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EDITOR **PROF. DR. MURAT KÜTÜK**

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CONTENTS

Chapter 1

PHYTOCHEMICAL AND PHARMACOLOGICAL ADVANCES IN *VERBASCUM* SPECIES: A COMPREHENSIVE REVIEW

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INTRODUCTION

The genus *Verbascum*, commonly known as mullein, belongs to the Scrophulariaceae family and encompasses approximately 360 species of biennial or perennial herbaceous plants distributed worldwide (Dong et al., 2022). In Turkey, *Verbascum* is particularly significant due to its high species diversity, with around 250 species, of which approximately 50% are endemic, making Turkey a global hotspot for this genus (Yılmaz and Dane, 2012). These species are predominantly found in Mediterranean and Irano-Turanian phytogeographic regions, thriving in diverse habitats such as rocky slopes, steppes, and disturbed areas. Globally, *Verbascum* species are distributed across Europe, Asia, North Africa, and parts of North America, with notable concentrations in the Mediterranean Basin and the Middle East (Alipieva et al., 2014). Compared to the global distribution, Turkey's high endemism rate highlights its unique role as a center of *Verbascum* diversity, likely due to its varied topography and climate. The genus has been a cornerstone in traditional herbal medicine across various cultures, valued for its efficacy in treating ailments ranging from respiratory disorders to skin conditions. Recent advances in phytochemical research have significantly expanded our understanding of the chemical diversity and therapeutic potential of *Verbascum* species, driven by the integration of cutting-edge analytical technologies and bioassay-guided approaches. These efforts have not only validated the ethnomedicinal uses of the genus but also highlighted its potential in modern pharmaceutical and nutraceutical applications. Over the past few years, studies have employed advanced chromatographic and spectrometric techniques to uncover the complex profiles of secondary metabolites, shedding light on their roles in antioxidant, anti-inflammatory, antimicrobial, and anticancer activities. In this review, we summarize and critically analyze recent developments in the phytochemical research on *Verbascum*, focusing on the major classes of secondary metabolites, the advanced methodologies used for their characterization, and the biological activities associated with these compounds (Blanco-Salas et al., 2021; Cambaz and Çördük, 2023; Fadel et al., 2020).

1. PHYTOCHEMICAL CONSTITUENTS OF *VERBASCUM* SPECIES

A. Phenylethanoid Glycosides

Phenylethanoid glycosides, most notably verbascoside and its analogues, have attracted significant research interest due to their wide-ranging bioactivities, including antioxidant, anti-inflammatory, and anticancer effects (Temporiti et al., 2020). Verbascoside, often detected in high concentrations in species such as *Verbascum* thapsus and *Verbascum sinuatum*, has been comprehensively profiled using high-performance liquid chromatography coupled with diode array detection and electrospray ionization mass spectrometry (Luca et al., 2019). Its structural analogues, including isoverbascoside and other phenylethanoid glycosides, have also been identified in similar studies, reinforcing their potential as chemotaxonomic markers and as lead compounds for therapeutic development (Luca, 2021). These compounds significantly contribute to the antioxidant potential of *Verbascum* extracts and are believed to be responsible for mitigating oxidative stress in various biological systems (Donn et al., 2023). For example, verbascoside and glucopyranosyl-(1→6)-martynoside isolated from *Verbascum bugulifolium* have shown potent antioxidant activity in DPPH, ABTS, and CUPRAC assays (Gökmen et al., 2021).

B. Flavonoids

Flavonoids in *Verbascum* species encompass a broad spectrum of compounds including flavones, flavonols, and *O*-methylated derivatives. Luteolin, apigenin, quercetin, and their glycosides are consistently reported across different species, contributing to notable antioxidant and anti-inflammatory activities (Öztürk et al., 2019). Studies on *Verbascum sinuatum*, for example, have demonstrated that the total flavonoid content correlates with strong free radical scavenging activity as measured by DPPH and other assays (Donn et al., 2023). Advanced LC-MS/MS techniques have facilitated the simultaneous qualitative and quantitative determination of these flavonoids, some of which are increasingly being considered as markers for the genus due to their distinct distribution patterns (Luca et al., 2019). The bioactivity of these compounds is also linked to their capacity to modulate key enzymes involved in inflammatory processes, a property that has been corroborated through both in vitro assays and *in silico* predictions (Blanco-Salas et al., 2021). In *Verbascum bugulifolium*, flavonoids such as luteolin and quercetin 3-*O*-rutinoside exhibited significant anti-inflammatory activity, with luteolin showing 54.1% inhibition in a LOX assay (Gökmen et al., 2021).

C. Iridoid Glycosides

Iridoids represent another major class of secondary metabolites in *Verbascum*. Detailed structural studies have confirmed the prevalence of catalpol-derived iridoid glycosides including aucubin, ajugol, and various acylated derivatives (Fadel et al., 2020). These compounds are associated with a range of biological effects, including modest antioxidant capacity and anti-inflammatory activities, although their contribution to the overall bioactivity of *Verbascum* extracts appears to be less pronounced than that of phenylethanoid glycosides and flavonoids (Fadel et al., 2020). Notably, some research indicates that while iridoids may exhibit weaker *in vitro* enzyme inhibitory activity, they are still relevant in the context of traditional medicine (Fadel et al., 2020). Their structural elucidation has been advanced by the use of high-resolution NMR and LC-MS/MS techniques, which have improved the detection of minor iridoid constituents in complex plant matrices (Keaveney et al., 2020). For instance, *Verbascum bugulifolium* contains catalpol, specioside, and ajugoside, which contribute to its pharmacological profile (Gökmen et al., 2021).

D. Saponins, Alkaloids, and Other Minor Constituents

In addition to the major compounds discussed above, *Verbascum* species also contain triterpene saponins, spermine alkaloids, and polysaccharides that further contribute to their pharmacological profile. Triterpene saponins, although less extensively studied than phenylethanoid glycosides and flavonoids, have been isolated from selected species and are known to possess surface-active properties that may enhance the absorption of co-administered compounds (Keaveney et al., 2020). Spermine alkaloids, such as verbacine and verballocine, have a much more limited distribution but are of interest due to their potential cytotoxic activities (Keaveney et al., 2020). Furthermore, emerging studies have begun to focus on other classes of compounds, including neolignans and oleanane-type triterpenes, which add to the chemical complexity of *Verbascum* (Luca, 2019). Although essential oils are less frequently the subject of detailed phytochemical profiling in recent investigations, there is evidence suggesting that they may also contribute to the antimicrobial properties of certain *Verbascum* species (Yabalak et al., 2020). For example, *Verbascum thapsus* contains terpenoids like stigmasterol and squalene, which enhance its bioactivity (Gupta et al., 2022).

2. ADVANCED ANALYTICAL METHODOLOGIES

The rapid evolution of analytical techniques over the past few years has greatly enhanced our ability to profile and quantify the diverse array of secondary metabolites in *Verbascum* species. High-performance liquid chromatography (HPLC) coupled with diode array detection (DAD) and electrospray ionization quadrupole time-of-flight mass spectrometry (ESI-Q-TOF-MS/MS) has become the gold standard for the detailed profiling of complex plant matrices (Luca et al., 2019). This methodology allows for the rapid and sensitive detection of compounds such as verbascoside and its analogues, as well as a broad spectrum of flavonoids and iridoids (Luca et al., 2019). In another study, advanced LC-MS/MS techniques were employed to achieve simultaneous quantification of multiple phytochemical classes in *Verbascum sinuatum*, illustrating the capacity for high-throughput analysis and structural elucidation (Donn et al., 2019). Coupled with these techniques, the use of gas chromatography-mass spectrometry (GC-MS) has been instrumental in characterizing the volatile fractions and essential oils extracted from *Verbascum* species (Nadeem et al., 2021). Recent innovations in sample preparation processes, including green extraction methods such as microwave-assisted (MAE), ultrasound-assisted (UAE), and pulsed electric field extraction (PEF), have improved the yield and stability of thermolabile phytochemicals while reducing solvent consumption (Donn et al., 2019). These state-of-the-art analytical approaches not only enhance compound detection and quantification but also facilitate the comparison of phytochemical profiles across different *Verbascum* species, thereby supporting chemotaxonomic studies (Keaveney et al., 2020).

3. BIOLOGICAL ACTIVITIES AND PHARMACOLOGICAL POTENTIALS

A. Antioxidant Activity

A considerable body of evidence supports the potent antioxidant activity exhibited by *Verbascum* extracts, which is primarily attributed to the high content of phenolic compounds, flavonoids, and phenylethanoid glycosides. In *Verbascum sinuatum*, for instance, studies have measured considerable total phenolic (29.03–54.77 mg GAE/g DW) and flavonoid contents that correlate strongly with their free radical scavenging capacity, as estimated by DPPH, ABTS, and ferric reducing antioxidant power (FRAP) assays (Nadeem et al., 2021). Similarly, extracts of *Verbascum thapsus* have demonstrated significant antioxidant properties that are comparable to synthetic antioxidants, supporting their potential application in food preservation and therapeutic formulations (Zhang et al., 2023). The antioxidant efficacy of these extracts has been further validated by *in vitro* studies, which indicate a dose-dependent inhibition of lipid peroxidation and free radical generation (Fadel et al., 2020). *Verbascum bugulifolium* extracts, particularly those containing verbascoside, showed IC_{50} values of 20–90 µg/mL in antioxidant assays (Gökmen et al., 2021).

B. Anti-Inflammatory and Enzyme Inhibition Activities

The anti-inflammatory potential of *Verbascum* species is another aspect that has garnered attention in recent studies. Flavonoids such as luteolin, apigenin, and their glycosides are known to modulate inflammatory signaling pathways, including the inhibition of proinflammatory cytokines and enzymes such as cyclooxygenase and lipoxygenase (Öztürk et al., 2019). *In silico* studies have further supported these observations by predicting favorable binding affinities of *Verbascum* flavonoids to targets such as LOX and NADPH oxidase, suggesting a mechanistic basis for their traditional use in treating inflammatory disorders (Blanco-Salas et al., 2021). Additionally, *Verbascum* extracts have been shown to inhibit acetylcholinesterase activity, an effect that may have implications in the management of neurodegenerative diseases such as Alzheimer's (Zhang et al., 2023). Such enzyme inhibition properties have been comprehensively analyzed using chromatographic techniques coupled with bioassays, thereby providing a direct link between the chemical composition and the pharmacological potential of these plants (Akman, 2025).

C. Antimicrobial and Antibiofilm Effects

Verbascum species have also demonstrated significant antimicrobial activities, which are primarily ascribed to their phenolic and volatile constituents. For example, ethanol extracts of *Verbascum pseudoholotrichum* have exhibited strong activity against a broad spectrum of microorganisms, including Gram-positive and Gram-negative bacteria, as well as fungal pathogens (Yabalak et al., 2020). The antibacterial efficacy of these extracts is often quantified by the determination of minimum inhibitory concentration (MIC) values and supported by biofilm inhibition assays (Öztürk et al., 2019). Furthermore, recent studies have demonstrated that certain secondary metabolites, particularly phenylethanoid glycosides, contribute to the disruption of microbial biofilms, thereby enhancing the effectiveness of traditional antimicrobial treatments (Taleb and Saeedi, 2021). These findings underscore the potential of *Verbascum* extracts as natural alternatives in the fight against antibiotic-resistant pathogens and as supplements in wound-healing formulations (Hacıoğlu et al., 2021). *Verbascum bugulifolium*'s n-hexane extract was notably effective against *Pseudomonas aeruginosa* (MIC = 125 µg/mL) (Gökmen et al., 2021).

D. Cytotoxic and Anticancer Properties

Several recent studies have investigated the cytotoxic and anticancer potential of *Verbascum* species. For instance, extracts of *Verbascum ovalifolium* have been evaluated for their selective antiproliferative effects on tumor cell lines while exhibiting minimal cytotoxic effects on non-tumor cells (Luca et al., 2019). These anticancer properties are thought to be mediated by key secondary metabolites such as verbascoside, which can modulate apoptosis-regulating proteins and interfere with cell cycle progression (Luca et al., 2019). In addition, *Verbascum lasianthum* has been shown to possess significant cytotoxic activity, with studies reporting selective inhibition of cancer cell proliferation accompanied by favorable selectivity indices (Akman, 2025). Together, these studies suggest that the bioactive compounds isolated from *Verbascum* may serve as promising leads for the development of novel anticancer agents and warrant further investigation through both in vitro and in vivo models.

4. *IN VITRO* CULTURE AND BIOTECHNOLOGICAL APPROACHES

Beyond the conventional extraction and isolation methods, recent research has also focused on the application of plant cell and tissue culture techniques to enhance the production of bioactive secondary metabolites in *Verbascum* species. Callus and organ cultures have been developed for species such as *Verbascum scamandri*, leading to significant improvements in the yield of phenylethanoid glycosides and flavonoids (Cambaz & Çördük, 2023). These in vitro systems not only provide a controlled environment for optimizing production but also offer the potential for large-scale biotechnological exploitation (Amini et al., 2024). Researchers have successfully employed elicitors, such as UV radiation and chemical agents, in cell suspension cultures to further stimulate metabolite synthesis, thereby bridging traditional phytochemical extraction with modern biotechnology (Temporiti et al., 2020). This integration of tissue culture with advanced analytical techniques represents a promising strategy for sustainable and reproducible production of high-value compounds from *Verbascum* in a manner that is both eco-friendly and cost-effective (Cambaz and Çördük, 2023). Additionally, the use of *Verbascum sinaiticum* extracts in the green synthesis of silver and zinc-ferric nanoparticles highlights their biotechnological potential in nanotechnology applications (Geyesa et al., 2024; Dinakarkumar et al., 2024).

5. CHEMOTAXONOMY AND COMPARATIVE STUDIES

Comparative phytochemical studies among different *Verbascum* species have provided valuable insights into the chemotaxonomic relationships within the genus. Detailed profiling efforts have revealed that while phenylethanoid glycosides, flavonoids, and iridoid glycosides are consistently present across many species, their relative abundances and specific structural features vary considerably (Jamshidi-Kia et al., 2020). For instance, investigations of Iranian *Verbascum* species have identified distinct chemotypes characterized by differential levels of rutin, luteolin, and apigenin, which appear to correlate with geographic and environmental factors (Jamshidi-Kia et al., 2020). Such studies have been instrumental in distinguishing species with high medicinal value from those with lower bioactive content, thereby aiding in the selection of candidate species for further pharmacological evaluation (Blanco-Salas et al., 2021). In addition, the integration of modern metabolomic approaches and chemometric analyses has allowed researchers to classify *Verbascum* species based on subtle variations in their secondary metabolite profiles, providing a robust framework for both quality control and taxonomic differentiation (Keaveney et al., 2020).

6. FUTURE PERSPECTIVES AND CHALLENGES

Despite the significant progress achieved in recent years, several challenges remain in the phytochemical study of *Verbascum* species. One major issue is the variability in metabolite composition due to factors such as geographical origin, seasonal variations, and differences in plant parts used for extraction (Jamshidi-Kia et al., 2020). This chemical heterogeneity often complicates the standardization of extracts and hampers the reproducibility of biological assays. Consequently, a concerted effort is needed to develop standardized harvesting, extraction, and analytical protocols that can reliably capture the true phytochemical diversity of the genus (Blanco-Salas et al., 2021). The development of eco-friendly extraction methods, as seen with *Verbascum* *sinaiticum*, is a step toward sustainable practices (Geyesa et al., 2024). Moreover, while significant advancements have been made using chromatographic and spectrometric techniques, there is still room for improvement in the resolution and sensitivity of these methods to detect minor compounds that may contribute to the therapeutic effects of *Verbascum* extracts (Keaveney et al., 2020). The integration of information-rich technologies such as NMR-based metabolomics and high-resolution LC-MS/MS with robust statistical and chemometric tools is expected to further enhance the depth of phytochemical analyses in the coming years (Keaveney et al., 2020). Another promising avenue is the application of in silico techniques to predict the biological targets of bioactive compounds. Recent studies have employed molecular docking and SwissTargetPrediction to link specific *Verbascum* metabolites with their potential pharmacological activities, providing a mechanistic basis for their medicinal properties (Blanco-Salas et al., 2021). However, these predictions require experimental validation through comprehensive in vitro and in vivo studies, which remains an important challenge for future research (Zhang et al., 2023). In addition, while the majority of studies have focused on the identification and quantification of non-volatile secondary metabolites, there is a noticeable gap in the detailed characterization of essential oils and other volatile fractions from *Verbascum* species. Given that essential oils can contribute significantly to the antimicrobial and anti-inflammatory activities of herbal preparations, further research that combines traditional extraction techniques with GC-MS will help fill this gap and broaden our understanding of the full chemical spectrum of *Verbascum* (Yabalak et al., 2020).

Finally, from a biotechnological perspective, the optimization of in vitro culture systems, including elicitation strategies and genetic transformation techniques, represents a promising approach to enhance the production of valuable secondary metabolites (Amini et al., 2024). Future research in this domain should aim to develop scalable bioprocesses that can consistently yield high concentrations of target compounds, thereby overcoming the limitations associated with wild harvesting and environmental variability (Cambaz and Çördük, 2023). The success of nanoparticle synthesis using *Verbascum* extracts further underscores their potential in innovative industrial applications (Geyesa et al., 2024; Dinakarkumar et al., 2024).

CONCLUSION

Recent phytochemical studies on *Verbascum* species have provided a wealth of information on the diversity, structure, and biological activities of several key classes of secondary metabolites, including phenylethanoid glycosides, flavonoids, and iridoid glycosides. Advanced analytical methodologies such as HPLC-DAD-ESI-Q-TOF-MS/MS and GC-MS have greatly enhanced our ability to characterize these compounds with high sensitivity and specificity, thereby contributing to a deeper understanding of the medicinal potential of this genus (Donn et al., 2023; Luca et al., 2019). Furthermore, integrated approaches combining tissue culture and modern biotechnological methods have opened new avenues for the sustainable production of bioactive substances (Amini et al., 2024). Comparative chemotaxonomic and metabolomic analyses have also laid the groundwork for standardizing and quality-controlling *Verbascum* extracts, which is essential for their successful implementation in clinical and industrial applications (Jamshidi-Kia et al., 2020; Blanco-Salas et al., 2021). The diverse applications of *Verbascum* in pharmaceuticals, cosmetics, food preservation, and nanotechnology, as demonstrated by species *Verbascum sinaiticum*, highlight their versatility (Geyesa et al., 2024; Dinakarkumar et al., 2024).

Despite these advances, challenges including variation in chemical composition and the need for improved analytical resolution continue to persist. Future efforts should focus on standardizing extraction methods, enhancing analytical techniques, and integrating in silico predictions with experimental validation to fully harness the therapeutic potential of *Verbascum* species (Keaveney et al., 2020; Zhang et al., 2023). The promising biological activities observed—ranging from potent antioxidant and anti-inflammatory effects to significant antimicrobial and anticancer properties—underscore the value of *Verbascum* as a rich source of invaluable natural products (Öztürk et al., 2019; Taleb and Saeedi, 2021).

In summary, the integration of modern analytical chemistry, bioassay-guided fractionation, and biotechnological approaches has significantly advanced our understanding of the phytochemical complexity of *Verbascum* species over the past few years. These efforts not only validate traditional uses but also pave the way for the discovery of novel compounds that could serve as leads for drug development and other industrial applications (Donn et al., 2023; Keaveney et al., 2020). Continued interdisciplinary research is essential to address the remaining challenges and to fully realize the potential of *Verbascum* as a reservoir of bioactive secondary metabolites for future therapeutic innovations (Yagi et al., 2024; Blanco-Salas et al., 2021).

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Chapter
2

EXAMINATION OF UNIVERSITY STUDENTS' OPINIONS ON THE STATUS OF THE WORLD POPULATION AND PRECAUTIONS THAT CAN BE TAKEN

Nurcan Özkan[1]

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INTRODUCTION

Today, the world population continues to increase despite falling fertility rates. According to United Nations estimates, the global population has increased by approximately 83 million people each year, reaching 7.3 billion in mid-2015 (United Nations-UN, 2015a), and has now surpassed that figure. This growth in population, which is expected to continue at a slower pace in the 21st century, can be considered as a result of the momentum created by the rapid increase in the 1980s and the fact that those born during this period have now reached the age of having children (Özgür, 2017).

Due to the population explosion and rising longevity experienced worldwide during the 20th century, the world now has both a younger population and the largest elderly population in history. The global population is represented by a large cohort of young people (those under 25), a product of high fertility on every continent except Europe. The number of young people has increased rapidly in recent years, reaching 3.1 billion in 2015, which corresponds to 42% of the world population (UN, 2015b). Africa, Asia and Latin America are home to 90% of the world's youth population. Even if the decline in global fertility rates is expected to continue in the future, it is estimated that the dynamics of the young population structure in African and Asian countries will increase the population under the age of 25 in absolute terms, reaching 3.4 billion by 2050. In this regard, creating health, education and employment opportunities for young generations is considered the most fundamental axis of the sustainable development agenda for poor countries and population groups (UN, 2015b).

A growing and urbanizing human population with expanding production and consumption patterns is triggering rapid global environmental change manifested by large-scale biodiversity loss, climate change, deforestation and land degradation, resource (water and soil) scarcity, altered biogeochemical flows and pollution. While the second half of the twentieth century saw major global health gains, the future of global health faces new challenges, including the increasing burden of noncommunicable diseases, increasing nutritional problems, exposure to new infectious diseases, displacement, injury, and mental health risks. A global action plan is being planned to address planetary health, revealing the links between human-induced disruptions in Earth's natural systems and their impacts on human health (Bulduk and Piyal, 2024; Veidis et al., 2021).

Technological developments after the industrial revolution, on the one hand, caused changes in consumption habits and an increase in consumption amounts, while on the other hand, it caused the rapid depletion of limited natural resources. The need to protect natural resources and environmental balance, which are important for the continuity of human life, the desire to maintain the consumption levels of both present people and future generations, and the desire for a better life reveal the necessity of sustainable consumption. Although many concepts (green consumption, savings, waste, media, etc.) are used together with the concept of sustainable consumption, it can be said that one of the most commonly used concepts is the concept of environment (Alma Savaş, 2022).

For decades, many population research groups have examined population size as one of the determinants of environmental degradation (Campbell, 1998). Pessimists, such as neo-Malthusians, focused on the role of population size in environmental collapse and participated in contemporary debates about the relationship between population and environment from the perspective of classical economics and the natural sciences (Hunter, 2000). In contrast, optimistic researchers argued that population growth and critical population densities essentially motivated agricultural innovations and changes (e.g., Boserup, 1965). They emphasized the importance of increasing crop productivity, rationality in the food production and consumption chain, and sufficient arable land potential in many developing countries (Johnson, 1984).

Since the ecological footprint is determined by population, per capita consumption, resource and waste density, ensuring ecological sustainability on a global scale requires that the environmental impact in the model remain within the limits determined by the carrying capacity of nature (Wackernagel et al., 2002). Figures comparing the world's biological productivity with humanity's demand for natural capital for each year since 1961 provide evidence that human activities have exceeded the capacity of the biosphere since the 1980s. In 2006, total global demand exceeded supply by approximately 40%, meaning that "humanity consumed enough food to meet its own needs to consume 1.4 Earths" (Ewing, et al., 2009). In 2007, the ecological footprint of per capita consumption in Turkey was 2.7 KHK, approximately 50% higher than the global biocapacity per capita. If all individuals in this world consumed as much as an average Turkish citizen, this would mean that humanity would need 1.5 planets. Türkiye is among the ecologically indebted countries because it consumes its natural resources faster than they can renew themselves. The main reason why the country has turned from a country with excess biocapacity to a country with a biocapacity deficit is seen as population growth (WWF, 2012a). WWF's study suggests that while the footprint per capita in Turkey increased relatively little over the years between 1961 and 2007, the 2.6-fold increase in the population in Turkey (from approximately 28 million to 73 million) during the same period caused a rapid increase in the total footprint (increasing 2.9-fold, from approximately 68 million kha to 197 million kha) (WWF, 2012a).

In addition, there is other data showing that resources are being over-consumed by the population, that too much waste is being produced, thus threatening life support systems. Every year, 6 million hectares of ecologically productive land in the world are becoming deserts; 17 million hectares of forests are losing their existence; oxidation and erosion of soil exceed soil formation by 26 billion tons per year; fishing grounds are collapsing, pollution and depletion of groundwater are increasing in many parts of the world, and ozone in the stratosphere continues to deplete (Aslan, 2010).

In addition to population growth, poverty reduction, employment creation and food security in the context of development also depend on increasing economic output in agriculture and beyond. The increase in these outputs has the power to intensify the pressures on all natural resources such as climate, soil, water and forest cover. Even if more people mean more jobs, it also means more water, food and energy; clothing, housing and infrastructure; and health and education services. Therefore, production needs to be increased significantly to meet the nutritional and other needs of the 2.4 billion people who will be added to the world population by 2050.

In this study, Trakya University Faculty of Education, Department of Science, senior students' perception of the world population situation and their opinions on what can be done about it were evaluated with open-ended questions.

MATERIAL AND METHODS

This research was conducted as a phenomenological design (Yıldırım and Şimşek, 2006) study. In the study, data were collected using qualitative research methods, using the opinions of Trakya University Faculty of Education, Department of Science Education students on the theme of human population in our world.

Data were obtained from 27 senior students in the Environmental Education course during the fall semester of the 2024-2025 academic year. In the course, the human population in the world and its ecological effects were first examined. Then, students were given A4 paper and asked two open-ended questions and were asked to explain their thoughts accordingly. The questions are as follows: 1. What do you think about the current state of the world population? 2- What can be done about it? Explain.

Then, each of the papers collected from the students was given a random code between 1-27. Of these codes, students with codes 3, 15, 16 and 19 are male, and the others are female. The statements made by the prospective teachers in the classroom environment about the situation of the world population and what could be done were then evaluated using the content analysis technique.

RESULTS AND DISCUSSION

This section includes the statements of senior science education students about the state of the world population and then their comments on what can be done about the population.

1. Student Statements About World Population

In this section, the opinions of 27 students about the world population were evaluated and discussed in code order.

Student coded 1 states that the world population will continue to increase in line with developments in business and medicine: "*The increase in the world population with the industrial revolution shows that as job opportunities increase, the number of people also increases. The continuous development of medicine shows that people are in good health. If we consider both, in terms of jobs and health, if these positive developments continue, the human population will continue to increase rapidly.*"

The student with code 2 touched more on the natural resource shortages in the world that arise based on population: "*The world's population has surpassed 8 billion, and this number continues to grow rapidly. Population growth is increasing the pressure on essential resources such as agriculture, water resources, and energy. Social problems are also increasing with urbanization. Housing shortages, resource constraints, and inadequate education and healthcare services are common. Inadequate healthcare services also increase the risk of disease spread. Moreover, this population growth is not balanced. Some areas are densely populated, while others are empty. This imbalance significantly impacts quality of life. In densely populated areas, challenges like pollution, overuse of resources, unemployment, and infrastructure problems affect people much more than in less densely populated areas. As a result, greater difficulties await people in the future, and planning must be made for this.*"

The student with code 3 expresses the problems the world has experienced and will experience in terms of resources from the past to the present and into the future. "*The problems of increasing the world population began to manifest themselves during the colonial period. The increasing need for raw materials has pushed states to seek other lands and exploit them. The largest outbreak occurred during World War I. Medical advances during the wars led to a decrease in mortality rates and an increase in birth rates. Population growth has been rapid. This has led to inequality and injustice in society and has led to the misuse of our planet for raw materials. In addition, due to uncontrolled and uneducated population growth, many people have begun to waste away in the "born, work and die" cycle. Although some studies say that the population growth rate decreases after a certain period, raw material resources will still decrease and humanity will face more difficult times. No matter how much control is attempted, I believe that humanity will reach a dead end due to the human population.*"

The student with code 4 discussed the pressure on natural resources and environmental degradation: "*The world's population has now surpassed 8 billion. As the population continues to grow rapidly, pressure on resources and environmental degradation (such as pollution) are increasing.*"

The student with code 5 explains the inequality in population between rich and poor countries: "*The world population is increasing rapidly, especially in developing countries. This is due to various socioeconomic factors. People are migrating to urban centers for better living conditions. However, this situation causes overcrowding and infrastructure problems in urban areas, and there is no solution to this problem. On the other hand, population growth creates greater inequality between rich and poor countries. While developed countries are in a more advantageous position in terms of access to resources, the situation is not the same in poor countries. At the same time, population growth increases environmental problems such as climate change and depletion of natural resources, and necessary precautions cannot be taken.*"

The 6-code student is addressed from different perspectives: "*While a growing population means a larger workforce and potential economic growth, it can also create problems with the adequacy of resources such as water, energy and food. For example, it is being discussed that tensions may arise in the future regarding water resources, and even that it may lead to wars.*"

The student with code 7 discusses the population growth rates and urbanization from past to present: "*The world population increased by only 1 billion between 1830 and 1930. In the following period, the world population increased by 1 billion in just 30 years. In the sixties (1960-1975), the time required for a billion was reduced to 15 years. According to this trend, the population tends to be 6x109 in 2000 and 8.2x109 in 2020. The distribution of the 6.2 billion population in the world in 2000 is also interesting. Of this population, 5 billion are in developing countries and 1.2 billion are in developed countries. Population growth has led to rapid urbanization in the second half of the 20th century, especially in developing countries. In the 35 years after 1960, the urban population in developing countries quadrupled, with an increase of 3.95%. As of 2023, the world's population is around 6 billion. Asia is the world's most populous continent.*"

Student coded 8 focuses on the positive and negative aspects of world population growth: "*The world's population has increased rapidly in recent years, bringing many opportunities but also challenges. Thanks to improved health services, agriculture and technology, life expectancy has increased and infant mortality has decreased; however, this rapid increase poses serious risks to the sustainable use of resources and environmental balances. Rural areas are becoming increasingly depopulated, while the density of population in large cities puts pressure on infrastructure. Global problems such as climate change,* *food security and water resources are becoming more complex with population growth. It is of great importance to manage this population growth in a more balanced and sustainable way in the future."*

Student coded 9 mentioned that people need to meet their basic needs and that this will lead to an increase in environmental problems: "*The world's population is increasing rapidly. The first thing that comes to mind for me is the struggle to meet people's basic needs. People first want to be full and never thirsty. Everyone must meet these needs to survive. As more people try to quench their thirst, water resources decrease, and as more people try to meet their food needs, plants and animals decrease. In addition, more factories are needed to process plants and animals. The same goes for water. An increase in factories means an increase in industrial zones. This will lead to environmental pollution. The increase in industrial zones will also lead to an increase in diseases. As people's natural resources dwindle and their own territories become polluted and diminished, they will begin to search for new lands. This may even lead to wars. Terrorism will increase and there will be nuclear competition. At the same time, there will be poverty and famine among the people. I said that people will first try to meet their basic needs, one of which is shelter. More people means more homes, buildings, and businesses. This will lead to increased car use and air pollution. When the air is polluted, the water will be polluted, and when the water is polluted, the soil, that is, the plants, will be polluted, and from there, the people and animals that eat these plants will become sick. In short, as the world population increases, the world will become uninhabitable."*

Student coded 10 includes some problems that occur due to resource consumption: "*The increase in the world population causes excessive consumption of natural resources, water and food security problems, increased energy demand and increasing environmental pollution day by day. This situation increases the pressure on climate change, threatens ecosystem stability, and can lead to problems such as poverty, access to education, and healthcare."*

Student coded 11 discussed the effects of the decrease in natural resources along with the population: "*Increasing population indicates that resources may be depleted further and environmental problems may increase. The rapid increase in the human population confronts us with the fact that resources on earth are limited. This situation may make it difficult to meet basic needs in the future. The human population is dependent on nature and resources, and it has its limits. The abundance of people reflects the challenge of uncontrolled population growthIt also increases the risk of competition and conflict between people as the population grows uncontrollably. Consequently, this increase can increase the likelihood of more conflict and harm between people, leading to higher crime rates."*

Student coded 12 addresses the problems that may arise with population growth: “*I think the world population is increasing unconsciously. As a result of this increase, it brings with it problems such as income-expense inequalities, environmental problems, climate change and resource depletion. Poverty increases, making access to healthcare particularly difficult. The risk of infectious diseases increases. It can also lead to famine in the future.*”

Student coded 13 focused on the environmental impacts of world population growth: “*World population growth has accelerated dramatically over the past few centuries. Birth rates have risen and death rates have risen. This has led to a rapid increase in the world's population. This growing population is increasingly putting pressure on global resources, increasing the risk of depleting resources like clean air. This intense pressure on ecosystems also deepens problems such as climate change and environmental pollution. Rapidly growing cities can make it difficult to provide basic services such as infrastructure, healthcare and education.*”

Student coded 14 discusses the negative and positive aspects of the increasing population: “*The world's population has been growing at an extraordinary rate in recent years. This population growth, fueled by medical advances, increased food production, and modern technologies, has placed immense pressure on global resources. Our world is now having difficulty supporting this dense population. This population growth has created an unprecedented increase in demand for essential resources such as water, energy, food and living space. In particular, the intensive use of water resources is causing us to move towards a water crisis. Food production requires vast land and water resources to meet growing demands. However, arable land in the world is limited, which brings about the expansion of agricultural areas and the destruction of natural forests. These pressures pose a threat not only to human health but also to the entire ecosystem. While energy consumption is increasing* rapidly, carbon emissions caused by fossil fuel use are accelerating climate *change. Currently, people are consuming the world's resources faster than nature's renewal rate. This rapid depletion of natural resources is becoming unsustainable. The growing population is also having a complex impact on the economy. Unemployment rates are rising. Educational opportunities may be inadequate, especially in areas with a young population. This means that young people cannot access economic opportunities. It also accelerates urbanization, driving up housing prices in major cities. This deepens social inequality. Overpopulation also threatens global biodiversity. The use of natural areas for agriculture and settlement causes the habitats of animal species to shrink and some species to disappear. The loss of biodiversity creates a situation that disrupts the balance in nature and affects the functioning of ecosystems, thus actually putting human life at risk. Population growth can sometimes present an opportunity for some countries. A young and dynamic population can contribute to economic growth. It can create new business opportunities. However, this only* *applies to areas where education, healthcare, and employment opportunities are adequate. In other words, population growth can only contribute to the welfare of society when it is well managed.*"

Student coded 15 expresses the unsustainable increase in population and the problems it will bring: "*The world's population is growing uncontrollably and exponentially. This uncontrolled and exponential growth is placing a serious burden on the world. If population growth continues at this rate, our resources will be depleted very quickly and we will experience serious resource difficulties. In such a situation, we would be in an unsustainable structure. Such population growth also brings with it environmental problems. Production is required for increasing needs, and this production has a negative impact on the environment in every area.*"

Student coded 16 also draws attention to the problems that arise with the increasing population and the precautions to be taken: "*The world's population is growing rapidly, creating many challenges. Issues such as resource overuse, environmental problems, and social injustices are becoming more pronounced. However, the existence of a young population provides economic potential. It can also pave the way for innovative solutions. For a better world, investments must be made in areas like education and healthcare. Everyone must contribute to a balanced future. Furthermore, a growing population is causing accelerated depletion of natural resources and environmental degradation. To overcome this situation, we need to turn to renewable energy sources and be more careful to protect the environment.*"

Student coded 17 discussed the pressures that the increasing population creates on sustainability: "*The rapid increase in the world population creates significant pressure on the sustainability of natural resources. We see that population growth, especially in developing regions, together with urban expansion and over-consumption of natural resources, leads to major environmental problems. We can list the problems caused by the increasing population as follows: Urbanization and urban expansion, decrease in water resources, decrease in food resources, increase in fossil fuel use along with energy demand, decrease in biodiversity.*"

Student coded 18 took into account the various environmental pressures created by world population growth: "*The rapid growth of the world population creates many critical problems, especially in a world where resources are limited. Rapid population growth further complicates global problems such as poverty, migration and climate change. It is becoming more and more difficult to meet the basic needs of more and more people, to protect the environment and to provide sustainable living conditions. I think that rapid population growth has shaken the economic and social balances. Underdeveloped and developing countries are having difficulty carrying this burden and are losing qualified manpower due* *to reasons such as brain drain. In developed countries, aging populations are increasing the demand for a young workforce. Migration and human mobility have also increased in parallel with the global population growth over the last 50 years. In cities experiencing migration, demand for housing, infrastructure, and government services is rapidly increasing. Urban sprawl and infrastructure problems are rapidly increasing in areas where migrants settle. As a result, the rapidly growing global population brings with it numerous problems, including poverty, soil infertility, deforestation, environmental pollution, and the erosion of social harmony. This growth is dragging us into a vicious cycle.*"

Student coded 19 touched upon the problems that human population growth may cause: "*The world's population continues to grow exponentially every day. This creates challenges. The population currently exceeds 8 billion. As the population grows, the world's resources decrease at the same rate. At the same time, social services are restricted. For example, there is a backlog in areas where people need basic services, such as education and healthcare. I think population density will be one of the biggest problems in the world in the future.*"

Student coded 20 expresses the negativities of population growth in countries: "*Our current population has surpassed 8 billion. According to the United Nations World Population Prospects Report, the world population could exceed 10 billion by 2100. The world's population is currently growing at a rate of nearly 2%. This rapid growth brings with it economic and social problems. It's undeniable that major global problems like poverty, migration, and climate change are linked to rapid population growth. Furthermore, rapid and unplanned population growth exacerbates global problems.*

According to calculations, if people are assumed to consume 6000 calories of energy per day, the world can feed a maximum of 11 billion people. However, once this number is reached, food supply won't be the only problem. As the soil approaches its carrying capacity, migration to cities increases. This exacerbates urban infrastructure problems, reducing urban safety, increasing the risk of theft and homicide, and exacerbating poverty and environmental pollution. *The impact of environmental problems on human health is undeniable. Heavy metals like DDT and mercury, when introduced into the food chain at any trophic level, significantly impact the highest-level consumer. Since the consumer does not eat a single food, these harmful substances, which are found in small amounts in every food, accumulate in high-level consumers and negatively affect their health.*

The number of individuals in developed countries is much less than those in developing countries. The biggest reason for this is the position and development level of women in society in developed countries. This difference in numbers was revealed in the study conducted in 2000, when the world population was 7.2 billion. Of this number, 5 billion are in developing countries, while only 1.2 billion people are in developed countries. In addition, an individual living in a *developed country puts 25-50 times more pressure on the environment than an individual living in a developing country. Due to the high consumption levels of industrialized countries, even a small population increase in these countries will cause environmental stress to reach its highest level.*

As the anti-growth Thomas Robert Malthus said, the production/consumption ratio is gradually decreasing because the human species is increasing at a geometric rate and food production is increasing at an arithmetic rate. Although the protective and restrictive controls he proposed for this purpose were perceived as catastrophic thoughts at the time, they are very accurate thoughts for today. Since the ideas of growth advocates are only valid for developed countries, if we look at the values from the 2000s, they were created for only 1.2 billion of the 7.2 billion people. This clearly demonstrates their invalidity.

Even though it is not visible in the population growth graphs, the population has started to slowly decrease. It is thought that the growth rate will decrease to 1.1% in 2040. This confirms the demographic transition hypothesis. In other words, as per capita income increases, population growth decreases. This hypothesis explains why poor countries are overpopulated."

Student coded 21 evaluated the effects of the population in developed and underdeveloped countries: "*The world's population has been increasing year by year. This population growth has both positive and negative aspects. On the positive side, for example, population growth in developed countries can be considered advantageous because they have more technological devices at their disposal or more organizations to contribute capital to. At the same time, food resources, shelter and economic conditions, which are important for people, are good. The increase here can increase the efficiency of the resources they have. For example, highly developed countries can leverage their technological know-how to turn their inadequacies into advantages. This requires human resources to do the same. Young, dynamic, knowledgeable people who should work here can contribute to the country. It can develop the country in any situation. For example, since the most basic aspects are health, education, economy, and justice, it can reach a much better level. It is the same in agriculture; the number of people working here and the development and abundance of the tools they will use are important. Having more people to use what they have means having more resources. But this situation is a serious problem for developing and underdeveloped countries. The situation here is even more serious than in developed countries. Population growth is higher in underdeveloped countries. Because for people here, having children is about having someone to care for their parents when they get old, and they think that having more resources will make it easier than the few they have. The population growth here causes the resources there to run out very quickly, the environment to become more polluted, and the economic income to decrease even further. After a while, they will even fall into a state of starvation due to the excessive increase. The countries here are generally* *poor, lack advanced technological equipment, or have not discovered the valuable lands in their countries, or lack the necessary equipment to enable this discovery even if they are discovered. Even if the number of people is high, if the resources are not available, this means that the country cannot develop. For developing countries, population growth may not be enough to cope with the situations they may encounter since they are starting something new. Collapses in these countries may become inevitable. Population growth in the world has caused many debates. A lot of research has been done on it, and as a result, forward-looking predictions have been made. Most predictions say that overpopulation will continue to increase over the years and that after a while, our world will not be able to sustain it."*

Student coded 22 discusses the world population in terms of natural resources and health: "*The world's population is still growing, especially in developing countries. This growth is accompanied by production and consumption. The use of natural resources is increasing and as the years go by, the resources we have will also be depleted. Natural resources aren't the only problem. As the world's and countries' populations grow, significant health problems will also arise. Most recently, in the pandemic we experienced in 2020, countries with very high populations, especially China and India, could not provide enough treatment for their patients and suffered high numbers of deaths. While population growth may seem positive from a humanitarian perspective, it needs to be brought under control. The Earth we live on will host us for a limited time."*

Student coded 23 addressed the opportunities and challenges of population growth: "*The growth of the world's population presents both opportunities and challenges. A growing population may increase the diversity and innovation of human resources, but it also increases the demand for basic services such as education, health and employment. In addition, problems such as environmental impacts, resource consumption and climate change are becoming more apparent."*

Student coded 24 examined the challenges brought by population growth: "*The world population has increased very rapidly in recent years and has exceeded the 8 billion level. This rapid growth continued due to factors such as high birth rates in developing countries and longer life expectancy in developed countries. Rapid population growth is increasing demand for natural resources, putting pressure on water, energy, agricultural land, and other resources. At the same time, it exacerbates problems such as environmental pollution, deforestation and climate change. Countries with a high proportion of young people also have a high proportion of working-age individuals. This can support economic growth, but it can also lead to increased unemployment in places where employment opportunities are limited. The rapidly growing population places a huge burden on health and education systems, especially in developing countries. This can restrict communities' access to basic services such as education and healthcare. Since world population growth is not evenly* *distributed, the concentrated population burden in some regions can lead to social and political tensions."*

Student coded 25 discussed the continuity of population growth and the problems it creates: "*The world population reached 6 billion in 1999, 7 billion in 2011 and 8 billion in 2022. The world population is growing. I believe it will increase even more if the necessary precautions are not taken. I think that the current world population is too high and that we will experience problems in economic, health, etc. areas due to this situation. If this situation continues, our natural resources may no longer be sufficient."*

Student coded 26 also expressed her views on the problems that have arisen and may arise as follows: "*It's a crucial issue for humanity. Today, the population has surpassed 8 billion, and this number continues to grow every year. This growing population increases the demand for water, food, energy, and other natural resources. This can lead to resource depletion and environmental problems. Urbanization rates are increasing, putting pressure on housing and transportation systems. Population growth also puts pressure on social security systems and health services. Since this rate is high in countries with young populations, it presents great opportunities and challenges in areas such as education, employment and social services. While population growth can contribute to economic growth, it can also lead to problems of excess demand and inequality. Therefore, understanding population dynamics and developing appropriate policies is critical. Investments in areas such as education, healthcare and women's empowerment can reduce the negative effects of population growth."*

Student coded 27 specifically addressed the negative aspects of global population growth as follows: "*The world population has increased rapidly in recent centuries and today has exceeded 8 billion. I anticipate further increases in the coming years. Rapid population growth presents many challenges and opportunities. For example: Rapidly increasing population leads to environmental problems such as excessive use of natural resources, pollution and loss of biodiversity; as the population increases, land fertility begins to decrease as land is used more intensively; population growth increases unemployment rates; increasing population causes faster depletion of basic resources; with increasing population, the population becomes dynamic; and with increasing population, production increases."*

2. What can be done about the world population?

The opinions put forward by students regarding the control of human population growth in the world are grouped and presented in Table 1.

Table 1. Student opinions on possible actions to be taken regarding reducing the world population

Precautions that can be taken	**Student codes**
Easy access to birth control	1, 2, 3, 4, 5, 6, 7, 9, 10, 12, 13, 14, 18, 19, 20, 22, 23, 24, 25, 26, 27
Family planning programs and awareness-raising activities	2, 3, 4, 6, 7, 8, 9, 10, 11, 12, 13, 14, 17, 18, 19, 20, 22, 23, 24, 25, 26, 27
Increasing reproductive health services	7, 23, 26
Limitation on population	1, 2, 15, 21, 23, 25, 27
The level of education should be increased	4, 7, 9, 10, 18, 20, 24, 25
Providing economic opportunities to families with few children	2, 9, 19, 20, 25
Higher taxes on families with more children	2, 20
Economic opportunities and job opportunities	4, 26
Reviewing migration policies for population balance in developed countries	5, 21, 22, 27
Promoting sustainable development through environmentally friendly policies	6, 7, 8, 11, 13, 14, 15, 16, 17, 18, 20, 21, 23, 24, 25, 26
Reducing food waste for increasing nutritional needs	6, 10, 17
Strengthening girls' education	7, 8, 10, 18, 20, 22, 23, 24, 25, 26
Implementing urbanization projects and increasing green areas	7, 10, 13, 14, 16, 17, 23, 24, 26
States breaking the cycle of poverty	8
Access to health services should be facilitated and improved	8, 9, 13, 26
Legal regulations preventing early marriage	8, 9, 25
Increasing productivity in agriculture	10, 17, 24
Developed countries providing support to developing countries	10, 21
Ensuring cooperation on international platforms for population and environmental problems	10
Regulations for the protection of ecosystems	10, 11, 14, 16
Achieving gender equality	18
Supporting rural development projects	18
Preventing population growth by increasing income per person	20, 22, 26

CONCLUSIONS

All developments have shown that humans can no longer be independent beings from the environment and that human societies are ecosystem-dependent. A new paradigm is needed. A shift from a human-centered understanding of the environment to a nature-centered understanding of the environment is necessary. For this reason, humans and the environment should be viewed within "Ecological Integrity" or "System Integrity" (Özerkmen, 2002). Findings obtained from student data support this situation. With population growth, ecosystems and natural resources are damaged in many ways and problems are increasing day by day.

The transition to a sustainable food system that can provide healthy diets for an estimated 10 billion people by 2050 is an unprecedented challenge. The Commission emphasises that today's data are sufficiently strong to warrant urgent action and that any delay would increase the likelihood of serious, even catastrophic, consequences. They noted that this transition can be achieved through a combination of significant dietary shifts towards mostly plant-based dietary patterns, dramatic reductions in food losses and waste, and significant improvements in food production practices. Such a "Great Food Transformation" will not happen without widespread, multi-sectoral, multi-level action guided by scientific goals (Anon, 2018; Haysom et al., 2019; Hidalgo et al., 2022).

For most of humanity's evolutionary history, such expansionist tendencies have faced negative consequences. On the other hand, scientific revolutions and the use of fossil fuels have given humanity opportunities for exponential growth and enabled it to reach its full potential, reducing the impact of many negative consequences. Humanity's excessive consumption of resources and constant short-term thinking are accelerating the sixth mass extinction that the Earth is estimated to be facing. It is predicted that food shortages, habitat loss, war and diseases will begin to reduce the population (Ören, 2023).

For this reason, underdeveloped or underdeveloped countries must either slow down their population growth rate or accelerate their economic development or their efforts to make the best use of their country's resources. While being able to do both is the ideal solution, the first option is easier. As the students stated, developed countries should help underdeveloped countries. Because this imbalance shown by the figures needs to be eliminated with serious measures before it causes major explosions around the world (Çamurcu, 2005). Overexploitation of natural resources combined with population growth will inevitably shrink the global economy, and humanity will experience a major population 'reclamation' in this century.

The precautions that teacher candidates focused most on in preventing the increasing world population were family planning programs and awareness-raising activities with 22 individuals. It seems that more work needs to be done on this issue, especially by the Ministry of Health and family medicine. It also states that 21 individuals should have easy access to contraceptive protection. In fact, while health centers used to provide support to families regarding birth control in the past, it was abolished in later years.

Prospective teachers (15 individuals) attach importance to promoting sustainable development through environmentally friendly policies. In this way, the lives of future generations are also safeguarded. Without sustainable policies, the lives of the growing population are also at risk. This will ensure the protection of natural resources, in particular.

One of the important issues that students focus on regarding population control is education, especially the education of girls. Educated families are more likely to do family planning and think about preparing a good future for their children. In the following years, there have been improvements in gender equality in education. Between 2000 and 2015, the number of girls for every 100 boys in primary education increased from 92 to 97, and from 91 to 97 in secondary education.

Between 2000 and 2015, the number of countries achieving gender equality in both primary and secondary education increased from 36 to 62. But some parts of the world, including Sub-Saharan Africa and South and West Asia, are not making the same progress.

According to the Education Commission's 2016 Learning Generation report, only two out of 35 countries in Sub-Saharan Africa have equal numbers of girls and boys in school, the lowest ratio among gender-equal countries. In many countries such as Afghanistan, Pakistan and Nigeria, there are attacks on girls' education and threats of school closures (URL 1).

Teacher candidates with codes 7, 8, 10, 18, 20, 22, 23, 24, 25, 26 support the need to strengthen the education of girls. Girls' education has many positive effects on society. Education is important for ensuring gender equality and establishing a civilized society. As educated girls grow into adults, they contribute to their families and their countries through their success in their professional lives. They also become more aware of health, the environment, sustainability, and culture. This contributes to individuals in society acting more consciously. This issue is of great importance for a society that is sensitive to women's rights, gender equality and human rights. Educated girls are sensitive to justice and equality. Therefore, they contribute to the creation of a peaceful and tolerant society where everyone is respected (URL 2).

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Chapter
3

PHYTOREMEDIATION POTENTIAL OF AZOLLA SPECIES FOR HEAVY METALS, POLYCYCLIC AROMATIC HYDROCARBONS, AND MICROPLASTICS: A REVIEW

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INTRODUCTION

Azolla, a genus of free-floating aquatic ferns, is renowned for its rapid growth, high biomass productivity, and symbiotic relationship with nitrogen-fixing cyanobacteria (*Anabaena/Nostoc/Trichormus azollae*), which collectively enable efficient nutrient accumulation and pollutant uptake from contaminated waters (Ahmad and Tariq, 2021; Akhtar et al., 2021). These attributes position *Azolla* as a promising candidate for phytoremediation, offering a cost-effective, eco-friendly, and sustainable approach to remediating polluted aquatic environments. Extensive research has demonstrated the efficacy of *Azolla* species, including *A. pinnata*, *A. filiculoides*, and *A. caroliniana*, in bioaccumulating heavy metals such as lead, cadmium, and mercury from industrial and municipal effluents (Akhtar et al., 2021; Jayasundara, 2022). Additionally, studies have highlighted *Azolla*'s ability to biodegrade polycyclic aromatic hydrocarbons (PAHs), such as naphthalene and phenanthrene, through metabolic transformation and microbial interactions (Kösesakal and Seyhan, 2023a, 2023b). Recent investigations have also begun to explore *Azolla*'s potential in addressing microplastic and nanoplastic pollution, revealing both phytoremediation capabilities and physiological impacts (Bottega et al., 2024; Dainelli et al., 2024; Kırkıncı et al., 2024). However, the limited data on microplastic remediation underscores a critical research gap. This review synthesizes recent advances in *Azolla*-based phytoremediation, covering heavy metal uptake, PAH degradation, and emerging perspectives on microplastics, while discussing challenges and future research directions to enhance its practical application.

1. HEAVY METAL PHYTOREMEDIATION STUDIES USING *AZOLLA*

Azolla species have been widely documented for their excellent heavy metal removal capabilities in polluted aquatic systems. Their rapid biomass accumulation coupled with significant biosorption and bioaccumulation capacities makes them suitable for the removal of heavy metals such as lead (Pb), cadmium (Cd), mercury (Hg), chromium (Cr), nickel (Ni), copper (Cu), and zinc (Zn) (Ahmad and Tariq, 2021; Akhtar et al., 2021).

1.1 Mechanisms of Heavy Metal Uptake

Heavy metal removal by *Azolla* involves several mechanisms. The living plants often absorb metals through their roots, an approach that is enhanced by high concentrations of pectins in their cell walls, which serve as binding sites for metal ions (Akhtar et al., 2021). In many studies, the biosorption process has been identified as the primary mechanism accounting for metal uptake. In addition, live *Azolla* plants engage in phytoextraction, whereby metals are absorbed into plant tissues and sequestered safely in non-photosynthetic tissues or within vacuoles (Akhtar et al., 2021; Herath et al., 2023). The induction of metal-detoxifying proteins, such as phytochelatins and metallothioneins, further enhances the plant's tolerance to metal toxicity and contributes to the sequestration and stabilization of heavy metals (Talebi et al., 2019). Both living and dead (dried) biomass are effective, with dead biomass sometimes outperforming live plants due to higher tolerance to extreme conditions and the absence of metabolic restrictions (Akhtar et al., 2021).

1.2 Experimental Studies and Performance Data

Multiple studies have evaluated heavy metal removal using *Azolla* in both batch and continuous treatment systems, with key findings summarized in Table 1. In one study, *A. pinnata* achieved removal efficiencies of up to 97.12% for lead, along with 92.84% for cadmium and 76.82% for nickel over 15 days, when exposed to metal concentrations of 5 to 25 mg/L (Jayasundara, 2022). Other investigations have documented bioconcentration factors (BCFs) in *Azolla* roots significantly exceeding those in the fronds, indicating a highly effective immobilization process in the root matrix (Akhtar et al., 2021; Jayasundara, 2022). *A. filiculoides* demonstrated high metal uptake efficiency from industrial wastewater by accumulating copper, zinc, cadmium, and nickel at rates that far exceed those observed in other aquatic plants, with Pb accumulation reaching 1.8% of dry biomass (Akhtar et al., 2021; Kosakivska, 2022). Similarly, *A. pinnata* showed up to 90% removal efficiency for Hg and Cd from industrial effluents (Khan et al., 2020). Experimental setups, including batch reactors and fixed-bed systems, have reported improvements in physicochemical parameters such as pH stabilization, reduction of chemical oxygen demand (COD), and decreased total dissolved solids (TDS) in the presence of *Azolla* (Kumar et al., 2020; Talebi et al., 2019). Comparative studies also show *A. pinnata* and *Lemna gibba* achieving high heavy metal absorption in rice farming systems (Herath et al., 2023). *A. imbricata* exposed to waste metal-cutting fluid accumulated Al (540 mg/kg), Cd (282 mg/kg), Cr (71 mg/kg), Fe (1645 mg/kg), Pb (2494 mg/kg), and Zn (1110 mg/kg), acting as a hyperaccumulator for Zn (Amare et al., 2023).

Table 1. *Summary of heavy metal removal efficiencies by Azolla species*

Species	**Metals Targeted**	**Removal Efficiency (%)**	**Conditions/Notes**
A. pinnata	Pb, Cd, Ni	76–97	5–25 mg/L, 15 days (Jayasundara, 2022)
A. filiculoides	Cu, Zn, Cd, Ni	High accumulation	Industrial wastewater (Akhtar et al., 2021)
A. pinnata	Hg, Cd	Up to 90	Effluents (Khan et al., 2020)
A. imbricata	Al, Cd, Cr, Fe, Pb, Zn	70–90+ (hyper for Zn)	Waste fluid; stress factors (Amare et al., 2023)

1.3 Molecular and Physiological Responses

On a molecular level, heavy metal exposure in *Azolla* triggers significant gene expression changes that enhance detoxification and tolerance. Specifically, genes encoding *metallothioneins* (MT) and *phytochelatin synthases* (PCS) are upregulated in response to metals such as copper, cadmium, and nickel, facilitating the sequestration of these metals into less toxic forms (Talebi et al., 2019). Metallothioneins are cysteine-rich proteins that bind heavy metals, reducing their cytotoxicity, while phytochelatin synthases catalyze the synthesis of phytochelatins, peptides that chelate metals and sequester them in vacuoles, thus preventing cellular damage. Transcriptomic analyses, including *de novo* assembly for non-model organisms like *Azolla*, have revealed that these genes are highly expressed under metal stress, contributing to the plant's hyperaccumulation capacity (Talebi et al., 2019). This molecular response is complemented by physiological adaptations, including the increased production of antioxidant enzymes such as superoxide dismutase (SOD) and catalase (CAT), which mitigate oxidative stress caused by metal toxicity (Ahmad and Tariq, 2021; Akhtar et al., 2021). These combined molecular and physiological mechanisms underscore the robust capacity of various *Azolla* species to act as hyperaccumulators of heavy metals, supporting their use in both remediation of contaminated wastewater and improvement of soil quality in agricultural settings (Kumar et al., 2020).

2. PHYTOREMEDIATION OF POLYCYCLIC AROMATIC HYDROCARBONS (PAHs)

Alongside the removal of heavy metals, *Azolla* species have been explored as phytoremediators for polycyclic aromatic hydrocarbons—an important group of organic contaminants derived from petroleum products and combustion processes.

2.1 Mechanisms Behind PAH Uptake and Biodegradation

A. filiculoides has emerged as a potent candidate in the removal of PAHs such as naphthalene and phenanthrene. The process involves not only physical adsorption onto plant surfaces but also metabolic transformation facilitated by the production of secondary metabolites like flavonoids and phenolics that play a defensive role under pollutant stress (Kösesakal and Seyhan, 2023a, 2023b). Experimental work has shown that exposure to naphthalene leads to elevated levels of photosynthetic pigments and secondary metabolites, suggestive of stress response mechanisms that concurrently enhance biodegradation (Kösesakal and Seyhan, 2023a). Al-Baldawi et al (2023) demonstrated that *A. filiculoides* achieves 100% removal of pyrene and phenanthrene (10–30 mg/L) over 10 days, with accumulations of 0.007–0.011 mg/g fresh weight (FW) for phenanthrene and 0.048–0.079 mg/g FW for pyrene, confirmed by Gas chromatography–mass spectrometry (GC-MS) detection of 10 biodegradation by-products, though growth parameters declined.

2.2 Experimental Evidence on PAH Remediation

Recent laboratory experiments utilizing *A. filiculoides* under controlled conditions have demonstrated that the fern can biodegrade up to 94% of naphthalene at low concentrations (25–50 mg/L) within 10 days (Kösesakal and Seyhan, 2023a). Phenanthrene studies reported biodegradation efficiencies of 88%, 69%, and 60% for initial concentrations of 1, 5, and 10 mg/L, respectively, over 14 days, with rapid uptake in the first hours and pigment stability at lower doses (Kösesakal and Seyhan, 2023b). GC-MS analyses confirmed naphthalene absorption and biodegradation, with rates reaching 94% after 10 days (Kösesakal and Seyhan, 2023a). Petroleum hydrocarbon remediation in soil-water systems was also effective, highlighting *A. filiculoides*'s versatility.

2.3 Interactions with Associated Microorganisms

Intrinsic to the effectiveness of PAH phytoremediation by *Azolla* is its association with microbial communities in the rhizosphere. These microorganisms are specialized in degrading complex PAHs. The close proximity of these microbial populations to the roots of *Azolla* creates a microenvironment that enhances the biodegradation process, leading to more efficient removal of PAHs from contaminated water (Kösesakal and Seyhan, 2023a, 2023b). The interplay between plant exudates and microbial enzyme systems thus constitutes a critical component of the overall remediation strategy, making *Azolla*-based systems highly promising for the treatment of PAH-contaminated waters (Kösesakal and Seyhan, 2023b).

3. EMERGING PERSPECTIVES ON MICROPLASTICS

The phytoremediation potential of *Azolla* species has been well-documented for heavy metals and polycyclic aromatic hydrocarbons (PAHs) due to their high pectin content and efficient adsorption mechanisms. However, their role in addressing microplastic (MP) and nanoplastic (NP) pollution—defined as plastic particles less than 5 mm and less than 100 nm in diameter, respectively—remains a largely unexplored frontier. Micro- and nanoplastics pose significant ecological threats due to their persistence and potential for bioaccumulation in aquatic ecosystems. The physicochemical properties of *Azolla*, such as the high pectin content in its cell walls, suggest potential interactions with various pollutants, yet the particulate nature of micro- and nanoplastics introduces unique challenges distinct from the adsorption of dissolved ions or hydrophobic organic compounds.

3.1 Interactions of *Azolla* Species with Micro- and Nanoplastics: Phytoremediation Potential, Physiological Impacts, and Symbiotic Disruptions

A recent study by Kırkıncı et al. (2024) provides experimental evidence of *Azolla caroliniana* Willd.'s capability to address MP pollution in freshwater systems. The study utilized a controlled growth medium containing fluorescent red polyethylene microspheres (250–350 µm) at concentrations of 1, 5, and 10 mg/L to assess the phytoremediation potential of *A. caroliniana*. Removal efficiency and bioconcentration factor (BCF) were measured on days 3, 5, 7, and 14. Notably, the microplastics were not absorbed by the plant due to their size but adhered to the roots and submerged leaf surfaces, facilitating their removal from the water column. After 14 days, *A. caroliniana* achieved average MP removal efficiencies of 27.6%, 23.8%, and 21.1% at 1, 5, and 10 mg/L concentrations, respectively. The highest BCF values were observed at lower concentrations, with 0.50 and 0.45 for 1 and 5 mg/L, respectively. These findings indicate that *A. caroliniana* can contribute to MP removal through surface adhesion, though efficiency decreases with higher MP concentrations.

Complementing these findings on microplastics, Bottega et al. (2024) investigated the interaction of *Azolla filiculoides* Lam. with polystyrene nanoplastics (NPS, 30 nm, green fluorescent) at a concentration of 50 mg/L under optimal (25°C) and high (35°C) temperatures for 7 days. Using fluorescence microscopy, the study demonstrated NPS adsorption primarily on root surfaces at 25°C, with signals in outer protective layers. At 35°C, uptake increased, extending to vascular tissues in roots and becoming more prevalent in fronds, particularly along leaf edges. This suggests that elevated temperatures enhance NPS penetration and translocation within the plant.

The study by Bottega et al. (2024) also revealed adverse physiological effects. Photosynthetic pigments decreased in a temperature- and NPS-dependent manner: total chlorophyll dropped to 0.24 mg/g FW at 35°C with NPS (from 0.59 mg/g FW in controls at 25°C), with reductions in chlorophyll a/b ratio (to 1.45) and carotenoids/total chlorophyll ratio (to 0.11). Oxidative stress markers showed hydrogen peroxide (H2O2) increasing significantly at 35°C with NPS (to ~0.35 µmol/g FW), while thiobarbituric acid reactive substances (TBARS, indicating lipid peroxidation) rose at 25°C with NPS but declined at 35°C. Antioxidant responses varied: enzymatic activities (ascorbate peroxidase, guaiacol peroxidase, catalase) decreased at higher temperatures, phenols and proanthocyanidins (condensed tannins) reduced with NPS, but proline peaked at 0.74 µmol/g FW at 35°C with NPS. Histochemical analyses confirmed heightened H2O2 and lipid peroxidation in shoots and roots under combined stress. Root morphology altered, with scarcity of root hairs near tips and shortened meristems at high temperatures and with NPS.

Further insights into the effects of nanoplastics on *Azolla* come from Dainelli et al. (2024), who examined the impact of polyethylene terephthalate micro-nanoplastics (PET-MNPs, mean size 190–230 nm) at environmentally relevant concentrations (0.05 and 0.1 g/L) on the symbiotic system *Azolla filiculoides-Trichormus azollae* over 10 days. No visible toxicity symptoms, growth disorders, or alterations in leaf anatomy and chlorophyll fluorescence parameters were observed. However, treated plants exhibited decreased chlorophyll content (chlorophyll content index, CCI, reduced by ~20-30% at higher concentrations) coupled with a reduction in Nitrogen Balance Index (NBI, ~25-35% lower), indicating impaired nitrogen status. Ionome analysis revealed substantial declines in essential elements: Ca (~40% reduction), Mg (~30%), Co (~50%), and Mn (~35%) at 0.1 g/L. Root integrity was compromised, with physical damages in the rhizodermis, cortex, and vascular system, potentially affecting nutrient uptake.

Dainelli et al. (2024) also reported disruptions in the symbiosis: a DNA-based estimation showed a decreasing trend in the relative abundance of *T. azollae* (normalized via qPCR using RPL25 and ITS loci, with 2^(-ΔCt) values ~20-30% lower in treated samples). Microscopy revealed phenotypic alterations in the cyanobacteria, including a reduction in the number of vegetative cells between consecutive heterocysts (from ~15-20 in controls to ~10-15 in treated) and heterocyst size (diameter reduced by ~15-20%). These changes suggest potential negative effects on nitrogen fixation and supply to the fern, with implications for ecosystem nitrogen cycling and biofertilization applications.

Despite these promising results on surface adhesion for MPs and adsorption/uptake for NPs, the mechanisms underlying plastic removal by *Azolla*—primarily particle capture and sedimentation—differ significantly from those involved in metal or organic pollutant adsorption (Jayasundara, 2022). The current literature lacks comprehensive studies on the optimization of *Azolla* for micro- and nanoplastic phytoremediation, such as through chemical or physical modifications of its biomass to enhance adsorption capacity or integration with other remediation technologies targeting particulate pollutants. Furthermore, the observed removal efficiencies suggest that *Azolla* alone may not suffice for large-scale remediation, highlighting the need for further research into synergistic approaches. The findings from Bottega et al. (2024) underscore potential risks, as increased NP uptake at higher temperatures could exacerbate toxicity and facilitate entry into food chains, particularly relevant for *Azolla* species used as feed or fertilizer. Similarly, Dainelli et al. (2024) highlight symbiotic disruptions that could impair nitrogen fixation, affecting agricultural yields and evolutionary dynamics.

In conclusion, while *Azolla caroliniana* demonstrates potential for microplastic phytoremediation through surface adhesion, and *Azolla filiculoides* shows nanoplastic adsorption with temperature-dependent uptake and symbiotic impacts, their effectiveness is limited compared to their well-established role in heavy metal and PAH remediation. The findings of Kırkıncı et al. (2024), Bottega et al. (2024), and Dainelli et al. (2024) mark critical steps forward, but significant knowledge gaps remain. Targeted studies exploring biomass modification, long-term plastic removal dynamics, hybrid remediation systems, the implications of climate change (e.g., rising temperatures), and symbiotic interactions are essential to fully harness *Azolla* species for addressing the global challenge of micro- and nanoplastic pollution while mitigating risks to ecological and agricultural systems.

4. CHALLENGES, LIMITATIONS, AND FUTURE DIRECTIONS

The promising laboratory and pilot-scale results for *Azolla*-based phytoremediation of heavy metals, PAHs, and microplastics highlight its potential as a sustainable remediation strategy. However, several challenges and limitations must be addressed to enable its widespread practical application, particularly in diverse environmental settings and for emerging pollutants like microplastics.

4.1 Environmental and Operational Limitations

Azolla's remediation efficiency is highly dependent on environmental conditions, including temperature, light intensity, pH, and nutrient availability (Akhtar et al., 2021; Peyda et al., 2023). For instance, high concentrations of heavy metals or microplastics can induce phytotoxicity, reducing growth rates and remediation performance (Jayasundara, 2022; Sabreena et al., 2022). Studies by Bottega et al. (2024) and Dainelli et al. (2024) demonstrate that nanoplastic exposure, particularly at elevated temperatures, exacerbates physiological stress in *A. filiculoides*, leading to reduced chlorophyll content and impaired nitrogen fixation. Similarly, excessively contaminated environments may overwhelm *Azolla*'s tolerance thresholds, limiting its efficacy in heavily polluted sites. Field-scale applications require robust strategies to maintain optimal growth conditions, such as controlled nutrient inputs or shading systems, to ensure consistent performance across varying climatic and pollution scenarios.

4.2 Biomass Disposal and Secondary Pollution

The accumulation of heavy metals, PAHs, and microplastics in *Azolla* biomass necessitates careful management to prevent secondary pollution. While conversion of contaminated biomass into bio-oil via pyrolysis or bioenergy feedstock has been proposed (Ahmad and Tariq, 2021; Kumar et al., 2020), the presence of microplastics introduces additional complexity. Unlike metals or PAHs, microplastics are not biodegradable, and their disposal requires specialized handling to prevent re-release into the environment (Bottega et al., 2024; Dainelli et al., 2024). The economic and environmental feasibility of these downstream processes remains underexplored, particularly for microplastic-laden biomass, which may pose unique challenges in thermal processing or recycling due to its persistence and potential to release toxic additives.

4.3 Limited Efficacy for Microplastics and Symbiotic Impacts

While *Azolla* demonstrates high removal efficiencies for heavy metals (up to 97%) and PAHs (up to 100%) (Akhtar et al., 2021; Al-Baldawi et al., 2023), its efficacy for microplastics is significantly lower, with *A. caroliniana* achieving only 21.1–27.6% removal for polyethylene microspheres (Kırkıncı et al., 2024). The reliance on surface adhesion rather than uptake or degradation limits its capacity for large-scale microplastic remediation. Additionally, Dainelli et al. (2024) highlight that PET micro-nanoplastics disrupt the *A. filiculoides-T. azollae* symbiosis, reducing cyanobacterial abundance and nitrogen fixation capacity, which could compromise *Azolla*'s utility as a biofertilizer. These findings underscore the need for strategies to enhance microplastic capture and mitigate symbiotic disruptions, particularly in agricultural applications where nitrogen fixation is critical.

Given the complex nature of environmental pollution, no single remediation strategy is likely to provide a complete solution. The inherent limitations of *Azolla*-based phytoremediation – including issues of biomass saturation, sensitivity to extreme contaminants, and limited capacity for handling particulate pollutants such as microplastics – suggest that integration with complementary treatment methods may be necessary (Akhtar et al., 2021; Ali et al., 2020). For example, a combined system that integrates *Azolla* phytoremediation with constructed wetlands or membrane filtration might offer a more comprehensive solution to mitigate the effects of heavy metals, PAHs, and potentially microplastics concurrently.

4.4 Integration with Complementary Technologies

The complex nature of environmental pollution necessitates integrated remediation approaches. *Azolla*'s limitations, such as biomass saturation and sensitivity to high pollutant concentrations, suggest that standalone phytoremediation may be insufficient for comprehensive pollutant removal (Akhtar et al., 2021; Ali et al., 2020). Combining *Azolla*-based systems with constructed wetlands, microbial bioreactors, or membrane filtration could enhance the removal of heavy metals, PAHs, and microplastics concurrently. For instance, constructed wetlands could leverage *Azolla*'s surface adhesion for microplastics while microbial systems degrade organic pollutants, creating a synergistic framework for multi-pollutant remediation.

4.5. Future Research Directions

To address these challenges, several research directions are proposed:

1. **Molecular and Genetic Enhancements**: Expanding research on molecular mechanisms, such as metallothionein and phytochelatin synthase gene expression (Talebi et al., 2019), could lead to genetically modified *Azolla* strains with enhanced tolerance and accumulation capacities for heavy metals and microplastics. Exploring genes involved in stress responses to nanoplastics could mitigate physiological impacts observed by Bottega et al. (2024).

2. **Biomass Modification for Microplastics**: Systematic studies on chemical or physical modification of *Azolla* biomass, such as surface functionalization, could enhance its affinity for microplastics, improving removal efficiencies beyond the current 21–27% reported by Kırkıncı et al. (2024). Research into bio-based adsorbents derived from *Azolla* could offer sustainable solutions for particulate pollutants.

3. **Field-Scale Studies**: Long-term field studies under diverse ecological conditions are essential to assess the scalability and persistence of *Azolla*-based remediation systems (Khan et al., 2020; Kosakivska, 2022). These studies should evaluate *Azolla*'s performance in real-world settings, including its response to fluctuating temperatures and pollutant mixtures, as highlighted by Bottega et al. (2024).

4. **Techno-Economic and Environmental Assessments**: Comprehensive analyses of the economic viability and environmental impacts of *Azolla*-based systems, particularly for biomass disposal and microplastic management, are needed to ensure sustainable implementation (Ahmad and Tariq, 2021; Kumar et al., 2020).

5. **Symbiotic Interaction Studies**: Further research into the effects of microplastics on the *Azolla-T. azollae* symbiosis, as noted by Dainelli et al. (2024), is critical to preserving nitrogen fixation capabilities, especially in agricultural contexts. Strategies to protect cyanobacterial populations from nanoplastic stress could enhance *Azolla*'s dual role as a remediator and biofertilizer.

5. CONCLUSION

Azolla species, including *A. pinnata*, *A. filiculoides*, and *A. caroliniana*, have demonstrated remarkable phytoremediation potential for heavy metals and PAHs, driven by their rapid growth, high biomass productivity, and robust biochemical and molecular mechanisms (Ahmad and Tariq, 2021; Akhtar et al., 2021). Removal efficiencies exceeding 90% for metals like lead, cadmium, and mercury, and up to 100% for PAHs like pyrene and phenanthrene, underscore their efficacy in controlled conditions (Jayasundara, 2022; Al-Baldawi et al., 2023). Emerging studies on microplastics reveal that *A. caroliniana* can remove 21–27% of polyethylene microspheres via surface adhesion, while *A. filiculoides* adsorbs polystyrene nanoplastics, with increased uptake at higher temperatures (Kırkıncı et al., 2024; Bottega et al., 2024). However, microplastic remediation efficiencies are lower than those for metals and PAHs, and nanoplastic exposure induces physiological stress and symbiotic disruptions, reducing chlorophyll content, nutrient uptake, and nitrogen fixation (Bottega et al., 2024; Dainelli et al., 2024).

The challenges of environmental variability, biomass management, limited microplastic removal capacity, and symbiotic impacts necessitate innovative solutions. Integrating *Azolla* with complementary technologies, such as constructed wetlands or bioreactors, could enhance multi-pollutant remediation (Ali et al., 2020). Future research should focus on genetic enhancements, biomass functionalization, field-scale studies, and techno-economic assessments to overcome these limitations and extend *Azolla*'s applicability to microplastics (Talebi et al., 2019; Khan et al., 2020). By addressing these gaps, *Azolla*-based phytoremediation can evolve into a sustainable, scalable strategy for mitigating heavy metals, PAHs, and emerging pollutants like microplastics, contributing to global efforts to restore polluted aquatic ecosystems and support sustainable agriculture (Peyda et al., 2023; Kosakivska, 2022).

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