JADAR PROJECT

POSSIBLE HARMFUL IMPACTS ON WILDLIFE AND HUMAN HEALTH

Milena Kataranovski
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Contents

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Summary

Jadar is an agricultural region in Serbia. Several years ago, the Serbian Government decided to open a boron and lithium mine in the area. This region is renowned for its ecological and biological diversity, with 101 habitat types reported. The geological heritage, representing the area's diversity, is situated within a broader zone of the planned underground mine Jadar, which includes a few natural protected areas. More than half of the landscape types belong to the complex of arable land and scattered forests. Additionally, there are also vast pastures and hunting grounds featuring an extraordinary diversity of flora and fauna.

This publication is the result of the initiative and work of 20 experts, addressing challenges in carrying out the official Environmental Impact Assessment (EIA) and Health Impact Assessment (HIA) as a part of the EIA for this Project, such as lack of transparency, absence of expert, scientific, and public debate before decisions were made, no HIA conducted for this project in the Strategic Impact Assessment, no HIA included in the Draft EIA, and no HIA ever carried out in EIA procedures for any plans and projects in Serbia. This is despite the fact that an EIA is required for new plans and projects expected to have negative impacts on the environment and human health.

The mining of metals and metalloids generates significant environmental pollution, including tailings from the extraction process, which contain heavy metals and chemicals. Water from the metal mine contains heavy metals that accumulate in the soil and aquatic environments, resulting in harmful effects on aquatic organisms and their habitats.

The mining of lithium and boron could lead to widespread devastation of the Jadar region, including pollution of the main natural resources, loss of biodiversity, destruction of landscape diversity, and loss of ecosystem services and amenity values. The Jadar region also contains large reserves of groundwater used for water supply in wider areas of Serbia, which

is especially important for the future supply in light of climate change and increasing water scarcity. This area is also prone to flooding.

There is still insufficient data provided by the investor, Rio Tinto, on the exploitation of the jadarite mine for a competent HIA. Decisions about the Project Jadar were made without applying the Act on the Plan System of the Republic of Serbia, adopted in 2018, and there are no studies that would reveal a national consensus on the necessity of the Project Jadar.

The entire environmental impact of the project must be considered in an integrated manner, relying on currently available data. The Ministry of Environmental Protection has granted permission to Rio Tinto to perform a partial EIA, limited to mining activities. This excluded ore extraction, tailings management, and a risk assessment of the overall technology applied. Such an approach does not allow a high-quality analysis of all the impacts of the project as a whole. Public knowledge about the risks associated with the mine's operation and the extraction of lithium and boron is crucial for decisions concerning the welfare of Serbia in a broader context.

Preface

Milena Kataranovski, Tamara Rakić,
Ivan Pavlović, Elizabet Paunović,
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Jadar is a region in Serbia situated on the River Jadar. Its southern starting point is the town of Osečina. It consists of two subregions: the Upper Jadar (around Osečina), which is part of the larger Rađevina region, and the Lower Jadar, a part of the similarly larger Podrinje region. The key town in the Lower Jadar region is the city of Loznica, situated approximately 10 km southwest of the River Jadar. The River Jadar belongs to the Drina River basin, into which it flows as a tributary. The Drina, through the rivers Sava and the Danube, belongs to the Black Sea basin. The area, specifically the River Jadar Valley, is surrounded by the mountains Vlašić, Cer, Gučevo, and the Sokolske Mountains. The Lower Jadar is a lowland agricultural area, while the Upper Jadar was, in the past, an important mining area, with the antimony mine at Zajača being one of the most historically polluted areas in Serbia, together with Bela Crkva.

The geological heritage, representing the diversity of the area, is situated within a broader zone of the planned underground mine Jadar, where several natural protected areas also exist. In the Lower Jadar area, there are nine distinct local landscape types, whose structure is primarily shaped by relief features. These landscape types extend from the River Jadar's alluvial plain with elongated aspects, to the undulating relief of gullies and ravines with aspects ranging from very enclosed to open. More than half of the types belong to the complex of arable land and scattered forests. Additionally, there are extensive pastures and hunting grounds, featuring an extraordinary diversity of flora and fauna.

This area is renowned for its ecological and biological diversity, largely determined by the habitats it supports. There are 101 habitat types re-

ported, 31 of which are priority habitat types. The protective measures were issued after two independent studies. The first, which encompassed aquatic and terrestrial ecosystems and species diversity in the River Jadar Valley, was conducted from 2016 to 2020. In 2020, a consortium of experts from several universities and scientific institutes in Serbia investigated habitat types, dispersal areas of key animal and plant species, and the potential impact of mining.

A few years ago, the Serbian government decided to open a boron and lithium mine in the agricultural region of Lower Jadar. The presentation of the effects of jadarite ore (a mineral containing boron and lithium) mining in this volume was intended for risk assessment on natural habitats, wild and cultivated plants, and domestic animal species as integral elements of the environment, as well as for assessing risks to human health in the Jadar region. People who live and work there directly rely on natural resources. Hence, the overall condition of those resources directly impacts not only their health, but also the health of those who consume their products, and, through the food chain, it impacts a much wider area. Analysis of the potentially adverse effects of mining on wildlife and people aligns with the "One Health" concept, a worldwide, multidisciplinary approach that recognizes the harmful effects of pollutants and develops responsive strategies. This publication is a result of the initiative and work of 20 experts, considering challenges in carrying out Environmental Impact Assessment (EIA) and Health Impact Assessment (HIA) as a part of EIA for this Project, such as lack of transparency, absence of expert, scientific, and public discussions before decisions were made, no HIA conducted for this project in the Strategic Impact Assessment, no HIA included in the Draft EIA, and no HIA ever carried out in EIA for any plans and projects in Serbia, even though an EIA is requried for new plans and projects. In addition, according to published data and analyses, Serbia shares the same difficulties and challenges in this respect with other developing countries, as the vast majority of published HIAs come from highly developed countries.

In a broader sense, it is important to note that, in addition to the specific negative circumstances in Serbia related to HIA, there are several other difficulties that are not exclusive to the Jadar Project and are not limited to Serbia. Common difficulties in projects involving boron and lithium mining include the introduction of new and emerging technologies, as well as the first-time execution of certain mining operations in agricultural and densely populated areas. All projected new lithium mines in inhabited areas of Europe share similar traits with the Jadar Project to some extent.

Different technologies will be employed, but the issues remain the same, based on the currently available data. Other systemic issues facing HIA in this context include methodological constraints, data deficiencies, and the intricacies of governance in this specific Project. These were the main motives for the experts to present their work in this book.

The greatest and longest-lasting source of environmental pollution generated by the mining of metals and metalloids is the tremendous amount of tailings from the extraction process. This waste material remains after the rock is ground down to fine particles and the target metals are extracted using various chemicals. A very large quantity of this milled waste rock, mixed with water in the form of sludge, is expelled in the vicinity of the mine, gradually forming a tailings landfill. After decades of mine operation, such landfills occupy vast areas and can exceed 100 m in depth, as is already the case in Upper Jadar, with the tailings of the closed antimony mine at Zajaca. Since a tailings landfill consists of fine metalliferous rock particles rich in metals, it poses a pollution threat to surrounding ecosystems and arable land, contaminating them with metals and processing chemicals. As the landfill is rich in metals but lacks essential nutrients for plant growth (e.g., nitrogen, phosphorus, and potassium), it is highly unsuitable for vegetation. Consequently, particles are easily spread by wind to surrounding farmland and natural vegetation, polluting soil, surface water, and wildlife. Additionally, the particles in the landfill have an enormous total surface area, and gradual chemical disintegration of minerals occurs within them. Metals released from the minerals are mobilized and dissolved in water from atmospheric precipitation. In this way, tailings landfills and wastewater from mines represent a long-lasting source of pollution to the surrounding soil, surface water, and groundwater, containing heavy metals and chemicals used in the extraction process.

Water that originates from a metal mine, whether as wastewater or as precipitation filtering through the tailings and reaching the groundwater, typically contains large amounts of heavy metals. Owing to their long-term presence in the environment, heavy metals accumulate in the soil. In aquatic environments, they cause a variety of harmful effects on aquatic organisms and habitats.

Mining and processing of the jadarite ore will not only increase the concentrations of arsenic, boron, and lithium in the soil, but also of other metals from the host rock. Severe pollution by arsenic, boron, and lithium has already been detected in water from probe drills. It must be noted that their content in soil would accumulate over time, even from long-

lasting, low-level pollution, to amounts potentially toxic for plants and all soil-dwelling organisms, which must be taken into account in EIA.

The potential impact of lithium and boron mining is the overall devastation of the Jadar region, initially through the pollution of its main natural resources (e.g., soil, water, and air), and subsequently through the loss of most, if not all, of its biodiversity. This would make it impossible to continue traditional economic activities, primarily agriculture, which produces healthy and high-quality food essential to life in the region. No less important is the destruction of landscape diversity across the Jadar region and the loss of all ecosystem services and amenity values it supports. Last but not least, as the Jadar region contains reserves of groundwater, endangering groundwater quality through mining activities would have far-reaching consequences not only for the Jadar region but also for the broader area.

There is still insufficient data from the investor, the Rio Tinto company, on the exploitation of the jadarite mine for a competent HIA. Providing such data in accordance with international and national legal acts, as well as expert methodological recommendations from the World Health Organization, would allow the impacts to be assessed with greater reliability. Therefore, the investor should provide the necessary data obtained through relevant methodology to demonstrate that impacts can be mitigated and the lowest possible risks to human health guaranteed.

Decisions about the Project Jadar have so far been made without applying the Act on the Plan System of the Republic of Serbia, adopted in 2018. The Development Plan of the Republic of Serbia is lacking, as is the Investment Plan. For these reasons, there are still no studies that reveal a national consensus on whether the Project Jadar is necessary, whether it aligns with other prioritized activities, and whether it is economically feasible. In the approval procedures for the Strategic Environment Impact Assessment Study and the Spatial Plan of the Special Purpose Area, there were neither sufficiently comprehensive public debates nor serious consideration of the numerous objections submitted. Consequently, the Strategic EIA Study was made incompletely and non-transparently.

It is currently impossible to predict the full extent of pollution or the size of the affected area, or to estimate the number of people who will be impacted. This is especially true given that the Ministry of the Environment, the legal authority responsible for setting the conditions for the EIA study, allowed Rio Tinto to prepare only a partial EIA, covering mining activities but not the overall project impact, which contradicts the basic

principles of EIA and HIA. In addition, social and economic considerations should take into account both intangible and tangible losses caused by such environmental and health effects, including the long-term impossibility of remediating groundwater reserves in both the Jadar region and wider areas (Mačva, Posavina, and Belgrade), which provide drinking water for hundreds of thousands, if not millions, of Serbian citizens.

Given the scope, multiplicity and expected magnitude of the Project Jadar impacts on the environment, both directly on its components and indirectly through the interactions of pollutants within ecosystems, food chains, and wider food webs, as well as the anticipated joint, combined, and synergic effects on plants, animals, and citizens in the project area, we believe that all of this must be assessed on the basis of currently available data. Certain knowledge about the mechanisms and impacts of pollutants such as arsenic and boron has long been established. However, there are still many unknowns, especially concerning lithium in the environment. The global expansion of lithium exploitation over the past few decades stimulated research and produced some results, which we have attempted to present in this volume, based on available data. Considering the adverse effects expected from the operation of the jadarite mine and the extraction of lithium and boron, the public has every right to be informed of all the challenges, as required by international agreements and Serbian national legislation. Being aware of all the risks we face, or will face, due to the implementation of this project is crucial for deciding about our welfare in Serbia and in a broader context.

Chapter 1

Legislative, International, and Professional Methodological Framework for Health within Environmental Impact Assessments

Elizabet Paunović

What is a Health Impact Assessment (HIA) within an Environmental Impact Assessment (EIA)?

The World Health Organization (WHO) defines Health Impact Assessment (HIA) as "a practical approach used to evaluate the potential health effects of policies, programs, or projects on a population, particularly on vulnerable groups or disadvantaged groups" (1). The goal of the assessment is to provide recommendations to decision-makers and stakeholders to maximize the positive health effects and minimize the negative ones.

The HIA approach can be applied across various economic sectors, using quantitative, qualitative, and participatory techniques. As Cole and Fielding note, it is "an activity that helps policymakers understand health as a concept much broader than the healthcare system and treatment" (2). This principle is present in numerous international documents, not only through the WHO definition of health as "a state of complete physical, mental, and social well-being, and not merely the absence of disease or infirmity" (3), but also through the concept of "health in all policies" (4). These principles are also embedded in Serbian public health legislation, which will be discussed in greater detail below.

An HIA provides a structured and legally defined process for engaging the public, particularly those groups directly affected by a given propos-

al. It also helps decision-makers consider alternatives and improvements for disease or injury prevention while actively promoting health. The approach is grounded in four interconnected values: democracy (ensuring stakeholder participation), equity (addressing impacts across the entire population), sustainable development, and the ethical use of evidence.

International Legally Binding Framework for the Implementation of Environmental Health Impact Assessments

Convention on Environmental Impact Assessment in a Transboundary Context (5)

The convention, informally known as the Espoo Convention, was adopted under the United Nations Economic Commission for Europe (UNECE). It was signed in the city of Espoo, Finland, in 1991 and entered into force in 1997.

The list of activities subject to mandatory international environmental impact assessment is provided in the Annex to the Convention. Point 14 of the Annex specifies that large mining operations, direct extraction, and the processing of metallic ores or coal require an assessment of their effects on health and the environment. Annex 3 outlines general criteria for application, specifying the types of effects that must be analyzed and emphasizing activities with complex or potentially severe consequences. These include significant impacts on human health, threats to endangered species and organisms, risks to existing or potential uses of damaged areas, and additional burdens that exceed the environment's capacity to recover.

Evidently, the assessment of effects on human health is explicitly required under the Convention.

Strategic Environmental Assessment (SEA) – Protocol on Strategic Environmental Assessment under the Convention on Environmental Impact Assessment in a Transboundary Context (6)

Strategic Environmental Assessment (SEA) is carried out much earlier in the decision-making process than Environmental Impact Assessment (EIA) and is therefore considered a key instrument for ensuring sustain-

able development. The Protocol also establishes the obligation of substantial public participation in government decision-making across various development sectors.

It is essential to begin assessing the health impacts of new environmental plans and projects at an early stage, prior to implementation, in order to prevent negative outcomes. The public must not only be informed in a timely manner but also enabled to actively participate in the discussions. To this end, the member states of the Espoo Convention adopted the Kyiv Protocol in 2003, which further extends the provisions of the Espoo Convention to the strategic level of decision-making (strategies and plans).

The Protocol commits member countries to "recognizing the importance of integrating environmental issues, including health, into the preparation and adoption of plans, programs... ensuring public discussion... integrating, through these means, environmental issues, including health, into measures and instruments adopted to contribute to sustainable development."

Accordingly, the Kyiv Protocol establishes that health impacts must be an integral part of studies assessing the effects of all new plans or programs.

Amendments to EU Directive 2014/52/EU

The amendments to Directive 2014/52/EU of the European Parliament and Council, adopted on 16 April 2014 (7), are particularly important for the implementation of health risk assessments as they explicitly prioritize population and human health.

As a candidate country for EU membership, the Republic of Serbia has committed through the Stabilization and Association Agreement to harmonize its legislation with EU regulations, making this Directive directly applicable to the Jadar project.

Article 3 of the Directive specifies that:

1. The environmental impact assessment will identify, describe, and appropriately evaluate, in light of each individual case, the direct and indirect significant effects of the project on the following factors:

(a) population and human health;

(b) biodiversity, with special attention to species and habitats protected by Directive 92/43/EEC and Directive 2009/147/EC;

(c) land, soil, water, air, and climate;

(d) material goods, cultural heritage, and landscape;

(e) the interaction between the factors listed in points (a) to (d).

In addition to this internationally binding strategic framework, it is important to mention the following document regarding the process of implementing and assessing HIA within EIA:

Gothenburg Consensus Paper (8)

Within the professional community concerned with assessing the health impacts of projects and programs that alter the environment in ways posing risks to human health, a professional consensus was reached in the late 1990s.

Every expert engaged in HIA is expected to be familiar with this methodology and to apply its recommended phases and activities. Undertaking activities that do not fundamentally adhere to this approach is considered unprofessional and does not constitute a valid health impact assessment under the aforementioned international documents or recognized scientific practice. The World Health Organization, in its subsequent documents related to this field, underlines the Gothenburg Consensus Paper as the gold standard for professional methodology. Further elaboration will follow in the section pertaining to this methodology.

The following text will reference these WHO documents.

Legislative Framework in the Republic of Serbia

The Convention on Environmental Impact Assessment in a Transboundary Context was ratified by the Republic of Serbia in 2007 (9).

The Protocol on Strategic Environmental Assessment, in conjunction with the Convention on Environmental Impact Assessment in a Transboundary Context, was ratified by the Republic of Serbia in 2010.

Additionally, the principles of these international documents have been largely incorporated into Serbian legislation governing strategic impact assessment and environmental impact assessment, as well as into laws and strategies regulating public health.

The Law on Strategic Environmental Assessment (11) is based on the Kyiv Protocol and follows its core principles. Article 15, paragraph 4, stipulates:

> "The assessment of the potential impact of plans and programs on the environment shall consist of the following elements:
>
> 4. The way in which the environmental elements have been taken into consideration in the impact assessment, including the data on: air, water, soil, climate, ionizing and non-ionizing radiation, noise and vibrations, flora and fauna, habitats and biodiversity, protected natural assets, population, human health, cities and other settlements, cultural and historical heritage, infrastructure, industrial and other structures, or other man-made values."

Despite this framework, the official Spatial Plan related to the Jadar Project contains only one paragraph on page 78 addressing health impacts, and even then, only in terms of monitoring after project implementation, rather than assessing risks before they occur. Therefore, this document lacks professional grounding, as it is not based on an impact assessment (it merely suggests monitoring respiratory diseases). Furthermore, its objective is undefined, and it is unclear which diseases it addresses and why. This is a significant omission, given that the goal of the strategic assessment is to predict and prevent negative impacts. That essential component is missing from this document.

The Law on Environmental Impact Assessment (13), which governs the assessment of individual projects within the framework of the Spatial Plan, also requires the preparation of a study that evaluates the impact of each individual project. Article 2 of the Law on Strategic Environmental Assessment defines the terms and clearly states that the health impact assessment must be an integral part of this study:

> "The environmental impact assessment is a preventive measure for environmental protection based on the preparation of studies and the implementation of consultations with public participation and the analysis of alternative measures, with the aim of collecting data and predicting harmful impacts of certain projects on human life and health, flora and fauna, soil, water, air, climate and landscape, material and cultural goods, and the interactions of these factors, as well as determining and proposing measures to prevent, reduce, or eliminate harmful impacts, taking into account the feasibility of those projects (hereinafter referred to as 'impact assessment')."

The competent authority for carrying out these activities is the Ministry of Environmental Protection. This ministry initiated the process of

developing a study for the Jadar Project in 2021 and set the conditions that the Environmental Impact Assessment study should meet. The resulting resolution regarding the content of the study requires Rio Tinto to "comprehensively and thoroughly describe all possible significant impacts of the project on the environment, including cumulative effects on biodiversity, the population, and environmental factors... The description of possible significant impacts of the project on the environment includes both qualitative and quantitative representations of potential changes in the environment during the execution of the project, regular operations, and in the event of accidents [...] concerning the health of the population. Additionally, among other things, it requires the listing of measures related to the protection of public health [...] as well as a presentation of the plan for monitoring the health of the population during the implementation of the project."

In the meantime, while the process of preparing the environmental impact assessment study for the Jadar Project is ongoing, the National Assembly of the Republic of Serbia adopted new laws on Strategic Environmental Assessment (15) and Environmental Impact Assessment (16) during the same session and, importantly, without any debate. Although it remains unclear which law on impact assessment will be applied to the Jadar Project, it is important to note that both new laws prioritize the impact of new projects on health, as the provisions listing impacts that must be analyzed are aligned with the EU Directive 2014/52/EU of the European Parliament and Council of 16 April 2014. The new laws also specify the elements that the impact assessment must contain, which are also based on the text of the Directive and the expert recommendations outlined in this text. Accordingly, we can conclude that, regardless of which law is applied—old or new—it is essential to conduct an impact assessment of the Jadar Project on health.

In the draft of the impact assessment study posted on the Rio Tinto website (17), there is text on page 191 and pages 585–587 (out of 896 pages assessing various impacts) that pertains to health impacts, which does not meet the criteria set by the competent authority or the methodological professional requirements for health impact assessments that will be briefly described in the following chapter.

Since health impacts have not been assessed in the studies accepted so far, despite the provisions outlined here and Serbian legislation and international agreements ratified by Serbia, we strongly advocate that the forthcoming study include a health impact assessment, as should all other studies for new projects.

This area is evidently new for our experts from various fields (beyond toxicological expertise, which deals with chemical impacts, it is necessary to include a range of other specialists), as such complex health impact assessments have not been conducted in Serbia until now. Therefore, it is essential to consider an approach that enables the production of a high-quality health impact assessment study.

It is also important to note that the Public Health Law (18) recognizes the need to provide such expert health assessments in other sectors. Article 8 of this Law (Environment and Health) stipulates that public health institutes and other institutes are responsible for the following activities:

> "6) Providing opinions on documents related to spatial and urban planning, including technical documentation, in accordance with the law governing planning and construction as it pertains to public health;
>
> 7) Assessment of health risks to the population based on the register of pollution sources (cadastre);
>
> 8) Monitoring and analyzing the health status of the population and assessing health risks related to environmental impacts, including the evaluation of the epidemiological situation."

In the Public Health Strategy of the Republic of Serbia 2018–2026, 61/2018-6 (19), specifically in the chapter related to the environment and health, the relevant institutions commit to carrying out the following activities:

> "4.2.1.4. Ensuring a favorable environment for the active implementation of accepted international obligations in the field of environment and health (EIA—declarations, protocols, action plans, etc.);
>
> 4.2.1.5. Active implementation of accepted international obligations in the field of environment and health (EIA—Declarations, Protocols, Action Plans, etc.)."

As is evident from the above, the implementation of health impact assessment (HIA) activities within the aforementioned international obligations is also explicitly mentioned as a requirement in the Public Health Strategy, and the institutions responsible for its implementation are public health institutes.

Yet, as already emphasized, these activities have clearly not been implemented so far, despite a solid legal basis for them. It can be reasonably

assumed that undertaking activities in this area, as described here, poses a significant challenge for the responsible institutions. According to the text mentioned above, it is evident that some measurements of the current state were conducted by the Public Health Institute of Serbia; however, the assessment of the future impacts of the described technology through a health risk assessment has not been conducted. This gap is certainly compounded by the fact that the description of the technology to be applied through the implementation of this project, as outlined in the materials available on the Rio Tinto website, is insufficiently detailed. Therefore, the Ministry of Health and the Government of the Republic of Serbia could consider requesting technical assistance from the World Health Organization. Many countries face similar problems, and this field is relatively new, requiring multidisciplinary collaboration among a larger number of experts from various profiles, so assistance from countries with well-developed activities of this kind would be welcome.

The foundational methodological elements were not applied in the draft study.

The World Health Organization, in its most recent guidelines for conducting Health Impact Assessments (HIA) within Environmental Impact Assessments (EIA), states that the summary for decision-makers on health impact assessment and the inclusion of health in environmental impact assessment (20) also provides a recommended methodology. The initial step is the so-called screening, which determines who should conduct the HIA and how it should be performed, followed by an evaluation of whether potentially harmful health consequences can be predicted from available data. As noted earlier, all mining activities carry health risks and, in line with international and Serbian regulations, a health impact assessment is required as part of the EIA for all new mining projects. This initial step was not carried out in the draft study.

After the screening phase, which serves as an introductory stage, there are six additional, highly complex phases that require a larger multidisciplinary team of experts, including toxicologists. The materials published on the Rio Tinto website, in the few pages addressing health considerations, only provide general references to the possible negative or positive effects of the proposed technology. However, they fail to specify the extent of those impacts, meaning that no risk assessment has been conducted.

Specifically, the study omits answers to several key questions that must be addressed:

- Will the project's health impacts be significant in terms of the number of affected individuals and/or the magnitude of the impact?
- Are there adequate methods, expert analyses, and evidence available to assess the project's impact on health?
- What data sources can be used for this assessment?

Since these questions have not been answered, it was not possible to carry out the central part of the HIA, and therefore, such an assessment does not exist. Had it been undertaken, it would have needed to include the following components:

- the use and analysis of surveys, interviews, and focus groups;
- field observations;
- statistical analyses of health data and GIS mapping;
- interpretation of data;
- identification and citation of evidence supporting the recommended measures for reducing health risks.

It is evident that health risks are present, and the purpose of an HIA is to determine their magnitude, assess whether they can be mitigated, and evaluate whether the proposed technological processes guarantee the lowest possible level of risks. All of these elements are missing from the draft study.

The standard public health methodology includes epidemiological methods, monitoring of defined parameters, assessment of risks from workplaces and the environment, health promotion, and health management. Additionally, it also integrates medicine in a broader sense, psychology, and social and economic sciences. Crucially, this multidisciplinary public health approach ensures special attention to all population groups, with the identification of protective measures for the most vulnerable (WHO, 2023). These considerations are also absent from the draft study.

Broader Challenges and Considerations Related to HIA in EIA for the Jadar Project

Regarding the implementation of the Espoo Convention, the Kyiv Protocol, the EU legal framework, the Serbian legal framework, and methodological recommendations, several challenges arise in carrying out EIA and HIA as part of the EIA for this Project. These include a lack of trans-

parency, the absence of expert, scientific, and public discussions before decisions were made, the omission of HIA in the Strategic Impact Assessment, the absence of HIA in the Draft EIA, and the fact that HIA has never been conducted within EIA for any plans and projects in Serbia, despite being required for all new plans and projects. According to published data and analyses, Serbia faces the same difficulties and challenges as other developing countries. In contrast, the vast majority of published HIA studies originate from highly developed countries (89/11) (21).

In a broader sense, it is important to note that, in addition to the specific shortcomings in Serbia regarding HIA, there are other difficulties that are not exclusive to the Jadar Project. Common challenges in such projects include the introduction of new and emerging technology and the first-time implementation of mining operations in agricultural and densely populated areas. All projected new lithium mines in inhabited areas of Europe share certain traits with the Jadar Project. Although different technologies may be employed, the issues remain largely the same, based on the currently available data. Other systemic problems affecting HIA in this context include methodological constraints, data deficiencies, and complex governance structures specific to this Project.

Limitations in Methodology and Data for New and Emerging Risks

1. Lack of Uniform Frameworks

• There are gaps in assessing long-term health risks because life cycle assessments (LCAs) of technologies lack data on supply chain emissions, particularly during the disposal phase.

• There are no widely recognized metrics for quantifying health impacts across environmental domains such as soil pollution or biodiversity loss, especially for non-climate-related factors (L). The Jadar Project may have avoided this issue, but an EIA (and potentially an HIA, for the first time) was requested solely for the mining operations. Therefore, it is anticipated that, even if health risks are assessed, they will not be evaluated reliably enough to reflect the full range of impacts on human health.

2. Various Methods of Assessment

• Experience from developed countries shows a persistent conflict between research-focused HIA, which relies on numbers and models, and step-by-step HIA, which relies on stakeholder input, complicating the integration of EIA procedures. Given that the proposed technology is new and

that the EIA process has not been conducted transparently by the company, without an official expert or public discussion, it is likely that this issue will remain unresolved.

3. Expertise Gaps and Resource Limitations

• Serbia lacks sufficient expertise in multidisciplinary epidemiology, environmental science, and economics—skills essential for conducting integrated assessments (HIA within EIA). Even if a foreign consultancy were engaged by the implementing company to perform an HIA, without cooperation with national institutions, domestic experts, and the general public, such work conducted in a non-transparent manner would not provide high-quality expertise, especially since HIA was omitted from the Strategic Impact Assessment.

4. Considerations for Equity

• Vulnerable populations (including low-income communities, farmers at risk of losing their land and livelihoods, and biologically sensitive groups such as children and the elderly) face disproportionate exposure to environmental hazards. Yet they are often underrepresented in impact analyses. There is no available data on possible positive (or negative) economic impacts of the project.

• Environmental changes that displace families, particularly agricultural workers and farmers, often place additional burden on women, leading to gender-related disparities. These social determinants of health have not been examined in the available studies.

Policy and Governance Obstacles

5. Risks of Stakeholder Conflict

• Reliance on "information conduit" techniques, whereby industry-provided environmental data is assessed by industry-funded experts, may skew evaluations in favor of corporate interests. This concern must be addressed openly, as the project has already triggered mass public protests largely due to such concerns. The authors of this publication are a self-organized group of independent experts who, on their own initiative, analyze the current situation and its possible impacts on nature and human health, in response to legally required procedures that were conducted in a non-transparent manner.

Precautionary Principle

Even in the face of scientific uncertainty, particularly given that the proposed technology for the project is new, the precautionary principle in HIA, a component of EIA, requires that health hazards related to projects or emerging technologies be proactively evaluated and addressed. Safeguarding human health and the environment before irreversible damage occurs places a strong emphasis on prevention, prudence, and the application of the best available knowledge and technologies. This idea changes the role of HIA and EIA in environmental and public health governance from a reactive approach to a preventive one. As noted in the Strategic Impact Assessment, the only reference to health was that potential negative impacts may be considered if they appear after the mine begins operation. Such an approach is, from the perspective of human health protection, unacceptable.

Important Elements of the Precautionary Principle in HIA (22)

- Act even in the face of scientific doubt

According to the precautionary principle, preventive measures should not be delayed simply because complete scientific certainty is lacking. This means that even when cause-and-effect relationships are not fully established, precautionary measures should be applied whenever there is a reasonable risk of significant or irreversible harm to human health or the environment.

- Preventing serious or permanent damage

By assessing potential effects on human health and the environment prior to project approval, the principle seeks to prevent significant or irreparable harm. Instead of responding after damage occurs, this anticipatory approach requires proactive identification and mitigation of risks.

- Integration with health and environmental law

It is legally required to conduct impact assessments that take potential health risks into account and to apply the best available technologies and techniques in order to minimize them.

- Directing risk evaluation and decision-making

The principle has an impact on the EIA process by calling for assessment of uncertainties, emphasizing precautionary measures unless there

are compelling reasons not to apply them, and expanding the definition of significant environmental and health impacts. As a result, decision-making becomes more risk-averse.

- Transparency and public involvement

The principle requires transparency and inclusion of diverse perspectives on potential health and environmental risks, encouraging the participation of the public and stakeholders in the assessment process. This has not been applied to past or ongoing procedures.

- Application in public health

According to this principle, the burden of proof lies with those advocating potentially hazardous activities, who must demonstrate their safety. It promotes risk-benefit analysis, continuous surveillance, and the use of interdisciplinary methods to identify and address emerging risks at an early stage.

Conclusion

From the above, it can be concluded that the materials currently available on the Rio Tinto company's website, presented as the Draft Environmental and Health Impact Assessment study, contain no valid assessment of the Project's impact on health, as required by international and Serbian legislation and the methodological recommendations of the World Health Organization. A study that omits the evaluation of health risks should not be accepted by the Ministry of Environmental Protection, as this would be contrary to the application of Serbian laws.

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Chapter 2

Sources, Environmental Distribution, and Availability of Lithium, Arsenic, Boron, and Particulate Matter

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- Arsenic, lithium, boron, and particulate matter can enter the environment through natural processes, but anthropogenic activities (industry, mining, etc.) increase their concentrations.
- Complex chemical, physical, and biological processes govern the movement and distribution of arsenic in surface waters and soils, as well as its entry into groundwater.
- Lithium generated through human activities moves easily through soil and leaches into surface springs and groundwater.
- Wastewaters and industrial landfills are sources of boron in soil and water bodies.
- Due to their accumulation in many edible plants, arsenic, boron, and lithium from water and soil enter food webs.
- Dust (fine particles) has adverse environmental and health effects on living organisms and may cause serious health problems in humans.

ARSENIC (As) MOBILITY IN THE ENVIRONMENT

Environmental Arsenic Distribution

With the concentration of ~3mg/kg, arsenic ranks as the 20th most abundant chemical element in the Earth's crust (1). Arsenic is mobilized

into the environment through natural, geogenic sources, such as geological processes, and through anthropogenic sources generated by human activities. Geogenic sources include geothermal waters, arsenic-rich minerals, and aquifers. The concentration of As in geothermal waters can reach 2 mg/L (2). From there, arsenic ultimately reaches surface waters and river sediments. Arsenic is a major constituent of over 245 minerals, including carbonates, oxides, sulphides, and silicates (3). Arsenopyrite (FeAsS) is the most common arsenic-containing ore (4). Arsenic is released from ores by erosion, dissolution, and weathering (3). Depending on the parent rock, As soil concentrations vary from 5 to 10 mg/kg (5). From there, it reaches water through precipitation and leaching (6). Its occurrence in water depends on local geological and hydrological characteristics, as well as on the geochemical properties of aquifers (7). Arsenic reaches groundwater mainly from natural sources, but in some regions, both natural and anthropogenic sources contribute (8). However, compared to anthropogenic inputs, natural sources contribute less to environmental As. Due to their localization, As releases from rocks and minerals are generally limited. Their mining, however, significantly increases As occurrence and environmental mobility (9). Industrial activities like mineral exploration and processing, smelting, and manufacturing, along with other economic activities, have significantly increased As mobilization in the environment (10). These anthropogenic activities are major soil polluters (11) and also contribute to increased As concentrations in groundwater. This is particularly important in regions where groundwater serves as a source of drinking water and irrigation for agriculture (12). Mining is among the most significant anthropogenic sources of arsenic pollution (9). Arsenic concentration in acid mine waters can be very high, ranging from 2 to 13 mg/L (13). Elevated As concentrations have also been recorded in surface waters in the vicinity of metal mines (14, 15). In contrast, the World Health Organization recommends 10 μg/L as the safe concentration (16).

Arsenic can also enter the environment via the atmosphere. In rural areas, concentrations range from 0.02 to 4 ng/m^3, whereas in regions affected by anthropogenic activities, concentrations may be fifty to one hundred times higher (3–200 ng/m^3) (10).

Arsenic Chemistry

Arsenic is a metalloid, exhibiting both metallic and non-metallic properties. It occurs in four oxidation forms (–3, 0, +3, +5): as As^{3-} (in arsine,

AsH_3), As^0 (elemental arsenic), As^{3+} [arsenite, As(III), occurring as arsenous acid, H_3AsO_3, and arsenic trioxide, As_2O_3], and As^{5+} [arsenate, As(V), occurring as arsenic acid, H_3AsO_4] (17).

Its electronic structure and bonding properties make arsenic chemically versatile, resulting in numerous inorganic and organic chemical forms. In aerobic conditions (in the presence of oxygen) in aquatic environments and soil, inorganic arsenic occurs predominantly as arsenic acid. In contrast, in the absence of oxygen, arsenous acid predominates (7). Redox reactions drive the transformation of inorganic arsenic As^{3+} to As^{5+} and *vice versa*. Biological processes in aquatic and terrestrial organisms enable the generation of organic forms of arsenic (18). The most common organic arsenic species are methylated forms, such as monomethylarsonic acid [MMA(V)], monomethylarsenous acid [MMA(III)], dimethylarsinic acid [DMAA(V)], and dimethylarsenous acid [DMAA(III)] (19). These compounds are formed by the substitution of hydroxyl (-OH) groups with methyl ($-CH_3$) groups in inorganic arsenate and arsenite. Substitution of methyl or hydroxyl groups with larger groups, such as lipid, sugar, or cyclic groups, produces more complex As organic forms (19). Examples include arsenobetaine, arsenocholine, and arsenosugars (20).

The toxicity of arsenic depends on its chemical speciation. Generally, As(III) is more toxic than As(V), while methylated As forms are, with some exceptions in fish species, less toxic than inorganic As forms (21). The toxicity of As(III) is related to its strong affinity for sulphydryl (-SH) groups of biomolecules, which reduces their activity (22). The structural analogy of arsenates with phosphates affects their activity in numerous biochemical reactions (23). In contrast, complex organic As forms are weakly toxic to living organisms.

Physicochemical Processes and Arsenic Environmental Mobility

Arsenic mobilization to the environment and its mobility in soil and water are influenced by complex, interrelated physicochemical processes such as precipitation and dissolution, sorption/adsorption to solid phases, and leaching/desorption, as well as chemical transformation through oxidation and reduction (24). These processes depend on environmental parameters such as acidity/basicity, oxygen presence or absence (redox conditions), and the presence of inorganic substances and organic matter (25). Arsenic mobility is greater in environments affected by human activities than in those influenced by geogenic sources (26).

During mobilization, a variety of arsenic compounds are generated, ranging from simple forms such as arsenic trioxide (As_2O_3) to more complex compounds containing oxides and metals. As^{3+} and As^{5+} form complexes predominantly with oxides and sulphides, respectively (27).

Arsenic mobility or retention (sorption) depends on bonding with other elements and redox state (28). The processes that determine environmental As mobility are not fully understood, and most available data is derived from As mobilization into groundwater (29). Arsenites and arsenates are well adsorbed to the surface of iron oxide-containing minerals, which results in their precipitation. However, adsorption of arsenites is weaker, making them more mobile (19). Arsenic adsorption intensity depends on the iron redox state, environmental pH, and oxygen availability (29). Arsenites are well adsorbed under increased basicity (pH > 8), whereas in mildly acidic conditions (pH around 6), adsorption of arsenates is stronger. Dissolved organic matter (30), phosphates from fertilizers, and bicarbonates formed through erosion and dissolution of carbonates from sediments can all increase As desorption, thereby increasing its mobility to groundwater (7).

Arsenates are stable and adsorbed to sediments in oxygen-rich waters (7). By contrast, increased concentrations of organic matter lead to arsenic desorption and its mobility (30). In surface water near metal mines, arsenic is distributed in the form of As-bearing colloids containing iron (FeIII), Fe(oxy)(hydr)oxide, or dissolved organic matter (31). These colloids can remain dissolved and transported, particularly during storms and heavy discharge periods. Arsenic mobility in soil depends on soil type and properties (26). In iron-rich soils, As can accumulate through adsorption to Fe(III)oxides (32). Changes in soil redox potential, pH value, and organic matter decomposition or dissolution, especially during extreme weather conditions such as drought or flood, influence As chemical speciation and mobility. Under anaerobic conditions, growing redox potential due to organic matter decomposition leads to As release from iron oxides and increased soil concentrations (31).

Biotic Processes and Environmental Arsenic Distribution

Microorganisms

Prokaryotes, simple one-cell organisms whose cells lack a nucleus, such as bacteria and archaea (formerly known as archaebacteria), along with certain algae species, as well as some eukaryotes (organisms that pos-

sess a clearly defined nucleus), such as microscopic fungi, are biotic factors that influence arsenic fate (33). For example, in soil, microorganism concentrations can reach 10^{10} cells per gram, and their mere presence can affect As adsorption. The transformation of arsenic, as well as other microelements, including iron and sulfur, by microorganisms affects their mobility in the environment (34). Compared to abiotic As transformation, which is transformation by physicochemical processes in the absence of microorganisms, biotic transformation is faster (35). Given the environmental abundance of microorganisms, the contribution of biotic processes to As speciation is substantial (36). Thus, biotransformation plays a key role in environmental As mobility and toxicity (37).

Arsenic can inhibit microbial growth and lead to their death. In response, microorganisms have evolved a variety of mechanisms to overcome arsenic toxicity, such as restriction of As entry into the cell, As extrusion from the cell, enzymatic detoxification, chelation, and precipitation (38). For some microorganisms, arsenic and iron serve as sources of energy required for growth (34). Microbial activity alters the physicochemical properties of the environment, thereby affecting abiotic processes of arsenic and iron chemical speciation (29).

Electrostatic interactions between chemical groups on bacterial cell walls enable the sorption of As to the bacterial surface and cell entry (37). Major microbial As transformations include oxidation, reduction, methylation, and demethylation. The bacterial enzyme arsenite oxidase transforms arsenites into arsenates (34, 39). In some microorganisms, this reaction is part of detoxification reactions, conferring tolerance to high arsenite concentrations; in others, it provides energy for growth (39). Arsenate reduction, carried out by certain aerobic bacteria, is part of detoxification and arsenic resistance acquisition mechanisms. In some anaerobic bacteria, arsenate reduction occurs via respiration, where As(V) serves as the terminal electron acceptor (33). Arsenate reduction decreases adsorption to Fe(III)oxide, thereby mobilizing As. In iron-rich soils, microbial reduction of iron increases arsenic mobility (32).

Arsenic methylation has been demonstrated in diverse anaerobic and aerobic microorganisms from both aquatic and terrestrial environments, involving enzymes such as reductases, transferases, and methyltransferases (26, 37). Microbial methylation results in monomethylarsonic acid (MMA), dimethylarsinic acid (DMAA), and trimethylarsinoxide (TMAO), while demethylation converts them back to inorganic forms (40). Methylated As species are more mobile due to their lower adsorption capacity (41).

Further microbial activity leads to the conversion of methylated As forms to methylarsine, dimethylarsine (DMA), and trimethylarsine (TMA) (40). These gaseous arsines are highly mobile and can disperse widely into the atmosphere.

Plants

Arsenic is accumulated in plants from soil, water, and air. In terrestrial environments, the plant root is the main site of As accumulation. Arsenic bioavailability in plants depends on numerous environmental factors, including the physical and chemical properties of soil, which determine the formation of different As chemical forms. Dissolved organic matter facilitates the mobilization of inorganic arsenites and arsenates into soil solutions, thereby increasing their availability for plants (42). As^{3+} is the dominant species in floodplain soils and is readily bioavailable to plants from these areas (43). The presence of iron in the rhizosphere (thin soil region surrounding plant roots) facilitates arsenic sorption and subsequent absorption. In plant roots, arsenic is absorbed as As^{5+}, and under anaerobic conditions as As^{3+}. Methylated As forms can also be taken up by plants (44).

Inside plants, arsenic undergoes oxidation, reduction, and methylation. Some species can accumulate As, which is used as a method to reduce As soil concentrations (soil phytoremediation). However, arsenic accumulation in plants allows its entry into food chains through the consumption of contaminated plants by animals and humans (44). Arsenic transfer into plants *via* the root system is the initial trophic level in terrestrial food chains (45).

Arsenic toxicity to plants, particularly edible species, may reduce the plant quality and overall productivity (46). From contaminated crops and fodder, As enters food chains and poses risks to animal and human health (46). There is thus a clear need for research on As bioavailability and chemical speciation in plants to improve understanding of food contamination pathways.

Invertebrates

Inorganic arsenic or methylated organoarsenicals are detected in invertebrates (47). Arsenic enters their bodies by ingestion or *via* cutaneous contact. The mechanisms of As excretion are not fully understood, but studies confirm As accumulation within invertebrate tissues. Being prey

for higher animals such as frogs, birds, and small mammals, invertebrates comprise an important part of terrestrial food webs (45).

Aquatic Environments

Due to atmospheric inputs, geothermal waters, and human-induced pollution, arsenic concentrations in freshwater are higher than in marine waters. According to available data, inorganic arsenic, As(V), is the only arsenic form present in phytoplankton, whereas organoarsenic compounds MMA(V) and DMAA(III), and arsenosugars are the main As species in zooplankton (48). Zooplankton from contaminated lakes contain mostly inorganic arsenic.

Arsenic speciation in aquatic invertebrates varies between different species. In aquatic insects, DMAA is most common, while marsh snails contain not only DMAA but also arsenobetaine and arsenocholine (49). In freshwater fish, the dominant As species are methylated arsenicals [MMA(V), DMAA(V)], as well as organic forms, with arsenobetaine being the most prevalent (50). Higher As concentrations are found in fish species at lower trophic levels compared to those at higher trophic levels (51), which speaks in favor of biological reduction of As in food chains.

Animals and Humans

Numerous studies have investigated the fate of arsenic in animals and humans due to their high position in the food chain and the associated health risks. Since organisms at higher trophic levels consume more food or biomass to meet their energy needs, it is assumed that biomagnification, the biological increase of arsenic concentration in food webs, may occur. The primary exposure pathways for animals are through drinking water and the consumption of plants and animals contaminated with arsenic (24).

Humans are also occupationally exposed to arsenic (8). Arsenates enter animal and human cells *via* phosphate transport systems, and arsenites *via* transporters for small molecules such as glycerol. In the bloodstream, As^{5+} can be reduced to As^{3+}, which accumulates in the liver, where it is further transformed by reduction and oxidative methylation, forming MMA(III) and DMAA(III) (52, 53). Methylation of inorganic arsenic and conversion to less toxic organic forms is considered the primary detoxification pathway in animals and humans. The comparatively lower sensitiv-

ity of mammals to toxicity is thought to result from their greater diversity of arsenic methylation enzymes (54). Arsenic metabolites are excreted in urine in both animals and humans (55).

Lithium in the Environment

Introduction

Lithium is used in organic synthesis and in the production of plastics and glass (mainly optical glass). Global lithium production in 2008 was 27,400 tons, while in 2023 it was estimated at 180,000 tons (56). Global annual consumption and demand for lithium continue to grow with the development of new technologies (57).

The rapid growth of production is driven by the wide use of lithium in batteries for video cameras, computers, telephones, and wireless devices. Commercial and military drones use high-capacity lithium-ion batteries that are lightweight and have high energy density. Those batteries provide the power required for take-off, and their performance significantly affects flight time, speed, and stability. In addition, lithium is added to rocket fuels and lubricants. It is also used in the pharmaceutical industry, in drugs for the treatment of psychiatric and neurological diseases.

Awareness of lithium in the environment has recently increased due to its extensive use in the energy sector (58). It has therefore attracted the attention of the wider public and the scientific community and is increasingly considered a new environmental pollutant.

Distribution of Lithium in the Environment

Lithium is the lightest metal, widely and relatively uniformly distributed throughout the Earth's crust, with concentrations ranging from 20 to 60 mg/kg (0.002–0.006%) (59). It is most commonly concentrated in acidic igneous rocks and argillaceous sedimentary rocks (60). As a highly reactive cation with a relatively small ionic radius, it easily replaces cations with sufficiently similar ionic radii in geochemical processes, such as Mg^{2+}, Fe^{2+}, Al^{3+}, and Ti^{4+}. Lithium is highly mobile in geochemical processes and preferentially enters silicate minerals rather than sulfide minerals. In nature, it is primarily found in rocks, minerals, and ores in various concentrations (61, 62).

In nature, during weathering in oxidizing and acidic environments, lithium is easily released from primary minerals. It is then incorporated into clay minerals and Fe–Mn hydroxides, accumulated in phosphate rocks, and is also easily absorbed by organic matter. Natural processes in geological formations, hot springs, geothermal activities, and volcanic eruptions are the primary geogenic sources of lithium into the environment (63).

Soil formation conditions have more control over lithium content in soils than the initial content in parent rocks. Its distribution in soil profiles follows the general trends of soil solution circulation, although it can be very irregular.

The average lithium content in soils worldwide ranges from 13 to 28 mg/kg. By soil type, the lowest concentrations are in sandy soils (22 mg/kg), while the highest are found in clay soils (53 mg/kg) and limestone soils (56 mg/kg). Higher lithium content is found in deeper horizons, i.e., soil layers. The portion of lithium considered bioavailable, and the most mobile fraction in soils, is very low and does not exceed 5% of the total content (64). This exchangeable fraction of lithium is often strongly associated with calcium and magnesium in the soil.

Lithium can be deposited in the upper horizons in arid climatic zones along with easily soluble chlorides, sulfates, and borate salts. The texture of mineral soil is the most significant factor controlling soil lithium status, while other parameters, such as total soil organic matter content (SOM), cation exchange capacity (CEC), and pH, are of lesser importance (60).

Sources of Lithium in the Environment

Geogenic sources of lithium are: ores and deposits, weathering of rocks, lithium-rich coal, volcanic eruptions, geothermal springs, and natural mineral waters. The most significant anthropogenic sources include smelting and mining of ores, chemical production, and discarded lithium batteries, among others.

Chemical Forms

As a reactive element, lithium does not occur in free form in nature. Still, it is predominantly bound to mineral components such as apatite or aluminum silicates (60) and in various salts, including lithium carbonate (Li_2CO_3), lithium chloride (LiCl), and lithium hydroxide (LiOH). Lithium compounds are highly soluble (e.g., LiCl) and relatively chemically inert.

Concentrations in thermal spas that advertise lithium-rich waters range from 1 to 10 mg/L (65).

Mobility of Lithium

Lithium is naturally present in trace amounts in surface waters, groundwater, oceans, and the atmosphere, and only rarely occurs in elevated concentrations in water, soil, or sediments. Water-soluble forms of lithium in soils represent about 5% of the total lithium content. Consequently, lithium is likely to appear in groundwater in areas where bedrock and soils contain elevated lithium levels. The lithium content exceeds its natural environmental threshold mainly due to various anthropogenic activities (66). Lithium contamination in the environment, whether geogenic or anthropogenic in origin (62), can be further categorized into point and non-point sources, based on its exact location in the environment (i.e., air, water, and soil). A point source of pollution originates from a single source of air, water, and soil pollution. In contrast, nonpoint sources are dispersed, making it difficult to trace pollution. Examples of pollution caused by land runoff include precipitation, atmospheric deposition, drainage, leaks, and hydrological modifications (such as rain and snowmelt).

While geogenic lithium is moderately soluble, lithium released through anthropogenic activities is one of the most mobile cations in soil (67). Given its high mobility, lithium and its compounds can easily leach into surface and groundwater sources (68), thus becoming highly bioavailable with a tendency to bioaccumulate in certain microbiota. Lithium can also enter the environment through landfills and mine drainage (69). Anthropogenic sources of lithium emissions into the atmosphere include the combustion of lithium-enriched coal, dust, and fine particles released during ore processing activities, emissions from recycling centers handling household electronic waste, and waste incineration (70, 71). Dai et al. (72) found that the volatile loss of lithium during coal combustion is less than 5%. Yet, once released into the atmosphere, lithium can condense onto fine particles and be transported by wind over long distances, contributing to its deposition in terrestrial and aquatic environments.

Lithium in the Air

Lithium is a flammable metal and potentially explosive when suddenly exposed to a sufficient amount of air. Lithium dust particles, mainly

in the form of lithium hydroxide (LiOH), can explode when heated. In the presence of moisture, lithium hexafluoroarsenate may form hydrogen fluoride, a highly corrosive compound. Lithium concentrations in the air are generally low (2–4 ng/m^3) and vary by location.

Lithium enters the atmosphere through fly ash particles from coal combustion, with about 10% of lithium from coal combustion released as fly ash aerosol. Mining operations also use large quantities of saltwater in lithium production. For example, 750 tons of water are needed to produce one ton of lithium. In regions where lithium-containing brine evaporates, the lithium concentrations in the air may be higher (62).

During the recycling of lithium batteries, fine particles are released into the air and may harm the respiratory health of workers in recycling centers (73). Considering the rising demand for lithium and its potential to cause air pollution, an assessment of all possible toxic effects on the respiratory system is necessary.

Plants

Although lithium is not considered an essential nutrient for plants, there is some evidence that it can affect plant growth and development (74) and may play a metabolic role in halophytes.

Soluble lithium in soil is readily available to plants; therefore, the content of this element in plants can serve as a good indicator of the lithium status in the soil (75). There are significant differences among plant species in their tolerance to lithium concentrations and in their ability to absorb this element. Lithium concentrations in plants typically range between 0.2 and 30 mg/kg, depending on the species and location (76). Buendía-Valverde et al. (77) calculated the bioconcentration factor, i.e., the ratio of lithium concentrations in the plant and the surface soil layer. For plants of the *Rosaceae* family, with the highest average lithium content, this index is 0.6, while for *Polygonaceae* plants it is 0.04. The highest value of this index, 0.8, was calculated for *Solanaceae* plants, which are known to have the highest tolerance to lithium. In arid climatic zones, some species from this family accumulate more than 1,000 mg Li/kg (78). The highest lithium uptake has been recorded in plants growing on "Natric" soils or other soils with increased alkaline metal content. Lithium appears to share the K^+ transport carrier, making it easily transported into plants and mainly translocated in the leaves. The lithium content in edible plant parts shows that, compared

to the root or bulb, the leaves are the main site of lithium accumulation. However, a high lithium content is often reported in roots as well, indicating that differences in plant tolerance to lithium are mainly related to the mechanisms of biological barriers in root tissues. Plants in industrial regions exhibit a higher concentration of lithium, particularly noticeable in the above-ground parts of plants compared to roots; for example, the leaf-to-root ratio of lithium in dandelion is 0.8 in rural regions, while in industrial areas it rises to 5.0 (60). Wallace et al. (79) concluded that beans accumulated more lithium in shoots than in roots in most experimental treatments. They also reported that higher levels of lithium reduced zinc content in leaves while increasing the calcium, iron, and manganese content in all plant tissues.

An increased lithium content in soil can be toxic to certain plants (60). Citrus trees are probably the most susceptible to excess lithium, and their growth in lithium-enriched soil can be significantly reduced. Moderate to severe toxic effects have been observed when concentrations range from 4 to 40 mg Li/kg in citrus leaves. In corn growing on soils with a high lithium content, root tip damage, impaired root growth, and chlorotic and necrotic spots on leaves have been observed (60).

Microorganisms

Microorganisms play an important role in soil ecology and are involved in various biochemical cycles of carbon and other nutrients. Research examining the effects of lithium on microbial activity indicates that increasing lithium concentrations reduces their respiration (80). Reduced microbiological activity in soil leads to decreased bacterial productivity, destabilization of food chains, and changes in biogeochemical cycles.

Excess lithium in microbial cells causes harmful effects, including excessive production of reactive oxygen species, overproduction of proteins, reduced enzyme function, obstruction of food uptake, lipid peroxidation, and DNA damage. As a result of toxicity, microorganisms and the microbial communities suffer irreversible damage due to mutagenicity and/or cell death (81).

Although some microorganisms are relatively sensitive to increased levels of lithium, some fungi, such as *Penicillium* and *Aspergillus*, easily adapt and grow on media containing lithium.

Toxicity

Lithium is not classified as an essential element for humans because its deficiency does not cause symptoms. At high doses (approximately 17.5–24 mg/L blood concentrations), lithium becomes toxic to humans, causing nausea, visual impairment, and kidney dysfunction; doses above 24 mg/L cause cardiac arrest and coma (62). Studies of the effects of elevated concentrations of LiCl on rats show a direct impact on the function of testicular tissue, leading to male infertility through impaired steroidogenesis and spermatogenesis (82). In fish, lithium has been found to accumulate in the brain, gills, and kidneys, posing a risk by entering food chains (83). The proposed daily lithium intake for an adult is 14.3 μg/kg of body weight (61). Despite significant research efforts, exposure to critical concentrations of lithium and associated health risks remains insufficiently understood (84).

Lithium is used to treat bipolar affective disorder, so most toxicological studies are based on clinical observations of patients who have undergone lithium treatment. In humans, lithium is known to affect the kidneys, with nephrogenic diabetes insipidus being the most common such effect. Additional adverse effects on thyroid function, primarily asymptomatic hypothyroidism, have also been observed in patients treated with lithium (85). In both animals and humans, lithium is predominantly excreted through the kidneys; approximately 80% is reabsorbed in the proximal renal tubule, while about 20% is excreted in urine.

Boron in the Environment

Boron (B) is widely but unevenly distributed in the environment, with an average concentration of 15 mg/kg in the Earth's crust. Its content in magmatic rocks ranges from 5 to 30 mg/kg and increases with the acidity of the rocks. The content of B is higher in sedimentary rocks than in magmatic ones and is closely associated with the clay fraction.

In nature, boron occurs in oceans, sedimentary rocks, coal, and soil (86). Its most common minerals are borax ($Na_2B_4O_7 \cdot 10H_2O$), colemanite ($Ca_2B_6O_{11} \cdot 5H_2O$), ulexite ($NaCaB_5O_9 \cdot 8H_2O$), kernite ($Na_2B_4O_6 \cdot 3H_2O$), and tourmalines of highly complex composition. Borate deposits are rare worldwide and are mainly found in regions with arid climates and a history of volcanic or hydrothermal activity. The production of pure boron is technically demanding due to its high melting point and corrosive properties of its liquid form.

Although present in trace amounts, boron occurs in the hydrosphere and lithosphere and enters the environment through natural geogenic processes and anthropogenic activities (87).

Natural sources of boron include borosilicate minerals, volcanic eruptions, geothermal waters, groundwater, and seawater. In seawater, the boron concentration is up to 4.5 mg/L, which affects its accumulation in marine sediments and saline soils (88, 89). Boron-rich deposits are found in areas associated with volcanic activity or in regions where swamps and lakes have evaporated due to drought. Some of the world's central boron-rich regions are located in California, the high Andes, and the plateau of the Alpine-Himalayan system (86). It is estimated that approximately 300,000 tons of boron are emitted into the atmosphere annually by volcanic activities. Boron, in the form of soluble borate, is present in oceans, especially in tectonically active zones, where geological processes facilitate its release. Boron is the tenth most abundant element in the oceans.

Anthropogenic activities are another source of boron in the environment. Global boron consumption exceeds 1.5 million tons annually due to its wide range of applications in glass and ceramics production, pharmaceuticals, cosmetics, cleaning detergents, fertilizers, anticorrosive materials, and high-heat-resistance products (90). Although the glass industry is the largest consumer, the primary sources of pollution include contaminated irrigation water, effluents from wastewater treatment plants, boron-rich fertilizers, and ash from coal-fired power plants containing boron (91, 92). Boron concentrations are elevated in carbonaceous sediments and some coals, resulting in a high boron content in fly ash, with an average content estimated at 509 mg/kg (93).

Boron concentrations in the air range from 0.5 (and lower) to 80 ng/m^3 (average content is 20 ng/m^3), in soil from 10 to 300 mg/kg, with an average of 30 mg/kg. In surface waters, boron concentrations typically range from 0.1 (and lower) to 0.5 mg/L, although they can be considerably higher depending on the geochemical nature and location.

Chemical Forms of Boron

Boron belongs to the group of metalloids, chemical elements that exhibit properties of both metals and nonmetals. It is positioned in the 13th group (formerly IIIa) of the Periodic Table of Elements and has only three valence electrons (94). Boron is among the lightest and most mobile elements, with a tendency to form anionic complexes due to its high ionization potential.

In nature, boron has only two stable isotopes. They have atomic masses of 10 and 11 and occur in a ratio of 20:80, resulting in an average atomic mass of 10.81 g/mol (95). This fact can be used to identify the water source based on its boron isotope ratio. In other words, different water sources always have slightly different isotope ratios.

In nature, boron exists exclusively in the trivalent (+3) oxidation state. However, it does not occur as a trivalent cation under natural conditions; rather, it is always bound to oxygen (96). Also, boron can only form covalent bonds due to its small size and high ionic potential (94).

In aqueous solutions, the chemical behavior of boron is determined by the concentration and pH value of the solution. Under neutral and slightly acidic conditions in natural waters and soils, boron exists as undissociated boric acid (H_3BO_3), a weak Lewis acid that is well soluble in water. At higher pH values, boric acid forms borate ions $B(OH)_4^-$, while at high boron concentrations, polyborates are formed. During rock weathering, boron easily enters solution, forming a range of anions, such as BO_2^-, $B_4O_7^{2-}$, $H_2BO_3^-$, and $B(OH)_4^-$ (97).

Physicochemical Processes and Mobility of Boron in the Environment

Boron is released into the environment through various processes. It enters the atmosphere through emissions from chimneys during the production of ceramics, glass, and cellulose. Oceans are estimated to contribute 65%–85% of atmospheric boron, reflecting the average ocean boron content of 4.6 mg/L, ranging from 0.52 mg/L in the Baltic Sea to 9.57 mg/L in the Mediterranean. Boron enters surface waters through wastewater discharged from the glass, ceramics, and fertilizer industries, as well as from the production and use of detergents. A substantial amount reaches landfills through the disposal of glass and ceramic products. Once in a landfill, boron inevitably migrates into soil and natural waters. Boron originating from coal ash in landfills is highly mobile, so its migration into the soil is unavoidable (98).

Boron enters the soil through atmospheric deposition, rock weathering, decomposition of organic matter, leaching from landfills, and the use of fertilizers. The availability of boron depends on the physicochemical characteristics of the soil, including soil texture, pH, and moisture content. The boron concentration is generally higher in clay soils than in sandy soils. Boron is most mobile in surface horizons of sandy soils, but its mobility decreases with depth due to the increased clay content and reduced

water movement. Most of the total boron in the soil is bound to organic matter and released through microbial activity (99). However, in arid conditions, the activity of microorganisms is reduced, and boron remains fixed and unavailable to plants.

There are several mechanisms of interaction between boron and soil, including: 1) sorption of the borate ion, 2) sorption of molecular boric acid, 3) formation of organic complexes, 4) precipitation of insoluble borate with aluminum and silicon, and 5) incorporation of boron into clay structures. Boric acid is the dominant form at pH values below 7, while the borate ion is the more prevalent form at higher pH. Organic matter has the most significant impact on boron availability in soils with a pH below 7, and increasing organic matter content also increases boron availability (100).

Plants

Soluble forms of boron are readily available to plants, which can absorb boric acid and other forms of boron in the solution. Boron is one of the seven essential micronutrients, or trace elements, required for plant growth, and is therefore extremely important in the production of commercial crops. This significance has led to widespread research into all aspects of boron and its impact on crops (101). Studies indicate that boron plays a role in carbohydrate metabolism, sugar translocation, pollen germination, hormone action, and nucleic acid synthesis. The property of boric acid to form complexes with polysaccharides plays an important role in passive sorption. Boron absorption occurs through the plant's roots, whereby it is absorbed from the soil solution mainly as undissociated boric acid, which theoretically should be membrane permeable. Boron uptake in higher plants is achieved through passive transport and primarily depends on the boron concentration in the soil solution and the transpiration rate, although it is controlled by the permeable membrane and internal complex formation (60).

However, the primary functions of boron remain unclear. Recent research suggests that boron is involved in cell wall cross-linking, particularly through complexing with specific pectin components (102). Still, the range between its deficiency and plant toxicity is very narrow.

The critical level of water-soluble boron in soil is 0.5 mg/kg, with plant species classified into two categories based on their translocation ability: species with limited boron mobility and those with significant mobility.

One of the most critical factors affecting boron bioavailability is soil pH. The uptake rate is lowest at a pH of around 7, while in alkaline soil,

availability increases with pH. Uptake also depends on temperature and increases during warmer periods of the year.

Sensitive species include citrus fruits, stone fruits, and walnuts; semi-tolerant species are cotton, tubers, cereals, and olives, and most vegetable plants are tolerant (60). However, much rarer than deficiency, boron-rich soils can reduce crop yields in some regions of the world. Phytotoxicity typically occurs due to anthropogenic activities, including fly ash, boron-contaminated irrigation water, and excessive application of boron-rich fertilizers. Elevated boron concentrations in some crops (usually in cereals and sunflowers) most often occur in boron-contaminated soil due to: 1) irrigation with municipal wastewater or river water with high boron content, 2) ash application, and 3) foliar application of boron-enriched fertilizers in citrus and/or apple orchards. The boron concentration in irrigation water should not exceed 0.3 mg/L for sensitive species and 2–4 mg/L for plant species highly tolerant to boron.

Damage has been recorded near glass factories, where high atmospheric boron concentrations lead to chlorosis and necrosis of pine needles (*Pinus sp.*) (86). Municipal waste and coal ash, when composted together, act as particular sources of available boron, negatively affecting the growth of certain vegetables (60). In saline soils, foliar boron levels may be reduced due to high soil salinity (103). Plants that exhibit tolerance to high salt concentrations are usually tolerant to elevated boron concentrations in the growth medium.

Aquatic Organisms

The acute toxicity of boron to various fish species has been the subject of numerous studies. The most sensitive freshwater fish identified so far is the rainbow trout (*Oncorhynchus mykiss*). Large trout hatcheries typically use water containing up to 1 mg/L of boron without apparent problems (86, 92). In marine aquaculture systems, studies examining the toxicity of boron to shrimp (*Americamysis bahia*) in saltwater indicate that the no-observed-adverse-effect level (NOAEL) depends not only on boron concentration but also on water salinity.

Microorganisms

Toxicity thresholds (TTs) for various microorganisms are defined as the concentrations at which inhibition caused by a chemical produces a difference greater than 3% compared to the control group. Thus, for the

green alga *Scenedesmus quadricauda*, the TT is 0.16 mg/L, while for *Entosiphon sulcatum* it is 0.28 mg/L. In aerobic sludge, a significant reduction in chemical oxygen demand was observed at concentrations above 10 mg/L (93).

Dust in the Living and Working Environment

According to the International Standard Organization (ISO) (104), dust is defined as small solid particles, conventionally below 75 μm in diameter, which settle under their own weight but may remain suspended for some time.

Dust is associated with a variety of mining operations, including drilling, crushing, cutting, grinding, and blasting, required to reduce the size of rocks and minerals in open-pit and underground mines. Most of the dust is produced by crushing (105, 106).

Dust properties are affected by particle hardness, density, and moisture content. The mineralogical and chemical composition may differ from the parent rock, as other minerals can be crushed in the process (107). Dust particle size is an important parameter, as many dust features depend upon it. The particle diameter typically refers to the particle size. The most often used diameter is the aerodynamic diameter. It combines particle aerodynamic properties that determine a particle's transport and fate. Dust particle size is coupled with its origin and chemical composition (108), the latter of which depends on the type of ore being mined.

Depending on the size, dust particles are categorized as coarse, fine, or ultrafine. Coarse particles include total suspended particles (TSP) ranging from 10 μm to 100 μm, while 30 μm aerodynamic diameter is commonly applied to represent TSP (109) and particles (particulate matter) at 2.5 μm aerodynamic diameter to 10 μm ($PM_{2.5\text{-}10}$) (110). Fine particles are $PM_{2.5}$ or smaller, and ultrafine particles are smaller than 0.1 μm (109).

Coarse particles are produced by crushing. Particles above 30 μm settle near the dust source (within 100 m). During secondary crushing in open-pit mines, coarse particles are deposited 350 m from the dust (105). Occasionally, dust can spread several kilometers from quarries (111). Coarse particles PM_{10} can be carried by wind up to 100 km, remaining airborne for days, whereas $PM_{2.5}$ can be transported thousands of kilometers, staying in the air for weeks (112).

Total suspended and fine particles are the main dust constituents in underground mines, accumulating near extraction and processing areas (106). Larger particles, with a diameter less than 500 μm, settle near the dust generation site, whereas fine particles can be transported to other mine sections (113). In deeper mine sections, the spreading of fine particles is more difficult to predict, mainly due to inadequate mine ventilation (108, 114).

Diesel-operated mobile equipment used for haulage and transportation adds diesel exhaust emissions to underground mines. The *International Agency for Research on Cancer* (IARC) has classified exposure to diesel exhaust fumes as carcinogenic in humans (115). Diesel exhaust is a complex mixture of gas-phase and particle-phase emissions. The gas phase contains nitrogen oxides, carbon monoxide, and low molecular weight polycyclic aromatic hydrocarbons (PAHs), while the particle phase consists mainly of an elemental carbon core with large surface areas capable of absorbing a number of substances, such as metals and organics (116). Organic components include high-molecular-weight PAHs (116). According to certain data, diesel exhaust particles account for approximately 80%, and, in some cases, up to 98% of fine particles smaller than 2.5 μm (117).

In addition to size, dust can be classified according to its environmental, occupational health, and pathophysiological effects (118).

The environmental impacts of dust are as follows: 1) Generated dust is produced by mechanical processes of solid material breaking into smaller pieces, 2) Total suspended dust – generated dust entrained in the air (particles up to 50 μm), 3) Nuisance dust – coarse particles that can affect environmental amenity, diminish visibility, become irritant or damage machinery, 4) Fugitive dust – deposited outside the mine or far away from the source of dust.

Dust posing occupational health risks is classified into: total inhalable dust, thoracic dust, and respirable dust (119). Airborne particulate matter of total inhalable dust enters the nose and mouth during breathing; inhaled particles of thoracic dust penetrate beyond the larynx. In contrast, inhaled particles of respirable dust penetrate to the gas exchange lung region. Fine particles, $PM_{2.5}$ and smaller particles, can cross alveolar walls from the lungs into the bloodstream. From there, they can reach nearly every organ and induce various acute and chronic effects (119).

Regarding pathophysiological effects, dust is commonly classified as: a) toxic – can cause chemical reaction, damage lung tissue, and gain access to the bloodstream, b) cancerogenic – can cause cancer, c) fibro-

genic – can trigger long-lasting reaction with scar tissue formation and loss of lung elasticity, d) explosive, and e) nuisance (120). According to some authors, the classification of diesel exhaust particles and radioactive particles should be added to mine dust categories, based on their types of pathophysiological effects (114). Mine dust can also be classified into combustible/explosive dusts and non-combustible dusts, based on combustion properties, as well as into primary dust sources and secondary dust sources, based on the source of generation (114).

Regarding the environmental impacts, dust can be a nuisance, but it can also affect regional ecology and agriculture. Depending on particle concentration, distribution, deposition rate, and chemical composition, dust can alter soil chemistry, impair plant health, influence meteorological and local climate conditions, and penetrate vegetation (121). Beyond vegetation, dust can also affect animal communities (122). In humans, in addition to its most critical effects on the lungs, dust can also cause irritation of the skin and eye mucous membranes (120). Dust has been one of the leading occupational "killers" (123) mainly due to negative impacts on the respiratory tract. Depending on their physical and chemical properties, dust particles can cause adverse health effects not only through inhalation but also through skin and eye contact or *via* oral exposure (124). Dust generated by mining activities can cause a range of respiratory diseases, including pneumoconiosis, chronic obstructive lung disease, emphysema, and pneumonia, as well as coronary heart disease and skin allergies (125, 126). Due to lower visibility, danger, or the possibility of explosion, dust can compromise workplace safety. Dust accumulation on equipment reduces its lifespan and modifies product properties, which can reduce productivity (118).

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Chapter 3

The Effects of Elevated Concentrations of Lithium, Arsenic, and Boron on Algae and Aquatic Plants

Gordana Subakov Simić, Ivana Trbojević

- Lithium, boron, and arsenic have different effects on algae and aquatic plants.
- Low concentrations of lithium can have a stimulating effect on algae and aquatic plants.
- Arsenic is toxic to algae and aquatic plants.
- Boron is essential for algal growth and development in low doses, but is extremely toxic at higher concentrations.
- Elevated concentrations of all three elements have a negative impact on the diversity and abundance of species and the number of individuals in the population.
- Continuous monitoring of potentially endangered aquatic ecosystems must be mandatory.

Introduction

Lithium, arsenic, and boron have different effects on algae and aquatic plants, depending on the concentration of the element, the type of algae, and the ecosystem. Unfortunately, limited research has been conducted on the influence of these elements on the growth and development of algae and aquatic plants.

Lithium

Lithium is an element widely used in energy storage (lithium batteries), the military and aerospace industries (as a component of alloys), the glass and ceramics industries, and medicine, where it is a component of antidepressants. In recent years, the demand for lithium has increased mainly due to the widespread use of lithium batteries in the automotive industry, resulting in a rise in lithium production. Lithium-containing waste poses a major threat to the environment. Lithium dissolves readily in water and often forms free cations with a low electrical charge. Its concentration in natural waters varies: in unpolluted waters, it occurs only in traces, i.e., it is considered a rare element. Lithium concentrations range from 0.5 to 91 μg/l in surface waters, from 0.05 to 150 μg/l in groundwater, and from 170 to 190 μg/l in seawater (1).

Lithium is not considered an essential element for plant growth and development, although some studies have demonstrated its beneficial effects. Due to the physicochemical similarities between lithium and sodium, potassium, and calcium, plant transport systems readily absorb all these elements (1).

At low concentrations, lithium can have a stimulating effect, while at higher concentrations it exerts an inhibitory and toxic effect. As with most other toxic substances, increased lithium concentrations reduce the diversity of algae in aquatic ecosystems.

Phytoplankton algae form the basis of the food chain. Being often unicellular organisms, their entire body is exposed to the active uptake of all dissolved substances from the surrounding water, including lithium, which they accumulate in their biomass. Even if the negative impacts of bioaccumulation are not noticeable, caution is still required due to the possible effects of biomagnification (the progressive increase in the concentration of dangerous compounds at higher levels of the food chain).

The effects of lithium on aquatic plants, the mechanism of absorption/accumulation, and the negative consequences remain poorly understood. To date, only one study has investigated the effects of elevated lithium concentrations on the species *Salvinia natans*. The results demonstrated that *S. natans* can accumulate lithium from the aquatic environment and that exposure to high lithium concentrations reduced potassium content and photosynthetic pigments in the plants (1, 21). As primary producers, macrophytes together with phytoplankton, form the basis of food chains in aquatic ecosystems, and biomagnification effects must be considered with caution.

Boron

Boron is a metalloid and an essential micronutrient required for the growth and development of vascular plants, marine algae, diatoms, flagellates, and cyanobacteria, as it plays a role in the maintenance of cell structure and function (2). Boron is widely used in various industries and contributes to water pollution worldwide. However, there is limited scientific data on its toxicity and effects on aquatic life. Available studies indicate that elevated boron concentrations in water negatively affect photosynthesis and growth of tested aquatic plants (3).

In undisturbed natural surface waters, boron concentrations should not exceed 0.1 mg/l. However, mining and mineral exploitation can lead to significant increases in boron levels in some surface waters (4). Boron is highly soluble in water, so its concentration is increased in waters around the world (5).

Many algal species have a low tolerance threshold for boron toxicity (6). Consequently, high boron concentrations in water inhibit the growth of sensitive phytoplankton (microalgae), thereby reducing their diversity.

Boron-containing chemical compounds accumulate in phytoplankton and enter the food chain. Phytoplankton, for example, is eaten by zooplankton, which is eaten by fish, which in turn are consumed by other larger fish, birds, mammals, and humans. Through biomagnification, boron compounds can accumulate in significantly higher concentrations in the bodies of animals and humans, posing a serious health threat.

Beyond the observation that elevated boron concentrations induce photooxidative stress and impair the growth in representatives of the macroscopic algae of genus *Chara* (14), the effects of arsenic and lithium on charophytes, many of which are rare and endangered species, remain unknown.

The floating aquatic plant species *Lemna gibba* has been shown to efficiently accumulate boron residues from contaminated waters, and numerous emergent aquatic plants are recognized as hyperaccumulators of this element. The submerged species *Myriophyllum elatinoides* is another potential hyperaccumulator. Studies demonstrate that boron damages the cells of *M. elatinoides*. The target organelles are the chloroplasts in leaf cells, mitochondria in stem and root cells, and nuclei in root cells. In the species *Salvinia natans* and *Lemna minor*, even a low boron concentration reduces the growth rate and photosynthetic capacity of the plants (27, 28, 29).

Arsenic

There is very little data on the effects of arsenic on algae. The available literature, however, indicates that arsenic is highly toxic to algae. At elevated concentrations, arsenic has a negative effect on the photosynthesis process, resulting in reduced growth and proliferation of algae. Like other toxic elements, arsenic diminishes biodiversity in aquatic ecosystems and favors more tolerant species.

Several submerged aquatic plant species, *Hydrilla verticillata*, *Ceratophyllum demersum,* and *Potamogeton malaianus*, were tested as bioaccumulators of arsenic from water, and all three species were able to absorb arsenic forms. *Hydrilla verticillata* was the most efficient, and all species showed a drastically reduced biomass compared to the control groups. Similarly, floating aquatic plants *Eichhornia crassipes* and *Pistia stratiotes* have also demonstrated the ability to bioaccumulate various forms of arsenic from the water (21, 26).

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Chapter 4

The Impact of Lithium, Arsenic, Boron, and Particulate Matter ($PM_{2.5}$; PM_{10}) on Lichens

Slaviša Stamenković

- The diversity and ecophysiology of lichens are among the best biological indicators of air quality in both natural environments and those modified by anthropogenic activity.
- Lichens are particularly sensitive to the presence of nitrogen and sulfur oxides in the air, as well as to other physicochemical substances.
- Although relatively scarce, research into the relationship between arsenic (As), boron (B), lithium (Li), and "particulate matter" ($PM_{2.5}$), and lichens suggests that these chemical elements have a negative impact on their vitality, leading to a reduction in lichen diversity.

Introduction

Lichens are a community of two different organisms—algae (photobiont) and fungi (mycobiont). Both members benefit from this symbiosis, as they functionally complement each other and enable mutual survival. The alga performs photosynthesis and supplies the fungus with necessary organic matter, while the fungus uses hyphae to absorb water containing mineral salts from the substrate, providing the elements for the alga's photosynthesis, as well as creating a humid environment vital for its survival.

The exchange of substances between the algae and the fungus takes place through their cell walls. The fungus is an essential part and builder of the lichen, forming the majority of its biomass. The body (thallus) of a lichen resembles neither a fungus nor an alga. The basic types of lichen thalli are: crustose (crustose lichen), leafy (foliose lichen), and bushy (fruticose lichen). In relation to their substrate, these organisms are categorized as saxicolous, when they occur on the surface of a stone or rock, or endolithic, when they penetrate the interior of a rock or stone. Terricolous lichens grow on the soil surface, while corticolous/lignicolous lichens inhabit woody substrates. Lichens growing on organic substrates serve as better biological indicators of air quality (environment) than those that inhabit inorganic substrates. In this sense, the best indicators of air quality are cortical lichens, which inhabit the bark of woody trees.

Lichens are highly sensitive to changes in air quality, a fact first noted as early as 1866 (1). At that time, their ability to respond to harmful substances in the air was observed and attributed to the effects of factory smoke. The complete disappearance of lichens from the Luxembourg Gardens in Paris was linked to the transition from wood to coal for domestic heating. In the 1970s and 1980s, lichens were also observed to have either completely disappeared or undergone significant changes in their composition in areas characterized by reduced air quality. Subsequent research linked air pollution to lichen composition (2), which led to the development of scales for assessing lichen sensitivity/resistance to reduced air quality (pollution).

Unlike other organisms, lichens lack a protective surface layer on their thallus (body), which makes them extremely sensitive to pollution, as pollutants can penetrate the thallus unhindered. However, this makes lichens excellent bioindicators of air quality. Lichens feed by absorbing nutrients directly from the air through water vapor. Their metabolic activity increases in the hydrated (wet) state, making them more sensitive than when they are dry. The hydrated state most often occurs when precipitation "washes" harmful substances from the air and brings them to the lichen thallus. The accumulation of harmful substances in the lichen thallus is facilitated by the fact that lichens lack the ability to excrete/release them, and what they once absorb (including harmful substances) remains permanently in their bodies. Unlike plants, lichens cannot shed body parts, such as leaves, and therefore cannot eliminate the accumulated harmful substances. Another important feature is that lichens are active (biologically, metabolically) throughout the year, yet grow very slowly. As a result, the negative effects

of harmful substances cannot be remedied quickly; however, this means that lichens are suitable for long-term monitoring (3).

Not all lichens are equally sensitive to air pollution. More tolerant (resistant) species are characterized by a smaller thallus surface area and a relatively faster growth rate, while those with a larger surface area of a lichen thallus (relative to its volume) are more sensitive to pollution. In terms of thallus type, bushy lichens, with a large surface area exposed to external influences, are more sensitive and, as a rule, are the first to disappear from polluted environments. They are followed by species of the leafy thallus type, while those of the crusty thallus type are the most resistant (3).

Lichen Response

Lichens are considered not only the best biological indicators of sulfur oxides (SO_x), nitrogen oxides (NO_x), and carbon oxides (CO_x) in the air, but also the best biological indicators of the negative effects of heavy metals (4) and other chemical pollutants known to harm other types of organisms. Results from a study of the influence of trace elements on lichens conducted in the Netherlands (5) support the hypothesis that this influence is relatively small, with the exception of antimony (Sb), which has a significantly negative influence on the abundance of several lichen species. The study concluded that lichens can serve as accumulator organisms in studies of air pollution, i.e., the environment, provided that the concentration in the thallus reflects the concentrations found in the air. A similar conclusion was drawn in a 1995 paper (6) regarding the "trace elements" cobalt (Co), scandium (Sc), and zinc (Zn). By contrast, the effects of other trace elements such as lithium (Li), arsenic (As), and boron (B) on lichens are relatively poorly documented, particularly in the case of lithium, for which no data are available in the literature.

Results on arsenic uptake/absorption and biotransformation in *Hypogymnia physodes* (7) suggest that metabolic and biochemical transformations occur in this lichen species, including at least two types of arsenic detoxification mechanisms: arsenite excretion and methylation. Another study on *Xanthoria parietina* (8) showed that even low concentrations (0.01 ppm) of arsenic induce physiological stress in both the fungus and the alga, resulting in cell membrane damage. This damage was observed as an increase in the electrical conductivity in cell water, which is the parameter most affected by arsenic treatment. Higher concentrations (10 ppm) of

arsenic cause a significant decrease in photosynthetic efficiency and consequently lead to their death.

The ecophysiological effects of arsenic salts on the lichen *Pyxine cocoes* were examined by transferring ("transplanting") the lichen from an unpolluted to an arsenic-polluted environment under experimental conditions (9). Higher concentrations of arsenate were shown to be detrimental to lichen photosynthesis, as both chlorophyll fluorescence and pigment abundance were significantly reduced. In addition, high arsenic concentrations inhibited the activity of antioxidant defense enzymes. A study on the physiological effects of excess boron in the lichen *Xanthoria parietina* (10) reported a decrease in lichen vitality, measured as the intensity of respiratory activity. By contrast, the contents of the protein and hydrogen peroxide (H_2O_2), as well as photosynthesis and chlorophyll, were not altered. From these results, it can be concluded that the fungus is more sensitive to excess boron than the alga, and since the fungus constitutes the majority of lichen biomass, the functioning of the entire lichen organism is disrupted, ultimately leading to its decline. Taken together, these findings indicate that arsenic and boron can negatively affect the functioning and survival of certain lichen species. However, due to the small number of studies, these results are only "hints" that indicate the need for more detailed and comprehensive research into the potentially harmful effects of chemical elements in small quantities, especially lithium, arsenic, and boron, introduced through anthropogenic activities such as mining and other forms of exploitation of natural resources.

In addition to "trace elements," air quality is also affected by the quantity and concentration of particulate matter smaller than 2.5 micrometers (μm) ($PM_{2.5}$) and 10 μm (PM_{10}). A study conducted in urban areas in Chile (11) examined the relationship between the amount and concentration of $PM_{2.5}$ microparticles in the air and the lichen diversity index, demonstrating that total lichen diversity can serve as an ecological indicator of $PM_{2.5}$ microparticle levels. Specifically, the lichen diversity index is significantly inversely correlated with $PM_{2.5}$ levels, meaning that the higher the concentration and amount of $PM_{2.5}$ particulate matter, the lower the lichen diversity index. Similar results were obtained in a study in Medellin, Colombia (12), where 110 lichen species were studied, with *Phaeophyscia chloantha* and *Physcia poncinsii* being the most common. Here too, lichen species richness (and lichen population abundance) and anthropogenic impacts were inversely correlated with $PM_{2.5}$ levels. This indicates that the greater the tree coverage by lichens and the higher the diversity of the lichen com-

munity, the greater the probability of a lower amount and concentration of $PM_{2.5}$ particulate matter in the air. The degree of lichen diversity is higher in areas with better air quality and more favorable microenvironments, suggesting that the most sensitive lichen species can only persist in environments where declines in air quality are lower.

Conclusion

Lichens are the best living indicators of overall air pollution and the negative impact of certain chemical and physical substances on living organisms. Although relatively limited, research on the relationship and impact of arsenic, boron, lithium, and $PM_{2.5}$ particles on lichens indicates adverse effects on their vitality and a consequent reduction in diversity.

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Chapter 5

The Impact of Elevated Lithium, Boron, and Arsenic Concentrations on Plants

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Similar to the physiological requirements of humans, the normal functioning of plants depends on certain trace metals that play an essential role in cell structure and metabolic functions. These elements are required in very low concentrations in the plant organism, such as zinc and copper, and are therefore referred to as micronutrients. However, their increased concentrations in the environment and subsequently in plants can lead to physiological stress and functional impairment in plant systems. In contrast, non-essential and highly toxic metals such as lead, mercury, and arsenic have no known biological function and can exert harmful effects even in low concentrations. Prolonged exposure to elevated concentrations of heavy metals, whether from contaminated soil or water sources, can disrupt important metabolic pathways in plants. This disruption typically results in physiological disorders leading to morphological damage to roots and leaves, impaired water and nutrient absorption, reduced biomass yield, premature leaf senescence and shedding, impaired reproductive development, fruit malformation, and ultimately plant death.

The Impact of Elevated Lithium Concentrations on Plants

Given the increasing use of lithium in modern technology and its subsequent release into the environment, it is particularly important to

understand the effects of lithium accumulation on living organisms. The study of its phytotoxic effects is therefore essential to assess the risks to plant health and ecosystem stability. Unlike most other metals, lithium (Li) is highly soluble and mobile in soil, making it readily available for plant uptake. Plants absorb lithium efficiently through their root system and accumulate it throughout their tissues. Consequently, in areas where lithium concentrations in soil or surface waters are elevated—whether due to mining activities or other forms of pollution—lithium is rapidly absorbed and incorporated into the flora of both natural ecosystems (such as forests, meadows, lakes, and rivers) and agricultural systems, thereby entering the food chain. This implies that herbivores feeding on lithium-enriched plants begin to accumulate elevated lithium concentrations themselves, which may subsequently be transferred to humans through dietary intake. The main sources of lithium exposure to humans in such regions, therefore, include contaminated drinking water, plant-based foods grown on lithium-contaminated soils, and animal products derived from animals raised on contaminated feed and water (1–6). The average lithium concentration in agricultural soils in Europe is about 11.4 mg/kg (7). In contrast, lithium concentrations in plants from uncontaminated environments are usually very low, typically only a few milligrams per kilogram of dry plant biomass (8, 9). For example, plant samples from uncontaminated regions in Russia and New Zealand contain lithium in concentrations between 1.5 and 4 mg/kg (10, 11). Research has shown that plant species differ in their ability to absorb lithium, with species-specific differences in sensitivity to lithium accumulation. Species belonging to the Asteraceae, Rosaceae, and Solanaceae families exhibit a relatively high tolerance to accumulated lithium. In contrast, other species, such as citrus plants, are highly sensitive and show signs of damage even at low levels of absorbed lithium (12–14). Members of the Brassicaceae family (e.g., mustard and cabbage) are particularly known for their ability to accumulate significant amounts of metals. For example, oilseed rape has been shown to accumulate up to 300 mg of lithium per kilogram of dry leaf mass under experimental conditions when grown in lithium-contaminated soil (15). Other plant species showing high lithium accumulation under experimental conditions include lettuce (1000 mg/kg), spinach (1131 mg/kg), beet (5500 mg/kg), barley (1131 mg/kg), and sunflower (3292 mg/kg) (15–18). In contrast, lithium concentrations in seeds and fruits of these plants are significantly lower (19).

So far, no physiological or biochemical processes have been found in plants that are related to or dependent on lithium, which clearly indicates

that this metal is not essential for plant life. Nevertheless, lithium is readily taken up from the soil by plant roots and transferred to above-ground parts (19). As a result, the lithium content in plant parts is often a reliable indicator of the lithium concentration in the soil. One of the most important factors influencing lithium availability to plants is the pH of the soil: lithium is more bioavailable in acidic soils than in neutral or alkaline soils (11–13, 20). This implies that soil acidification—whether by acid rain or by the influx of acidic water—can significantly increase the bioavailability of lithium, as well as other metals such as boron, for plants and soil organisms (8, 21).

Why do plants absorb lithium so easily, even though it has no known metabolic function? Studies comparing lithium concentrations with those of other cations studied in plant tissues—such as potassium, calcium, and magnesium—suggest that lithium probably uses the same transport pathways across cell membranes and within the plant as these essential cations (K^+, Na^+, Ca^{2+}, Mg^{2+}) (12, 22). The ability of lithium to replace essential elements in plants is mainly attributed to the similarity of its ionic radius to that of important cations such as potassium, calcium, and magnesium, as well as to its strong polarization power (23, 24). Detailed studies of the structure and function of sodium transport channels in plant cell membranes have shown that these channels conduct both sodium and lithium ions with comparable efficiency. When the lithium concentration in the soil is elevated, plants absorb lithium intensively, often at the expense of sodium uptake (25). In addition, plants growing in lithium-contaminated soils may take up lithium instead of calcium and magnesium, reducing the levels of these vital nutrients in plant tissue (18, 26). Once inside the cell, lithium frequently binds to biomolecular sites normally occupied by potassium, sodium, calcium, or magnesium, and can even displace these cations if they are already bound. This substitution disrupts the structure and function of various cellular components—including proteins, membranes, and cell walls—that naturally depend on these ions. As a result, lithium impairs critical cellular functions such as photosynthesis, cellular respiration, signaling, and gene expression (12, 13, 22–24, 26–30). For example, disturbances in intracellular calcium levels impair calcium-dependent processes, particularly intracellular signaling pathways that enable the cell to respond appropriately to environmental stimuli (18). Additionally, lithium is one of the metals that readily binds to ligands with available oxygen atoms. These interactions can lead to structural and functional alterations in cellular components, resulting in extensive metabolic disturbances, oxi-

dative stress, DNA and membrane damage, and can ultimately trigger premature cell death (17, 30, 31). Research conducted over the last two decades has clearly demonstrated that elevated lithium concentrations in plants exert multiple detrimental effects. These effects disrupt fundamental biochemical and physiological processes within the plant organism, causing numerous negative outcomes. In particular, high lithium concentrations are associated with reduced seed germination rates, root damage, and leaf damage, manifesting as brown necrotic and desiccated leaf areas. In addition, plants show visibly stunted growth, significantly impaired fruit and seed production, and reduced overall survival rates (9, 15, 18, 22, 26, 27, 32–35). Some data on the stimulatory effects of low lithium concentration in plants are based on short-term experiments. However, further detailed studies are needed to gain insight into the long-term effects of low lithium concentrations on individual plant species (growth and development, biomass yield, fruit quality, seed productivity, and germination) as well as on plant populations and communities.

The Impact of Elevated Boron Concentrations on Plants

Boron (B) is a micronutrient required by plants in very low concentrations (36, 37). Despite this minimal requirement, plants readily take up and accumulate boron as it is efficiently transported from the roots to all aerial parts of the plant, including the stems, leaves, and fruits. Different plant species and varieties vary in their boron requirements: crops such as maize, wheat, and barley thrive at lower boron concentrations (1 to 6 mg/kg plant dry biomass), while most dicotyledonous plants perform best at boron concentrations between 20 and 70 mg/kg dry matter. However, when boron is present in high concentrations in soil or irrigation water, it is readily absorbed by plant roots and transported to above-ground parts where it exerts toxic effects (38, 39). Visible symptoms of boron toxicity usually appear first on mature, older leaves, often in the form of necrotic, *burnt* edges, where the highest boron concentrations accumulate (38, 40). Given the strong phytotoxicity of boron at elevated concentrations, soils and water sources with elevated boron concentrations pose a serious threat to crop yields and quality, particularly during drought periods. Excess boron in plant tissues disrupts metabolism by binding to various biomolecules, causing their structural and functional damage, and conse-

quently disrupting essential biochemical and physiological processes. This metabolic imbalance manifests in characteristic symptoms of boron toxicity in the aerial plant parts—stems, leaves, and fruits. Affected plants typically exhibit smaller, irregularly curled leaves that become necrotic and appear scorched, particularly at the tips and edges. Interveinal bleaching and premature leaf death are also common (41). In addition to foliar symptoms, boron toxicity reduces overall plant vigor and growth rate. Other symptoms often include bark necrosis, stem dieback, reduced flower bud and fruit formation, and the formation of smaller, misshapen fruit with reduced weight and often poor flavor quality (41–47).

Even before visible symptoms of boron toxicity appear, numerous cellular damages occur in the plant tissue. Commonly observed effects include reduced chlorophyll content, reduced photosynthetic efficiency, alterations in protein synthesis, structural damage to certain enzymes, leaf transpiration disorders, oxidative stress, oxidative damage, and increased cell membrane permeability. These effects have been documented in the leaves of lemon, orange, pear, kiwi, maize, and *Arabidopsis thaliana* (46–60). Although not all negative effects of excess boron on plant metabolism have been fully elucidated, current research suggests that boron-induced damage is due to several mechanisms: (a) impairment of cell division and development, (b) disruption of key metabolic processes due to the binding of boron to ribose—either in its free form or as a structural component of important biomolecules such as RNA, ATP (the energy currency of the cell) and NADH (koenzyme central to metabolism, critical cofactor in redox reactions) and NADPH (involved in redox reactions, critical cofactor in biosynthetic processes including Calvin cycle, lipid and DNA synthesis), (c) alterations in cellular pH, and (d) changes in gene expression activity. When boron binds to ATP and NADPH molecules, they become non-functional, leading to cellular energy deficits. Consequently, affected plants exhibit reduced sugar production, disturbed sugar distribution, and reduced starch accumulation—phenomena observed in the leaves of sugar beet, tomato, and barley (48, 60–65). Excess boron also negatively affects the biosynthesis, structure, quantity, and functionality of many cellular proteins. It often binds directly to enzymes or their substrates, thereby interfering with enzymatic reactions (41, 61). The cell membrane is another likely target of boron toxicity. Boron can bind to the hydroxyl groups of glycoproteins, glycolipids, and membrane-associated proteins, leading to structural and functional membrane damage. Overall, these disturbances indicate that elevated intracellular boron concentrations impair or partial-

ly inhibit numerous cellular processes (62). As a result, oxidative stress and oxidative damage occur in plant cells—a phenomenon observed in barley, wheat, maize, chickpea, potato, pumpkin, cucumber, lettuce, grapevine, apple, and other species (50, 55, 57, 58, 65–71).

In addition to the visible damage to the above-ground parts of the plant, excess boron also has toxic effects on the root system. In roots, high boron concentrations lead to thickening of the hypodermis, thickening of the cell walls, deposition of suberin in the root cortical cells, and lignification and hardening of the root tissue—as observed in crops such as tobacco and soybean (72–74). Similar changes have been reported in orange leaves, where boron toxicity induces the formation of additional mechanical tissue (48). By forming these protective structural barriers, root cells effectively "defend" themselves against the external environment and increase their ability to bind excess boron in tissues where it causes less damage (48, 56). However, excess boron also causes chromosomal damage, abnormal mitosis in root meristem cells, and oxidative stress. These cellular disturbances impair root development, as demonstrated in maize, wheat, barley, and fava beans (41, 71, 75–78). Reduced root growth impairs water uptake capacity, often leading to plant desiccation. This has been frequently observed in wheat, barley, maize, tomatoes, and grapevines (68, 75, 77, 79).

As far as the maximum permissible boron concentrations in irrigation water are concerned, the threshold values vary depending on the sensitivity of plants. For boron-sensitive species—such as apricot, peach, sour cherry, fig, apple, pear, bean, and walnut—the recommended boron concentration is 0.3–1.0 mg/L. Moderately sensitive species, such as sweet potato, pumpkin, oats, corn, wheat, barley, olive, tomato, and potato, can tolerate 1–2 mg/L. Boron-tolerant species, including carrots, lettuce, onions, alfalfa, and sugar beet, can tolerate concentrations between 2–4 mg/L (80).

It is important to note that plants can store excess metals in specialized cellular compartments—such as vacuoles and the cell wall—which are unique to plant cells. This mechanism helps isolate toxic metals and metalloids from metabolically active cellular compartments, thereby reducing their harmful effects. In contrast, animal cells—including human cells—lack these structures and consequently do not have this protective mechanism. As a result, animal and human cells are more susceptible to the toxic effects of excessive metal accumulation.

Boron enters the human body daily through the consumption of drinking water and fresh or processed foods of plant and animal origin

(81). Detailed information on the occurrence, potential sources of human exposure to elevated boron concentrations, and associated health effects can be found in a U.S. EPA document (82). The total daily intake of boron should not exceed the established safety thresholds. To ensure the protection of public health, it is important to monitor the concentrations of boron, arsenic, and other potentially toxic metals in water, soil, and food for human consumption. These levels must remain below the prescribed upper safety limits to avoid adverse health effects.

The Impact of Elevated Arsenic Concentrations on Plants

So far, there is no evidence that arsenic (As) is an essential element for plants. On the contrary, it is known to be highly toxic to most plant species, even in low concentrations (83). Despite numerous excellent studies on the effects of As on plant metabolism, the exact nature of toxicity remains insufficiently understood, as the molecular, biochemical, and physiological mechanisms of arsenic toxicity are still not fully clarified (83). Available data show that the toxic effects of As depend on its chemical form and concentration, the molar ratios between As and mineral nutrients, substrate pH, plant species, the plasticity of plant metabolic pathways, and the protective mechanisms. Consequently, this metalloid can negatively impact plant growth, development, and metabolism, ultimately reducing productivity in various plant species, including important agricultural crops. Understanding the effects of arsenic on plants is therefore critical for managing contaminated environments and ensuring food safety, particularly given the extreme toxicity of arsenic to humans.

The estimated concentration of arsenic in the Earth's crust ranges between 1.5 and 3 mg/kg (84). To date, over 200 arsenic-bearing minerals have been identified (86), with the most common ore minerals being sulfides such as realgar (As_4S_4), arsenopyrite (FeAsS), and orpiment (As_2S_3). Arsenic concentrations in uncontaminated soils can vary considerably. According to various sources, arsenic levels in soils range from 1 to 40 mg/kg, with the lowest concentrations typically found in sandy soils and soils on granitic bedrock, while much higher values are often found in alluvial soils (86, 87). In the surface layers of European soils, the average arsenic concentration is about 7 mg/kg (88). However, in gold (Au)-enriched metalliferous zones in southwestern Poland, concentrations can reach up to

18,100 mg/kg (89). In addition to natural processes, various anthropogenic activities contribute significantly to the release of arsenic into the environment, estimated at 52,000 to 112,000 tons per year. These activities include mining and ore processing, coal combustion, the use of contaminated irrigation water, and the application of phosphate fertilizers, herbicides, and pesticides, all of which promote the mobilization and spread of arsenic in agricultural soils and aquatic ecosystems (91). Nearly 60% of arsenic released annually from anthropogenic sources originates from coal combustion and copper smelting (88).

Soil contamination with arsenic is therefore a serious environmental threat, particularly in agricultural regions (90). Once arsenic is absorbed from the soil, it can accumulate in the edible parts of plants, posing a potential risk to human health through dietary exposure (83).

Depending on the physicochemical conditions in the environment, arsenic occurs in different chemical forms that significantly affect its bioavailability and toxicity to plants (91). In the environment, arsenic occurs in different oxidation states, with arsenite (As(III)) and arsenate (As(V)) being the most common forms found in soils. Organic species, such as mono-, di-, and trimethylarsenates, are also present (92, 93). In terms of toxicity to plants, the inorganic forms—arsenite and arsenate—are the most important, as they are readily taken up by root cells.

Although both inorganic forms of arsenic can interfere with plant metabolism, the mechanisms of their action differ (83). Arsenite (As(III)) is up to 60 times more toxic than arsenate (As(V)) due to its higher solubility and mobility in water, which facilitates its uptake by plants from the soil (94). Until recently, little was known about the mechanisms of arsenite uptake. However, numerous independent studies have demonstrated that aquaporins—membrane proteins that form pore channels for water transport—are involved in arsenite uptake. Silicon transporters in rice have also been shown to be significantly involved in arsenite transport (95). Understanding these mechanisms is particularly important for plants growing in flooded soils, where reducing conditions prevail and arsenic is predominantly present as arsenite (96).

In contrast, arsenate (As(V)) occurs predominantly in well-aerated soils (97). Due to its chemical similarity to phosphate, arsenate is absorbed by plants instead of phosphate and transported through the plant via phosphate transporters, leading to disturbances in phosphorus metabolism (98). Phosphorus is an essential nutrient for plants involved in critical processes such as energy transfer, photosynthesis, and respiration. Under conditions

of low phosphate content, arsenate is absorbed by the roots instead of phosphate and transported into the cells due to the antagonistic relationship between the two elements. Thus, As causes phosphate deficiency in the plants and disrupts the above-mentioned processes, resulting in reduced plant growth and poor crop yields. For example, arsenate competes with phosphate in phosphorylation reactions, producing unstable arsenate-containing compounds that interfere with cellular energy production. This disruption reduces ATP production, which is essential for plant metabolism. A deeper understanding of phosphate transporters and their regulation in plants will undoubtedly lead to a better insight into the mechanisms of arsenate uptake and transport (97). High arsenic concentrations disrupt water uptake in plants, induce the formation of reactive oxygen species (ROS), damage lipid membranes, and lead to oxidative stress (91, 94). Arsenic pollution also affects carbon metabolism, amino acid and protein balance, as well as metabolic pathways involved in nitrogen and sulfur assimilation. Emerging research approaches utilizing transcriptomics and proteomics have greatly improved our understanding of the effects of arsenic on plant metabolism (83). Most of these analyses have been conducted in rice. Transcriptomic studies reveal that exposure to arsenite (As(III)) alters the expression levels of numerous genes, including those involved in the synthesis of various transporters, plant hormones, and fatty acid metabolism. Elevated arsenic concentrations also alter key metabolic pathways related to plant growth and development and activate many genes involved in oxidative stress responses (99). Arsenic also reduces chlorophyll content, disrupts the structural integrity of chloroplast membranes, and consequently lowers the rate of photosynthesis. It interferes with enzymatic interactions, displaces essential ions in the ATP molecule, and causes other biochemical disorders. As a result, plants exposed to arsenic often develop fewer, smaller, deformed, and necrotic leaves and flowers, have poorly developed roots and stems, and produce smaller quantities of inferior quality fruit (100).

Interestingly, several studies on non-hyperaccumulating and hyperaccumulating plant species (*Arabidopsis thaliana, Pteris vitata*) have reported that trace amounts of As can have a stimulating effect on plant growth under certain substrate pH conditions and adequate phosphate nutrition. In these two species, this paradoxical response is most likely explained by the interaction of low arsenic concentrations with phosphate uptake, which can transiently stimulate growth under certain conditions (83, 101, 102).

Once absorbed, arsenic generally exhibits low mobility within plants, as shown by numerous studies, including those carried out on cultivated species such as tomato. The low efficiency of arsenic translocation from the roots to the aerial parts is attributed to the rapid reduction of arsenate to arsenite in the root zone. Arsenite then forms complexes with thiols and is sequestered in the vacuoles of the root cells (97). By contrast, arsenic transport from roots to shoots is highly efficient in a small group of arsenic hyperaccumulator plants. These species possess the remarkable ability to accumulate arsenic in their aerial parts in exceptionally high concentrations while simultaneously employing effective detoxification mechanisms (98). One of the world's best-known arsenic hyperaccumulators is the Chinese brake fern (*Pteris vittata*). However, only a relatively small number of plant species have proven to be tolerant to elevated arsenic concentrations in the soil.

The phenomenon of mycorrhiza and the role of mycorrhizal fungi in promoting arsenic tolerance in plants is remarkable, and a variety of mechanisms may be involved. Symbiosis with mycorrhizal fungi may lead to the suppression of high-affinity phosphate transport systems, thereby reducing arsenate uptake. The fungi may also contribute to arsenic efflux into the external environment and may increase the phosphorus uptake efficiency, which stimulates plant growth while reducing arsenic concentrations in plant tissue. These fungi may also inhibit the translocation of arsenic from the roots to the aerial parts of the plant. However, further studies are needed to confirm this hypothesis (103–110).

The uptake of arsenic by plants is influenced by a variety of factors, particularly plant species. A study examining the effects of increasing arsenic concentrations in irrigation water on arsenic accumulation in tomato and cabbage plants found that arsenic concentrations were higher in the roots of both species than in their aerial parts. However, the arsenic concentration in the edible parts of cabbage was up to 20 times higher than in tomato fruits. The study also showed the significant influence of soil type on arsenic uptake in both species, particularly under conditions of increased arsenic concentrations in irrigation water (111). These findings highlight the critical importance of monitoring arsenic concentrations not only in agricultural soils and irrigation water, but also in plants intended for human and animal consumption. Such monitoring is essential to prevent arsenic from entering the food chain.

Although this discussion focuses primarily on agricultural crops, it is important to note that long-term contamination of natural ecosystems

(such as forests, grasslands, and aquatic habitats) with metals and/or metalloids will inevitably negatively impact the growth and survival of wild plant species. The species sensitive to elevated metal concentrations will likely be the first to disappear from contaminated areas, jeopardizing the stability of complex food webs and leading to a gradual loss of other species due to the lack of food and habitat.

In all regions where mining and ore processing are carried out, contamination of the environment with heavy metals and associated chemicals is inevitable. Elevated concentrations of heavy metals are found not only in agricultural crops, but also in locally produced milk, cheese, eggs, and meat—foods regularly consumed by both adults and children in such areas (112–114). To limit food contamination by heavy metals, international organizations—including the Food and Agriculture Organization of the United Nations (FAO), the World Health Organization (WHO), and the International Organization of Vine and Wine (OIV)—have set maximum permissible levels for certain metals in food and water.

In view of this, it is essential to prioritize regular and mandatory monitoring of environmental quality (air, soil, and water) and food safety. Particular attention must be paid to the practical implementation of measures aimed at preserving environmental quality and protecting ecosystems and public health.

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Chapter 6

Analysis of the Effects of Arsenic, Lithium, and Boron on the Fauna of Aquatic Invertebrates

Ivana Živić, Vidak Lakušić, Milenka Božanić

- The negative effects of pollutants (arsenic, lithium, and boron) on aquatic invertebrates are reflected in their inability to feed (due to morphological deformations of the mouthparts) and in a decreased reproduction rate (leading to a reduction in abundance and diversity), which over time results in the disappearance of aquatic invertebrates from aquatic habitats.

Introduction

Springs, streams, and rivers are not only centers of biodiversity and endemism, but also habitats for a large number of endangered species (1). The most threatened groups of aquatic invertebrates are mollusks (44% endangered in Europe) (2), crustaceans (one third threatened with extinction globally) (3), and insects (33% of aquatic species compared to 28% of terrestrial species) (4). A similar situation is observed in Serbia, where these three groups are considered the most endangered (5, 6). Together with other groups of aquatic invertebrates, they represent the main route for the uptake of heavy metals into food chains and directly influence the trophic structure of the aquatic environment, since, as first-order consumers, they feed on primary producers that readily incorporate metals (7). In

addition, they are bottom-bound, have long life cycles, and move slowly (8, 9), making them particularly suitable for monitoring long-term changes in aquatic environments through their presence or absence. They are used as model organisms at all levels of biological organization, from the molecular level to the population and the ecosystem as a whole (8, 9, 10). Aquatic insects and other invertebrates are not only essential to aquatic food chains but also play a key role in energy transfer from aquatic to terrestrial ecosystems (11). Adult insects serve as prey for other organisms (predators) living in riparian zones, acting as "transporters" of pollution from water bodies into agricultural areas (11).

The larvae of aquatic insects (mainly Ephemeroptera, Plecoptera, and Trichoptera) accumulate heavy metals and tolerate their low to moderate concentrations. Consequently, the metal concentrations in their bodies correlate with those in the aquatic environment and sediment (12, 13). In addition, metals are eliminated from insect bodies more slowly than from non-living components of river ecosystems (14). Importantly, heavy metal concentrations in sediments, rather than in water bodies, correlate with concentrations in aquatic invertebrates (11, 12). The accumulation of heavy metals in the sediment has a greater impact on the aquatic invertebrate communities than their concentration in the water (15). For example, a positive correlation has been found between heavy metals in the larvae of *Ephemera danica* (Insecta: Ephemeroptera) and corresponding metal concentrations in the sediment (15). Therefore, following alterations to riverbeds and shorelines during the construction of mines and ore processing, an even greater danger to wildlife arises from changes in sediments due to the deposition of metals (As, B, Li, Zn, Cu, Cr, Fe). These metals reach watercourses through wastewater from mining plants and accompanying infrastructure, and their increased concentrations directly affect the diversity of aquatic invertebrates. The intensity of the impact depends on the degree of pollution and may range from minor changes in the composition and structure of benthic communities to their complete disappearance from water bodies in cases of high inflows of heavy metal-rich effluents from ore processing. This is particularly important in areas where mining activities are planned in habitats containing species subject to legal protection (strictly protected and protected species) or species listed as vulnerable (e.g., crayfish) or endangered (e.g., river mussel) in international lists (IUCN, Habitat Directive). Likewise, many rare and endangered species of aquatic insects across several orders (Ephemeroptera, Plecoptera, Trichoptera, Odonata) occur in such areas. Therefore, continuous monitoring of

aquatic ecosystems using macroinvertebrates as bioindicators (one of the measures) is required during mine construction, ore extraction, and after mine closure (16).

In view of these facts, this chapter provides a review of the literature on the negative effects of arsenic, lithium, and boron on aquatic invertebrates. These effects are manifested as the decline in diversity, abundance, and biomass of macroinvertebrate fauna downstream of mines, the occurrence of oxidative stress, morphological asymmetries and deformities (in mouthparts, body size, tentacles, etc.), reduced food consumption (sometimes even a complete cessation of feeding), as well as decreased growth, survival, and reproduction rates. Over time, these effects lead to the disappearance of aquatic invertebrates from affected habitats.

The Impact of Arsenic on Aquatic Invertebrates

In freshwater ecosystems, natural arsenic concentrations are generally below 1 μg/L (17). However, human activities such as agriculture, industry, and mining can significantly increase arsenic concentrations in the environment, sometimes reaching up to 28,000 μg/L (18). In aquatic systems, arsenic may occur in the more toxic inorganic trivalent and pentavalent forms, or in organic forms. The trivalent form is considered more toxic than the pentavalent form because it reacts with the -SH group of proteins, thereby disrupting their normal functions (19). Studies have shown that arsenic, together with copper and nickel, negatively affects the activity of antioxidant defense enzymes in the endangered mussel species *Unio tumidus*. For example, an increase in arsenic concentration in water between 1.30 and 1.55 μg/L leads to a decrease in the activities of catalase (CAT) and glutathione S-transferase (GST) in the tissues of this mussel (20), which may result in increased production of reactive oxygen species (ROS) and the onset of oxidative stress.

In addition to these effects on the physiology of aquatic invertebrates, higher arsenic concentrations negatively influence the morphological development of certain invertebrates, such as aquatic insect larvae from the Chironomidae family. Laboratory studies have demonstrated that increased exposure of the cultivated species *Chironomus tentans* to elevated arsenic concentrations increases the frequency of deformities of the oral apparatus and reduces the body size of their larvae (21). High arsenic con-

centrations of 260 µg/L dry weight cause 20% deformities, including the fusion of adjacent teeth. Furthermore, the head width of larvae was also significantly smaller at medium and high arsenic concentrations (0.4 mm) compared to those from control groups (0.8 mm). Body length was also reduced in aquaria with medium (8 ± 3 mm) and high arsenic concentrations (7 ± 1 mm), compared to larvae from the control aquaria (11 ± 2 mm). Elevated arsenic and heavy metal concentrations in sediments have also been shown to increase the frequency of deformities in Chironomidae larvae (22). At arsenic concentrations of 117 to 134 mg/kg, deformities were recorded in 10.3% to 26 % of the chironomid population, compared to only 2.2% under control conditions with uncontaminated sediment (22). Under laboratory conditions, species such as *Deleatidium* spp. (order Ephemeroptera) and *Zelandobius* spp. (order Plecoptera) were found to be sensitive to increased concentrations of pentavalent arsenic. The lethal concentration (LC50, the concentration at which 50% of the organisms die) during a 4-day exposure was 2.1 mg/L for *Deleatidium* spp. and 2.4 mg/L for *Zelandobius* spp. (23). In addition, sensitivity to arsenic increased with prolonged exposure: the 14-day LC50 for *Deleatidium* spp. was 0.36 mg/L, while the 4-day LC50 was 2.10 mg/L (23).

In addition to the direct impact of elevated arsenic concentrations on individual organisms, negative impacts have also been observed on the population structure of species, i.e., on the entire biocenosis of aquatic invertebrates. For example, Chi et al. (18) investigated the influence of elevated arsenic concentrations (trivalent or pentavalent, inorganic or organic) on the structure of the benthic invertebrate communities in a river system consisting of three rivers in China. Macrozoobenthos (large invertebrates) were sampled from five sites, two of which were located downstream of a mine, revealing elevated total arsenic concentrations of 28.29 mg/L and 0.57 mg/L, respectively. The results showed that sites with increased total arsenic concentrations had fewer species, lower diversity, abundance, and biomass of macrozoobenthos compared to unpolluted sites (18). Additionally, even the most pollution-tolerant groups (Oligochaeta and Hirudinea) showed declines in abundance compared to the control site, while the most sensitive tax—Ephemeroptera, Plecoptera, and Trichoptera (EPT taxa)—were completely absent, which was expected as they are recognized as bioindicators of clean and unpolluted waters (24, 25). Due to their detritus feeding, which exposes them to the highest concentration of arsenic, scraper, collector, and filter-feeder species were also the most sensitive to pollution, while predatory groups were the most abundant at polluted sites due to their ability to remove heavy metals through excretion (18). As a consequence of

the disappearance of aquatic invertebrates that decompose organic matter, arsenic pollution disrupts the energy cycle of aquatic ecosystems. The results of this study are consistent with those of Mori et al. (19), who reported similar findings in a river in Corsica (France), where arsenic concentrations reached 3.010 µg/L in water and 9.450 µg/L in sediment due to mining activities. At contaminated sites, there was a decrease in the number of species and the abundance of macrozoobenthos. The negative effects of elevated arsenic concentrations on EPT taxa were further confirmed by Valenti et al. (26) in a US stream, where arsenic concentration reached 7.900 µg/L due to the proximity of a mine that ceased operations a hundred years ago. Upstream of the mine, EPT taxa accounted for over 20% of the species in the community, while downstream they represented less than 4% (26). As organisms from the EPT taxa group play a key role in the fragmentation and decomposition of coarse organic matter into finer particles more accessible to other aquatic organisms, the disappearance of the most sensitive EPT taxa from the benthic communities disrupts the circulation of matter in food chains and alters the structure of benthic communities (26).

The Impact of Lithium on Aquatic Invertebrates

In freshwater ecosystems, natural lithium concentrations are generally lower than 0.04 mg/L (27). The mechanisms of lithium action are complex, multi-layered, and still poorly understood. One of the better-studied pathways of lithium action is based on the similarity of its ionic properties to those of the biologically important magnesium ion (Mg^{2+}). This similarity enables lithium to bind directly to the magnesium binding sites of several enzymes, preventing Mg^{2+} binding and thereby inhibiting enzyme activity through a mechanism of competitive inhibition. Lithium is known to bind to two highly conserved enzymes in the vertebrate central nervous system: Glycogen synthase-3 (GSK3) and inositol monophosphatase (IMPA) (28). These two enzymes are also found in the invertebrate species *Hydra viridissima* (Cnidaria: Hydrozoa), suggesting that lithium toxicity may be exerted on these enzymes (29). Under laboratory conditions, increased lithium concentrations in the model organism green hydra (*Hydra viridissima*) lead to degenerative changes (reduction in body length, tentacles, or changes in tentacle shape) that prevent feeding and ultimately cause death (29). Lithium toxicity has also been studied in the freshwater snail (*Elimia clavaeformis*) under laboratory conditions (30). Experiments demonstrated

that a lithium concentration of only 0.15 mg/L leads to a 50% decrease in food intake, while exposure to 0.3 mg/L completely stopped feeding (30).

The model organism *Daphnia magna* (Crustacea: Branchiopoda) has been shown to be moderately sensitive to lithium exposure, with LC50 values of 10.2 mg/L and 4.1 mg/L (31). Prolonged chronic exposure progressively reduced somatic growth and reproductive capacity, ultimately threatening the survival of the population at the ecological level. Elevated lithium levels induced biological responses that were insufficient to avoid oxidative stress and neurotoxicity, as evidenced by changes in lipid peroxidation and antioxidant enzyme activities. Lithium exposure led to a decrease in protein and glycogen content in *D. magna*, negatively affecting demographic characteristics and possibly leading to changes in fitness, as increased energy expenditure is required to survive stressful situations (31). Martins et al (32) also examined the effects of lithium using *D. magna* as a model organism. The results of the study showed that a 21-day exposure to different concentrations of lithium (0.02, 0.04, 0.08 mg/L) significantly reduced the adaptive capacity (i.e., the ability to survive and reproduce) of *D. magna* by 67%. This indicates that lithium has a significant negative impact on the health and reproductive capacity of *D. magna*. The toxicity of lithium in *D. magna* was also investigated by Kim et al (33), who found significant changes in gene expression after exposure to lithium hydroxide (LiOH) at concentrations corresponding to the LC50. The exposure resulted in both increased and decreased gene expression in *Daphnia magna*. Increased expression occurs in genes encoding cuticle-associated proteins, suggesting that the organisms have adapted to maintain the shape of the exoskeleton in a contaminated environment. Conversely, reduced expression was observed in the gene responsible for synthesizing the enzyme GAPDH (glyceraldehyde-3-phosphate dehydrogenase, which regulates enzyme levels in the cell), which could indicate changes in metabolism. This study indicates the complex responses of *D. magna* gene expression to the toxic effects of lithium (33).

The Impact of Boron on Aquatic Invertebrates

Boron concentrations in freshwater ecosystems are typically below 1 mg/L (34). Most existing studies have focused on determining the toxic concentration of boron in various aquatic invertebrates, but not on the mechanism by which it affects the organisms themselves.

In the study by Hall et al. (34), a reduction in survival rate was reported in the aquatic Oligochaeta species *Lumbriculus variegatus* and in the freshwater clam *Lampsilis siliquoidea.* The LC25 values of the boron concentration, at which a 25% reduction in survival or growth occurs, were 12.7 and 45 mg/L, respectively (34). Similarly, Maier and Knight (35) demonstrated that *Daphnia magna*, when exposed to certain concentrations of sodium tetraborate, had a 48-hour LC50 value of boron concentration of 141 mg/L. As these organisms play a key role in the food chain, such toxicity can trigger a domino effect throughout the aquatic ecosystem.

When Diptera larvae of the family Chironomidae were used as model organisms, an LC50 value of 1.376 mg/L was found for *Chironomus decorus* (35) at 48-hour exposure, with a significant reduction in growth rate under chronic exposure of 20 mg/L. In *Chironomus riparius,* boron concentrations of 180 mg/kg in sediment or 32 mg/L in water were found to be toxic (36). Later studies reported that chronic toxicity for this species at 37.7 mg/kg in sediment (37). Boron also leads to a lower reproduction rate in aquatic invertebrates. For example, Soucek et al. (39) concluded that the freshwater crab *Hyallela azteca* (Malacostraca: Amphipoda) experienced a reduction in reproductive rate at boron concentrations of 13 mg/L (38).

Emiroğlu et al. (39) investigated the effects of boron in the Kirkuk region of Turkey on the biocoenosis of a habitat (Sejdi stream) with elevated boron concentrations due to the proximity of a mine. The results showed that the site closest to the boron mining complex had the highest boron concentrations in water and sediment (mean 3.45 ± 0.33 mg/L and 32.72 ± 4.63 mg/kg, respectively), with no aquatic invertebrates being detected at this site (39). In contrast, aquatic invertebrates were found at two downstream sites, but they exhibited elevated tissue boron concentrations (Chironomidae larvae – 0.44 and 0.38 mg/kg, Tipulida larvae – 0.84 and 0.63 mg/kg, Ephemeroptera larvae – 1.98 and 0.60 mg/kg, and *Helobdella* sp. – 1.07 and 0.92 mg/kg, at the second and third sites, respectively). Notably, *Gammaridae* and *Donax* sp. (Bivalvia) were absent at the second site, but present at the third site, with tissue boron concentrations ranging from 1.08 (*Gammaridae*) to as high as 14 mg/kg in the *Donax* sp. (39).

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Chapter 7

Risk to the Aquatic Ecosystem of the River Jadar Owing to the Exploitation of Boron and Lithium in the Project Jadar

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- The risk to the aquatic ecosystem of the River Jadar depends on the composition and amount of wastewater that would be processed and discharged into the stream. This study assessed the effects of untreated wastewater on aquatic ecosystem components, specifically fish, by simulating an accidental release.
- The methodology relied exclusively on the publicly available documents issued by the investors, Rio Tinto and Rio Sava Exploration d.o.o., concerning the planned boron and lithium mine in the Jadar region of Serbia. These documents provided the only available data on the concentration of mineral contents in groundwater obtained from probe drills.
- The results revealed that in the event of a spill caused by the failure of proper wastewater processing, concentrations of arsenic ranging from 0.26 to 969.64 μg/L, boron from 3.43 to 12058.83 mg/L, and lithium from 0.70 to 2452.50 mg/L in water of the River Jadar would pose a serious risk to the aquatic ecosystem at low-, medium-, and high-water levels.
- These concentrations would inevitably cause toxic, genotoxic, and other pathological effects in aquatic organisms. Studies using the relevant *in vitro* fish models have revealed that when concentrations of arsenic, boron, and lithium in the stream exceed safe con-

centrations, the entire ecosystem is seriously harmed, particularly the fish, which are the most sensitive to metal pollution.

- Due to the lack of direct field data, it remains difficult to predict either the full extent of harmful effects on downstream sections or the period the ecosystem would need to recover from such high levels of pollution.

Introduction

Jadarite, a lithium sodium borosilicate mineral [$LiNaSiB_3O_7(OH)$], was discovered in 2004 southeast of the city of Loznica in western Serbia (Figure 1) and was named after Jadar, the toponym of that area. The Jadar Project was initiated with the aim of producing battery-grade lithium carbonate (Li_2CO_3), a critical mineral used in large-scale batteries for storing energy in various electric vehicles. In addition, the project is expected to yield substantial amounts of borates (BO_3^-) and sodium carbonate (Na_2CO_3) as by-products, owing to their high contents in the mineral rock deposits and groundwater. Borates are essential for the development of renewable energy technologies, including solar panels and wind turbines (1). The proposed project includes the construction of an underground mine with associated infrastructure and equipment, and a beneficiation chemical processing plant to produce battery-grade lithium carbonate, expected to operate no earlier than 2027. The exploitation period of this mine is expected to be at least 40 years, during which an estimated 2.3×10^6 t of Li_2CO_3 would be produced.

As reported in Anonymous (2), the contents of arsenic (As) (271.5 µg/L), boron (B) (2404.7 mg/L and 2865.9 mg/L), and lithium (Li) (2735 mg/L and 269.5 mg/L) were measured in groundwater sampled from exploration drills at depths of 482 m. Additionally, concentrations of 4600 mg/L of B and 1200 mg/L of Li were recorded in groundwater sampled from deep piezometers, indicating very high levels. If the mine were to be exploited, such water would become wastewater. During the exploitation period, 0.038 m^3/s of groundwater is expected to enter the mine corridors. For safety reasons, this wastewater will have to be pumped out to the surface (2). According to the same source, during most of the exploitation period, groundwater inflows into the mining corridors are expected to range from 0.02 m^3/s (if the spent, i.e., exhausted corridors are backfilled after the exploitation period) to 0.1 m^3/s (if they are left hollow, i.e., unfilled).

In deep drill samples of groundwater, the contents of As, B, and Li were reported as 53.29–172.70 μg/L, 537.73–846.70 mg/L, and 283.38–319.15 mg/L, respectively (3). These values are slightly lower or approximately similar to those cited above (2), but vary more than those in the data used here.

The reported absolute maximum discharge of the River Jadar was 192 m^3/s in June 2001, with an average annual discharge of 8.21 m^3/s and an average high water level discharge of 105.4 m^3/s (4). Another report (5) documented an absolute maximum discharge of 219 m^3/s in May 2014, an average annual discharge of 7.79 m^3/s, and a minimal discharge of 0.03 m^3/s in October 2012. Another source (6) reported an average discharge of 6.8 m^3/s, with minimum values during summer droughts decreasing to 0.12 m^3/s. Records of specific water levels and their corresponding discharges in the River Jadar drainage basin for the period 1990–2010 are also available (4).

During the period of exploitation, the wastewater treatment facilities are projected to operate within a capacity range of 0.145 to 0.290 m^3/s, with a maximum of treated wastewater output of 0.23–0.53 m^3/s discharged into the River Jadar (6). For most of the exploitation period, the expected volume of wastewater released into the River Jadar as a local recipient would be 0.25 m^3/s. However, the risk of pausing wastewater purification facilities for any reason was not addressed at all in the investor's documentation (2).

The fish fauna in the area affected by Project Jadar is documented in two sources (2, 7). Both sources indicate fish fauna typical of small-to-medium-sized streams flowing through valleys at low altitudes, characterized by moderate slope and flow velocity. While the composition of fish communities may vary slightly seasonally and annually, it generally consists of chub *Squalius cephalus* (Linnaeus, 1758), spirlin *Alburnoides bipunctatus* (Bloch, 1782), common barbel *Barbus barbus* (Linnaeus, 1758), Balkan barbel *B. balcanicus* (Kotlík, Tsigenopoulos, Ráb & Berrebi, 2002), nase *Chondrostoma nasus* (Linnaeus, 1758), stone loach *Barbatula barbatula* (Linnaeus, 1758), Balkan loach *Cobitis elongata* (Heckel & Kner, 1858), and Danube loach *C. elongatoides* (Băcescu & Mayer, 1969), along with several other species occurring occasionally.

Arsenic is released through natural processes such as mineral dissolution, volcanic eruptions, and deep groundwater discharge to the surface, as well as through anthropogenic sources, including herbicides, wood- and metal-protecting chemicals, oil refining, and fossil fuel combustion. Once in the aquatic environment, this element accumulates in fish, caus-

ing biochemical disorders and damaging the gills and liver, reducing fertility, and leading to tissue damage, lesions, necrosis, and cell death due to pronounced oxidative stress. In aerobic environments, the dominant form of inorganic As is arsenite [As(V)], which is highly toxic, particularly at elevated water temperatures. Organic arsenobetaine [AsB], derived by biomethylation of inorganic As, is the most toxic form for freshwater fish. Most As enters fish through the gills. Contents of As compounds in water below 0.08 mg/L are considered harmless for fish (8), while changes in behavior quickly appear at concentrations over 2.25 mg/L. Arsenic intoxication of fish manifests in genotoxic and neurotoxic effects, sensory system irritation, swimming either back-and-forth or side-to-side, leaping out of the water, and accelerated ventilation movements of the gill covers, whereas acute and subacute effects at concentrations above 9.64 mg/L affect the respiratory, circulatory, gastrointestinal, and nervous systems (8). A detailed survey of lethal concentrations of various inorganic and organic forms of As (9) implies that many fish species are considerably more sensitive to As than other freshwater biota.

Although there are reports that B can be considered an essential micronutrient at concentrations below 2 mg/L, higher concentrations produce a variety of toxic effects, regardless of pH or dH (water hardness) values. Regarding toxicity, the groundwater contains chlorides (Cl^-) at 4058 mg/L (2), which may enhance the toxic effects of B on aquatic organisms, depending on the species (ANZG, 2021). The intake of B occurs either through passive diffusion or active transport at lower concentrations. In the cell, the toxin inhibits the mitochondrial enzymes. Chronic toxic effects for particular aquatic organisms have been reported at concentrations of 1.8–14 mg/L for fish, 2.4–29 mg/L for shrimps, and 15–56 mg/L for amphibians (10, 11). The lowest concentration considered harmless for aquatic organisms is 1 mg/L; however, experimental studies show that some can survive longer at concentrations up to 10 mg/L. The most sensitive species reported is the flathead minnow (*Pimephales promelas)*, which exhibits clear signs of intoxication at concentrations below 13 mg/L (10, 11, 12). Experiments exposing zebrafish (*Danio rerio)* to boric acid and borax at concentrations of 1–64 mg/L for 24–48 hours have revealed a prominent genotoxic effect on erythrocytes, as assessed by the comet assay (13). Direct effects, including oedema, inflammation, degeneration of parenchymatic tissues, and histopathological changes, have been recorded in rainbow trout (*Oncorhynchus mykiss)* muscles, kidneys, and gills at B concentrations ranging from 10^2 mg/L to 10^3 mg/L (14).

As for the toxicity of Li in the aquatic environment, an experiment on juvenile rainbow trout exposed to a Li concentration of 1 mg/L demonstrated its high bioavailability (15). The rapid intake of Li into the organism increased its content in the blood serum within eight hours and in the brain within two days of exposure. The Li concentration in the brain reached that of the blood serum within the following four days, illustrating the ease with which Li passes through the blood-brain barrier. Simultaneously, the concentration of cation electrolytes (e.g., Na^+, Ca^{2+}, Mg^{2+}, K^+, and NH_4^+) was markedly reduced in the blood serum and the brain, severely affecting the functioning and survival of the fish. The assumed mechanism of Li's effect involves altering the level of arachidonic fatty acid in the brain, which participates in active transport through the brain cell membranes and acts as a mood stabilizer (15).

In considering the impact of mining activities on flora and fauna (2), a lethal effect was assumed to occur only in fish species. A lesser impact on aquatic invertebrates was expected to result from wastewater discharge and structural modifications of the river channel (e.g., siltation of deposits), excluding the effects of the canalization and changes in water flow direction. Harmful effects on the aquatic ecosystem, air and soil pollution, and habitat disturbance caused by mining and associated activities were considered as possibly deleterious to amphibians, aquatic reptiles, and mammals.

The circumstances surrounding the Jadar Mine Project became controversial shortly after its initiation. To date, there has been no detailed report on the anticipated effects of mine operation on the aquatic environment of the River Jadar. Wastewater consisting only of natural groundwater would be extracted from the mine corridors, processed, and released into the River Jadar. The present study aimed to assess the effects of untreated wastewater on aquatic ecosystem components, particularly fish species, by simulating an accident in the most realistic and conservative way possible.

Materials and Methods

The research was carried out in 2021, when documents containing data on the concentrations of As, B, and Li in groundwater obtained from deep drilling were published by the mine investor, the Rio Tinto company, and became publicly available. Owing to the scarcity, variability, or even contradictions in certain data from the available literature sources, the

approach applied was as conservative as possible. This was accomplished both by presuming the least possible effect of wastewater and by working with mean values (instead of either maximal or minimal) for the parameters studied, e.g., the depth of groundwater sampling horizons and the concentrations of As, Li, and B in wastewater expected to be pumped from the mine corridors. To ensure an unbiased assessment of effects on the aquatic ecosystem (primarily fish species), the data on the average discharges in the River Jadar, the foreseen recipient of wastewater, were taken for periods of high-, medium-, and low-water levels available in the literature. The reports from Rio Tinto (2, 6) did not include As, Li, and B concentrations in intact surface waters, i.e., the unpolluted River Jadar. Our starting presumption was that those values were sufficiently low to pose no harmful effect to aquatic organisms in the River Jadar, as no such events had ever been previously reported. This was subsequently confirmed by a published report (3). Therefore, only the concentrations of As, B, and Li in groundwater that would be released as untreated wastewater into the River Jadar were considered. Potential releases of wastewaters from the mine's tailings site were not taken into account. For the quantity of groundwater afflux into the mine corridors and, consequently, the volume of wastewaters discharged into the River Jadar during most of the exploitation period, the two given values were used (2).

Fish sampling from the section of the River Jadar in the area where the Project Jadar is to be implemented was conducted by electrofishing on 11 August 2021. Sampling was performed by wading with a backpack-battery-powered electrofishing gear, an AquaTech device IG200/1 (input 12 V per maximum 15A DC, output 500 V, and frequency 65 P/s). Three locations were sampled (Figure 1): Lopatara in the Krivajica village area (N 44°26'10.36"; E 19°26'43.80"); Draginac village area (N 44°30'19.07"; E 19°24'44.76"); and Kozjak in the Gornji Dobrić village area (N 44°34'51.16"; E 19°18'02.0").

Each sampling stretch was approximately 100 m in length, covering the entire width of the river at low water levels and discharge, and included all types of habitats (e.g., glide, pool, riffle, undercut bank, little cascades, etc.), under very high air (30–35°C) and stream water temperatures. All sampled fish were immediately identified to the species level, and their standard length (*SL*) and body mass were quickly measured on site. Measurements were taken using a field-adapted ruler graduated to the nearest 0.5 cm and a digital balance with a precision of 1 g. All fish were returned alive to the stream.

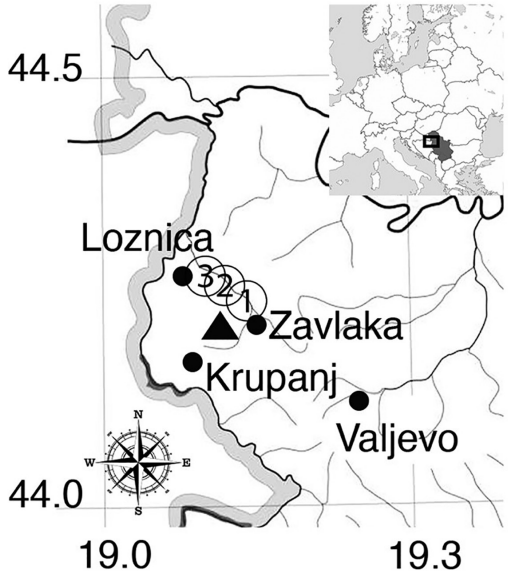

Fig. 1. Sampling locations on the River Jadar (1 Lopatara; 2 Draginac; 3 Kozjak; representing the planned mine's location)

Concentrations of As, B, and Li from groundwater samples obtained from deep drilling at two depths and deep piezometers were taken from the investor's data (2, 15) and used to assess the potential impact of untreated wastewater. Average concentrations of 271.5 μg/L, 3617.65 mg/L, and 735.75 mg/L for As, B, and Li (C_W), respectively, were assumed throughout the lifetime of the mine (16). The calculation of As, B, and Li concentrations in the River Jadar water (C_S) was conducted for three average discharge values of the stream (D_S). Average minimum and medium discharges of 0.07 m^3/s and 7.60 m^3/s, respectively, were derived from three literature sources (4,5,9), while a discharge of 105.4 m^3/s (4) was used for the medium-high flow level. Two wastewater discharge rates (D_W), 0.1 m^3/s and 0.25 m^3/s, were obtained from the literature (2, 6). Instream concentrations (C_S) of the three elements were calculated using the following expression:

$$C_s = \frac{D_W}{D_S} C_W,$$

where D_W/D_S denotes the dilution rate, and C_W represents As, B, and Li contents in the wastewater.

Results

The analysis revealed that releasing untreated wastewater at a discharge rate of 0.1 m^3/s into the River Jadar at three water flow rates would lead to arsenic (As) concentrations ranging from 0.26 μg/L to 387.86 μg/L, boron (B) concentrations ranging from 3.43 mg/L to 4823 mg/L, and lithium (Li) concentrations ranging from 0.70 mg/L to 981 mg/L. The release of untreated wastewater at a discharge rate of 0.25 m^3/s into the River Jadar, under three different water flow conditions, would lead to As concentrations ranging from 0.64 μg/L to 969.64 μg/L, B concentrations ranging from 8.58 mg/L to 12059 mg/L, and Li concentrations ranging from 1.74 mg/L to 2452.5 mg/L (Table 1).

Table 1. Contents of As, B, and Li in the River Jadar water at low, medium, and high average water flow rates, and at two discharge rates of untreated wastewater.

Wastewater discharge rate D_W (m^3/s)		0.1	0.25	0.1	0.25	0.1	0.25
Concentrations in wastewater C_W		271.5 μg/L		3617.65 mg/L		735.75 mg/L	
Water levels - Average flow rates D_S (m^3/s)		$C_{S\,(As)}$ (μg/L)		$C_{S\,(B)}$ (mg/L)		$C_{S\,(Li)}$ (mg/L)	
Average low D_S	0.07	387.86	969.64	4823.53	12058.83	981.00	2452.50
Average medium D_S	7.60	3.57	8.93	47.60	119.00	9.68	24.20
High D_S	105.40	0.26	0.64	3.43	8.58	0.70	1.75

The faunistic composition was very similar across all three fish sampling sites (Table 2), comprising a total of eight fish species from five families, as expected for that type of stream. The majority of fish were in size classes below 10 cm *SL*; only a few specimens of chub and common barbel reached up to 20 cm *SL*, whereas all nase specimens measured 15–19 cm *SL*.

Table 2. Structure of fish samples in the River Jadar and numbers present at three localities, presented in the order of upstream to downstream, situated close to the mining site of the project Jadar.

Species	Locality		
	Draginac	Lopatara	Kozjak
FAMILY CYPRINIDAE			
Common barbel *Barbus barbus*	10	6	18
FAMILY LEUCISCIDAE			
Chub *Squalius cephalus*	13	11	6
Nase *Chondrostoma nasus*	6		
Spirlin *Alburnoides bipunctatus*	18	8	7
Roach *Rutilus rutilus*			1
FAMILY GOBIONIDAE			
Gudgeon *Gobio obtusirostris*			3
FAMILY ACHEILOGNATHIDAE			
Bitterling *Rhodeus sericeus*	4		7
FAMILY COBITIDAE			
Danube loach *Cobitis elongatoides*			1

Discussion

All data of As, B, and Li used in this assessment originated from literature sources published by the investor, the Rio Tinto company, and its affiliate in Serbia, Rio Sava. Additional relevant records on the River Jadar water discharges and various types of harmful effects of As, B, and Li on aquatic organisms, primarily fish species, were obtained from available scientific literature.

The average discharge in the River Jadar of 7.6 m^3/s was determined based on three similar and one slightly different records. Similarly, the average minimal discharge of 0.075 m^3/s was derived. To establish a conservative and unbiased approach, the average maximum discharge value of 105.4 m^3/s was used, despite the existence of higher values that would likely

have made an assessment more favorable for the aquatic environment. The value we selected for groundwater inflow that would constitute wastewater during most of the exploitation period was 0.1 m^3/s, considering that the literature source stated that filling the exhausted mine corridors and the consequent decrease in groundwater inflow would occur only after the mine's exploitation period. The second value of 0.25 m^3/s that we also used was reported as the wastewater discharge into the River Jadar. Concentrations of B and Li in the groundwater samples from the depths of 482 m were 2404.7 mg/L and 2865.9 mg/L, and those of Li were 273.5 mg/L and 269.5 mg/L, respectively. In deep piezometer samples, concentrations of 4600 mg/L for B and 1200 mg/L for Li were reported in groundwater. From these data, we used average values of 3617.65 mg/L for B and 735.75 mg/L for Li to represent concentrations expected in groundwater extracted from the mine corridors during the exploitation period.

The estimated concentrations of As, B, and Li in the River Jadar after the release of untreated groundwater as wastewater were high (Table 1). According to Anonymous (6), based on groundwater influx and low filtration coefficients, the water-bearing capacity of the Jadar site deposits is weak, especially considering the size and features of the mine. A prominent feature of the groundwater from horizons between 375 m and 613 m deep is its high total salinity of 15 g/L. This groundwater is suitable only for industrial use, as it contains 1.3×10^4 t of salt that would be precipitated as a by-product annually. Such chlorine-sodium-magnesium deep groundwater, in horizons dating back to the early Mesozoic (i.e., the Lower Triassic), is assumed to derive from fossil oceans and meteorites, and is always under high pressure, posing a threat to Cenozoic aquifers at shallower horizons (17). The planned location for releasing the treated wastewater is at Veliko Selo, 1.5 km from the mine site (2).

We applied a discharge rate of 0.25 $m^3 s^{-1}$ for wastewater during the exploitation period (2, 15), instead of the lower value, about ten times lower, reported in Anonymous (6). This decision was based on the higher relevance of the literature source reporting the stated, higher value. It should also be noted that the amount of wastewater is expected to increase up to about 50 times compared with the first year of mine operation (we used the maximum value). In addition, it is stated that the concentrations of B and Li in the wastewater are expected to increase with the depth of the horizons where the mine corridors are planned to be built. This progression would intensify the harmful, particularly toxic effects of As, B, and Li on fish populations, moving from initial weaker, chronic impacts

to subsequent sublethal and lethal effects. None of the referenced reports clearly specifies which wastewaters will be treated: whether all wastewater, including infiltrating groundwater pumped from the mine corridors, or only the wastewater coming from the extraction of B and Li and sulphuric acid (H_2SO_4) used in processing of the jadarite mineral. Any accident at the solid tailings disposal site would release its dissolved content into the recipient aquatic ecosystem. Under such conditions, it would be impossible to intervene or remedy such high concentrations of As, B, and Li (Table 1). The common toxic effect would be devastating, regardless of season or the immediate meteorological, hydrological, and idioecological conditions in the River Jadar ecosystem.

Statements claiming that there would be no additional risk from solid tailings deposits because concentrations of heavy metal microelements from jadarite mineral processing would be similar to those occurring in soil elsewhere (6) are highly questionable. The composition of soils and rocks at depths of over 375 m, near the largest deposits of jadarite, differs significantly from the surface soils (2). Depositing such large quantities of tailings would extend the single spot of potential pollution across the surrounding area and into adjacent surface waters. As for the water quality, the investor stated that their wastewater treatment facilities employing ultra-filtration, double reverse osmosis, and ion exchanger technologies worth $40 million, would process wastewaters to meet class II quality standards reported for River Jadar. However, their claims that water discharges in the River Jadar would be about 300 times greater during high water periods, and five times greater during low water levels periods, compared with the expected discharge of the released wastewater, cannot negate the risks of such high concentrations of As, B, and Li, should untreated wastewater be released for any reason (e.g., power supply failure at the wastewater treatment facilities, etc.). The investors further stated that the daily release of treated wastewater into the River Jadar would be about 70% less than the amount of communal wastewater released from the city of Loznica into the River Drina (6). However, the relevance of this statement is questionable given the size and discharge of both recipient rivers (Drina and Jadar), the character of pollution involved, and the ecosystem's resilience, i.e., the self-purification capability of the two ecosystems against these two completely different kinds of pollution. In contrast to the findings presented here, the investors' assessment reporting only a moderate environmental impact of the mine's groundwater, despite such high values of As, B, and Li contents in the groundwater, appears unrealistic, particularly when the risk from accidents is considered.

In the event of a malfunction at the wastewater treatment facility, our assessment indicates that concentrations of As, B, and Li (Table 1) would rise far above levels considered harmless for aquatic organisms in the River Jadar (Figure 2). Such concentrations would likely exert toxic, genotoxic, or histopathological effects on fish and other organisms within the aquatic ecosystem. This statement may be applied with caution only to As and Li concentrations at high water levels and large average stream flows in the River Jadar at the beginning of mine operation (Table 1); however, even such concentrations of Li within the range of 0.70–1.75 mg/L, if sustained over a longer period, could result in chronic intoxication.

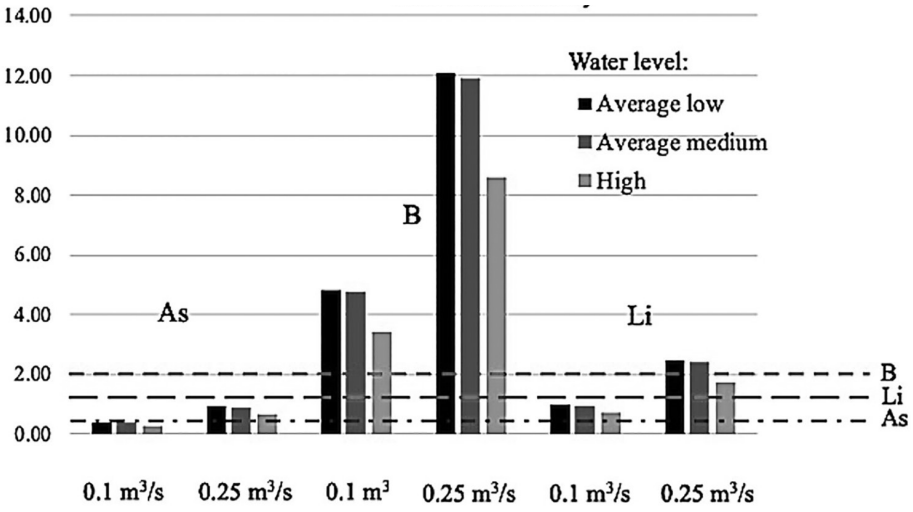

Figure 2. Contents of As (μg/L), B (mg/L), and Li (mg/L) in the River Jadar after a spill of untreated waste groundwater at discharge rates of 0.1 and 0.25 m^3/s, at average low (in 10^3), average medium (in 10^1), and high water levels; dashed lines denote toxicity thresholds.

Not only would this affect rainbow trout, a cold-water species highly sensitive to pollution (13,14), but the levels of B and Li pollution assessed in this research would also affect more tolerant species such as zebrafish and flathead minnow (10, 12). Although the tolerance of native fish species in the River Jadar to increased concentrations of As, B, and Li in natural aquatic ecosystems is currently unknown, their general ecology and

natural distribution suggest similar sensitivity to that of other fish species. The fish sampling conducted in August 2021 at three localities along the River Jadar revealed the composition of fish fauna (Table 2) closely resembling that reported in previous studies (7, 2). The only difference was the absence of several fish species from this sample, e.g., lamprey *Eudontomyzon* sp., bleak *Alburnus alburnus*, Danube barbel *Barbus balcanicus*, Balkan loach *Cobitis elongata*, Balkan spined loach *Sabanejewia balcanica,* and streber *Zingel streber*. Their absence at the time of sampling was likely due to seasonal circumstances, such as high air and water temperatures, very low water level, reduced discharge and oxygen content, and their inactivity or temporary retreat into the cooler nearby tributaries during the harsh summer conditions. The fish community structure observed at the time, along with middle-rithron fish communities recorded in the foothill streams of the River Sava drainage area (18), is most comparable to those found in foothill streams and ponds of mid-western North America containing flathead minnow. The harsh summer conditions may also roughly correspond to the natural habitats of tropical zebrafish, another commonly used model fish species. Therefore, their tolerance of B content, as reported in the literature (11, 12, 13), may also be relevant when considering the effect on those native fish species in the River Jadar. The simultaneous spill of Cl^- and HCO_3^- could, in certain quantities, intensify the harmful effects, particularly at medium and low stream discharges and water levels that dominate in the River Jadar throughout the year (4). This implies that a spill of mine wastewater would pose a pollution risk to nearly all aquatic animal species, regardless of the level of pollution. Although all aquatic species would be equally threatened from the ecosystem's point of view, particular strictly protected fish species (19), such as brook lamprey *Eudontomyzon* sp. (2) and streber *Zingel streber* (7) would face especially serious threats due to their still, sedentary lifestyle in their specific habitats. They would be strongly limited in their ability to avoid any pollution wave or escape downstream in its path. Even the more vagile fish species, such as nase, chub, and spirlin, would be unable to cope effectively, so such a wastewater spill would most likely result in a fish kill, while the River Jadar ecosystem would suffer heavy pollution and face a long recovery period. Such a massive fish kill would likely be accompanied, to some extent, by the death of other aquatic biota. Their carcasses would decompose swiftly, especially at high water temperatures, consuming large amounts of dissolved oxygen and leading to its depletion in the stream. Additional intoxication from ammonium released by

decomposed organic material would further exacerbate the initial lethal effect on the remaining aquatic organisms, despite their higher tolerance to the original pollution from As, B, and Li compared to fish. It remains difficult to estimate the distance downstream at which the intensity of pollution would sufficiently decrease below lethal or chronic toxicity thresholds. Equally uncertain is the capacity of the River Jadar ecosystem to recover from this type of pollution.

Conclusion

The risk posed by the mining of the mineral jadarite in the Jadar Region of Western Serbia to the River Jadar, as the principal recipient of treated groundwater, is very high. Elevated concentrations of As, B, and Li in groundwater would significantly impact the ecosystem of the River Jadar, regardless of whether untreated water was released at low-, medium-, or high-water levels and discharges. The concentrations of As, B, and Li that would occur in the River Jadar under such circumstances would greatly exceed the limits considered harmless for species in the fish community of the River Jadar and would most likely result in large-scale fish mortality, as well as the death of other aquatic organisms. Due to their sedentary lifestyle, the most vulnerable species would be the brook lamprey and the streber, both of which are designated as strictly protected species under national legislation. The mass mortality of aquatic organisms and the decomposition of their carcasses would severely deplete oxygen in the stream water and increase ammonium content, thereby further exacerbating the initial lethal effect. The downstream extent of pollution, as well as the duration required for the recovery of the ecosystem, remains difficult to predict.

Acknowledgment

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Chapter 8

Toxic Effects of Lithium, Boron, Arsenic, and Particulate Matter on Amphibians in Aquatic and Terrestrial Ecosystems

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Exposure of amphibians to lithium, boron, or arsenic, particularly in aquatic ecosystems, leads to a wide range of adverse effects, both in individuals at different developmental stages and in entire populations. Lower survival rates in juveniles and reduced fecundity in adults have been observed in several species studied. Taken together, these changes lead to a decline in local population sizes.

Possible Effects of Pollutants on the Body Structure and Functions of Amphibians

Various chemicals present in the environment influence the relationship between behavior, nutrition, and energy metabolism of organisms. Most amphibians have a biphasic life cycle: the first, which they spend as larvae in aquatic habitats, and the second, which they spend as juveniles and then as adults on land. This dual life cycle makes them doubly vulnerable, since the two habitat types differ in terms of the presence of chemicals that adversely affect amphibians (1, 2). For this reason, these vertebrates can be important indicators of environmental pollution.

Amphibian eggs are not resistant to potential pollutants in the aquatic environment. The permeable and gelatinous egg coat provides only a limited protective barrier for the embryo (3). In addition, adult amphibians residing in aquatic environments lack body structures that fully protect them from chemicals dissolved in water, making them effective indicators of the presence of pollutants. Where soil pollution is widespread in the terrestrial habitats, amphibians are chronically exposed (4). During the terrestrial phase of their life cycle, however, amphibians can avoid exposure to pollutants by altering their behavior.

As most amphibian species are a group of vertebrates that spend at least part of their lives in water, their survival depends entirely on the presence and quality of freshwater habitats—puddles, ponds, lakes, marshes, and other small lentic habitats, as well as streams and rivers (5). Therefore, the negative anthropogenic impacts on these aquatic habitats (reduction of aquatic habitats, drainage for agriculture or construction, and water and soil pollution) inevitably affect amphibian populations in these areas. Consequently, increased inputs of chemicals into water bodies can be linked to rising reports of abnormal development in amphibian larvae (6). In addition, the ongoing intensive destruction of natural habitats, recognized as one of the four major anthropogenic impacts on biodiversity, poses a major threat to the survival of contemporary amphibians (7). Various chemical substances released into the environment and accumulated in the soil of terrestrial ecosystems and/or in sediments of running and stagnant waters are ingested by amphibians. Metals and metalloids are a source of pollution for amphibians when environmental concentrations exceed the recognized species-specific thresholds, which may also vary among different compounds of the same metal/metalloid.

Most amphibian species in Serbia exhibit early developmental stages called larvae and tadpoles—the typical terms for the early stages of tailed amphibians (newts) or tailless amphibians (frogs and toads), respectively. Amphibian larvae and tadpoles, as well as the adults of some species, directly absorb metals and metalloids that have previously entered and dissolved in the water. During the aquatic stages, amphibians deposit these substances in their bodies by absorbing them through their permeable skin. Juveniles and adults of many species live mainly on land, so they take up metals and metalloids in their habitat mainly through food, and less frequently through accidental ingestion of soil/sediment particles or drinking water; additional quantities may be absorbed directly from the water or air through the skin surface (8).

The Effect of Lithium on Different Age Stages of Amphibian Species

Early stages of amphibian development —fertilized eggs, embryos, larvae/tadpoles

It has been shown that the exposure of fertilized eggs and embryos of various amphibian species (salamanders: *Ambystoma punctatum* – now *A. maculatum*, *A. tigrinum*, both in the family Ambystomatidae; frogs in the family Ranidae: *Rana clamitans* – now *Aquarana clamitans*, *R. sylvatica* – now *Boreorana sylvatica*, *R. catesbeiana* – now *Lithobates catesbeianus*/ *Aquarana catesbeianus*, and *R. pipiens* – now *L. pipiens*) to dissolved lithium salts (concentration range 0.17%–0.7%) leads to microcephaly (a markedly reduced head region associated with a smaller brain size, and weaker motor function observed, which consequently reduces individual survival rates) (9). Lithium exposure is known to cause teratogenic changes in early embryonic stages of amphibians (10). Exposure of 4-cell and 32-cell embryos, as well as early blastula stage embryos of the marsh frog (*Rana ridibunda* - now *Pelophylax ridibundus*, also in the family Ranidae) to a lithium chloride solution resulted in changes in the composition of plasma membrane proteins. The researchers concluded that such alterations affect interactions among embryonic cells, or rather that they may misalign, leading therefore to changes in the body shape of individuals exposed to lithium (10).

The toxic effects of lithium on amphibian embryos have also been studied on commercially bred African clawed frog embryos (*Xenopus laevis*, family Pipidae), a commonly used amphibian model because it easily breeds in the laboratory. Exposure of embryos to lithium chloride at concentrations ≥ 100 mg/L led to disturbances in the development of the nervous system: the eyes failed to form or remained fused into one large eye in the center of the head (cyclopia) (11). A study by Cook and Smith also showed that exposure of early embryonic stages of the clawed frog to lithium ions disrupted embryonic development (12). Embryonic development proceeds at a certain pace—chemical signals determine the development of certain anatomical systems and the establishment of physiological functions. Disruption of normal development leads to permanent changes in the organism, such as anatomical or physiological disorders.

In studies on the exposure of amphibian larval stages to lithium chloride, the American species *L. catesbeianus* (now *A. catesbeiana*) from the family of true frogs (Ranidae), a species related to the European green frogs, has often been used as a model organism (in Serbia there are two species of green frogs – *P. ridibundus*, the marsh frog, and *P. lessonae*, the pool frog, as well as a hybrid species – *P.* kl. *esculentus*, the edible frog) (13). Due to its well-studied physiology and its ability to easily adapt to laboratory conditions, *L. catesbeianus* is suitable for studying the effects of chemicals likely to occur in its habitat (14, 15). Local populations of *L. catesbeianus* have become established in some European countries, as this species was introduced from the Americas for cultivation for human consumption due to its larger body size compared to related European species. Escaped individuals have successfully established local populations in the wild. *Lithobates catesbeianus* negatively impacts native amphibians, including green frogs, leading to their local extinction, and is considered an invasive species in Europe. The above facts show that experimental results from *L. catesbeianus* can be extrapolated to the European green frog species, and thus also to the species inhabiting Serbia.

Pinto-Vidal and colleagues studied the exposure of *L. catesbeianus* tadpoles to lithium chloride dissolved in water at a concentration of 2.5 mg/L over a period of three weeks (16, 17). After 21 days, they observed that the total thyroid surface area of the exposed individuals was 26% smaller than that of the control group (individuals not exposed to lithium chloride), while the thyroid follicles were 55% smaller and their number was 48% lower than in the control group; furthermore, reduced activity (lethargy) in the exposed tadpoles was observed after only seven days of exposure to the same lithium chloride concentration (16). The second part of the study focused on metabolic, immunologic, and histopathologic responses in the liver of *L. catesbeianus*: tadpoles exposed to the indicated lithium chloride concentration for three weeks showed the following: increased glucose and triglyceride utilization in the individuals from the exposed group compared to the control group, accompanied by a decrease in fat stores; various liver parenchymal tissue disorders and a general toxic effect of the solution on liver tissue; and an immune response reflected in an increased number of certain cell types (16).

Another species used to examine the effects of lithium chloride ingestion at the tadpole stage is the South American species of the toad family (Bufonidae), *Rhinella arenarum*, a species widely distributed in the area it

inhabits. Its occurrence in local aquatic habitats can be compared with that of the European common toad (*Bufo bufo*), also in the family *Bufonidae,* and one of the most common anuran species in Serbia (18, 19). *Rhinella arenarum* tadpoles were exposed to concentrations of lithium that are officially considered environmentally acceptable (2.5 mg/L water) (20). The results showed genotoxicity (damage to genetic material – DNA), increased thyroid-hormone secretion, cardiotoxicity (weakened heart function), loss of body energy reserves due to digestive system dysfunction (after 48 hours of exposure), and decreased function of antioxidant enzymes. The lowest lithium chloride concentrations that reduced tadpole survival were 119.09 mg/L of water after 72 hours of exposure and 56.51 mg/L of water after 96 hours of exposure, while the concentrations causing 50% population mortality were 319.52 mg/L of water after 72 hours and 66.92 mg/L of water after 96 hours of exposure. A decline in the survival rate of individuals began as early as 48 hours (two days) after the start of treatment at 412.5 and 321.75 mg/l water.

Adult developmental stage – adult frogs, salamanders, and newts

Studies on the effects of exposure to lithium compounds in adult amphibians were not available to us; however, we noted the results from a study of metal accumulation in the bodies of amphibians from Serbia: lithium was found to be deposited mainly in the skin of the marsh frog (*P. ridibundus*) (21).

The effect of boron on different age stages of amphibian species

Early stages of amphibian development —fertilized eggs, embryos, larvae/tadpoles

The effects of exposing African clawed frog (*X. laevis*) embryos to relatively low concentrations of boric acid (5.0, 7.5, 10.0, and 15.0 mg boron/L water, corresponding to 28.5, 42.8, 57.0, and 85.5 mg boric acid/L water) were investigated (22). The results did not confirm the presence of developmental disorders in embryos of this frog species.

Adult stage of development —adult frogs, salamanders, and newts

The effect of boric acid on the reproductive process, as well as on the function of the endocrine system (organ system of glands with internal secretion) of adult African clawed frogs (*X. laevis*) was investigated by exposing them to different concentrations of boron dissolved in water (23). The results showed that boron in the aquatic environment at a concentration of 50 mg/L resulted in males with lower testis weight than in the control group and in females with an increased number of necrotic eggs. When females were exposed to boron concentrations of 100 mg/L water and 500 mg/L water, their fertilized eggs developed into embryos with abnormal development and a lower survival rate.

Interestingly, the offspring of males exposed to the same boron concentrations as females in the control group (which were not treated with boric acid) showed no signs of impaired embryonic development or reduced viability. Exposure of males to boric acid concentrations ≥ 500 mg/L water resulted in the production of fewer spermatozoa, with a non-functional structure in a certain number of males. Females exposed to the same environmental conditions had lower ovarian weights and fewer oocytes than those in the control group. Finally, at 1,000 mg/L, males developed testicular necrosis and were unable to initiate mating. Females exposed to the same boron concentration in the aquatic environment exhibited necrosis of the ovaries and all oocytes, and were also unable to mate. Further studies on boron toxicity (as boric acid) in adult amphibians have again focused on features related to the reproductive process and the function of the endocrine glandular organ system. The effects of exposure of adult African clawed frogs (*X. laevis*) to relatively low boric acid concentrations (5.0, 7.5, 10.0, and 15.0 mg boron/L water, corresponding to 28.5, 42.8, 57.0, and 85.5 mg boric acid/L water) were studied (22). It was found that females exposed to a concentration of 15.0 mg/L water had a higher proportion of immature oocytes in the ovaries (i.e., fewer mature oocytes), while males exposed to the same concentrations showed a reduced spermatozoa count and an increased frequency of sperm with non-functional structures. No other general or endocrine disorders were observed.

Based on these results, it can be concluded that the highest concentration of dissolved boron without negative effects on reproduction in the African clawed frog was 10 mg/L of water. These results indicate that bo-

ron can be toxic to amphibians when present in high concentrations in the aquatic environment, but at the tested levels, it did not affect the function of the endocrine system.

The effect of arsenic on different age stages of amphibian species

Early stages of amphibian development —fertilized eggs, embryos, larvae/tadpoles

The effect of the sodium salt of arsenic—sodium arsenite, $NaAsO_2$—on fertilized eggs, embryos, and larvae was studied in two tailed amphibian species of the family Hynobiidae: the East Asian salamander (*Hynobius leechii*) and the Fisher's clawed salamander (*Onychodactylus fisheri*), collected from different arsenic-contaminated sites near mines in South Korea (24). Eggs and larvae were treated with sodium arsenite in the laboratory. It was found that the accumulation of arsenic in the body led to DNA damage and a carcinogenic mutation (p53 mutation). Visible damage included skin changes, abnormally curved tails laterally or upwards, and/or general disruption of embryonic development.

The effects of arsenic (as disodium hydrogen arsenate (HNa_2AsO_4) at concentrations ranging from 10 to 1.000 μg/L of water) on embryos and tadpoles of the North American leopard frog *Rana pipiens* (family Ranidae) were studied by dividing the individuals into five experimental groups, each exposed to a different concentration: 10 μg/L, 20 μg/L, 150 μg/L, 500 μg/L, and 1000 μg/L (25). These developmental stages had slower swimming speeds compared with the control group after exposure to pentavalent arsenic.

The lethal concentration of arsenic in water, as sodium arsenite, was determined for embryos of a toad species in Argentina (*Rhinella arenarum*) (26). In local aquatic habitats, arsenic occurred at concentrations between 0.01 mg/L and 15 mg/L. The mean lethal arsenic concentration across all stages of embryonic development was 24.3 mg/L. The researchers concluded that embryos of this toad species are more sensitive to arsenic at early stages of development and that mortality is caused by relatively high concentrations. However, even lower, though still above-average concentrations, had negative effects, including oxidative stress. When exposing the embryo to sublethal arsenic concentrations, overall antioxidant potential was markedly

reduced, while a biochemical protective mechanism was activated to prevent further oxidative damage. Previously, exposure to arsenite ($NaAsO_2$) for 4 to 17 days and at concentrations of 46 mg/L to 50 mg/L resulted in 50% mortality of the tadpoles in this species (27). When tadpoles were exposed to a slightly lower concentration of arsenite (30 mg/L, in the form of $NaAsO_3$) for the same duration, their growth was completely inhibited.

The effect of arsenic ($NaAsO_2$) in concentrations of 100 to 400 µg/L on the development of tadpoles of the Indian cricket frog (*Rana limnocharis*, family Ranidae) was investigated, from Gosner stages 26 to 28 through metamorphosis (28, 29). It was found that exposure of tadpoles to sodium arsenite concentrations of 200 µg/L and 400 µg/L accelerated metamorphosis, producing small, terrestrial juveniles, a number of which had fully or partially underdeveloped limbs. In addition, exposure of tadpoles to $NaAsO_2$ concentrations of 100 µg/L and 400 µg/L resulted in DNA damage.

The effects of arsenic were also examined in tadpoles of the frog *Rana hexadactyla* (family Ranidae) in India (30). The results showed 50% mortality among tadpoles after 96 hours of exposure to an arsenic concentration of 0.249 mg/L.

A group of scientists observed the uptake of a mixture of arsenic and antimony during embryonic development in tadpoles of the North American toad *Anaxyrus boreas* (family Bufonidae) from heavily contaminated wetlands (31). Tadpoles accumulated very high amounts of these metalloids in their bodies (3866.9 mg/kg body weight, or 315.0 mg/kg dry weight). Although the metamorphosis process of the tadpoles was not interrupted, it lasted longer than usual, and individuals were smaller at completion. Ingestion of contaminated sediment contributed far more to metalloid accumulation than mere exposure to an aqueous solution containing metalloids. The presence of organic arsenic compounds in tadpole tissues indicated that they can biomethylate inorganic arsenic compounds. These data suggest that tadpoles of this toad species can survive exposure to very high concentrations of arsenic and antimony, probably due to specific physiological processes that enable tolerance.

Adult stage of development—adult frogs, salamanders, and newts

The effects of the sodium salt of arsenic, sodium arsenite ($NaAsO_2$), were also tested on adult East Asian salamanders (*H. leechii*) and Fisher's clawed salamanders (*O. fisheri*) at sites in South Korea contaminated

with arsenic from nearby mines (24). Abnormally developed individuals with visible skin damage found in mine water contaminated with heavy metals and arsenic carried the p53 mutation, a carcinogenic alteration associated with arsenic accumulation in the body. Based on these results, it was suggested that individuals of these salamander species, showing the described morphological anomalies in the vicinity of arsenic mines, can be considered as potential ecological indicators of arsenic-contaminated ecosystems.

The cytotoxicity of arsenic released into the environment by gold mining was studied in adult rice paddy frogs (*Fejervarya limnocharis*, family Dicroglossidae) in Thailand (32). The average arsenic concentration in adult animals from the contaminated area was 0.35 mg/kg body weight, and chromosomal aberrations were observed.

Effects of particulate matter on amphibians

Experiments on the American green frog *R. catesbeiana* (now *L. catesbeianus*), in which isolated palates of individuals were exposed to solutions containing different concentrations of particles 2.5 μm in diameter, have shown that particles of this size significantly reduce the thickness of the mucous membrane of this frog species even at the lowest doses tested (33). It is assumed that similar responses to exposure at this particle size can be expected in green frog species living in Serbia. A reduction in mucosal thickness increases the likelihood of ulcer formation and, in the long term, more severe damage.

Conclusion

The literature data presented here indicate that exposure of amphibians to lithium, boron, arsenic, and particulate matter, particularly in aquatic ecosystems, leads to a range of adverse effects on both individuals at different developmental stages and entire populations. Embryos and juveniles (tadpoles, larvae, and metamorphosed juveniles) are affected, resulting in lower survival rates. Reduced fecundity in adults has been observed in some species. The combined effect of all these changes is a decrease in the size of local populations, and small populations are highly vulnerable to extinction due to reduced genetic potential and greater susceptibility

to random fluctuations in environmental and demographic parameters. A number of negative changes, such as a decrease in the number of juveniles due to increased mortality, a predominance of older individuals, and an unequal number of males and females in the population (which negatively affects the number of newborns in future generations), often lead to the extinction of small populations.

A detailed list of known localities of amphibian species in Serbia can be found in the literature (34–38). The data reviewed here indicate that environmental exposure to lithium, boron, arsenic, and particulate matter would have negative effects especially on local populations of amphibian species in the families of true frogs (Ranidae) and toads (Bufonidae), which are also the most widespread amphibians in Serbia, as most of the data presented here were obtained by studying representatives of these two families (39).

Long-term observations of individual populations of widespread amphibian species in Serbia in the vicinity of human settlements have shown that the numbers in some populations fluctuate greatly from year to year, probably also under the influence of climate change. This implies that the beginning and end of the breeding season, when individuals are exposed to an increased risk of predation, are influenced by fluctuations in certain weather parameters (40, 41). Sudden population declines increase the likelihood of extinction. Therefore, exposure of populations of these species to lithium, boron, and arsenic compounds would impose additional stress and increase the likelihood of their extinction.

It should be noted that certain amphibian species, including those living in Serbia, are a valuable source of various biologically active substances that are or could be used in the pharmaceutical industry; in Serbia, these are members of the toad family (Bufonidae) and the fire-bellied toad family (Bombinatoridae) (42). The skin of amphibians contains numerous glands whose products play an important role in cutaneous respiration, the regulation of water balance in the body, reproduction, and defense against predators, microorganisms, and fungi. Studies have demonstrated their importance for pharmacological and medical research (42–45).

Due to their position in the food chain, amphibians are undoubtedly valuable components of the ecosystems they inhabit. Their importance for biomedicine further underscores that the conservation of local amphibian populations is far more important for long-term human well-being than the limited economic benefits yielded, for example, by mining these metals/metalloids, which have undeniably harmful effects that threaten the biodiversity of the area.

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Chapter 9

What is Known about the Effects of Lithium, Boron, and Arsenic Extraction and the Spread of Particulate Matter on Local Reptile Populations?

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Current evidence on the effects of lithium and boron on reptile populations suggests that lithium released from soil into the environment disrupts local populations and, in the long term, may lead to their extinction. Arsenic accumulated in the bodies of females is transferred to their offspring. In snakes and lizards, arsenic accumulation has been associated with negative effects on survival traits.

Introduction

Reptiles are the oldest group of vertebrates that have fully adapted to life in terrestrial habitats. The young of many species develop in eggs laid by females in the external environment, and the outer egg membrane protects the embryo from desiccation; in addition, reptiles breathe exclusively through lungs (1). The skin of reptiles is characterized by a surface layer consisting mainly of rows of small keratinized plates, scales, and/or large keratinized plates, and it contains relatively few glands grouped in specific areas of the skin (2). This structure somewhat reduces the skin's permeability to environmental pollutants, though not invariably. Both the

physiology and behavior of reptiles are highly dependent on certain environmental factors (ambient temperature and humidity) during particular stages of their life cycle or throughout life (3).

Ecological Characteristics of Reptiles and Their Importance in The Ecosystem

Reptiles are important components of many ecosystems because, like amphibians, they are often positioned in the middle of the food chain. Compared to endothermic (warm-blooded) vertebrates—birds and mammals—reptiles consume significantly less of the energy contained in their prey, meaning that more of this energy is transferred to the next level of the food chain (4). Although reptiles do not have physiological mechanisms to maintain a constant body temperature (their body temperature fluctuates according to variations in environmental parameters), they can still influence their body temperature through various behaviors (thermoregulatory behavior). This makes reptiles sensitive to environmental changes, as pollutants can alter their thermoregulatory behavior, usually leading to excessive increases in body temperature. For example, some pesticides have been found to increase body temperature in certain reptiles, leading to the onset of fever (5).

Reptiles are predominantly carnivorous and, over the course of evolution, have developed a number of complex adaptations, including morphological, physiological, and behavioral traits, as well as diverse prey-capturing strategies. All of these traits may be sensitive to increased concentrations of environmental pollutants. In the presence of pollutants, the organism activates defense mechanisms that consume substantial energy, thereby reducing growth, energy expenditure for movement, and egg or offspring production, among other similar processes. This indirectly threatens the reproductive success of populations and could ultimately lead to their extinction.

Although reptiles have a less complex life cycle compared to amphibians, their embryos develop either in eggs laid in the external environment or in the mother's body (oviparous and viviparous reproductive patterns). The reproductive pattern can vary both among species and within a species, with ambient temperature having a significant influence on offspring quality. In some species, it even determines the sex of the newborn individuals (temperature-dependent sex determination). It is assumed that temperature-dependent sex determination can be altered by the presence of pollutants (6).

Threats to Reptiles

Reptile populations worldwide are declining in number and size. The main threats to their survival include partial or complete habitat destruction, climate change, invasive species, diseases/parasites, and pollution (7). Reptiles can be easily exposed to pollutants through ingestion of contaminated food, absorption (through the skin), inhalation, maternal transmission to eggs/offspring, or ingestion of pollutants from their immediate environment (8). Despite their ecological importance, very little is known about the contaminant effects across different reptile species, as this vertebrate group has been largely neglected in ecotoxicological research in recent decades (9, 10).

Ecological risk assessments usually do not consider the effects of pollutants on reptiles, assuming that data obtained for birds and mammals are sufficient. However, due to the unique physiological and biological characteristics of reptiles, the effects of pollutants observed in other vertebrates cannot simply be extrapolated to them (11). Previous studies on the effects of pollutants in reptiles have primarily focused on measuring the concentration of these substances in tissue samples of reptiles collected in the wild (12–15). Although such data are useful for understanding past exposure of specific populations to contaminants during defined periods, actual risks and population-level effects remain largely unknown and insufficiently studied. Greater attention to this aspect of reptile ecology is therefore needed.

The Impact of Lithium on Different Life Stages of Reptile Species

The effects of lithium and its compounds have not been studied in reptiles. However, one published study investigates whether inorganic pollutants from mining waste accumulate in the body of the endangered African lizard species *Smaug giganteus* (Cordylidae family) (16). Blood samples from individuals collected near the most polluted mine contained significantly higher concentrations of lithium and all other ore-derived elements (sodium, aluminium, sulphur, silicon, chromium, manganese, iron, nickel, copper, tungsten, and bismuth) compared to individuals of this lizard species from an unpolluted site. No statistically significant correlation was found between body mass and the heavy metal concentrations, but all cor-

relations were consistently negative, meaning that the individuals were always lighter where metal concentrations were higher. In addition, the sex ratio in the populations at the contaminated site deviated from the optimal (1:1), with more females than males, which negatively impacts the number of new young individuals in future generations and population survival. When females outnumber males, the same male will mate with multiple females, increasing genetic relatedness among their offspring. Subsequent inbreeding reduces the survivability and/or fertility of their offspring due to the high relatedness between the parents, leading to population decline over generations, and ultimately to the extinction of the population.

The Impact of Boron on Different Life Stages of Reptile Species

A review of the available literature has revealed a lack of data on the effects of boron exposure in reptiles (17). In general, the toxic effects of boron have been observed in animals when the ingested amount of boron exceeds the threshold of 100 μg/g body weight (17).

The Impact of Arsenic on Different Life Stages of Reptile Species

Studies have shown that a significant increase in environmental pollutant concentrations, including arsenic, leads to changes in the concentration of certain biological markers of oxidative stress in the blood of the dice snake (*Natrix tessellata*), a water snake widely distributed in Serbia. These results also indicate the occurrence of uncontrolled oxidative processes and oxidative damage to biomolecules, cells, and tissues in this snake species (18). Reduced activity of the enzyme acetylcholinesterase, which is often used as an indicator of neurotoxicity in the aquatic environment, was also observed, attributable to prolonged exposure of the organism to dissolved metals. A similar study was conducted with another water snake species, *Nerodia fasciata*, which lives in the southeastern parts of the United States and resembles the dice snake in its predominantly aquatic lifestyle and feeding habits (19). This species was found to be exposed to not only increased concentrations of arsenic, cadmium, and selenium in water and sediment (soil), but also to these elements by feeding on contaminated

prey. In these snakes, the standard metabolic rate (acceleration) was 32% higher than in populations from an uncontaminated area. This means that the organism exposed to these pollutants has fewer energy resources for growth, reproduction, and fat storage. Consequently, birth rates declined and mortality increased, negatively affecting the population size and survival of these snakes in the long run. In experiments with the subspecies of the water snake *Nerodia fasciata fasciata*, individuals were fed fish containing selenium and arsenic. Both groups of water snakes fed with contaminated fish (in which concentrations of arsenic and selenium were 8.3 and 22.8 times higher than in the control diet) developed liver fibrosis. This provides the first evidence of histopathological changes in the organs of reptiles that ingest pollutants through their diet (20).

An important observation is that arsenic accumulated in the mother's body can be transferred to the offspring, as observed in the Australian viviparous (oviparous) western tiger snake (*Notechis scutatus occidentalis*) (21). In addition, the eggs of many oviparous reptile species have permeable shells that can absorb pollutants from contaminated soil (22, 23). For example, higher arsenic concentrations were found in the eggshell of the chameleon (*Chamaeleo chamaeleon*) than in the embryo (23). Experimental studies have shown that arsenic absorbed in this way can negatively impact offspring development, as demonstrated by a 2004 study on a species of lizard from the family Lacertidae that lives on the Iberian Peninsula (*Lacerta monticola cyrenni*, now *Iberolacerta monticola*) (22). The eggs of these terrestrial lizards develop buried in the substrate so that they are exposed to the pollutants accumulated in the soil, which considerably impair their development. Lizard eggs are permeable to gases such as oxygen and carbon dioxide and can absorb a large amount of water. In an experiment where eggs of this species were placed in artificial substrate contaminated with different concentrations of arsenic (50 ppb, 100 ppb, 250 ppb, and 500 ppb), eggs absorbed, and the embryos accumulated significant amounts of this metalloid. As in chameleons, arsenic concentrations were significantly higher in shells than in embryos, although in this case, embryos also accumulated considerable quantities of arsenic. These results show that the shell does not fully protect reptile embryos from the pollutants in the immediate environment. The arsenic concentrations tested had no effect on incubation duration, survival, or size of the offspring. However, increased arsenic concentrations reduced hatchling running speed, diminishing their ability to escape, find food, and consequently survive (23). It should be noted

that other metalloids and metals were also present in the environment (at low concentrations) and accumulated in eggshells and embryos of the lizards. Therefore, the observed association between arsenic concentration in the tissues of the embryos and reduced locomotor speed must also consider possible simultaneous effects of a greater number of heavy metals accumulating in the organism.

Pollution from chemical elements such as arsenic is problematic because such substances remain in the environment for a long time and thus enter the food chain. This was observed in two lizard species inhabiting an area where a mining tailings dam burst in 1998, releasing several million cubic meters of toxic sludge and acidic water into the Guadiamar river basin in southern Spain. The clean-up of the toxic sludge spill created aerosol clouds that were carried by air currents and contaminated a wider area. A few years after this event, studies measured heavy metal concentrations in reptile species from the broader area. One of the species studied was the gecko, *Tarentola mauritanica*, common in this region. It was found that the concentrations of arsenic, lead, and cadmium in geckos varied with the distance of collection points from the tailings dam (24). The areas affected by the mining spill remained contaminated, and three years after the incident, elevated metal concentrations were still found in all organisms within the local food chain. The persistence and long-term nature of this pollution were further confirmed in a study conducted eight years after this environmental disaster. The study found significantly elevated concentrations of heavy metals and arsenic in individuals of another lacertid species (*Psammodromus algirus*) compared with conspecifics from a nearby, unpolluted region (25).

Arsenic is a metalloid known to cause cancer, reproductive and developmental disorders, endocrine disruption, impaired immune function, kidney and liver dysfunction, and neurotoxicity in reptiles (26).

Of particular concern is that arsenic accumulated in the bodies of female reptiles is transmitted to their offspring. This suggests that, for example, in areas contaminated by mining processes and products that release arsenic into the environment, arsenic concentrations in the bodies of local reptile species may increase with each successive generation. Arsenic accumulation in the body leads to liver damage in snakes and reduced reproductive success in both snakes and lizards. In these two groups of reptiles, negative effects of arsenic accumulation on survival traits have been observed. It has also been shown that arsenic accumulated in the soil can penetrate lizard eggshells and accumulate in embryonic tissues.

The impact of particulate matter on reptiles

A literature search revealed no publications dealing specifically with the effects of particulate matter (PM) on reptiles. However, there are data on the accumulation of metal and metalloid particles 10μm or less in diameter in the bodies of certain lizard species from industrial and urban areas (27). This lack of data emphasizes the need for adequate research to determine whether the release of particulate matter into the environment threatens the survival and reproductive success of local reptile populations.

Conclusion

The limited data on the effects of lithium and boron on the body condition and population structure of various reptile species highlight the need for detailed experiments to determine whether, and to what extent, there are negative effects on populations in areas where these two metals are mined. These analyses must be carried out before decisions are made on jadarite mining activities in a given area, in order to assess in a timely manner whether the planned activities will jeopardize species survival and local biodiversity. Existing studies indicate that the release of lithium from soil into the environment disrupts local reptile populations and can lead to their extinction. Reptiles are crucial links in the food chains of ecosystems in Serbia, as they regulate the abundance of many invertebrate and small vertebrate species through predation. These prey animals also include species that negatively affect agriculture by damaging crops. Furthermore, initiating anthropogenic activities in a given area without assessing their potential negative impact on components of local biodiversity constitutes a violation of the Convention on Biological Diversity (28).

There is considerably more data on the effects of arsenic release from mining activities on local reptile populations. Despite uneven data coverage, it can be concluded that the release of lithium, boron, and arsenic into the environment would pose a major threat to local reptile populations and would likely lead to their extinction in the long term. Possible indicators of lithium, boron, and arsenic concentrations in the environment in this part of Europe are mainly aquatic snakes, the dice snake (*N. tessellata*) and the grass snake (*N. natrix*), which are recognized as bioindicators as they occur both in water and on land. Snakes are considered susceptible to bioaccumulation of environmental pollutants, as they are second- and

third-level predators and, in some cases, even the first predator in the food chain (29). Aquatic snakes also play an important role in the food chain by mediating between terrestrial and aquatic carnivorous species through their feeding relationships (30). Therefore, they can provide information on the movement of pollutants through communities of organisms living in the same area (29).

Due to their relatively large distribution area in Serbia, the following reptile species could be indicators of increased concentrations of the metals and metalloids mentioned in the soil: the European green lizard (*Lacerta viridis*), the sand lizard (*L. agilis*), the common wall lizard (*Podarcis muralis*), Hermann's tortoise (*Testudo hermanni*), aquatic snakes (grass snake – *Natrix natrix* and dice snake – *N. tessellata*), the smooth snake (*Coronella austriaca*), the Aesculapian snake (*Zamenis longissimus*) and the Caspian whipsnake (*Dolichophis caspius*). However, it remains to be investigated whether increased environmental concentrations of lithium, boron, and arsenic lead to declines in reproductive success in these species. Particular attention should be paid to the potential negative impact of mining these metals/metalloids on populations of reptile species important to the pharmaceutical and medical industries. In Serbia, these include venomous snakes in the viper family (Viperidae) (31). The negative impacts of these pollutants on populations of venomous snakes in Serbia would diminish their numbers, thereby reducing the supply of raw materials for antivenom production and other pharmaceutical preparations derived from snake venom. Conserving the populations of these species allows for the sustainable/renewable use of natural products, such as *in situ* venom collection with minimal disturbance to the animals. Such a national strategy would bring long-term benefits to the country, as opposed to the exploitation of non-renewable natural resources, which is associated with significant negative environmental change.

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Chapter 10

Toxicity of Lithium, Arsenic, Boron, and Heavy Metals on Birds

Ivan Pavlović

The effects of lithium, arsenic, boron, and other heavy metals have been studied to a lesser extent in birds than in mammals. Biomonitoring of organisms inhabiting potentially polluted areas yields an assessment of overall ecosystem health. Many current biomonitoring efforts rely on samples taken from aquatic organisms because of possible human consumption risks. However, many terrestrial organisms also consume resources from contaminated aquatic ecosystems, exposing them to such pollutants. For these reasons, higher trophic-level organisms, such as piscivorous birds and raptors, have been extensively studied and monitored for the presence of heavy metals (1, 2, 3, 4). Heavy metal contamination from water sources can bioaccumulate to levels sufficient to negatively affect organisms at lower trophic levels (5).

Lithium concentrations in surface and ground water, in some instances, can exceed standard levels in places with lithium-rich brines and minerals, and in areas where lithium batteries are disposed of. This metal has numerous effects on humans and other organisms, but there is no direct evidence of its effects on birds. In general, the type and severity of lesions depend on the level of lithium accumulation, which is influenced by diet, intensity of exposure, and time spent in a habitat. Once inside the body, metals accumulate in internal tissues, are excreted in feces, or are deposited in feathers (3). Metal concentrations in feathers can indicate body burdens at the time of molt and feather growth (8). Metal levels in blood

provide an indication of recent, short-term dietary exposure before being excreted or accumulated into other tissues (9). Their presence indices various kinds of physiological dysfunctions (6,7), any of which can modify the severity of toxicity and consequent lesions.

Feathers obtained from migratory species indicate exposure to contaminants throughout the year, as these birds molt and regrow feathers between breeding and wintering grounds (10). Concentrations in resident birds are likely sourced locally and can be used to identify contaminated areas (11). With very large home ranges, migrants are better adapted to handling toxic compounds (12). Migratory birds have a higher basal metabolic rate (BMR) than non-migratory birds (13), which could suggest that heavy metals are absorbed more rapidly into the body in migrants, resulting in lower blood concentrations, although this hypothesis remains underexplored.

Songbirds (Oscines) are low- to mid-trophic level organisms and can accumulate heavy metals through air, food, or water resources, and most commonly through dietary intake (9, 14). Assessing their exposure to environmental pollution can be indicative of overall ecosystem health from an ecotoxicological standpoint and provide information on the presence of heavy metals in their habitats (15, 16, 17, 18, 19). Various species like blackbird (*Turdus merula*), great tit (*Parus major*), blue tit (*Cyanistes caeruleus*), chaffinch (*Fringilla coelebs*), greenfinch (*Carduelis chloris*), house sparrow (*Passer domesticus*), willow warbler (*Phylloscopus trochilus*), reed warbler (*Acrocephalus scirpaceus*), segde warbler (*Acrocephalus schoenobaenus*), and reed bunting (*Emberiza schoeniclus*) have been examined. These small passerine species are ideal bioindicators because they are common and widely distributed. Many passerine species have fast metabolic rates and forage in small home ranges, often near residential areas, making them ideal for monitoring point-source contamination. In addition, data on barn swallows (*Hirundo rustica*) have been used (20, 21, 22). Results indicated flycatchers were exposed to differing heavy metal levels during feather formation on their wintering grounds, as compared to their recent exposure (through blood samples) on breeding grounds.

Heavy metals have also been detected in seabirds, such as seagulls. Several tissues have been examined to evaluate heavy metals, including arsenic concentrations in seabirds. During analyses of liver, kidneys, and feathers, Pb, Se, and As levels were also determined, albeit to a lesser extent. Feathers should be used cautiously as indicators of pollutant accumulation, since the procedure during analysis may lead to controversial results (23).

There is limited evidence concerning the effects of lithium on different bird species. It has been reported that lithium can be accumulated in the organs of birds, with a higher rate of lithium accumulation in terrestrial birds than in aquatic ones (24).

Lithium-induced polydipsia (excessive thirst, or drinking) in birds (25) was examined in experiments with a single administration of LiCl (lithium chloride) on drinking in pigeons, budgerigars (*Melopsittacus undulatus*), and ducks, and on electrolyte excretion in pigeons. The first experiment confirmed that LiCl resulted in an immediate, substantial, and prolonged increase in water consumption in pigeons. The polydipsia was dosage-dependent and did not involve disruption of normal drinking patterns. In the second experiment, an even greater polydipsic effect of LiCl was observed in budgerigars, a desert-dwelling species with a well-developed fluid retention system. In the third experiment, a transient polydipsic effect was noted with a low dosage of LiCl in ducks, a species with an efficient sodium-secretion system. Finally, the fourth experiment revealed that, following LiCl injection, Li^+ concentration in the excreta was elevated for 2 days, K^+ concentration showed an initial rise followed by depression to subnormal levels for approximately 16 hours, and Na^+ concentration remained normal. Considered together with the polyuria produced by LiCl, these results suggest that the birds may have been losing sodium, perhaps through a suppression of the renin-angiotensin-aldosterone system. The return of Li^+ concentration to normal level before water intake returned to baseline suggests that lithium may also exert a polydipsic influence centrally.

The potential use of lithium to reduce feather-damaging behavior was examined in Quaker parrots (*Myiopsitta monachus)* with feather damage (26). Lithium is freely filtered by the glomeruli since it is not bound to serum proteins, and in the proximal tubules, it is handled similarly to sodium. Approximately 80% of the lithium filtered by the glomeruli is reabsorbed, with the remainder excreted in the urine. Of the filtered lithium, 60% is reabsorbed in the proximal tubules and 20% between the loops of the collecting ducts.

The first complete experiment on the clinical and pathological effects of lithium poisoning in broilers after experimental lithium consumption was conducted by Rasooli et al. (2018). Broiler chicks received 200 ppm lithium carbonate daily in their water for 20 days, while the control group received water without lithium. No pathological changes were found in the organs of chickens from the control group. When ingested in exces-

sive amounts, lithium primarily affects the GI, central nervous system, and kidneys (28). Consistent intestinal lesions, including hyperemia and hemorrhages with infiltration of mononuclear cells in the submucosa and lamina propria of the gastrointestinal organs, were noted in experimental chickens.

Histological examination of the liver revealed that hepatocytes in experimental birds showed varying degrees of fatty infiltration and focal necrosis. At the same time, significant differences between the experimental and control groups were observed in the levels of Serum Glutamate Pyruvate Transaminase (SGPT), Serum Glutamate Oxaloacetate Transaminase (SGOT), along with an increase in uric acid (27). Enhanced serum concentration of the SGPT and SGOT in the lithium group likely indicates liver and intestinal damage, and in skeletal muscle, heart muscle, brain, and kidneys as well, given the aminotransferase level in these organs

Lithium is excreted almost entirely by the kidneys (29). It is freely filtered by the glomeruli, since it is not bound to serum proteins, and in the proximal tubules, it is handled similarly to sodium (29). This was confirmed when degenerative and necrotic changes were observed in the cellular lining of the renal tubules in chickens from the experimental group.

An impact of heavy metal bioaccumulation in two passerines with differing migration strategies was studied in the USA (30). The objectives of this study were to survey terrestrial heavy metal contamination at potentially bioavailable contaminated (PBC) sites through blood and feather samples from resident northern cardinals (*Cardinalis cardinalis*) and migratory great crested flycatchers (*Myiarchus crinitus*). Neither species showed sex-related differences in bioaccumulation, whether for recent exposure (e.g., through blood samples at nesting grounds) or earlier exposure (e.g., through feather samples at wintering grounds). Biomonitoring studies on songbirds rarely report the sex of individuals, though sex-specific differences in physiology and behavior could affect heavy metal bioaccumulation (31, 32). For example, during the breeding season, passerine foraging behavior often differs between sexes in relation to space use: males tend to forage higher near song perches, while females forage lower at nest heights. This contrast between sexes can explain the relationship between differing feeding habitats and bioaccumulation/detoxification rates. In addition, females have been shown to deposit trace elements into eggs and eggshells, providing an alternative excretion pathway compared to males during the breeding season (32, 33) and thereby decreasing body burden. However, female-to-egg metal deposition rates are not consistent among species.

Arsenic (As) is a toxic and carcinogenic element (34). The toxicity of As compounds depends not only on total concentration but also on chemical speciation (35). The inorganic forms of As—arsenate, As(III), and arsenate, As(V)—are predominant in the environment and are considered more toxic than simple methylated As species such as monomethylarsenic acid (MMA) and dimethylarsenic acid (DMA) (34). Organisms can accumulate, retain, excrete, and transform arsenic by their own metabolic pathways in the body when exposure occurs through diet or other routes, such as water, soil, and particles (36). Due to its toxic and biological effects, monitoring chronic exposure of animals and humans to As in As-contaminated areas is a global concern (37, 38).

In birds, exposure to toxic metals can affect reproduction, causing smaller clutch sizes, reduced fertility, and increased nesting mortality (39, 40, 41). Passerines have successfully been used in biomonitoring of environmental pollutants due to their broad distribution and sensitivity to environmental changes. Koch et al. (42) first reported both total As concentration and As speciation in terrestrial birds from Yellowknife, Canada, finding that the primary As species were As and B in two bird species. The accumulation and speciation of As in passerines have otherwise rarely been documented (43,44). Arsenic speciation in terrestrial biota is quite complicated, as As can be biotransformed from inorganic As to organic As, from simple to complex As compounds within organisms, or complex forms can be directly obtained from the diet or prey (45). However, knowledge of As biotransformation in wild birds remains relatively limited (46).

Mining and smelting activities have been reported as major sources contributing significantly to increased environmental As levels (47, 48). The extent to which elevated environmental As in mining areas affects bioaccumulation and speciation in birds remains uncertain. Since birds are usually considered to occupy top positions in environmental food chains, As from the environment can enter their bodies mainly through diet or prey. It is therefore reasonable to assume that birds in mining regions exposed to large and long-term amounts of this metalloid may show high accumulation of multiple As forms.

Boron (B) has many beneficial functions in biological, metabolic, and physiological processes in plants and animals. In plants, it supports the transport of organic compounds (mostly sugar) through the plant and influences the creation of elements for their reproduction. In animals and humans, it is necessary for the even distribution of calcium in the body. Together with calcium, magnesium, and vitamin D, it regulates metab-

olism, growth, and development of bone tissue. Its deficiency causes calcium loss and bone demineralization. Conversely, excessively high boron levels cause cellular damage and toxicity in humans and various animal species (49).

Under natural conditions, birds rarely come into contact with boron, but in controlled settings (farms, aviaries, etc.), they may come into contact with two boron derivatives—borax and boric acid (49).

Although borax and boric acid are considered practically non-toxic to birds, this does not mean they are completely harmless. They are distinct chemical compounds with different properties and, if ingested, can be dangerous. Borax has a wide range of applications and is most often used as an ingredient in detergents, cosmetics, and related industries. Boric acid is used as an antiseptic and insecticide and, industrially, in the production of fiberglass, pyrotechnics, and other products. While boric acid is considered virtually non-toxic to birds, larger amounts of borax can have a toxic effect (50). This means that relatively high doses are required to cause significant adverse effects. Studies indicate that birds have a high tolerance for boric acid. For example, testing shows that the acute oral lethal doses in chickens are relatively high. This, however, does not justify irresponsible handling. Even if borax and boric acid do not directly poison birds, they may still pose indirect threats (50).

Birds can ingest boron through contaminated food, for instance, by consuming insects killed by borax or boric acid. Although the amount present in such insects may be small, repeated exposure can lead to boron accumulation, particularly in smaller bird species. There are also implications for the food chain: extensive use of borax-based insecticides can reduce insect populations, which serve as a vital food source for many bird species. However, this represents more of an ecological concern than a direct toxicity issue. Excessive boron intake can also occur through habitat contamination, where excessive use of borax or boric acid leads to contamination of bird food and water sources. This is of particular concern for ground-feeding or pond-drinking birds (50).

Although direct poisoning is rare, it is essential to recognize potential signs of boron exposure in birds, like lethargy and weakness, sudden decrease in activity levels, and loss of appetite (reduced or absent feeding behavior). Uncoordinated movements, difficulties in flying, perching, or walking, vomiting or diarrhea, and signs of gastrointestinal distress should also be observed. Feather loss or abnormalities in feather condition or growth are reported only rarely (50).

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Chapter 11

Mammals and Environmental Pollution – The Impact of Lithium (Li), Arsenic (As), and Boron (B)

Jelena Blagojević, Ivan Pavlović

- Small mammals have short lifespans, produce numerous litters, and inhabit all environments, making them ideal for monitoring environmental pollution.
- Destruction and fragmentation of small mammal habitats reduce genetic diversity of species and threaten their survival.
- Pollutants cause DNA damage, as well as tissue and organ damage in both developing and adult individuals.
- The content of heavy metals in the tissues of domestic mammals and game serves as a bioindicator, providing data on their presence in the ecosystem.
- Lithium (Li) and arsenic (As) can accumulate in animal organisms and, through the food chain, in humans. At high concentrations, they cause serious health problems.
- Boron (B) has a wide range of positive physiological effects on mammalian biological systems at low levels, but is toxic when its concentration in tissues and blood is high.

The Effects of a Polluted Environment on Small Mammals

Due to their position in the food chain, small mammals are used as bioindicators of environmental health. As they are food for larger mam-

mals, any harmful substances that accumulate in their bodies are transferred to higher trophic levels. Habitat type plays an important role as it determines the exposure to pollution through habitat structure, agricultural practices, and the presence of environmental pollutants. For example, small mammals living near agricultural habitats are more exposed to pesticides and other agrochemicals than small mammals in forest habitats (1).

Mice, voles, and shrews are frequently used in studies on the effects of pollution on wildlife. They are widespread, have a limited distribution area (0.5 hectares or less), and have relatively high population densities. These mammals have short lifespans and produce numerous offspring. They are found all around us, in clean and polluted habitats, enabling comparisons of pollutant levels detected in their organs. Such comparisons provide information about the degree of bioaccumulation of pollutants and their effects. Species diversity and abundance offer additional information when assessing the sustainability of small mammal habitats at a site. Pollution can reduce species diversity, creating conditions for population increases in certain species or for dominance of an invasive species that occupies an empty ecological niche. Habitat loss is the most common cause of population endangerment in a given area. In addition, microclimatic changes due to pollution can cause some organisms to disappear from affected sites. The impact of humans on wildlife species, including small mammals, is most evident in the fragmentation and destruction of their natural habitats. When a large population is divided into several smaller ones due to habitat fragmentation, the number of individuals within each of these populations is reduced and movement is constrained, which indirectly leads to a decline in genetic diversity. Large natural populations are characterized by high genetic diversity, which enables them to adapt easily to environmental changes without jeopardizing their survival. When genetic diversity decreases, organisms become more susceptible to parasites and diseases, and their ability to adapt to environmental changes, such as extreme weather conditions, is reduced. Pollution studies are mostly conducted in areas of intensive human activities, such as industrial and mining areas, urban areas, and along major roads. Animal samples collected from these areas are usually analyzed for the presence and content of various pollutants, including heavy metals and various toxic organic and inorganic compounds. The distribution and concentrations of heavy metals in various small mammal tissues correspond to those found in humans. Therefore, small mammals are used as surrogate mammals for humans (2), i.e., they

are an adequate substitute for analyses that cannot be performed on people. The concentration of pollutants can be measured in individual organs (liver, heart, lungs, spleen, brain, muscles, bones, skin) or in the entire organism, after which the harmfulness of pollutants can be assessed.

Both abandoned and active mines pose risks to wildlife. The effects of arsenic and cadmium have also been studied in slightly larger, more mobile species such as the muskrat (*Ondatra zibethicus*) and the red squirrel (*Tamiasciurus hudsonicus*) near a mine in Canada (3). Although skeletal abnormalities were present, the study could not unequivocally link arsenic and cadmium contamination to significant bone pathology or changes in bone mineral density. Only subtle changes in the fat content of muskrat bones suggested a link to environmental pollutants. Arsenic and cadmium are xenobiotics, chemical elements that are not naturally found in the body as building blocks and are often present as pollutants.

The routes by which pollutants can enter the mammalian body are diverse and include ingestion through food and water, inhalation, and dermal absorption. The physical and chemical characteristics of the pollutants themselves are also very important in determining their effects on living organisms. Small mammals can be exposed to pollutants from multiple sources simultaneously. For example, in France, animals in a former gold mine were exposed to arsenic through air, dust, water, food, and soil (4). Partial remediation (restoration to the previous state) was carried out at the site, and arsenic in the soil ranged from 29 to 18,900 μg/g. In the four small mammal species examined (the wood mouse *Apodemus sylvaticus*, the Algerian mouse *Mus spretus*, the common vole *Microtus arvalis*, and the large white-toothed shrew *Crocidura russula*), arsenic concentrations varied largely and independently of soil concentrations. These results indicate that arsenic accumulates in animal organisms from multiple sources. A significant correlation has been observed between arsenic concentrations in tissues and body condition. In addition, increased masses of certain organs (liver, kidneys, and lungs) were associated with increased arsenic concentrations, indicating possible pathological changes in the tissues.

The International Agency for Research on Cancer, a United Nations (UN) agency, classifies arsenic as a Group 1 carcinogen (5). In England, a study was conducted on the most common free-living small rodents, the wood mouse (*Apodemus sylvaticus*) and the bank vole (*Clethrionomys glareolus*), at six locations with varying levels of arsenic contamination (6). In contrast to previous studies, arsenic concentrations in the stomach contents, liver, kidney, and whole body of both species of small mammals

reflected the differences in contamination levels between locations. Exposure of juveniles and adults was similar, but in voles, females were more susceptible to arsenic accumulation. In studies conducted so far, the results of analyses of arsenic bioaccumulation in animal tissues are conflicting: some report accumulation, some do not, and others find accumulation only in certain organs (7–10). Inorganic arsenic has been extensively studied as a teratogen in animals—substances that can cause structural or functional abnormalities in embryos or fetuses. Animal studies have shown that arsenic toxicity causes developmental problems, including malformations (developmental deformities), death, and growth retardation in four small mammal species: hamsters, mice, rats, and rabbits (11). The pattern of malformations is characteristic, and toxicity effects depend on dose, route of ingestion, and fetal age. Animal studies have not identified effects of arsenic on fertility in either sex. Studies in natural populations remain few due to the heterogeneity of environmental factors, as well as the variability of population dynamics, which complicate interpretation. Boron is another element that can have harmful effects on small mammals, particularly in areas where boron levels are elevated due to natural deposits or human activity, primarily mining. Exposure to high boron concentrations negatively affects reproduction, that is, fertility and offspring survival, as demonstrated in experimental conditions in house mice (*Mus domesticus*). In one study, twenty adult males were divided into two groups: one received boron in water at a dose of 12 mg/L, and the other at a twenty-fold lower dose of 0.6 mg/L. Numerous changes in testicular tissue and size were observed, resulting in male sterility (12). Boron can also elicit physiological responses under stress, which are manifested as changes in behavior, feeding patterns, and habitat use. In addition, elevated boron concentrations can affect population dynamics by altering survival rates and reproductive success, which in turn leads to changes in community structure.

The effects of lithium on small mammals have been little studied, but available work reports behavioral changes, such as increased aggression, as well as physiological alterations. In addition, lithium-induced behavioral changes have been implicated in shifts in feeding and predator avoidance strategies (13). Similar to heavy metals, elevated lithium concentrations can induce physiological stress (14), disrupting growth and reproduction. As with other pollutants, lithium's effects on living organisms are context-dependent and vary with environmental factors, including the availability and presence of other pollutants. Unique combinations of pollutants and variations in their concentrations produce unique effects in living organ-

isms. Sulphur dioxide is a gas and a common pollutant emitted into the atmosphere during the combustion of fossil fuels (oil and its derivatives) or other sulphur-containing substances in industry. As an air pollutant, sulphur dioxide causes problems primarily with the respiratory tract. It does not remain only in the air, since it returns to the soil through acid rain. Citizens of Loznica still remember the smell of hydrogen sulphide (a sulphur compound), reminiscent of rotten eggs, which spread from the Viskoza factory, where sulphur dioxide was also present in high concentrations. Effects depend on concentration and on duration and frequency of exposure to sulphur dioxide, most commonly including irritation of the skin, mucous membranes, and eyes at high levels. In addition, there is experimental evidence that sulphur dioxide can damage genetic material in multiple tissues. When mice were injected with varying concentrations of chemical derivatives containing sulphur dioxide over seven days, changes were observed in the genetic material of cells in the brain, lungs, heart, liver, stomach, spleen, thymus, bone marrow, and kidneys (15). The extent of damage was related to the dose. Because of these effects, sulphur dioxide has been characterized as a systemic DNA-damaging agent, meaning that it damages DNA not only in respiratory cells but throughout the body.

DNA damage can further lead to carcinogenic changes, which are usually permanent, and if they occur in germ cells, there is a possibility of transmission to the next generation. Nitrogen dioxide (NO_2) is another gaseous air pollutant produced when fossil fuels such as coal, oil, methane (natural gas), or diesel are burned at high temperatures. It is one of six widespread air pollutants for which standards regulate permissible levels in the air. Thus, air quality in Serbia is categorized into five categories, from clean to heavily polluted, with a network of measuring and monitoring stations making pollution levels accessible to everyone. Nitrogen dioxide can also be produced indoors when fuels such as wood and gas are burned, and it is present in cigarette smoke. Reported effects of nitrogen dioxide in animals include respiratory problems, more commonly airway inflammation, and metabolic changes, with high concentrations even leading to death. Beyond the airways, which are most directly exposed, the immune system also undergoes significant changes. Although no carcinogenic effect of nitrogen dioxide has been recorded in animals, it has a mutagenic effect. For example, increased chromosomal aberrations were observed in a rat strain exposed to four different concentrations of nitrogen dioxide for three hours (16). In addition to the aforementioned gases, the operation of heating plants, power stations, industrial facilities, motor

vehicles, and other sources also produces fine particles ($PM_{2.5}$ and PM_{10}), which are emitted into the atmosphere and can penetrate the respiratory system. Although their harmful effects on humans are well documented, there is virtually no data on the effects on small mammals in natural populations. The study of small mammals is therefore an important tool for monitoring and evaluating pollution, providing valuable information on the condition of local ecosystems and potential risks to human health. The integration of data from small mammal populations into broader ecological studies can significantly contribute to biodiversity conservation and the improvement of environmental protection.

The Effects of a Polluted Environment on Large Mammals

The area of Jadar is known for its ecological and biological diversity, in both flora and fauna. It contains extensive pastures and hunting grounds with a rich biotic diversity. More than half of the landscape types belong to the arable land and agroforestry complexes (17). There is also a significant population of large mammals (carnivores, omnivores, and ruminants).

Among the large mammals inhabiting this area, the largest populations of domestic animals are kept on pasture. In the narrower sense, pastures are fenced areas of agricultural land on which domestic livestock—such as horses, cattle, sheep, or pigs—graze. The vegetation of cultivated pastures, fodder, consists mainly of grasses, with admixtures of legumes and other grass plants (herbaceous plants). Grazing on pastures occurs throughout the summer, unlike meadows, which are not continuously grazed or are used for grazing only after they are mowed to collect hay for fodder. Given that during their metabolism, plants adopt and incorporate lithium, arsenic, and boron from the soil, the concentrations of these elements depend on their natural presence in the habitat or subsequent environmental contamination. Animals metabolize these elements through their diet, incorporate them into their organism (in muscles, tissues, and bones), excrete and secrete them, with secretion being further used in human nutrition (milk, for example). As the meat, internal organs, milk, and products of these animals are used in human nutrition, the chemical elements present in them directly affect human health. Pastures and hunting grounds are areas where domestic animals and hunting game often co-occur, so these elements affect both groups of animals.

Game Animals

Within the Jadar area, the largest hunting ground, "Cer," is owned by *Srbija šume*, and several smaller hunting grounds in the vicinity are managed by local hunting associations. Mount Cer is classified as an area of exceptional features and a landscape of recognizable appearance with significant natural, biological-ecological, aesthetic, and cultural-historical values that have developed over time through the interaction of nature, the natural potential of the area, and the traditional way of life of the local population (18).

Large game in these hunting grounds include deer (*Cervus elaphus*), roe deer (*Capreolus capreolus*), and wild boar (*Sus scrofa*) (19). Smaller game species include the brown hare (*Lepus europaeus*), while game birds include the pheasant (*Phasianus colchicus*), grey partridge (*Perdix perdix*), and common quail (*Coturnix coturnix*). The most numerous of wild carnivores are the red fox (*Vulpes vulpes*), jackal (*Canis aureus*), wild cat (*Felis silvestris*), and badger (*Meles meles*), along with numerous species of martens and their relatives. The area is also inhabited by numerous bat species (20).

Domesticated Animals

Given that this area is largely rural, there are substantial populations of small ruminants (sheep and goats) and large ruminants (cattle) in rural households. There is also a large population of pigs in extensive farming; as omnivores, plant matter is one of the essential ingredients of their diet. Livestock production in this area is not only highly developed but also, in many respects, close to meeting the requirements for organic production. Ruminants are mostly kept on pasture, whose floristic composition fully meets all the criteria for good and high-quality grazing and for preparing autumn hay.

The Influence of Lithium, Arsenic, and Boron on the Population of Large Mammals

The effects of heavy metals on living organisms have been well studied and have shown numerous harmful impacts on the environment as a whole, as well as on individual plant and animal species. Industrial activities that contribute to environmental pollution with heavy metals are

mainly mining and metallurgical operations. Most of these industries use raw materials or auxiliary chemical substances in their processes.

Waste from these industries is often hazardous to people and the environment, even in very small quantities. Industrial and agricultural activities (mining, ore smelting, etc.) are frequently associated with local contamination of water, soil, air, and plants grown nearby. The accumulation of toxic metals in plants, water, and soil increases the risk of their transmission to domestic and wild mammals and game birds through ingestion of contaminated food (21, 22, 23).

Compounds that easily accumulate in living organisms and contain elements such as lithium, boron, arsenic, and mercury are particularly dangerous (24). This has been confirmed by numerous studies conducted worldwide in areas of mining, for example, in Australia, North America (USA), South America, China, and parts of Europe (e.g., North Macedonia) (25, 26, 27, 28). The results published in relevant studies unequivocally attest to the catastrophic consequences that the mining and processing of lithium and its accompanying elements have on plant and animal life, and consequently on humans, considering our position in the food chain and our presence in the habitats where these destructive activities occur.

The commissioned studies conducted by Rio Tinto (Jadar Project) and Rio Sava Exploration (Request to determine the scope and content of the environmental impact assessment study) did not provide an answer to the question of the extent to which mining and exploitation/processing of ore will affect mammals in this area.

Hunting Game and Domestic Animals as Bioindicators

Bioindicators are defined as organisms whose measurable changes in known characteristics can be precisely registered with the aim of assessing the extent of environmental contamination. On this basis, conclusions can be drawn about health implications for other species and the environment as a whole. The identification of heavy metal concentrations in the organs of wild animals indirectly provides data on the degree of environmental pollution, enabling an assessment of the degree of animal exposure to these elements in a given area.

Due to their biological characteristics (lifestyle, diet, relatively long lifespan) and relatively simple sampling procedure during regular hunting

seasons, numerous studies have used game, especially wild boar, deer, and roe deer, as bioindicators of environmental pollution over the last decade. The prolonged accumulation of contaminants during their lifetimes enables early detection of negative toxic effects in ecosystems, and therefore, timely warning (31, 32, 33). Heavy metal contents in game tissues can serve as a solid basis for providing important data on their presence in ecosystems (22, 34, 35, 36).

Lithium

Lithium (Li) can accumulate in the bodies of animals, and eventually humans, through the food chain, at concentrations high enough to cause serious health problems. The Australian Inventory of Chemical Substances (AICS, 2007) classifies lithium as a health, physicochemical, and/or ecotoxicological hazard according to criteria approved by the National Occupational Health and Safety Commission (NOHSC) for classifying hazardous substances (37). Lithium, lithium aluminum hydride, and lithium methanolate are listed as dangerous substances in Denmark (38).

Doses of 500–700 mg/kg were found to cause severe depression and ataxia (disorder of balance and movement coordination) in mixed-breed cattle (*Bos taurus*), as well as lithium accumulation in muscle (86.64 mg/L), kidney (66.97 mg/L), liver (68.57 mg/L), heart (79.15 mg/L), and brain tissue (51.7 mg/L) (1). Li_2CO_3 treatment significantly increased oxidative membrane damage (53%) in rats fed a protein-deficient diet (8%) compared to those fed a high-protein diet (30%), in which such damage was only 18%. The LC50 value of LiCl (lethal concentration at which 50% of animals die) for rats ranged from 526 to 840 mg/kg (39).

High doses of lithium have been found to cause weight gain and increased thirst, damage to the male reproductive system, significant reductions in plasma testosterone levels, and oxidative stress in the liver (40, 41, 42, 43).

Arsenic

Arsenic (As) is inherently poisonous. It occurs naturally at low concentrations in the soil, water, and air, and reaches people mainly through food. Arsenic tends to accumulate in tissue, and once ingested, it is elim-

inated very slowly from the body (44). Ingestion of large doses of arsenic leads to gastrointestinal symptoms, dysfunction of the cardiovascular and nervous systems, and ultimately to death (45, 46, 47).

Inorganic arsenic compounds, such as arsenite and arsenate, can contaminate groundwater and cause arsenic poisoning (48). In animals exposed to arsenic compounds, intense abdominal pain, diarrhea, bloody or mucoid stool, weak pulse, and dehydration are observed. Single, lethal doses of inorganic trivalent arsenic range from 1 to 25 mg/kg, depending on the species of animal. Among the tested species, cats are most sensitive, followed by horses, cattle, sheep, pigs, and birds (49, 50).

Trivalent arsenicals, or arsenites, are more soluble and therefore more toxic than pentavalent arsenate compounds. These include arsenic trioxide, arsenic pentoxide, sodium and potassium arsenite, sodium and potassium arsenate, and lead or calcium arsenate (51). The lethal oral dose of sodium arsenate in most species is 1–25 mg/kg, with cats often being more sensitive. In animal products (milk, meat, eggs), arsenites are 4–10 times less toxic than arsenate. The reduced use of these compounds as ingredients in pesticides, ant baits, and wood preservatives has made poisoning less common. Arsenates have been used to some extent as tick control dips. Lead arsenate has sometimes been used as an anthelmintic in sheep, causing environmental contamination and human poisoning (52, 53, 35). Many of these compounds are no longer used in the US, but may still be available in other countries.

High amounts of arsenic have been reported in foods of poultry and livestock origin, such as milk, egg yolks, egg whites, liver, and meat (54). In general, animals are exposed to arsenic through drinking water, animal feed, grasses, vegetables, and contaminated leaves. In endemic areas naturally rich in arsenic, water irrigation leads to soil contamination, subsequent accumulation in plants, and transfer to livestock, resulting in excessive arsenic levels in animal products (52, 54). A recent report found that arsenic bioconcentrates more rapidly in water than in rice straw, and when straw is used as animal feed, arsenic is detected mainly in cattle feces and tail hair (55). Arsenic-contaminated vegetables and grains are considered a primary exposure route for livestock and thus, through the food chain, for humans as well (56, 57, 58). Studies of the blood mineral status of dairy cattle and water quality in some high-altitude areas have revealed the presence of arsenic in blood, likely reflecting high arsenic levels in animal feed and water sources in that region. Thus, contaminated feed, cereals, and drinking water are considered important sources of livestock exposure to arsenic, with implications for food of animal origin (e.g., meat, milk, and eggs) (59).

Boron

Boron (B), at low concentrations, has a wide range of beneficial physiological effects on biological systems. In plants, it participates in the transport of organic compounds and the formation of their reproductive organs. In humans, it is important for proper skeletal development because it regulates metabolism, calcium distribution, and bone tissue development (60). Normal boron levels in soft tissues, urine, and blood generally range from 0.05 ppm (and less) to 10 ppm. However, when boron concentrations in tissues and blood are elevated, it becomes toxic (61). In cases of boron poisoning, boric acid levels in human brain and liver tissue have been as high as 2,000 ppm (62). Recent studies by the U.S. Environmental Protection Agency in Washington have shown that high boron intake can reduce fertility in male rodents fed diets containing 9,000 ppm boric acid (63, 64). Within days, boron levels in blood and most soft tissues rapidly reached a plateau of about 15 ppm. Experiments in dogs demonstrated that concentrations of 350 ppm resulted in reduced spermatogenesis and ejaculates lacking sperm (65). Bone boron did not appear to increase, reaching 47 ppm after 7 days on the diet. Cessation of dietary boron exposure led to a rapid decline in bone boron.

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Chapter 12

Experimental Studies of the Adverse Effects of Arsenic, Lithium, Boron, and Particulate Matter on Laboratory Species of Small Mammals

Milena Kataranovski

- During the processing of the mineral jadarite, many harmful substances would be released, predominantly arsenic, lithium, boron, and particulate matter.
- Experimental investigations have demonstrated a wide range of harmful effects of these substances on the organs of laboratory animals.
- Damage to the liver and kidneys causes dysfunction of these organs. Breathing becomes difficult due to lung damage. These substances also compromise cardiac function. Damage to the nervous system alters behavior, learning capacity, and memory. Changes in the reproductive organs reduce the likelihood of producing offspring. These substances also reduce immunity.
- If exposure is prolonged, the resulting changes cause various organ diseases and the development of cancer.
- Given that they provide insight into pathological changes in various organs and the mechanisms underlying these effects, research on laboratory species of mice and rats, as mammalian surrogates for humans, is important for understanding the risk of pollutant toxicity to human health.

ARSENIC (As)

General Notes

The harmful effects of arsenic are complex and intertwined. Owing to cell-membrane proteins that enable the transfer of different chemical forms of arsenic, this metalloid enters cells. Once inside, arsenic binds to enzymes involved in signal transduction that enable cellular responses to environmental stimuli. In this way, the activity of genes responsible for various cellular processes, such as cell division and multiplication, increase in cell number (proliferation), growth factor synthesis, and tissue structure, becomes disrupted (1). These disturbances may further lead to cellular transformation and/or cell death. Arsenic can also trigger processes that result in epigenetic changes, i.e., heritable changes in genes without affecting DNA (2).

Oxidative stress occurs due to the formation of reactive oxygen species (ROS), including superoxide anion and hydrogen peroxide. In biological systems, ROS are normally formed during reactions involving oxygen (oxidative reactions) in cellular respiration, as well as within various physiological reactions that regulate the stability and survival of the organism (homeostasis) (3, 4). Under typical conditions, ROS are naturally removed/neutralized, but their accumulation can occur in the case of increased production. An imbalance between ROS formation and removal leads to oxidative stress. Increased ROS concentrations can lead to changes in signaling pathways in the cell and cause oxidative damage to molecules necessary for biological processes, including proteins, lipids (fats), cell membranes, and the main molecule of genetic material, DNA. This, in turn, can result in loss of function, damage to cellular components, and even cell death (5). Arsenic-induced ROS formation is based on: 1) disruption of enzymatic reactions related to energy processes in mitochondria (a cellular organelle that is the principal site of cellular metabolism and respiration), and 2) reduction of cellular antioxidant defense, affecting both specific enzymes (e.g., superoxide dismutase, catalase, glutathione reductase), and components of non-enzymatic antioxidant defense (e.g., reduced glutathione) (6).

Tissue damage and oxidative stress caused by arsenic trigger various reactions that can lead to inflammation, enabling further development of pathological processes that culminate in numerous diseases, including cancer (6).

The described processes are responsible for damage to various body organs and disrupting their function.

Research on laboratory species of small mammals, mainly mice and rats, provides insight into arsenic toxicity, the resulting pathological changes in various organs, and the cellular and molecular mechanisms underlying these effects. These data are also important for understanding the risks of arsenic toxicity and other pollutants to human health.

Harmful Effects (Toxicity) of Arsenic on Tissues and Organs

Liver and Kidneys

The liver and kidneys are the main organs where arsenic accumulates (7). In response to cellular damage caused by this metalloid (1), the livers of mice and rats mount an inflammatory response, allowing scar tissue to form and replace the damaged one (8). Through the action of key inflammatory mediators, cytokines, and inflammatory stimulus receptors, specialized stellate cells produce components of the extracellular matrix of the scar fibrous connective tissue. Long-term arsenic exposure and the accompanying inflammatory response can lead to a pathological, fibrotic response, i.e., the formation of excess connective tissue and tissue thickening (11). Fibrosis can destroy liver structure and function.

In the kidneys of rats exposed to arsenic, inflammatory cells, primarily blood leukocytes, damage renal tubules, inducing oxidative stress with damage to biomolecules, such as DNA, as well as mitochondrial fragmentation (12). Mitochondrial fragmentation is associated with the development of renal fibrotic reaction (13). In the process of removing damaged mitochondria, inflammatory cytokines stimulate an increase in fibroblast numbers (14) and drive fibroblast production of collagen and other extracellular matrix components (12). Long-term accumulation of connective tissue leads to the formation of tissue scars, which impair kidney function (14).

Respiratory System

Inhalation of high concentrations of arsenic trioxide produces structural changes in the lungs of mice, including damage to the alveolar-capillary membrane (which maintains lung parenchyma and gas

exchange capacity), a decrease in blood oxygen concentration, and pulmonary edema formation (fluid in the lungs) (15). These changes center on an inflammatory reaction triggered by compounds that "leak" through damaged cell membranes, stimulating the production of cytokines that attract inflammatory cells (predominantly neutrophil leukocytes). Through their oxidative activity, leukocytes further damage lung tissue. The result is reduced lung capacity and ability to expand, which is characteristic of acute respiratory syndrome in humans (15). Prolonged intake (through drinking water) of arsenic in the form of arsenite in mice leads to a chronic inflammatory process and fibrotic scarring that interferes with breathing (16, 17).

Digestive System

Arsenic induces an inflammatory response in mouse intestines by activating intestinal immune system cells (dendritic cells and macrophages). Their activity damages the intestinal mucosa, altering normal intestinal permeability and allowing the passage of harmful substances from the intestine (18, 19). This inflammatory process is accompanied by indicators of cancerous changes in the colon (18, 19). Arsenic can also affect the complex community of intestinal microorganisms (the gut microbiota) (20). Mice exposed to arsenic through water or food show increased activity of bacteria that convert organic to inorganic chemical forms of arsenic (20) and an increased presence of bacteria that cause inflammatory processes in the intestines (18).

Nervous System

Mice and rats exposed to arsenite through drinking water exhibit impaired spatial learning and memory, short-term memory loss, and behavioral disturbances (reduced exploratory activity and depression-like behavior) (21, 22). Exposure of female mice to arsenic results in its accumulation in the fetus, primarily in the fetal brain (23). This results in impaired motor function, spatial learning, and memory in sexually mature offspring (24, 25). These effects are associated with morphological changes, including reduced brain weight, reduced numbers of nerve cells and glial cells (brain cells that support nerve cell function), and alterations in molecules involved in the transmission of nerve impulses (neurotransmitters) (21).

The mechanisms of arsenic neurotoxicity are not fully elucidated, but oxidative stress (26) and inflammatory response (27) are thought to underlie damage to cells in the hippocampus, the brain region responsible for short-term, long-term, and spatial memory. In mice exposed to arsenic in the form of arsenite, shrinkage of nerve cell nuclei, irregular shape and arrangement, vacuolation, degeneration, and neuronal death (22) have been observed.

Skeletal System

Bone is continually renewed through the processes of modeling and remodeling. Remodeling is driven by the coordinated activity of osteoblasts (bone cells that participate in the growth and development of bones through the synthesis of bone matrix) and osteoclasts (cells that break down bone tissue, creating space for osteoblasts to form new bone tissue). In rats, arsenic administered as arsenic trioxide or arsenite leads to a range of adverse effects on bone (28). The inability to replace cartilage with bone tissue during fetal development (endochondral ossification) is the basis for preventing bone tissue formation (29). Arsenic also reduces the synthesis of cytokines and proteins necessary for osteoclast and osteoblast maturation and activity, thereby disrupting bone tissue formation and reducing bone mineral density (30, 31).

Reproductive System

Arsenic in the form of arsenite in rats and mice leads to impaired spermatogenesis (32). This effect is attributed to oxidative stress, which has multiple effects on this process. These include damage to the seminiferous tubules, damage to specialized cells necessary for the early stages of spermatogenesis, and disruption of the sperm maturation process, storage, and transport (32). Oxidative stress induced by arsenic in the form of arsenite in rats leads to the development of benign and malignant prostate diseases, which compromise the motility of mature spermatozoa (33). Spermatogenesis is also affected by an inflammatory reaction that damages the testes (testicles) (34).

Oxidative stress damages the cell membrane proteins, enzymes, and DNA of mature spermatozoa, reducing their ability to fertilize an egg (32). This condition in mice negatively affects both the cells involved in the synthesis and the activity of sex hormones (35).

Arsenic causes changes in ovarian morphology and impaired ovulation (release of a mature egg) in sexually mature female rats and mice (36). These disarrangements can also occur in the offspring of exposed female animals and are transmitted to at least two subsequent generations (36, 37).

Immune System

Exposure of rats and mice to arsenite produces numerous changes, including quantitative alterations, such as the size of lymphatic organs and the number of immune system cells, and qualitative impairments of their activity and function. Examples include decreased weight of lymphatic organs (spleen, lymph nodes), numbers and individual subclasses of lymphocytes, reduction in the production of pro-inflammatory cytokines, and the movement and activity of leukocytes, as well as reduced antibody production (38, 39). These changes largely reflect arsenic effects on the activity of genes whose products participate in innate and cellular immune responses, as well as antibody-mediated immunity (38). A decrease in the activity of immune system components can lead to reduced resistance to a range of infections (40).

Carcinogenicity of Arsenic

The carcinogenic effects of arsenic in laboratory animals are manifested at high concentrations and with long-term exposure (41). In mice and rats, arsenic in drinking water induces lung and bladder carcinomas, while inhalation causes lung cancer in hamsters (41). Maternal exposure of mice to inorganic and organic forms of arsenic results in cancers of various organs in the offspring (42). At low concentrations, insufficient to be carcinogenic on its own, arsenic promotes various types of tumors induced by other chemicals (43, 44) or by physical agents such as ultraviolet radiation (44, 45). One of the basic mechanisms responsible for cancer occurrence is the stimulation of genes that control cell division and proliferation (46).

LITHIUM (Li)

Unlike elements such as sodium, potassium, calcium, magnesium, zinc, and iron, lithium is not essential for the major biological processes in the body. However, owing to its potential to affect various enzyme

systems, biochemical reactions, and cellular ion channels, lithium can influence many biological processes (47). These activities of lithium, initially recognized in experimental studies on guinea pigs (48) and later in controlled human trials (49), have led to the clinical use of lithium in the form of lithium carbonate (Li_2CO_3) in the treatment of certain psychiatric diseases. Although administered at high concentrations in these contexts, lithium has a beneficial effect in a certain number of patients (50). However, high doses of lithium in some patients are toxic to some organ systems, and a potential risk of renal disease has been reported (51). Consequently, there has been a trend towards reducing the use of lithium in humans (52).

By analogy with *in vitro* experiments, the effects of lithium salts in living systems exhibit a property called "hormesis" (53), where low concentrations have a positive effect. In contrast, higher concentrations have a negative effect. High doses of ≥ 100 mg per day (therapeutic doses) may produce harmful effects.

Toxic Effects of Lithium on Tissues and Organs

Liver and Kidneys

High doses of lithium carbonate in rats induce oxidative stress, which damages the most abundant cells in the liver (hepatocytes), disrupting normal hepatic function. This further leads to increased blood concentrations of liver cell enzymes and elevated cholesterol, triglycerides, and glucose in the blood (54). In the kidneys, oxidative stress is also thought to cause structural damage (increased size and number of epithelial cells and the surface area of the glomeruli) and changes in kidney function (the flow of filtered fluid through the kidney) (55, 56). Elevated reactive oxygen species activate signaling pathways in the cell that stimulate inflammatory responses that can lead to cell death within the renal system (57).

Nervous System, Endocrine System, and Reproductive System

Studies in mice have shown that lithium can disrupt endocrine functions, including hormone synthesis, and is therefore now classified among endocrine disruptors. Mice and rats exposed to high concentrations of

lithium exhibit decreased thyroid function, with reduced synthesis of the hormones triiodothyronine (T3) and thyroxine (T4). This disruption is caused by oxidative stress and inflammation that damage thyroid cells (58, 59). In addition to structural changes, lithium also causes functional impairment. Inhibition of enzymes that control thyroid cell activity results in the inability of thyroid cells to take up iodine, which is necessary for hormone synthesis (60). Prolonged inflammatory reactions can cause fibrotic changes in the thyroid gland (59).

High doses of lithium in pregnant female rats induce inflammatory changes in the thyroid gland and decrease thyroid hormone synthesis not only in mothers but also in newborn rats (61). Exposure of rats to lithium during gestation negatively affects the formation of the neural tube, which gives rise to the entire central nervous system during embryonic and fetal development. (62, 63). An altered ratio between the number of newly formed and dead cells is considered the main mechanism responsible for this effect of lithium (62, 63). Harmful effects on fetal development also include impaired formation of cranial and vertebral bones, as well as blood vessels (64), likely due to disruption in the synthesis of molecules necessary for maintaining normal cell membrane structure and function (64).

Heart

The administration of high doses of lithium in experimental animals alters contractility (contraction and relaxation) of cardiac muscle tissue and induces arrhythmias (65, 66). Pathological changes induced by lithium include degenerative changes and death of cardiac muscle cells (65, 67). At the heart of these damages and changes are oxidative stress and an inflammatory reaction with an influx of leukocytes (66, 67, 68). Products released by activated leukocytes damage cardiac cells. In intense inflammatory reactions, excessive collagen accumulation and cardiac fibrosis may develop (65).

Immune System

Reduced production of proinflammatory cytokines in the spleen of mice (69) and decreased blood lymphocytes in rats (70) indicate an immunosuppressive effect of lithium. In mice, lithium exerts a dual effect on the process of formation of mature blood cells (hematopoiesis) (71) by stimulating the formation of lymphocytes (lymphopoiesis) and reducing the

formation of myeloid cells (granulocytes and monocytes/macrophages). Increased lymphopoiesis may favor the development of autoimmune reactions, whereas a reduced number of myeloid cells can diminish the body's ability to defend against intracellular bacterial infections.

BORON (B)

Boron is an essential chemical element that plays an important role in basic physiological processes, including growth, embryonic development, metabolism, and hormone secretion (72). Low concentrations of boron, provided by a daily intake of 0.5 to 1 mg, have a beneficial effect on these biological processes (73), while higher concentrations are toxic (74). This means that boron, like lithium, has hormetic effects.

Tissue and Organ Toxicity

Rodents exposed to high concentrations of boron (≥ 40 mg/L) as boric acid accumulate this metalloid in almost all body organs (73). Reported signs include weight loss, decreased activity, and depression, muscle spasms and uncontrolled body tremors, diarrhea, nasal discharge, tissue damage, and reproductive disorders (73).

Degenerative changes in the liver and kidneys (75) are attributed to an inflammatory reaction and the activity of leukocytes that have reached these tissues (76).

Reproductive toxicity caused by high boron concentrations in rats is manifested by decreased activity of seminal enzymes required for sperm maturation (77) and reduced viability of Sertoli cells (78). Disturbances in the metabolism of lipids and amino acids necessary for synthesizing sex hormones also impair spermatogenesis (77). An imbalance between oxidative and antioxidant reactions is considered one of the causes of the negative effects of boron (77). In female rats, high doses of boron (over 116 mg/kg per day) lead to infertility by inhibiting ovulation (release of a mature egg). Lower doses (< 80 mg/kg per day) in mice and rats compromise fetal development, whereas higher doses cause fetal death (79). Following exposure of pregnant female mice to high boron concentrations, offspring exhibit low birth weight (80).

High boron concentrations adversely affect lymphatic organs in rats. In the thymus, a lymphatic organ where T lymphocytes are produced, ex-

cess boron decreases production of cytokines necessary for lymphocyte development and induces programmed cell death (apoptosis). Interference with intracellular signaling is one of the mechanisms underlying boron toxicity in the thymus (81). High boron concentrations reduce spleen size and lymphocyte count and alter the structure of the spleen tissue (82). As boron changes the ratio of lymphocyte subclasses, decreases the production of cytokines important for lymphocyte activity, and increases the frequency of lymphocyte death (83), it may also change the type of immune response.

PARTICULATE MATTER (PM)

Among inhaled particulates, the most toxic are fine particles, $PM_{2.5}$ (≤2.5 μm), as they can penetrate deepest into the lungs (84). The reactions they provoke can lead to a range of respiratory and pulmonary diseases. Because $PM_{2.5}$ can pass from the lungs into the bloodstream, these particles reach all tissues and organs, exerting toxicity in other organs, including the cardiovascular and digestive systems (85).

Toxicity of Particulate Matter to Tissues and Organs

In the lungs, phagocytic cells, mainly macrophages, ingest fine particles, which produce numerous inflammatory mediators, primarily cytokines (86, 87). They attract blood leukocytes and stimulate their activity, establishing an inflammatory reaction. The heightened oxidative activity of leukocytes in the lungs increases the concentration of reactive oxygen species, causing oxidative damage to biomolecules and ultimately cell death (88, 89). A prolonged inflammatory reaction can lead to a fibrotic reaction in the lungs (90).

In the cardiovascular system, fine particles can initiate a series of pathophysiological reactions, leading to heart rate variability, elevated blood pressure, increased coagulation and thrombosis, and metabolic disturbances (91, 92). Changes in heart rate can occur due to multiple negative effects on the autonomic nervous system, which controls the activity of many organs, including respiration and heart rate. PM-induced inflammatory changes lead to: (a) dysfunction of cells on the inner surface of blood vessels that maintain blood flow stability, (b) disrupted vascular tone and

elasticity, (c) fibrotic reaction and narrowing of blood vessels. Together, these effects contribute to increased blood pressure (93).

Evidence suggests that fine particles may disrupt hepatic metabolism of lipids and proteins (94), or sugars (glucose) and the hormones that regulate their levels in the blood (95). Some of these hormones act as stress mediators and can disrupt the circadian rhythm of animals (95). In experimental mice, particulate matter can alter the composition of the gut microbial community, leading to intestinal inflammation (96) and disturbances in blood glucose levels (97).

Particulate matter contains many organic and inorganic chemical species, including metals and metalloids, whose toxicity greatly contributes to the toxic potential of particulate matter (98, 99).

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Chapter 13

Possible Impacts of the Jadar Project on the Health of the Local Population

Petar Bulat, Zorica Bulat

- In the draft Study, health effects are only briefly mentioned, without a detailed overview of possible positive and negative effects of the facility's operations.
- Among many unknowns, the most significant is the new technology that the investor plans to implement in the exploitation of jadarite.
- Large quantities of industrial waste with high concentrations of arsenic, boron, and lithium are expected during jadarite processing, along with cadmium, chromium, lead, mercury, nickel, and zinc.
- The health of local residents and workers may also be affected by dust particles ($PM_{2.5}$ and PM_{10}), noise, and tremors from blasting, as well as large amounts of sulfuric acid and explosives. Taken together, these would have serious consequences for public health.
- Since no analysis of economic gain and damage, or of sociodemographic implications, has been conducted, no conclusions can be drawn about any potential positive aspects of the Jadar project.
- Given that most of the necessary data is not publicly available, it is currently not possible to make a definitive assessment of the impact of the Jadar project on the health of the local population.

Introduction

The initiative to begin lithium exploitation from jadarite has been accompanied by numerous uncertainties. Opposing views between the local population and investor representatives regarding the possible effects of jadarite exploitation on health and the environment have, over time, taken on a political dimension, making the relevant discussions even more complex. The lack of explicit data on the details of the planned lithium ore exploitation technology prevents more meaningful participation of experts in resolving these uncertainties and contributes to the polarization of public opinion. The situation is further complicated by the absence of a quality health impact assessment in the draft Environmental Impact Assessment Study for the underground exploitation project of lithium and boron deposits (Jadar), the ore processing plant, and the disposal of mining tailings (1). In the draft Study, there is almost no assessment of the impact on the health of the local population, and much of its content is largely irrelevant for evaluating such impacts. One illustration appears in the section titled "Potential association of exposure to significant pollutants and possible diseases," where page 586 includes a passage on hypertension stating:

> "Hypertension (high blood pressure) is a common condition in which the long-term pressure of blood on the artery walls is strong enough to eventually cause health problems, such as heart disease. Risk factors for hypertension include obesity, a diet high in fat or cholesterol, diabetes, physical inactivity, chronic kidney disease, obstructive sleep apnea, thyroid problems, and family history. Use of tobacco or certain illegal drugs is another risk factor for hypertension. Exposure to elevated concentrations of $PM_{2.5}$ is also associated with an increased risk of hypertension."

The quoted text does not mention pollutants that, based on current knowledge, are expected to appear in the environment and can contribute to hypertension, such as lead, cadmium, and indirectly arsenic, as well as noise. Furthermore, it offers no assessment of the impact of the only identified pollutant ($PM_{2.5}$) on hypertension in the local population. The omission of pollutants known to be present in the environment and potentially linked to increased hypertension, along with the lack of assessment of $PM_{2.5}$'s impact on disease incidence, can be attributed either to the incompetence of the authors of these chapters or to deliberate withholding

of information. This issue would be minor were it confined to the chapter on hypertension; unfortunately, all chapters addressing health effects are presented at a rudimentary level, mentioning only a subset of relevant pollutants and providing no estimation of their impact on disease incidence. It is particularly concerning that the text fails to mention the known carcinogenic properties of some pollutants that are realistically expected to be present in the environment.

The assessment of the health and environmental effects of lithium production can be approached from several perspectives. Among the most significant documents are certainly: "Draft Environmental Impact Assessment Study for the Underground Exploitation of the Lithium and Boron Deposit 'Jadar', Ore Processing Plant, and Tailings Disposal Resulting from Mining Activities" (1) and the "Spatial Plan for the Special Purpose Area for the Implementation of the Exploitation and Processing of the Jadarite Mineral 'Jadar'—Strategic Environmental Impact Assessment Report of the Spatial Plan" (2). Additionally, the document titled "Information Brochure—Answers to Questions about the 'Jadar' Project" (3) (published by Rio Tinto) contains data that can further contribute to understanding potential exposures of the local population and employees involved in jadarite exploitation. Conversely, the media report almost daily on the views of non-governmental organizations and various local initiatives, which are often diametrically opposed to the investor's position regarding possible health and environmental impacts.

When considering potential general environmental effects based on the Strategic Environmental Impact Assessment Report of the spatial plan, the facility exhibits the characteristics of a lower-tier Seveso facility (1) due to the presence of large quantities of explosive materials (ANE 130—Subtec Eclipse). The classification of the jadarite exploitation facility as a Seveso site warrants particular caution when making decisions about its development.

Lithium Toxicity

The available data on lithium toxicity derive primarily from the medical literature, since lithium has been used for decades in psychiatry to treat bipolar disorder. As this is a chronic psychiatric condition, lithium use is long-term, and therefore, the data on its health effects stem from its chronic use in this patient group. Available data indicate that therapeutic

use of lithium may lead to decreased kidney function (reduced glomerular filtration and concentrating ability), decreased thyroid function, and increased parathyroid hormone synthesis (4).

To date, the World Health Organization has not recommended any "safe" lithium levels in drinking water. In certain regions, such as northern Chile, northern Argentina, and the area around Graz in Austria, drinking water contains elevated lithium concentrations ranging from several dozen μg/L to over 1,000 μg/L (5). These data highlight the need for guidelines and recommendations, particularly in light of evidence regarding thyroid dysfunction in areas with high lithium levels in groundwater (6).

Given that large-scale lithium production began only in the 21st century, there is still very limited data on the intensity of lithium exposure in production processes, as well as on any potential effects on workers' health.

Heavy Metals Present in Jadarite

In addition to lithium, jadarite contains other heavy metals. Based on the plans to process between 1.6 and 1.8 million tons of ore annually, it is evident that processing will generate large quantities of industrial waste. This waste is expected to contain primarily arsenic, boron, cadmium, chromium, lead, mercury, nickel, and zinc.

Arsenic

Based on available data, significant amounts of arsenic can be expected in the industrial waste. From disposal sites, it may enter the atmosphere through dust particles and, through surface and groundwater, reach watercourses. Arsenic is absorbed by inhalation (23%) and ingestion (90%–95%). Once inside the body, pentavalent arsenic is reduced to trivalent arsenic, which is then subject to oxidative methylation by methyltransferase to form pentavalent organic arsenic compounds (7). The dominant metabolites of inorganic arsenic compounds—monomethylarsonic acid and dimethylarsinic acid—are relatively quickly excreted from the body via urine (8). Absorbed arsenic inhibits mitochondrial respiration and causes free-radical damage, producing toxic effects on the peripheral and central nervous systems. Polyneuropathy is associated with damage to the myelin sheath of peripheral nerves and to the cytoskeleton (9, 10). Encephalopathy is also linked to free-radical damage that results in cell death and accelerated deg-

radation of dopaminergic neurons, producing clinical manifestations similar to Parkinson's disease (10). Exposure to arsenic through contaminated food and water is associated with diabetes and obesity, via mechanisms involving not only free-radical damage but also the development of insulin resistance (11, 12). Arsenic exposure through drinking water is directly linked to cardiovascular complications, such as myocardial injury, cardiac arrhythmias, and cardiomyopathy (13). Chronic exposure to arsenic can lead to arteriosclerosis, progressive vascular blockage, gangrene of the lower limbs, and skin changes, including melanosis, increased keratosis, and "black foot disease" (14).

Arsenic has been classified as a human carcinogen by the International Agency for Research on Cancer (IARC), the World Health Organization (WHO), and the United States Environmental Protection Agency (US EPA).

Boron

Boron enters the human body daily through drinking water and fresh or processed foods of plant and animal origin (15). Detailed information on occurrence, potential sources of elevated exposure, and health effects can be found in a U.S. EPA document (16). Total daily boron intake should not exceed the established safety thresholds. According to the European Union Directive 98/83/EC on the quality of water intended for human consumption (17) and the Serbian Rule Book on the Hygienic Quality of Drinking Water, the boron concentration in drinking water must not exceed 1 mg/L (18). According to the World Health Organization, the acceptable daily boron intake for humans is between 1 and 13 mg (19). Exposure to high boron concentrations through oral intake causes acute effects such as nausea, vomiting, diarrhea, and dizziness. Chronic ingestion at high boron levels has been associated with decreased appetite, weight loss, reduced sexual activity, and reduced sperm motility (20). Occupational exposure to dust containing boron causes irritation of the skin and mucous membranes of the eyes, nose, mouth, throat, and respiratory organs, causing symptoms such as sore throat and productive cough (21). To avoid adverse health impacts, the concentrations of boron, arsenic, and other potentially toxic metals in water, soil, and food for human consumption should be monitored and kept below established upper safety limits.

Lead

According to available data, industrial waste generated during lithium production at the proposed facility may contain lead. Like other toxic metals, lead could reach watercourses and thereby cause environmental contamination. Since gastrointestinal absorption of lead in adults is relatively low (5%–10%), significant adverse health effects are less likely in the adult population. However, in children, gastrointestinal absorption of lead is significantly higher (about 50%), and the developing nervous system is particularly sensitive to the neurotoxic effects of lead, so the impact of lead on children's intellectual development cannot be ruled out. Current research indicates that lead-related neurobehavioral disorders (behavioral disorders) represent the so-called no-threshold effect, meaning there is no exposure level that can be considered definitively safe (22).

Cadmium

Based on available data on expected cadmium levels in industrial waste, direct toxic effects are not anticipated. However, since some plants concentrate cadmium (e.g., tobacco, St. John's wort, rice, and green vegetables), potential toxic effects cannot be entirely ruled out. After prolonged exposure, these effects may manifest in the kidneys (e.g., tubular damage, microproteinuria, calciuria, phosphaturia), bones (osteoporosis), endocrine system (cadmium is classified as an endocrine disruptor), and cardiovascular system (23). It is important to note that the International Agency for Research on Cancer (WHO–IARC) classifies cadmium as a human carcinogen, with evidence for lung cancer.

Presence of other metals in the living and working environment

Based on available data, the following metals can also be expected in industrial waste resulting from lithium production:

- Nickel (human carcinogen);
- Chromium (human carcinogen);
- Mercury;
- Zinc;
- Boron.

Other Hazards Expected During Jadarite Mining

Dust ($PM_{2.5}$ and PM_{10})

According to the draft Environmental Impact Assessment Study for the underground mining project of the lithium and boron deposit Jadar, the ore processing plant, and tailings disposal facility, elevated emissions of $PM_{2.5}$ and PM_{10} particles are expected due to: mining activities, transportation and unloading of industrial waste, wind-induced erosion of the tailings dump, movement of machinery on the dump, and emissions from internal combustion engines.

Exposure to $PM_{2.5}$ and PM_{10} is associated with respiratory tract irritation, coughing and/or breathing difficulties, reduced lung function, more frequent asthma attacks in asthmatics, and an increased incidence of non-fatal myocardial infarctions.

Noise and Vibrations

Based on the planned method of jadarite exploitation, as well as the expected quantities of explosives used at the facility, intensive daily blasting is realistically expected, especially in the first year. The available information does not specify any "quiet periods" at night when blasting or transport of industrial waste to the tailings facility would cease. Considering that the facility is being established in a rural area with no prior significant industrial activity—and therefore no notable noise or vibrations—adverse health effects from these phenomena may include:

- Sleep disturbances, leading to mood changes, fatigue, reduced work capacity, and headaches;
- Psychophysiological effects (high blood pressure, appetite and sexual function disorders, tension, and depression).

Before the Conclusion

As socio-economic determinants also influence health, the methodology for analyzing the impact of such large new projects should—before any decision-making process on the project implementation—include an assess-

ment of economic benefits and harms, as well as other socio-demographic implications of the project. This analysis must be conducted by the decision-maker, not the interested company. In this case, as in many other new projects in our country, that has not been the case. Such analyses would enable the implementation of only those projects that are sustainable and have predominantly positive economic and socio-demographic impacts. Since no such analysis was conducted prior to the decision to implement the Jadar project, no conclusions can be drawn regarding potential positive aspects of this project on the economic and socio-demographic determinants of health.

Conclusion

Since most of the necessary data are not publicly available, it is currently not possible to provide a definitive assessment of the impact of the Jadar project on the health of the local population. As a preventive measure, all precautionary actions should be applied to avoid possible negative consequences for the health of local residents.

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REVIEWS

Review

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Responding to the invitation to review the volume *Project Jadar—Possible Harmful Impacts on Wildlife and Human Health*, edited by Milena Kataranovski, Tamara Rakić, Elizabet Paunović, and Predrag Simonović, I have examined the submitted text and offer the following opinion.

The authors of the chapters, mostly affiliated with institutions of the Universities of Belgrade and Niš, the Scientific Institute of Veterinary Medicine, and the WHO, adopt an evidence-based approach, as supported by 619 relevant references.

Starting from the impact of arsenic, lithium, boron, lead, cadmium, and particulate matter, the authors strive to provide as comprehensive an insight as possible. In addition to the preface, the volume comprises 12 chapters addressing: (1) legislative, international, and expert methodological frameworks for assessing environmental and health impacts; (2) the origin and availability of lithium, arsenic, boron, and particulate matter in the environment; (3) the impact of their elevated concentrations on algae and aquatic plants; (4) lichens; (5) plants; (6) the impacts of arsenic, lithium, and boron on aquatic invertebrate fauna; (7) the risk posed by untreated groundwater from the Jadar mine to fish in the Jadar River, the principal recipient of mine wastewater; (8) the toxic effects of lithium, boron, arsenic, and particulate matter on amphibians in aquatic and terrestrial ecosystems; (9) current knowledge about the consequences of lithium, boron, arsenic extraction, and the spread of particulate matter on local reptile populations; (10) the impact of lithium, arsenic, and boron on mammals

and environmental pollution; (11) experimental studies of the harmful effects of lithium, arsenic, boron, and particulate matter on laboratory small mammals; and (12) the possible impacts of this project on the health of the local population.

I am particularly pleased to recommend this text for publication, as it serves both experts and interested lay readers. In short, this text is encouraging because this level of expertise, seriousness, and responsibility was needed from the very beginning of the problem. The authors themselves scrupulously conclude that, as "most of the necessary data are not publicly available, it is currently not possible to provide a definitive assessment of the impact of the 'Jadar' project on the health of the local population." Yet, the contributions of this kind are necessary and valuable steps toward the desired definitiveness.

Belgrade, 26 February 2025

Review of the publication The Jadar Project—Possible Harmful Impacts on Wildlife and Human Health

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In the chapter by Elizabet Paunović, "Legislative, International and Professional Methodological Framework for Health within Environmental Impact Assessment," it is emphasized that the Health Impact Assessment within the Environmental Impact Assessment is regulated by numerous international frameworks and national legislation. In contrast, the draft Environmental Impact Assessment of the Jadar project published on the Rio Tinto website does not contain a Health Impact Assessment.

The chapter "Sources, Environmental Distribution, and Availability of Lithium, Arsenic, Boron, and Particulate Matter," by Jelena Mutić and Milena Kataranovski, provides a detailed analysis of the movement of arsenic, lithium, and boron in the environment, including their passage through food chains from microorganisms to vertebrates. The possible toxicity of these elements, in relation to their chemical characteristics and to living things and humans, is highlighted. The impact of dust in the mining context on the environment and human health is also addressed in particular.

Authors Gordana Subakov Simić and Ivana Trbojević, in the chapter "The Effects of Elevated Concentrations of Lithium, Arsenic, and Boron on Algae and Aquatic Plants," note that data on the impacts of lithium, arsenic, and boron on algae and aquatic plants are limited, but that higher

concentrations of these elements are toxic. Mining can lead to increased concentrations of these elements; consequently, constant monitoring of freshwater ecosystems is essential.

In "The impact of Lithium, Arsenic, Boron and Particulate Matter ($PM_{2.5}$; PM_{10}) on Lichens," Slaviša Stamenković observes that there are no data on lithium impacts on lichens, which are otherwise exceptional indicators of air pollution. Limited scientific evidence on arsenic and boron suggests negative impacts on lichen growth when these elements are present in high concentrations. Elevated particulate matter concentrations are negatively correlated with the diversity and vitality of lichens.

Regarding the impact of elevated lithium, boron, and arsenic concentrations on plants, Tamara Rakić and Tomica Mišljenović report in "The Impact of Elevated Lithium, Boron, and Arsenic Concentrations on Plants" that plants readily absorb these elements, which then accumulate in plant organs, negatively affecting their growth and development. Of particular concern is the impact on humans and on domestic and wild animals that feed on contaminated plants. These elements mainly lead to metabolic disorders in plant cellular functions such as photosynthesis, cellular respiration, signal transduction, and gene activity. The authors emphasize that, for health safety, it is necessary to monitor lithium, boron, arsenic, and other metals in water, soil, and food intended for human consumption, ensuring that values do not exceed upper limit concentrations.

The impact of arsenic, lithium, and boron on aquatic invertebrates includes deformities and reduced reproduction rates, as noted by Ivana Živić, Vidak Lakušić, and Milenka Božanić in "Analysis of the Effects of Arsenic, Lithium, and Boron on the Fauna of Aquatic Invertebrates." Although the number of studies is limited, the authors present several relevant studies from several countries (China, Turkey, France, among others). They conclude that constant monitoring of aquatic ecosystems, using macroinvertebrates as bioindicators, is necessary during potential mine construction, throughout ore exploitation, and after the mine closure.

In the chapter "Risk to the Aquatic Ecosystem of the River Jadar Owing to the Exploitation of Boron and Lithium in the Project 'Jadar'," Predrag Simonović and Vera Nikolić consider risks to fish and related ecosystems stemming from groundwater that would be evacuated from the Jadarite mine shaft and discharged into the Jadar River as untreated wastewater with markedly elevated concentrations of arsenic, boron, and lithium. They note that less mobile, rare, and strictly protected species would be particularly endangered. The authors emphasize that any malfunction

or power outage at the wastewater treatment plants could lead to large quantities of arsenic, boron, and lithium entering the Jadar River. They further state that there is no known pathway and no practical possibility of recovering the Jadar ecosystem from this type of pollution. For a more complete picture of the impact of mining lithium, a detailed report on the possible effects of mining on the aquatic ecosystem of the Jadar River is still awaited.

Jelka Crnobrnja-Isailović and Bogdan Jovanović, in "Toxic Effects of Lithium, Boron, Arsenic, and Particulate Matter on Amphibians in Aquatic and Terrestrial Ecosystems," emphasize predominantly negative effects of the three elements on amphibian embryos, larvae, and adults. Existing literature indicates that exposure of amphibians to lithium, boron, arsenic, and particulate matter in aquatic ecosystems commonly produces a range of harmful effects both on individual developmental stages and on population levels, reducing survival and reproduction.

In "What is Known about the Effects of Lithium, Boron, and Arsenic Extraction and the Spread of Particulate Matter on Local Reptile Populations?" Jelka Crnobrnja-Isailović and Jelena Ćorović report that research on the negative effects of lithium and boron on reptiles is very limited. Somewhat more information exists for arsenic, which has been associated with liver damage in snakes and decreased reproductive success in both snakes and lizards. The authors emphasize that, before any activities related to jadarite excavation, detailed studies on the impact of lithium, boron, and arsenic on local reptile diversity must be conducted.

Ivan Pavlović, in "Toxicity of Lithium, Arsenic, Boron, and Heavy Metals on Birds," notes that there is limited evidence on the effects of lithium on different bird species, and reports that lithium can accumulate in avian organs, with higher accumulation rates in terrestrial birds than in aquatic birds. He further indicates that about 80 % of lithium filtered by the glomeruli is reabsorbed, with the remainder excreted in urine. Several examples highlight feather damage in various birds. The chapter also underscores the toxic effect of heavy metals in birds and, drawing on the literature, reiterates that arsenic (As) is a toxic and carcinogenic element. Although borax and boric acid are not directly poisonous to birds, they may pose indirect risks. Overall, the chapter provides a comprehensive review of the adverse effects of toxic metals on birds.

In "Mammals and Environmental Pollution—Impact of Lithium (Li), Arsenic (As), and Boron (B)," Jelena Blagojević and Ivan Pavlović summarize numerous scientific studies on the negative effects of toxic metals on

small and large mammals. In four species of small mammals, significant correlations were observed between the concentration of arsenic in tissues and the condition of the organisms, as well as between increased organ mass (liver, kidneys, lungs) and higher arsenic concentrations, indicating possible pathological changes in the tissues. Although findings on arsenic bioaccumulation in animal tissues are contradictory, numerous data show that arsenic toxicity causes developmental problems, including malformations, death, and growth retardation in four species of small mammals: hamsters, mice, rats, and rabbits. High boron exposure negatively affects reproduction, i.e., fertility and offspring survival, as demonstrated experimentally on house mice (*Mus domesticus*). The effects of lithium on small mammals have been little studied so far; available work reports behavioral changes, such as increased aggression, as well as physiological changes. The accumulation of toxic metals in plants, water, and soil increases the risk of their transmission to domestic and wild mammals, as well as to game birds, through dietary intake. In larger mammals, high lithium doses have been associated with increased body weight and thirst, damage to the male reproductive system, significant reductions in plasma testosterone levels, and oxidative stress in the liver.

Milena Kataranovski, in "Experimental Studies on the Adverse Effects of Arsenic, Lithium, Boron, and Particulate Matter on Laboratory Species of Small Mammals," lists a whole range of toxic effects of these elements on multiple organ systems in small mammals, including the carcinogenic effects of arsenic. She notes that tissue damage and oxidative stress caused by arsenic trigger various inflammatory reactions and pathological processes that lead to numerous diseases, including cancer. The chapter also addresses the hormetic effects of boron and lithium, which are beneficial at low concentrations but toxic at higher levels (as may occur near mines), and analyzes the toxicity of fine particulate matter ($PM_{2.5}$ and smaller).

In "Possible Impacts of the Jadar Project on the Health of the Local Population," Petar Bulat and Zorica Bulat note that the project's draft Study contains almost no assessment of impacts on local population health. Much of the text of the Study is largely irrelevant for assessing such impacts, and all chapters addressing health effects are presented at a very basic level, mentioning only some pollutants potentially linked to disease and without any assessment of their impact on disease incidence. The authors emphasize that the omission of known carcinogenic properties of certain pollutants, which are realistically expected to be present in the environment, is particularly concerning. The chapter summarizes the

known adverse effects of lithium, heavy metals in jadarite, noise, and dust on human health. In conclusion, the authors state that because most of the necessary data is not publicly available, a definitive assessment of the Jadar project's impact on local population health cannot be provided.

The publication is written in a clear, accessible style, suitable not only for professional and scientific audiences but also for a broad readership and citizens seeking more information about the risks to health, the environment, and wildlife posed by the lithium mining project in Jadar. Based on all of the above, I am very pleased to recommend publication of the edition *Project Jadar—Possible Harmful Impacts on Wildlife and Human Health*.

Belgrade, 18 March 2025

JADAR PROJECT
POSSIBLE HARMFUL IMPACTS
ON WILDLIFE AND HUMAN HEALTH

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