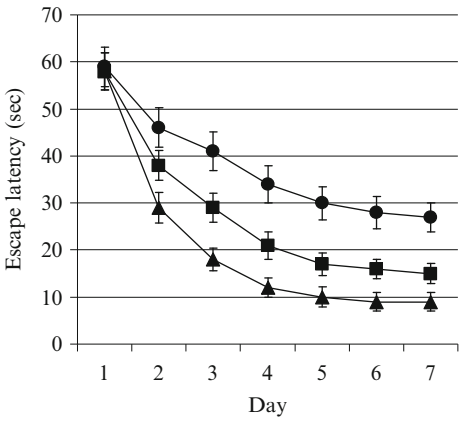

Fig. 22.2 Effects of chronic thinner inhalation on the acquisition of spatial learning in the Morris water maze. ▲ – control; ● – thinner-exposed group; ■ – thinner + melatonin treated group

similar in the first trial, which suggests that their motor performance (ability to swim) was unaffected by the thinner inhalation; however, the thinner-exposed group tended to use more time than controls in following trials. The overall rate of learning was significantly higher in control group than in thinner-exposed rats ($P < 0.02$). Although, melatonin administered rats learned the task faster than thinner-exposed rats ($P < 0.05$), the learning performance of control rats was better than melatonin-treated group ($P < 0.05$). The performance of thinner exposed and control rats in the trial with the visible platform were not different (latencies: 9.2 ± 0.8, 10.2 ± 1.2 and 10.1 ± 1.0 s in control, thinner-exposed and thinner + melatonin-treated rats respectively). Melatonin treatment largely prevents the impaired performance induced by thinner inhalation in the water maze.

Data from the probe trial of the Morris water maze study, which measures how well the animals had learned and consolidated the platform location during the 7 days of training, indicated significant differences between the groups ($P < 0.001$; Fig. 22.2). Thinner-exposed rats spent 39.6% more time in the target quadrant than the control group. On the contrary, the rats treated with melatonin spent significantly less time in the target quadrant than the thinner-exposed group in the probe test ($P < 0.05$). There was a greater significant difference between control and thinner groups ($P < 0.001$) than melatonin and thinner groups ($P < 0.05$).

The levels of NCAM were examined in different brain parts of thinner-exposed and control rats. The antibody against NCAM allowed the detection of bands at 120, 140 and 180 kDa in rat brain. Although there is no significant change in the expression of NCAM 120 and NCAM 140 levels, a significant reduction was found in NCAM 180 level in thinner-exposed rats. Melatonin administration significantly elevated the levels of the three NCAM isoforms both in hippocampus and cortex (Fig. 22.3).

Thinner is thought to generate ROS which induce oxidative damage in lipids, proteins and nucleic acids. Toluene has been shown to produce ROS both in vivo

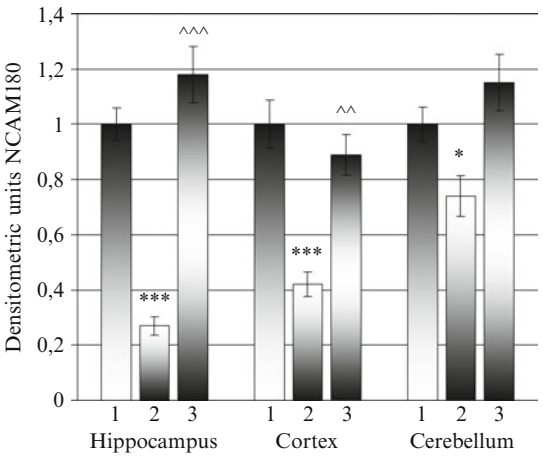

Fig. 22.3 Relative densitometric analysis of NCAM 180 kDa from control and thinner-exposed rats. 1 – control; 2 – thinner-exposed group; 3 – thinner + melatonin treated group. Values are mean ± S.D.; *P <0.05, ***P <0.001 vs. control; ^^P <0.01, ^^^P <0.001 vs. thinner group

and in vitro in many tissues including brain. Individuals working with paint thinner have been shown to have increased levels of MDA in their serum compared with unexposed subjects [17]. Recently, we showed [18] that thinner-exposure increases LPO in different brain regions and these elevations are inhibited by antioxidant melatonin. Consistent with the previous studies herein we demonstrate that thinner exposure significantly elevated the levels of LPO in several brain regions. Generation of ROS can lead to cell and tissue damage and alterations in functions of the brain resulting in cognitive deficits. In the present study, thinner exposure-related effects on cognitive functions were observed in both the Morris water maze and passive avoidance tests in rats. We found that exposure to high level of thinner caused spatial learning deficit, compared with control animals. These findings are in agreement with the results of other investigators who showed that toluene exposure stimulates ROS formation [3, 17] and that memory is impaired by oxidative stress [19]. The retention latency in the passive avoidance test was correlated with increased LPO levels in cortex and hippocampus ($r=-0.50$, $P<0.05$; $r=-0.55$, $P<0.01$ respectively). Furthermore, increased LPO levels within the cortex and hippocampus were also correlated with the swim time spend in the target quadrant ($r=-0.62$, $P<0.01$; $r=-0.67$, $P<0.001$ respectively). These findings indicate that oxidative stress induced by thinner exposure may be an important pathophysiological mechanism underlying learning and memory deficits.

Herein we also showed that the administration of melatonin significantly reduced the levels of LPO in hippocampus and cortex and considerably improved cognitive performance. This strongly indicates that thinner exposure impairs memory, an effect probably mediated by oxidative stress as treatment with the antioxidant melatonin prevented learning and memory deficits. Melatonin is highly effective in reducing oxidative damage in the central nervous system; this efficacy derives from its ability to directly scavenge a number of free radicals and to function as an indirect antioxidant [7, 8]. Furthermore, consistent with the previous studies melatonin

significantly increased GSH levels in brain regions. As learning and memory deficits are associated with increased brain oxidative stress after thinner exposure, and its reversal by antioxidants [20], our results suggests that, at least partly, the effects of melatonin in the improving cognitive deficits could be due to its antioxidant actions. This is in agreement with some previous studies [21].

The second important observation of the current study is the changes in the expression pattern of NCAM in different brain regions of thinner-exposed and melatonin administered rats. To our best knowledge this is the first report that documents an effect of melatonin on the expression of NCAM in thinner-exposed rats. NCAM contribute to the structural organization of the nervous system during brain development and also participate in synaptic modification in the mature brain. Furthermore, it has been shown that NCAM may contribute to the neural regeneration. In accordance with learning, expression of NCAM was impaired in the thinner-exposed rats compared with controls. Several recent studies indicate a role for NCAM in learning and establishment of long-term memory [22]. In the present study, we found that chronic exposure to thinner induced a significant reduction especially in the expression of NCAM 180 in both hippocampus and cortex.

The mechanism by which thinner exposure alters the pattern of NCAM expression is not known. Chronic stress exposure induces a considerable degree of structural plasticity in the adult brain, especially in the hippocampus where these changes are accompanied by impairments in cognitive performance [23]. It is postulated that chronic stress interferes with the mechanisms involved in the expression of NCAM [24]. Thinner exposure to rats provides a relevant example of chronic stress which generates ROS and induces structural and functional changes in the nervous system [3, 17]. Supporting this hypothesis, a significant elevation of lipid breakdown products was found in hippocampus, cortex and cerebellum. Loss of NCAM 180 has been found to be accompanied by cell injury following ototoxicant trimethyltin chloride, which involves ROS generation, exposure and to be related to cytoskeletal alterations and destabilization of cellular contacts [25]. We have previously indicated that NCAM is down-regulated in the hippocampus of rats under constant light leading to generation of ROS [10]. Thus, we speculate that generation of ROS induced by thinner exposure alters the pattern of NCAM expression and suggests that modification of NCAM could affect cognitive functions in rats. We found here that thinner exposure, like ototoxicant trimethyltin chloride, reduced the levels of NCAM 180 in cortex and hippocampus. The changes in the expression of NCAM may underlie the impaired cognitive performance induced by thinner exposure. One possible explanation for the learning and memory deficits in the thinner-exposed rats is that synaptic remodeling and plasticity require optimal NCAM concentrations that are permissive for activity-dependent synaptic sprouting [26]. Thus, thinner exposure causes down-regulation of NCAM 180 in hippocampus and this in turn inhibits the formation of new synapses required for learning and memory. NCAM 180 is the main isoform of NCAM and it is crucial for the stabilization of cell binding at synaptic sites [27]. NCAM 180, but not NCAM 140, is known to interact with spectrin via its cytoplasmic domain. This interaction was proposed to be important in signal transduction [28]. Augmented NCAM expression in the

hippocampus and cerebral cortex by pharmacological doses of melatonin indicates that these brain areas may be sites at which this indole modulates cognitive processes. In the brain, melatonin-binding sites have been found in regions implicated in cognition and memory [29]. Argyriou et al. [30] showed that melatonin facilitated short-term memory and that endogenous melatonin administration exerted a permanent facilitatory effect on memory processes.

22.4 Conclusions

Astrocytes play the key role in the regulation of neuron microenvironmental. Presented data show that melatonin can defend the neurons from toxic compounds through the protection of astrocyte surviving. Especially, melatonin may promote effectiveness neuroprotective effect of astrocytes by its antioxidant activity. The cellular and molecular mechanisms by which melatonin modulate cognitive functions are as yet unclear. The findings of the present study show that melatonin alters NCAM expression in the hippocampus, cortex, and cerebellum. Loss of NCAM 180 has been found to be accompanied by cell injury following toxicant exposure and to be related to cytoskeletal alterations and destabilization of cellular contacts. Thus, melatonin could stabilize neuronal connections by preventing the reduction of NCAM 180 expression.

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Chapter 23
Environmental and Drug Induced Renal Damage; The Way to Protect

Svetlana Babiy, Olga Dyomshyna, Tatiana Loskutova, and Natalia I. Shtemenko

Abstract Kidneys are targets of numerous toxicants due to anatomical, physiological and biochemical features of the organs. Factors contributing to the sensitivity of kidneys include large blood flow, presence of a variety of transporters, a lot of functionally necessary metabolizing enzymes, etc. This paper reviews some mechanisms of nephrotoxic action of widely distributed metal compounds and of an anticancer drug cisplatin as a model of drug induced renal damage. Cisplatin is known to induce nephropathy that is restricted primarily to the S_3, segment of the proximal tubule, with involvement of S_2, and S_1 segments at higher doses. This particularity appears to be derived from the distribution of enzymes and transport proteins important for uptake of cisplatin into proximal tubule cells: apical γ-glutamyltranspeptidase and the basolateral organic anion transport system. Regional distributions of transport mechanisms for binding proteins appear to be important in the expression of nephrotoxicity of cisplatin. According to the mechanism of damage the way to protect is proposed with application of antioxidants and mighty antioxidants such as cluster rhenium compounds with organic ligands that contain an unique quadruple bond are demonstrated as nephroprotectors in the model of tumor growth and cisplatin application.

Keywords Pollutants • Nephrotoxicity • Model of tumor growth • Acute failure • Antioxidants • Cluster rhenium compounds

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23.1 Introduction

For some heavy metals toxic levels can be just above the background concentrations usually found in nature [17, 30, 32, 35]. The symptoms of chronic exposure are very similar to the symptoms of common diseases and are often developed slowly over months or even years. The associations of the symptoms of chronic exposure actually abate from time to time, leading the person to postpone seeking treatment, thinking the symptoms are related to something else. The symptoms indicative of acute toxicity is not difficult to recognize because they are usually severe, rapid in onset and associated with a known exposure or ingestion [5, 19, 28, 35, 36]: cramping, nausea, and vomiting, pain, sweating, headaches, difficulty breathing, impaired cognitive, motor, and language skills, mania, and convulsions, etc. [19]. Several antineoplastic agents metal-based drugs exert potent nephrotoxicity with common symptoms. In cancer patients one must always be aware of additive or synergistic effects that may potentiate chemotherapy-induced nephrotoxicity [21]. Through induction of renal hypoperfusion and prerenal azotemia, these underlying processes enhance nephrotoxicity of many metal drugs and substances. In particular, renal hypoperfusion increases nephrotoxicity in drugs excreted primarily by kidneys (excessive drug dosing), in those reabsorbed in the proximal tubule (increased intracellular concentration), and in those that tend to be insoluble in the urine where crystal precipitation occurs within distal tubular lumens with sluggish flow [5, 15, 16, 19, 28, 36]. These processes have difficult mechanisms. The unusual susceptibility of the mammalian kidneys to the toxic effects of noxious chemicals can be attributed in part to the unique physiologic and anatomic features of this organ. Although the kidneys constitute only 0.5% of total body mass, they receive about 20–25% of the resting cardiac output. Consequently, any drug or chemical in systemic circulation will be delivered to these organs in relatively high amounts [28]. The processes involved in forming concentrated urine also serve to concentrate potential toxicants in the tubular fluid. As water and electrolytes are reabsorbed from the glomerular filtrate, chemicals in the tubular fluid may be concentrated, thereby driving passive diffusion of toxicants into tubular cells. Therefore, nontoxic concentrations in the kidney of chemical in plasma may reach toxic concentration in the kidneys. Progressive concentration of toxicants along the nephron may result in intraluminal precipitation of relatively insoluble compounds, causing acute renal failure secondary to tubular obstruction. Finally, renal transport, accumulation, and metabolism of xenobiotics contribute significantly to the susceptibility of the kidney (and specific nephron segments) to toxic injury [5, 16, 28].

23.2 Mechanism of Metal Compounds Toxicity

Acute renal failure is a common complication associated with the treatment of cancers and is connected with a marked increased risk for mortality [6]. It is associated with used metals in cancer therapy (Pt, V). They are accumulated in the mammalian kidneys, largely in the proximal tubule cells, and cause functional and structural

damage that result in reabsorbtive and secretory defects [25]. Most metal ions are essential enzyme cofactors. In the free form these ions in biological systems can facilitate the transfer of electrons to susceptible macromolecules such as proteins, lipids and DNA. It is important for organisms that free transition metals in biological fluids are restricted to very low levels. The exposure to redox-active transition metals is controlled primarily through the action of specific chelating proteins such as ceruloplasmin or transferrin [37].

According to the urinary data, proximal tubules seem to be the major site of metal-induced nephrotoxicity. Dependent on the severity of intoxication, functional defects are often accompanied with a plethora of structural damages in the proximal tubules epithelium, including loss of cell–cell contacts and detachment of cells from the basement membrane, blebbing, shortening and loss of microvilli, loss of basolateral invaginations, vesiculation of the cytoplasm, derangement of the cytoskeleton, swelling, vacuolation and fragmentation of mitochondria, swelling of lysosomes and whole cells, etc. [22, 23].

The mechanisms of platinum drugs and other heavy metal entry into the proximal tubules cells are poorly known. Recent studies have indicated that a significant uptake of Cd, Hg, Pb, and Pt (such as cisplatin) in proximal tubules and some other mammalian cells may proceed by ionic and molecular homology or 'mimicry', e.g., transported as free ions through calcium (Ca^{2+}) channels or ion-transporting proteins (Ca^{2+}-ATPase, DMT1, Mn and Zn transporter ZIP8), or bound to thiol (SH)-containing amino acids and organic anions and cations (methionine, cysteine, homocysteine, N-acetylcysteine (NAC), glutathione (GSH)), that are carried by specific transporters located in the basolateral and brush-border membranes [14, 29, 33]. In addition to segmental difference in transport, segmental differences in cytochrome P450, apical γ-glutamyltranspeptidase, and cysteine conjugate β-lyase activity also are contributing factors to the enhanced susceptibility of the proximal tubule. These enzymes are localized almost exclusively in the proximal tubule, with negligible activity in the glomerulus, distal tubules, or collecting duct. For example, cisplatin nephrotoxicity is released by P450, γ-glutamyltranspeptidase, and β-lyase-mediated bioactivation. Thus, primary effect this compound will most certainly be localized in the proximal tubule [25, 30, 31].

The nephrotoxicity following molecular mimicry of metal uptake via organic anion and cation transporters develops predominantly in the straight (S3) portion of proximal tubules, reflecting the segment-specific localization of the transporters. [3] For example, S3 segment is the primary site of transport and toxicity of cisplatin, and several lines of evidence suggest a strong correlation between the transport, accumulation, and nephrotoxicity of this metal compound. The higher doses of this drug can also damage S1, S2 sigments, distal tubule, and collecting duct [5, 25]. Once taken up and sequestered by proximal tubular cell, the nephrotoxic potential of metal drugs ultimately may be depended upon the intrinsic reactivity of the drug with subcellular or molecular targets [24, 30].

Finaly, proximal tubular cells appear to be more susceptible to ischemic injury than are other parts of the nephron. Therefore, the proximal tubular cells will be the primary site of toxicity for platinum drugs and environmental heavy metals, which interfere with renal blood flow, cellular energetic and mitochondrial function [30].

23.3 Metal-Induced Oxidative Stress

The mechanisms of nephrotoxicity at the cellular level of various toxic metals have been studied for decades, and are still known only in fragments; some more details have been collected for the actions of Cd, Hg, and cisPt [22]. A growing amount of results provide the evidence that toxic metals are capable of interacting with nuclear proteins and DNA causing oxidative deterioration of biological macromolecules [33].

Metal-induced formation of free radicals has some common mechanisms involving Fenton generation of the superoxide and hydroxyl radical appear to be involved for iron, copper, chromium, vanadium and cobalt (Me = metal) [4, 22, 33].

$$Me^{(n+1)+} + O_2^{\bullet -} \rightarrow Me^{n+} + O_2 \tag{23.1}$$

$$2O^{2\bullet -} + 2H^+ \rightarrow H_2O_2 + O_2 \tag{23.2}$$

$$Me^{n+} + H_2O_2 \rightarrow Me^{(n+1)+} + {}^{\bullet}OH + OH^- \left(\text{Fenton reaction}\right) \tag{23.3}$$

Fenton reactions are predominantly associated with mitochondria, microsomes and peroxisomes [29, 33].

Recent studies in vivo (experimental animals) and in vitro (renal cortical slices, various cell lines of the renal origin, isolated mitochondria) have indicated oxidative stress, apoptosis, and necrosis as common phenomena in the intracellular action of all toxic metals studied thus far. The best evidence supporting the hypothesis of the oxidative nature of metal-induced genotoxic damage is provided by a wide spectrum of nucleobase products typical for the oxygen attack on DNA in cultured cells and animals exposed to carcinogenic metals (Fig. 23.1) [17, 33].

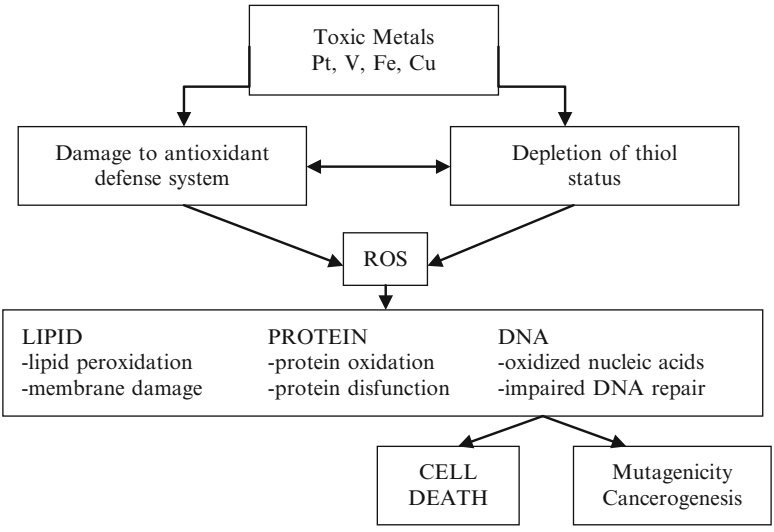

Fig. 23.1 Pathways of metal-induced oxidative stress [4]

A number of other phenomena, such as the inhibition of ion channels, ATPases and other transporters, enhancement of ion conductances, increase in intracellular (cytoplasmic) concentration of Ca^{2+} ($[Ca^{2+}]_i$) deranged metabolism, cytoskeleton and cell polarity, impaired endocytosis and intracellular vesicle recycling, increased synthesis of metalotheonines, upregulation of heat-shock proteins in the cell cytoplasm and multidrug resistance proteins in the brush-border membrane, selective loss of transporters from the brush-border membrane and basolateral membrane, destabilization and/or loss of the cell membrane integrity, and distinct structural and functional damage in mitochondria have been demonstrated in fragments in the course of action of Cd, Hg, and cisPt. Their appearance with other toxic metals has not been reported yet [14, 2, 33].

23.4 Role of Some Antioxidants

The metal anticancer drugs usually have electron-sharing affinities that can result in formation of covalent attachments [4]. These attachments are mainly formed between heavy metals and sulfhydryl groups of proteins [7]. The tripeptide, reduced glutathion (GSH), is found in mammalian kidney tissues at millimolar concentrations and, therefore, accounts for more than 90% of the total non-protein sulfur. Its physiological and pathological roles in metabolic regulation have been reviewed extensively [4]. Interaction of metal drugs with GSH metabolism is an essential part of the toxic response of many metals [10].

When GSH is depleted by any metal, GSH synthesizing systems start making more GSH from cysteine via the γ-glutamyl cycle. GSH is usually not effectively supplied, however, if GSH depletion continues because of chronic metal exposure [10, 20, 29]. Several enzymes in antioxidant defense systems may protect this imbalance. Unfortunately, most of these enzymes become inactive also due to direct binding of metals to the enzymes' active sites, if the sites contain sulfhydryl groups [20]. Furthermore, zinc, which usually serves as a cofactor of many enzymes, could be replaced by heavy metals, thereby making the enzymes inactive [4]. Conjugation to GSH is a means of detoxifying electrophilic compounds. However, as noted above, the glutathione-conjugates of some compounds can be further metabolized to nephrotoxins [31]. Proposes the hypothesis that the nephrotoxicity of cisplatin is the result of the metabolic activation of the cisplatin in kidneys to a more potent toxin. This activation is through a pathway including γ-glutamyltranspeptidase (GGT) and cysteine-S-conjugate beta-lyase [31].

Cisplatin is not a substrate for either GGT or cysteine-S-conjugate beta-lyase. However, cisplatin has been shown to form glutathione-conjugates spontaneously in solution [2]. Dissociation of one of the chlorines from the cisplatin molecule results in a positive charge on the platinum that will attract the negatively charged sulfur on the cysteine moiety of the glutathione molecule. Cisplatin-glutathione-conjugates have been isolated from cells treated with cisplatin and from the serum of cisplatin-treated rats [11]. Pretreating rats with an inhibitor of glutathione-S-transferases reduced the nephrotoxicity of cisplatin, suggesting that in vivo glutathione-S-transferases may

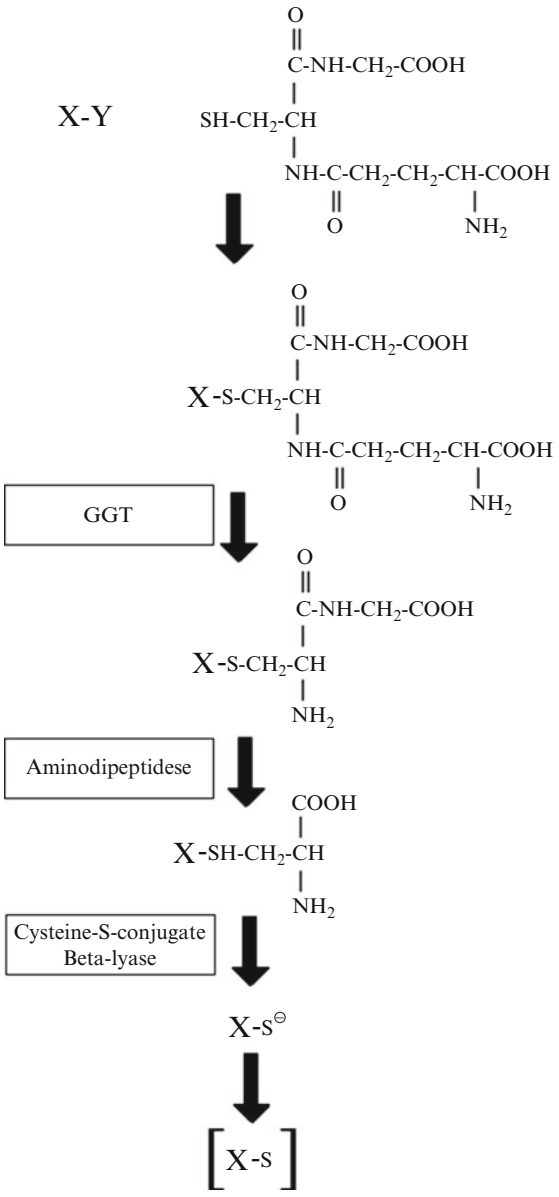

Fig. 23.2 Metabolic activation of glutathione-conjugates to reactive thiols (X represents the platinum or other metal and Y a halogen molecule: fluorine, chlorine, or bromine) have been shown to be metabolized to nephrotoxins via this pathway [31]

catalyze the conjugation of cisplatin to glutathione [24]. It is possible, that GGT cleaves the gamma-glutamyl group of the glutathione-conjugate, and aminodipeptidase cleaves the cysteinyl-glycine bond, resulting in a platinum-cystein-conjugate. Finally the cystein-conjugate is metabolized by cysteine-S-conjugate beta-lyase to a reactive thiol (Fig. 23.2) [31].

While conjugation of cisplatin to GSH is the first step in the activation of cisplatin to a nephrotoxin, it renders cisplatin inactive as an antitumor drug. Conjugation of cisplatin with glutathione reduces the formation of interstrand and intrastrand platinum DNA adducts, resulting in decreased toxicity of cisplatin in dividing cells [8]. Expression of GGT has opposing roles in the nephrotoxicity and antitumor activity of cisplatin. GGT expression is necessary for the metabolism of cisplatin to a nephrotoxin [9, 31].However, GGT expression in tumors decreases the antitumor activity of the drug [8]. These contradictory effects may be due to differences among tissues in the uptake of the cisplatin-cysteine-conjugates or in expression of the cysteineconjugate beta-lyase that converts the cisplatin-cysteine-conjugate to a reactive thiol [31].

Thiol compounds are used in clinical practice to mitigate cisplatin-induced nephrotoxicity. Some thiol compounds, such as diethyldithiocarbamate, are bound to cisplatin and inactive it, reducing both the antitumor and nephrotoxic activity of the drug [31]. We claim that these compounds block both the binding of cisplatin to DNA and the binding of cisplatin to glutathione, preventing its further metabolism to a nephrotoxin. A large number of sulfur-containing compounds have been shown to reduce the nephrotoxicity of cisplatin without inhibiting its antitumor effect [34]. Some of these agents, such as the prodrug Amifostine, are used in the clinic to protect against cisplatin nephrotoxicity [13]. Procainamide, an antiarrhythmic drug, also protects against the nephrotoxicity of cisplatin without altering its antitumor activity [31]. The formation of the procainamide-cisplatin complex increases the amount of platinum bound to DNA, which would explain the maintenance of the antitumor activity of cisplatin in the presence of procain amide [34]. The binding of procainamide to the cisplatin may prevent the formation of a cisplatin-GSH complex and thereby protect against the metabolism of cisplatin to a nephrotoxin. The thiol agents may be working by the same mechanism as procainamide, forming complexes with cisplatin that do not prevent the binding of the platinum to DNA but do prevent the formation of a GSH-cisplatin-conjugate [31].

23.5 Antioxidant and Nephroprotective Properties of Rhenium Cluster Compounds

Cancer patients are frequently treated with nephrotoxic platinum drugs. These patients also may present with immune complex-mediated glomerulopathy or paraprotein-felated nephropathy, and with amyloidosis as well as minimal-change nephritis. They may have direct infiltration of the kidney by tumor cells as well as an obstructive nephropathy caused by lower urinary tract invasion and compression [21]. Therefore, search of the new antitumor drugs with low nephrotoxic effects is a key problem.

An important paradigm for the development of new antitumor pharmaceuticals is represented by dinuclear paddle-wheel carboxylate complexes of rhodium, ruthenium and rhenium (Re) [18, 26, 27]. Of this group, the dirhenium complexes are the

most promising for clinical development because of their low toxicities as compared to other heavy metal-containing compounds [18]. The paddle-wheel structure of dirhenium (III) complexes provides very flexible approaches towards substitution and functionalization, including tuning the number of bridging carboxylate, amidate or amidinate ligands and additional monodentate coligands such as halogenides. It also allows introducing additional binding sites within the structure of the organic portion and varying the charge of the complex. These factors may be important considering the interaction of the Re species with nucleobases and binding to DNA. Similarly to dirhodium compounds, two initial steps of such interaction may involve coordination at the axial position of the cluster (which is trans-to the quadruple Re–Re bond) followed by trans/cis rearrangement with substitution of the equatorial group. Thus, the relative liability of the equatorial ligand (for example, chloride vs. bridging carboxylate) is essential for ease of substitution, while weak interactions at the axial positions are sensitive to the overall charge of the complex [27]. It is postulated that such species can bind to DNA and inhibit DNA replication in tumor cells, in a manner similar to cPt [27], with little effect on RNA or protein synthesis [18].

Our previous works have shown that different cluster rhenium compounds inhibit tumor growth by 20–30% when introduced in the liposome form to tumor-bearing animals. At the same time, these species reveal potential as biochemical modulators of cPt action, which enhance efficiency and decrease toxicity of the latter. The combined use of cPt and the Re component in nanoliposomal capsules (rhenium-platinum (Re-Pt) antitumor system) had more therapeutic effect in most of the experimental animals [26]. Experiments with Re compounds and Re-Pt system in the models of tumor growth have shown antiradical, anticancer, cells-supporting properties of this substance practically without nephro- and hepatotoxic effects [1, 12]. We showed, that GSH level was in twice higher and MDA level in three to four times lower in kidney homogenates under application of rhenium compounds together with cisplatin in comparison to cisplatin groups. We may conclude that rhenium cluster compounds lowered the oxidative stress in kidneys caused by cisplatin and activated GSH-defensive system. Rhenium compounds also activated GGT (in 1.8–1.9 times in comparison with control data) that may witness about high excretion of cPt-GSH conjugates in kidneys of these group.

23.6 Conclusions

The main way of metal nephrotoxicity is oxidative stress that led to lowering of GSH level and depletion of protective capacity of kidneys, such as excretory activity. Quadruple bond-containing rhenium compounds with organic ligands supported normal GSH level and GGT-activity due to antioxidant properties and diverse chemical potential. To our mind such application of such antioxidants is the way for kidneys protection and these substances are very promising in cancer therapy and in any circumstances followed by oxidative stress.

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Chapter 24
Conservation of Medicinal and Aromatic Plants in Croatia

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Abstract The use of medicinal and aromatic plants (MAP) in Croatia has a very long tradition. Commercial gathering was economically important source of income in many Croatian regions. However, gathering in the wildness has a negative impact on biodiversity, collected plant material is not uniform and its price is significantly lower. Current production of medicinal and aromatic crops in Croatia is very limited. The MAP cultivation on family farms is usually marginal activity. MAP producers and processors generally agree that marketing opportunities do exist in case of a number of species. Natural MAP populations in Croatia show great biodiversity in morphological, biochemical and genetic level. The assessment of biodiversity is a starting point for efficient conservation of plant genetic resources and its use in plant breeding programmes. The main aim of the management of plant genetic resources is conservation, characterization, evaluation and documentation of the existing genetic biodiversity. For the purpose of conservation of these valuable genetic resources, The Collection of Medicinal and Aromatic Plants has been established at the Faculty of Agriculture, University of Zagreb, Croatia. Characterization and evaluation of accessions in plant genebanks is traditionally based on morphological traits. Currently, the analysis of target species including Dalmatian sage (*Salvia officinalis* L.), Dalmatian pyrethrum (*Tanacetum cinerariifolium*/Trevir./Sch. Bip.) and basils (*Ocimum* spp.) has been carried out on morphological, biochemical and genetic level.

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24.1 Introduction

Croatia is located in Southeast Europe and occupies three different geographical regions and climatic zones: Pannonian in the northern part of the country, influenced by the continental climate; Mediterranean coastal region with Mediterranean climate and Mountainous region that occupies the central part of Croatia. Various types of climates, relief and soil are the main reason for the richness of medicinal and aromatic plant species (MAP). Moreover, natural medicinal and aromatic plant populations show great biodiversity on morphological, biochemical and genetic level. Mediterranean landscapes are remarkably heterogeneous, with sources of heterogeneity ranging from anthropogenic to biologically generated processes that self reinforce across spatial and temporal scales. Therefore, the Mediterranean region is considered as one of the biodiversity hotspots for conservation priorities. Gathering and use of medicinal and aromatic plants in Croatia has a very long tradition. Agricultural production of a number of medicinal and aromatic plant species was also economically important source of income in many Croatian regions. Commercial gathering in the wildness has a negative impact on biodiversity conservation, also collected plant material is not uniform and its price is significantly lower. Therefore, it is important to promote production of medicinal and aromatic crops in Croatia which is currently very limited. MAP cultivation is mainly organized on family farms as a marginal activity and only a few farms are specialized in MAP production. According to the Croatian Bureau of Statistics [12] MAPs are cultivated on an area of about 4,000 ha, which is far below Croatian potential due to favorable bioclimatic conditions. Since 1999, Croatian MAP producers and processors are organized in the Medicinal and Aromatic Herbs Production and Processing Group at the Croatian Chamber of Economy with the aim to encourage cooperation in this sector and to strengthen links between research institutes, private companies and government agencies. The Group is also involved in making regulations and legislation affecting production, processing, quality control, and trade of MAP species and products. MAP producers and processors generally agree that marketing opportunities do exist in case of a number of species but there are many problems to be solved and increased governmental support is clearly needed. Since there is no breeding program for MAP species in Croatia, additional problem is a lack of commercially available seeds of modern high-quality, high-yielding cultivars. Moreover, there is a need for education and training of necessary specialists for the establishment of medicinal and aromatic plants production. A training program on Production and Processing of Medicinal and Aromatic Plants has been organized by the Samobor Open University with the aim of promoting the production of MAPs as well as to raise awareness of the threats from the overexploitation of natural resources. This training program has become an influential forum bringing together family farm owners, wholesalers and processors of MAPs in Croatia [28].

24.2 Legal Protection of Plant Species in Croatia

The basic Croatian law regulating the issues of biological and landscape diversity preservation is The Nature Protection Act (Official Gazette 70/05; 139/08; 57/11). According to this Act the nature is an important part of the environment and under particular protection by the State. The purpose of this Act is to provide protected parts of nature, to preserve the exploitability of nature's resources, to safeguard the conditions for its conservation and development and to prevent any kind of nature destruction. Establishment of protected natural areas is the main principal of *in situ* biological conservation method. Protected natural areas are classified into nine categories: strict reserve, national park, special reserve, Nature Park, regional park, nature monument, significant landscape, Park Forest and park architecture monument. Currently, there are 461 protected areas designated in various categories covering approximately 7.95% of the Croatian State Territory. The largest portion of the territory is protected as nature parks, i.e. 3.71% of the total protected area. Apart from the protected areas, pursuant to The Nature Protection Act threatened plant species are equally protected either as strictly protected wild taxa, protected wild taxa and protected native domesticated taxa. The Ministry of Culture issued the Ordinance on the proclamation of protected and strictly protected wild taxa (Official Gazette 7/06 and 99/09), which entered into force in July 2009 and it lists a total of 768 strictly protected and 320 protected taxa. The Ordinance regulates protection of plant species which are on the Red List in Croatia. The Red List lists endangered taxa in the Republic of Croatia and represents one of the basic expert documents in nature conservation. Red Book of Vascular Flora of Croatia [20] provides the data of the endangered species in the Republic of Croatia including description and the biology of the species, distribution, ecological characteristics, ongoing legal protection, causes of threats and proposed conservation methods. It provides a list of 760 nationally threatened plant species with their appropriate categories of threat according to the IUCN criteria. Plant species are classified into ten categories: extinct (EX) (1), Extinct in the wild (EW); Regionally Extinct (RE) (10) Critically Endangered (CR) (90); Endangered (EN) (62); Vulnerable (VU) (71); Near Threatened (NT) (186); Least Concern (LS); Data Deficient (DD) (340); Not Evaluated (NT). According to the Red Book of Vascular Flora of Croatia, six plant species are endangered due to collecting as medicinal plants: arnica (*Arnica montana* L.), bear-grape (*Arctostaphylos uva-ursi*/L./Spreng.), yellow gentian *(Gentiana lutea* L. ssp. *symphyandra*/Murb./Hayek), pheasant's-eye (*Adonis annua* L.), mandrake (*Mandragora officinarum* L.) and bog-bean (*Menyanthes trifoliata* L). Other factors which also represent a threat for plant species are habitat loss due to road construction, drainage, dam building, clearing the land for agriculture, as well as pollution of earth, water and atmosphere and collecting in decorative purposes [27]. According to the Article 89 of the Nature Protection Act the approval from the Ministry of Culture should be obtained for gathering plants and their parts that are not protected by present or other law, if this is done for scientific research, processing, trading or commercial purposes. Gathering of the wild protected species is regulated by the Ordinance on the collecting of protected wild growing plants for the processing,

trade and other purposes (Official Gazette 154/08). The Ordinance defines the measures of protection and requirements for obtaining the approval from the Ministry for collecting wild species and their parts for medicinal, nutritional, decorative purposes or cultivation for further processing and trade. Upon request of natural or legal persons, Ministry of Culture issues permits where it sets quotas, plant parts approved for gathering and conditions under which the collecting must be conducted. Quotas depend on the endangerment and distribution of defined plant species. In the period from 2005 to 2009, curry plant (*Helichrysum italicum*/Roth/G. Don; above-ground parts), bay laurel (*Laurus nobilis* L.; leaves), dog rose (*Rosa canina* L.; fruit) and chasteberry (*Vitex agnus-castus* L.; leaves and flowers) are the species which have been collected in largest quantities. Other plant species which were also of great interest for collecting are heather (*Calluna vulgaris*/L./Hull), snowdrop (*Galanthus nivalis* L.), St. John's wort (*Hypericum perforatum* L.) and spineless butcher's broom (*Ruscus hypoglossum* L.) [3].

24.3 Conservation of Plant Genetic Resources

In 2004 Croatia joined SEEDNet (South East European Development Network on Plant Genetic Resources) supported by Swedish International Development Agency (SIDA). The main goal of SEEDNet is long-term conservation and utilization of the diversity of plant genetic resources within the South East European region through a well coordinated network of functional national programs. SEEDNet operates through seven working groups: Cereals and Maize, Medicinal and Aromatic Plants, Vegetables, Fruit crops and Vitis, Fodder crops, Industrial crops, and Documentation and Information. Within SEEDNet working group for Medicinal and Aromatic Plants (WG MAP) a project entitled 'Genetic Structure of Dalmatian Sage (*Salvia officinalis* L.) Populations: A Model for a Collaborative Research on MAP Genetic Resources' has been conducted. The objective of this project was the use of Dalmatian sage as a model species for the establishment of a collaborative network for the assessment of genetic variability of medicinal and aromatic plants within SEEDNet WG MAP. Ecogeographical surveys and collecting of Dalmatian sage populations have been successfully carried out in all SEEDNet partner countries including Albania, Bosnia and Herzegovina, Bulgaria, Croatia, Kosovo, Macedonia, Moldova, Montenegro, Romania, Slovenia and Serbia. Two collecting missions were organized in each partner country in order to collect leaf material of two Dalmatian sage populations in order to carry out biochemical and molecular analyses. Commission on Plant Genetic Resources has been constituted in 2006 by the Ministry of Agriculture, Forestry and Water Management. Commission prepared the Ordinance on the Conservation and Sustainable Use of Plant Genetic Resources approved by the Ministry in 2009 (Official Gazette 89/09). The main objective of the Commission is to coordinate all the activities regarding collecting, storage and regeneration, characterization and evaluation, documentation, distribution and utilization of Croatian plant genetic resources. Seven working groups covering all

plant species have been established including researchers affiliated to 12 Croatian institutions. The Collection of Medicinal and Aromatic Plants has been established in 1998, at the Department for Seed Science and Technology, Faculty of Agriculture, University of Zagreb. Through this collection, plant genetic resources of medicinal and aromatic plants are systematically collected, characterized, maintained, evaluated, regenerated and documented in order to be introduced into agricultural production and into breeding programs. From the year 2006 the Collection and the conservation of medicinal and aromatic plant genetic resources is supported through MAP Working Group within the framework of the Commission on Plant Genetic Resources. The procedure of integrating an accession into a collection involves cleaning, moisture determination, drying, viability testing, packaging and registration. Accessions are held in classical *ex situ* maintenance facilities for medium term seed storage, in a cold chamber at +4°C, in paper bags or glass jars. Seed viability of each accession is periodically monitored and when necessary the regeneration is performed. Currently the MAP collection holds 1961 accessions of 275 MAP species. Approximately 80% of the collected material represents wild material of Croatian origin, sampled during collecting missions or donated. The foreign accessions are obtained from other national gene banks, botanical gardens and research institutes. The most represented species in the MAP collection are: basils (*Ocimum* spp. mainly *O. basilicum* L.), Dalmatian sage (*Salvia officinalis* L.), St. John's wort (*Hypericum perforatum* L.), Dalmatian pyrethrum (*Tanacetum cinerariifolium*/ Trevir./Sch. Bip.) and oregano (*Origanum vulgare* L.). The main activity involving the existing MAP collection includes collecting a wide range of medicinal and aromatic plant species. Each year collecting missions are organized in order to carry out ecogeographical survey and seed sampling of wild populations of medicinal and aromatic plants, following the guidelines of Bioversity International, Rome (former International Plant Genetic Resources Institute, Rome). Ideally, seeds are collected at optimum maturity when seed vigor and longevity are expected to be the highest. For the purpose of conducting ecogeographical survey standardized medicinal and aromatic multicrop collecting form is used. The form includes data such as: accession identification (taxonomy, morphology, phenology, etc.), physical description of the collecting site (latitude, longitude, elevation, topographic landform, soil characteristics, etc.), vegetation description (local vegetation classification, dominant vegetation, etc.) and assessment of population diversity and threats of genetic erosion (spatial pattern, diversity status, abundance, causes of biodiversity loss, overexploitation status, etc.). Since 2001 a number of multi-species collecting missions have been conducted covering municipalities of Brckovljani, Donja Stubica, Duga Resa, Dugi otok, Ivanić grad, Karlovac, Koprivnica, Krapina, Kutina, Rab, Samobor, Senj, Split, Zadar and Zaprešić. Two species-specific collecting missions, focused on more extensive sampling of natural populations of Dalmatian sage (*Salvia officinalis* L.) and Dalmatian pyrethrum (*Tanacetum cinerariifolium*/Trevir./Sch. Bip.) have been carried out. Populations were collected throughout Adriatic coastal region, representing the whole geographical distribution of these two species. Characterization and evaluation of collected accessions is carried out according to the descriptor lists. The descriptor list includes data on qualitative, morphological

traits as well as quantitative, agronomic traits. One of the main problems for many medicinal and aromatic plants is the lack of suitable descriptor lists for characterization and evaluation of the collected material [27]. A number of field trials are conducted on the experiment field at the Department of Seed Science and Technology, Faculty of Agriculture in Zagreb and at the Institute of Adriatic Crops and Karst Reclamation in Split. So far, characterization and evaluation has been conducted for basils (*Ocimum* spp.) [10, 11, 19], Dalmatian pyrethrum (*Tanacetum cinerariifolium*/Trevir./Sch. Bip.) [15], Dalmatian sage (*Salvia officinalis* L.) [18, 33, 34], lemon balm (*Melissa officinalis* L.) [4], oregano (*Origanum vulgare* L.) [17], St. John's Worth (*Hypericum perforatum* L.) [23], and wild hops (*Humulus lupulus* L.) [25, 26]. The proper documentation of plant biodiversity is a crucial part of making it useful and available to scientists, breeders and producers. In order to establish a national documentation system, the Croatian Plant Genetic Resources Database (CPGRD) has been developed for recording and access to data on accessions collected and maintained in the framework of the Commission on Plant Genetic Resources. The database is available at cpgrd.zsr.hr and includes passport data (multicrop EURISCO descriptors), collecting data (multicrop collecting form), characterization data (crop-specific descriptor lists) and management data (data on storage, regeneration and distribution).

24.4 Enhancing Germplasm Utilization

Characterization and evaluation of accessions in plant genebanks is traditionally based on morphological traits, but modern conservation programs aimed at the promotion of germplasm utilization by plant breeders often include the analyses on biochemical and genetic levels. The research at the Department for Seed Science and Technology, Faculty of Agriculture, University of Zagreb is focused on the following target species: Dalmatian sage, Dalmatian pyrethrum, and basils, chosen due to its potentials for the introduction into agricultural production and further research concerning plant breeding.

Dalmatian sage is a perennial subshrub belonging to Lamiaceae family. Its native distribution is the northern coastal region of the Mediterranean, and it grows wild in large masses in the calcareous, karstic mountains of Dalmatia (Croatia), Herzegovina (Bosnia and Herzegovina), Montenegro and Albania, as well as in northern Greece and northern Italy [16]. Dalmatian sage is naturalized and cultivated in temperate climate all over the world. It is used as an herb with beneficial healing properties while its essential oil is used in pharmaceutical, food and cosmetic industry [30]. A total of 25 Dalmatian sage populations was sampled throughout its distributional area in the R. of Croatia and the R. of Bosnia and Herzegovina [18]. Plantlets established from cuttings were transplanted to set up a field trial at the Institute of Adriatic Crops and Karst Reclamation in Split. The essential oil yield ranged between 1.93% and 3.70% of the dry leaf weight. The chemical compositions of essential oils for each population were determined by gas chromatography and mass spectrophotometry

(GC/MS). GC/MS analysis allowed the identification of 58 compounds. Multivariate analysis of chemical variability enabled the classification of analyzed populations into three chemotypes [A: cis-thujone (β-thujone); B: trans-thujone (α-thujone); C: camphor]. Genetic diversity and structure of Dalmatian sage populations has been assessed by Amplified fragment length polymorphism (AFLP) markers [32]. Four primer combinations yielded 559 polymorphic markers. Analysis of molecular variance (AMOVA; [13]) revealed that most of the genetic diversity was attributable to differences among individuals within a population (92.31%). Genetic differentiation among populations showed typical pattern of isolation-by-distance [22].

Dalmatian pyrethrum is a perennial plant species endemic to the east coast of the Adriatic Sea. It is a valuable source of potent natural insecticide pyrethrin. Pyrethrin has highly unusual insecticidal activity, with the ability to control or repel numerous insect species, with little or no adverse impact on humans, worm blooded animals or environment [2]. Dalmatian pyrethrum was cultivated in Croatia from the end of nineteenth century. From 1930 the production gradually decreased, especially during the World War II. Commercial use of DDT (Dichloro-Diphenyl-Trichloroethane) chemical insecticides ended the production of Dalmatian pyrethrum in Croatia [1]. Nowadays, it is cultivated in more than 10 countries (Kenya, Australia, Tanzania etc.) [31]. Samples of 25 natural populations were collected along Adriatic coast and its islands representing its whole natural distribution area [14]. An optimized ultrasound assisted extraction with acetone was developed and therefore used for the extraction of pyrethrins from samples of dried pyrethrum flowers. High Performance Liquid Chromatography (HPLC) was used for qualitative and quantitative determination of six pyrethrin components and total pyrethrin. The total pyrethrin content ranged between 0.36% and 1.30% of the dry flower weight while pyrethrin I/pyrethrin II ratio ranged between 0.64 and 3.33 [15]. Multivariate analysis of chemical variability enabled the identification of five chemotypes having different pyrethrum extract quality. Amplified fragment length polymorphism (AFLP) markers were used to investigate genetic diversity within and among Dalmatian pyrethrum populations. Six primer combinations produced 935 polymorphic markers. Analysis of molecular variance (AMOVA) revealed that most of AFLP diversity was distributed among individuals within populations (85.89%). With growing interest in organic farming as well as an increased resistance of pests to synthetic pesticides and more strict environmental legislation, interest in pyrethrin has been expanding continuously in recent years. The results show that wild Dalmatian pyrethrum populations are valuable source of genetic variability to be exploited in plant breeding programs with great commercial value for agricultural utilization. The genus *Ocimum* L. comprises between 30 and 160 species [21]. The most widely grown are *O. africanum* Lour (= *O.* x *citriodorum* Vis.) *O. americanum* L. (= *O. canum* Sims.), *O. basilicum* L., *O. gratissimum* L. and *O. tenuiflorum* L. (= *O. sanctum* L.). Basils have been acclaimed for its diversity as a source of essential oils, its flavor and delicacy as spice, and its beauty and fragrance as an ornamental [24]. Currently, the MAP collection holds more than 150 accessions of 11 *Ocimum* species (mainly *O. basilicum*) that have been characterized on morphological [10, 11, 19], molecular [5–7, 29] and biochemical level [8, 9]. Future research will be aimed

at further assessment of biochemical (essential oil composition, pyrethrin composition) and genetic diversity (AFLPs, microsatellite markers) for association mapping studies in order to identify markers linked to quantitative trait loci (QTLs) related to biochemical traits. The expected results of ongoing research will provide the knowledge on available biochemical diversity and inherent genetic variability of MAP accessions and develop the technology to be used in future Marker-Assisted Selection (MAS) programs. The conservation of MAP genetic resources will be promoted by the continuous maintenance of the collection freely available to plant breeders and the scientific community.

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