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Table of Contents

A Commendation

Michael Brett-Crowther, 2025 Editor-in-Chief

pp. 1–2

Letters to the Editor

Relocating Nepal's Problematic Tigers to Suitable Habitats: A Lens of Management

Bhesh Kumar Karki, Thakur Silwal, Shukra Raj Paudel

pp. 3–6

Articles Section

Isolation of Cryptococcus from Pigeon Droppings in Central Casablanca

Chaimae El Karkouri, Safaa Sebbahi, Malika Naman, Hajar Boughroud, Samira Rais, Fatima Amarir

pp. 7–21

A Comparative Study of the Physicochemical and Nutritional Properties of Moroccan Picholine Olives and Related Varieties: A Pathway to Development and Improvement

Oumaima Mrabet, Bouchaib Bahlaouan, Said El Antri, Nadia Boutaleb

pp. 22–44

Biofilms in the Food Industry: Adhesion Mechanisms, Control Strategies, and Mastery

Ghita Radi Benjelloun, Othmane Chouhbi, Bouchaib Bahlaouan, Mohamed Bennani, Said El Antri, Nadia Boutaleb

pp. 45–82

Iraq's Desertification: The Complication of Environmental Security Issues

Salam Abdulqadir Abdulrahman

pp. 83–103

The Kakhovka Sea Before and After the Destruction of the Kakhovka Dam

Vitalii Pichura, Larysa Potravka, David Dent

pp. 104–133

Consequences of Sabotage of the Kakhovka Dam on the Waters of the Dnipro-Buh Estuary and the Black Sea

Vitalii Pichura, Larysa Potravka, Pavlo Kutishchev

pp. 134–160

The Biggest Explosions at Ammunition Depots During the Russia–Ukraine War

Viktor Vyshnevskyi

pp. 161–178

Book Review Section

The Heart of the Wild: Essays on Nature, Conservation, and the Human Future

Edited by B. A. Minter and J. B. Losos; contributions by B. Adams, J. Berger, S. Clayton, E. Crist, M. L. Crump, T. L. Fleischner, H. W. Greene, H. Herzog, E. Marris, K. D. Moore, G. P. Nabhan, P. H. Raven, C. J. Schell, R. Shine, and K. Whyte

Princeton University Press, 2024, 280 pp., £22.00 (hardback)

ISBN: 978-0-691-22862-4

Reviewer: Nadia Boutaleb

pp. 179–183

Garbocracy: Towards a Great Human Collapse

Sayan Dey

Peter Lang Group AG, 2025, XX + 128 pp., US\$72.95 (eBook)

ISBN: 978-1-80374-727-9 (PDF), DOI: 10.3726/b22308

Reviewer: Kanav Narayan Sahgal

pp. 184–186

Before They Vanish: Saving Nature's Populations – and Ourselves

Edited by Paul R. Ehrlich, Gerardo Ceballos, and Rodolfo Dirzo

Johns Hopkins University Press, 2024, 320 pp., \$29.95 (hardback)

ISBN: 978-1-4214-4969-2

Reviewer: Nadia Boutaleb

pp. 187–189

Managing Biodiversity in Agricultural Landscapes: Conservation, Restoration and Rewilding

Edited by Nick Reid and Rhiannon Smith

Burleigh Dodds Science Publishing, 2024, 543 pp.

ISBN: 978-1-80146-454-3 (hardback) £150

PDF: 978-1-80146-456-7; EPUB: 978-1-80146-455-0

Reviewer: David Dent

pp. 190–197

Biomass-based Clean Technologies for Sustainable Development: Sustainable Resources of Energy Production and Utilisation

Edited by Dan Bahadur Pal and Ashish Kapoor

Springer, 2024, 268 pp., €159.99 (hardcover)

ISBN: 978-981-97-0846-8

Reviewer: Ahmad Reza Maulana

pp. 198–200

A COMMENDATION

This journal's title has a functional purpose. It means to state that human health and the health of the environment with all its living components depends on its integration. As the complexity of life has increased so has the complexity of environmental problems. These are very often a multi-problem, a problematic; which means that this many-sided problem or multi-problem, or problématique contains its solutions. Waddington [1] made clear that anyone concerned with any of the world's major problems (e.g., population, food supplies, energy) would have to understand all of the major problems and how they interact in producing the problématique. Waddington also referred to the 'networks of networks' which face the researcher, and which require not so much specialised knowledge as general knowledge of the major problems for their solutions to be identified and applied [1, p. 10f.]

Waddington notes the work of Meadows *et al.* [2], and says that these researchers were the first to use computer simulation to study the 'World Problématique' [1, p. 281].

We may say that a worldview in which biology is a central feature must be adapted to solving the multi-problems confronting humanity. This would allow ethics and religion to have their values active in the attempted solving of problems, as much as a sharp awareness of the dangers of human aggression, which is everywhere related to the major problems. Ethics and religions must challenge human aggression and must try to restrain it.

A worldview featuring biology underlies the Sustainable Development Goals. These may be said to indicate necessary facts for social and economic development without adverse impact; e.g., agriculture with no-tillage (Conservation Agriculture) and reduced chemical inputs; development which raises the status of women and encourages girls to aim for an education; resource use which includes reuse and recycling in its system.

This journal, **Natural Built Social Environment Health (NBSEH)**, has a Middle East and North Africa orientation, but looks beyond. Many may feel that the hopes of a New Partnership for Africa's Development (NEPAD) have been dissipated. It is clear that in many respects many regions of the Earth are in transition. In France, the Ministère de la Transition écologique, de la Biodiversité, de la

Forêt, de la Mer et de la Pêche operates with the general idea that ‘la transition écologique et solidaire’ affects everyone with justice and a spirit of cooperation: a process in solidarity. It is easy to see many contrary approaches. Consumerism is a deeply rooted habit of over-consumption and personal indebtedness which has joined with the availability of mass credit (*i.e.*, possibility of incurring debts) and is associated with exuberant fossil fuel use and avoidance of alternative energy systems.

But global heating will not be universally beneficial. Impaired air quality is widespread. The pollution of soil is just as dangerous as the loss of soil by erosion and pollution. The pollution of water resources is dangerous in the everyday life of millions of people. A process in solidarity must have an objective towards which a society in transition moves with hope. NBSEH’s idea of the environment and health is worth considering.

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LETTER TO THE EDITOR

Relocating Nepal's Problematic Tigers to Suitable Habitats: A Lens of Management.

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Sir,

Relocating wild species is a practical and conservationist approach which addresses the growing problem of declining animal populations by moving them from captivity or the wild to areas where their populations are at risk or have gone extinct [1]. Relocation has aided some populations in the wild, but the benefits in conservation sustainability have been accompanied by significant challenges including inter and intra species disease transfer between the relocated and native one. Further, these schemes have been criticized for lack of success and substantial concerns on husbandry practices that produce genetically or behaviourally marginalized individuals [1,2]. Despite these limitations, Nepal may face challenges with individual tigers (*Panthera tigris*) that often enter human settlements and attack people and livestock because their habitat has become degraded or intra-species competition, where weaker tigers are pushed out of their habitats. One potential solution could be animal diplomacy with other countries.

Nepal's proposal to offer surplus tigers (tigers which have become problematic in their normal territory) to countries with suitable habitats represents a significant contribution to global wildlife conservation. Owing to successful conservation efforts, the tiger population has grown substantially, nearly tripling the target of doubling between 2010 and 2022. In 2022, the wild tiger population in Nepal reached 355, up from 121 in 2010 [3], dispersing across five protected areas covering 5,200 km² [4] and adjoining forests. Tigers are territorial animals requiring large territory areas to thrive with each requiring about 50 km² of territory & prey density of 150 animals/km² [5]. Over the past six years, tiger attacks have claimed 68 lives, with fatalities nearly doubling from 2018 to 2024 [6]. This underscores the challenges of coexisting with tigers in areas with high populations. With limited habitat [7] and rising human-wildlife conflict, a strategic solution is essential to balance conservation process with ecological and

social stability. Relocation with proper plans can reduce pressure on existing reserves and ensure tigers thrive in suitable areas, addressing both wildlife and human needs [8].

The selection of tiger relocation sites requires thorough assessments to ensure ecological suitability, with sufficient prey and habitat to support tiger populations, offering ample space and food [9]. The area must also have strong anti-poaching laws and a robust conservation infrastructure to protect the relocated tigers. Additionally, enhancing genetic diversity is crucial to strengthening the species' gene pool and preventing inbreeding, ensuring long-term sustainability. The relocation sites must comply with both national and international wildlife laws, and include local commitments to ensure the success and sustainability of the relocation efforts.

Tiger relocation from Nepal opens doors for global conservation partnerships [10]. Countries with similar climatic conditions and strong ecological infrastructures, facing declining tiger populations, could benefit from Nepal's surplus. Organizations such as Worldwide Fund for Nature (WWF), International Union for Conservation of Nature (IUCN) and the Global Tiger Forum can facilitate translocations, ensuring adherence to best practices. Relocation poses challenges such as transport stress, adaptation issues, and wildlife conflicts, requiring careful planning and proper monitoring to mitigate risks [9,11]. Countries like China (China's Panda diplomat – giant pandas loaned on careful terms) and India (an example of successful conservation with the Indian Lion and also the tiger) offer valuable lessons on habitat preparation and community involvement in wildlife translocation [12].

Moreover, local community support is crucial for successful translocations, and eco-tourism incentives can help foster cooperation [13]. In addition to tiger relocation, Nepal should continue expanding suitable habitats outside protected areas, strengthen anti-poaching laws, and promote community involvement in tiger-human coexistence. This can be achieved through dedicated conflict mitigation programs to increase community tolerance towards tigers. Local and global cooperation should be strengthened to ensure the long-term success of conservation efforts, creating a more sustainable future for both wildlife and local communities.

Nepal's proposed plan to relocate surplus tigers for various purposes including tiger diplomacy can support wildlife conservation and sustainable ecosystem management. By addressing the

ecological needs of tigers and the challenges of human-wildlife coexistence, this initiative seeks to balance biodiversity conservation with local community welfare. With careful planning, scientific rigor, local and international collaboration, this approach can serve as a model for other nations facing similar conservation issues. Furthermore, strengthening collaboration from the local level to global partnerships will be crucial to ensuring long-term success, as demonstrated by Nepal's achievement of more than doubling its tiger population within a decade. This initiative not only benefits tigers but also contributes to broader efforts to safeguard endangered species rather than resorting to population control measures like hunting, thereby ensuring the conservation of habitat and thus its suitable residents worldwide.

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Yours sincerely,

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Isolation of *Cryptococcus* from Pigeon Droppings in Central Casablanca

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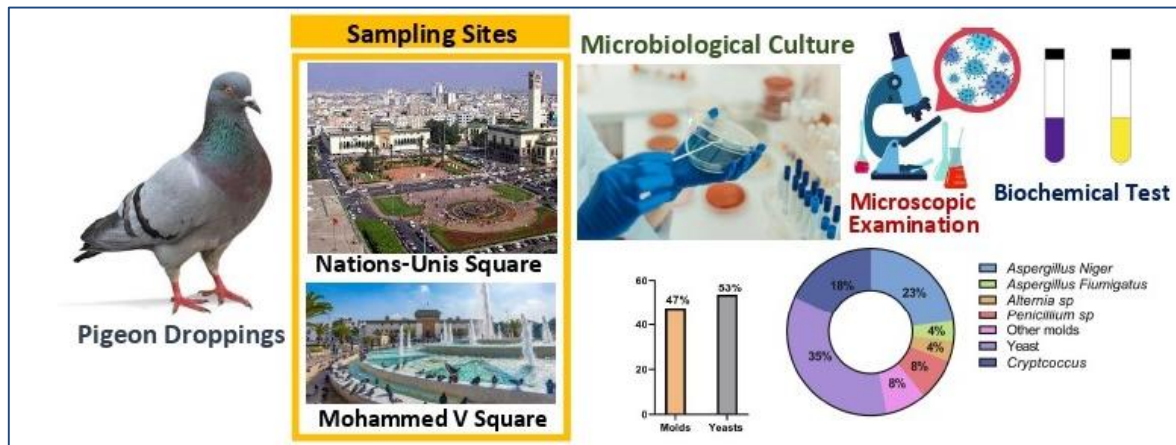
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Graphical Abstract

Cryptococcus sp. was detected in 20% of pigeon droppings collected in Casablanca, highlighting environmental exposure as a potential health risk for neuromeningeal cryptococcosis.



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Abstract: *Cryptococcus neoformans* is an encapsulated yeast that can cause potentially fatal neuromeningeal cryptococcosis in immunocompromised individuals. Contamination occurs through the inhalation of soil aerosols and pigeon droppings contaminated with *C. neoformans*. In Casablanca, neuromeningeal cryptococcosis remains a severe opportunistic infection in HIV patients (1.2%), with a high mortality rate estimated at 60%. The aim of this paper is to evaluate, for the first time, the rate of contamination in pigeon droppings in Casablanca. Samples of pigeon droppings were collected from different sites at Mohammed V Square and United Nations Square. All samples were inoculated onto Sabouraud Chloramphenicol medium without actidione and incubated at 37°C for 48 hours. Macroscopic examination of the culture revealed yeast colonies (53%) and opportunistic moulds (47%). Confirmation by urease test showed 20% positive samples. The preliminary isolation of *Cryptococcus sp.* in Casablanca is of major interest in identifying the *Cryptococcus* species and assessing the health risk to the population.

Keywords: *Cryptococcus*, *Cryptococcosis*, *Pigeon droppings*, *Meningitis Casablanca*.

Introduction

Cryptococcosis is a common opportunistic systemic mycosis caused by encapsulated yeasts of the *Cryptococcus* genus [1-3]. The primary aetiological agents of the disease are classified into two species, *Cryptococcus neoformans* (serotypes A, D, and AD) and *Cryptococcus gattii* (serotypes B and C) [4,5]. *C. neoformans* has been isolated from bird habitats and pigeon droppings, as well as from the intestinal tract and crop milk of pigeons [6,7]. *C. gattii* is associated with organic matter in trees and decaying wood [8]. The creatinine contained in pigeon droppings serves as a source of nitrogen for the fungus, promoting its development and facilitating its survival in the soil, with estimates of up to 50 million yeasts per gramme of pigeon droppings [9-11].

Human contamination occurs mainly via the respiratory route, through the inhalation of environmental propagules and aerosols from soil and pigeon droppings contaminated with *Cryptococcus neoformans* [12-14]. The fungus enters the body through the lungs, causing pulmonary disease, which, in most cases, heals spontaneously [15]. But, because of its neurotropic nature, *Cryptococcus* can spread haematogenously to the central nervous system, which is the main target organ [16]. This can lead to cryptococcal meningitis or meningoencephalitis, conditions that can be fatal, particularly in immunocompromised individuals, organ transplant recipients, patients with cancer, those on corticosteroid therapy, and even smokers and diabetics [17,18].

Before the AIDS pandemic, the incidence of cryptococcosis worldwide was approximately 0.2 to 0.8 cases per 100,000 inhabitants per year [19-21]. Today, there is a significant upsurge in this infection linked to the spread of AIDS, with 181,000 deaths annually [22]. In Morocco, neuromeningeal cryptococcosis represents the third leading cause of death after tuberculosis and intestinal cryptosporidiosis in HIV-infected patients (77%), with an estimated mortality rate of between 13% and 60% at the Ibn Rochd University Hospital in Casablanca [23,24]. Pigeons, which are abundant in Casablanca, are the primary hosts for the sexual reproduction of *Cryptococcus* [25,26]. They are implicated in the epidemiology of this infection, acting as both disseminators (through droppings and soil) and amplifiers of the infection [27]. For this reason, a preliminary exploration of public places frequented by pigeons is essential to identify potential sources of contamination for the population.

With this in mind, the aim of our study is to explore the prevalence of *Cryptococcus* in pigeon droppings at Mohammad V Square (“Sahat el Hmam”) and United Nations Square. These areas are frequented by children, visitors, and tourists and are known for their abundance of wild pigeons.

Materials and Methods

Sampling Sites

Casablanca, located in west-central Morocco along the Atlantic coast, approximately 80 km south of Rabat, spans an area of 384 km². As the economic capital of the country, it is renowned for its large population and forms part of the Casablanca-Settat region. Among its best-known landmarks are Mohammed V Square and United Nations Square. Mohammed V Square, often referred to as the "Square of Birds," is a bustling public square surrounded by key institutions, including the Prefecture, the Court, the Post Office, and Bank Al-Maghrib (the Kingdom's Central Bank). Situated just south of United Nations Square, these two locations reflect Casablanca's rich historical heritage and remain popular destinations for both residents and tourists.

Samples Collection

The study was conducted in April 2024, with a total of 10 pigeon droppings collected — five from Mohammed V Square (33° 35' 37" N, 7° 36' 08" W) and five from United Nations Square (33° 35' 41" N, 7° 37' 07" W) — sourced from various sites within these locations (Table 1). Sterile containers were labelled prior to sample collection. Using a disinfected tool, samples were carefully collected, placed into the containers, and securely sealed afterward (Figure 1).

Table 1. Key to sampling sites

Sampling location	Sample number	Sampling site	Type of sample
Mohammed V Square	1	Right-hand side before the fountain	Pigeon droppings
	2	Right side behind fountain	
	3	Left side behind fountain	
	4	Left-hand side before the fountain	
	5	At the entrance to the square	
United Nations Square	6	Wall	Pigeon droppings
	7	The earth	The ground
	8-9-10	1 and 2 and 3 from the ground	



Figure 1. Sampling Sites and Techniques: (a) Mohammed V Square, (b) Entrance to the Square, and (c) Sampling Methodology.

Sample Processing

The droppings were processed following a method adapted from Mariat's protocol for isolating *Sporothrix schenckii* from soil dust [28]. A total of 400 mg of droppings were weighed, finely ground in a mortar, and suspended in 10 mL of sterile distilled water in a tube. The suspension was vortexed for 10 min, then allowed to settle for 15 min (Figure 2).

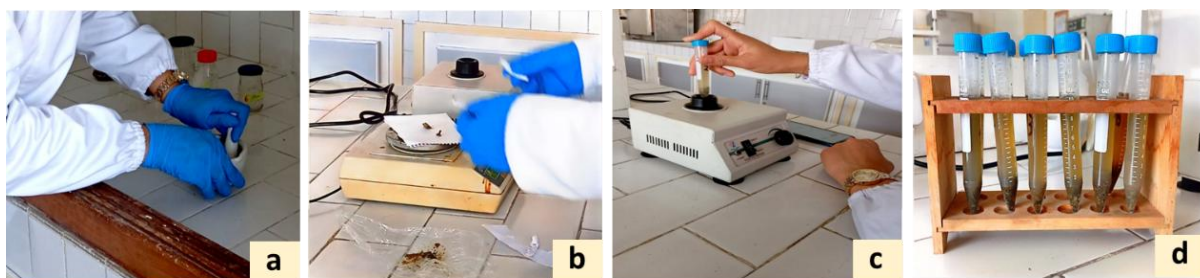


Figure 2. Sample Processing Stages: (a) Weighing, (b) Grinding, (c) Stirring, and (d) Sedimentation.

Seeding and Incubation

Inoculation is carried out in a fume hood by spreading 0.5 mL of the suspension over the Petri dishes and tubes containing the culture medium, using a flamed metal handle. The seeded tubes and Petri dishes were then incubated at 37°C (Figure 3).



Figure 3. Inoculation technique: (a) Flaming the loop on a Bunsen Burner, (b) Sample collection, and (c) Inoculation of media.

Microscopic Examination with India Ink

The slides are numbered and degreased. A drop of 1:5 diluted India ink is placed on each slide, followed by the addition of a well-isolated colony from the culture. The colony is mixed with the India ink and covered with a coverslip. The slides are then arranged in a grid and examined under a light microscope at 40X magnification.

Urease Test on Indole Urea Medium

The tubes were labelled, and 2 ml of Urea Indole medium was added to each. A yeast colony was then collected with a loop and suspended in the medium. The mixture was vortexed and incubated at 37°C for a minimum of 4 h. After incubation, results were interpreted: a colour change from yellow to pink indicated a positive test.

Statistical Analysis

The results are presented as mean values with corresponding standard deviations (SD). Statistical analysis and graph plotting were conducted using GraphPad Prism version 8.0.2 software.

Results

Macroscopic Examination

After culturing, the macroscopic appearance of the colonies was examined and described. The distinctive characteristics of each colony type, including shape, texture, colour, and any notable pigmentations, are systematically recorded in Table 2 below, which summarizes the typical macroscopic aspects of the isolated colonies.

Table 2. Typical macroscopic aspects of isolated colonies

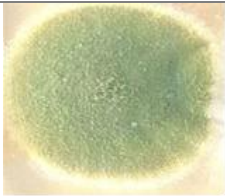
<i>Aspergillus Niger</i>	<i>Aspergillus Fumigatus</i>	<i>Alternaria sp</i>
		
Blackish granular to bushy colonies with a colourless underside, sometimes diffused with a bright yellow pigment.	Dark green to greyish-black colonies with a downy appearance.	Grey to black colonies with a fluffy to cottony appearance and a thick texture.
<i>Penicillium sp</i>	<i>Cryptococcus sp</i>	Yeasts (<i>Candida</i> and others)
		
Presence of grey-blue to grey-green colonies; the colonies are dense, powdery, and fluffy.	Smooth, mucous, shiny colonies, ranging from white to beige to ochre in colour.	White, creamy colonies with a smooth or finely downy appearance.

Figure 4 shows the distribution of fungal species based on macroscopic examination. Yeasts (53%) were more prevalent than moulds (47%), with *Cryptococcus sp.* accounting for 18% and *Candida* and other yeasts making up 35%. Among moulds, *Aspergillus niger* was the most common, comprising 23%.

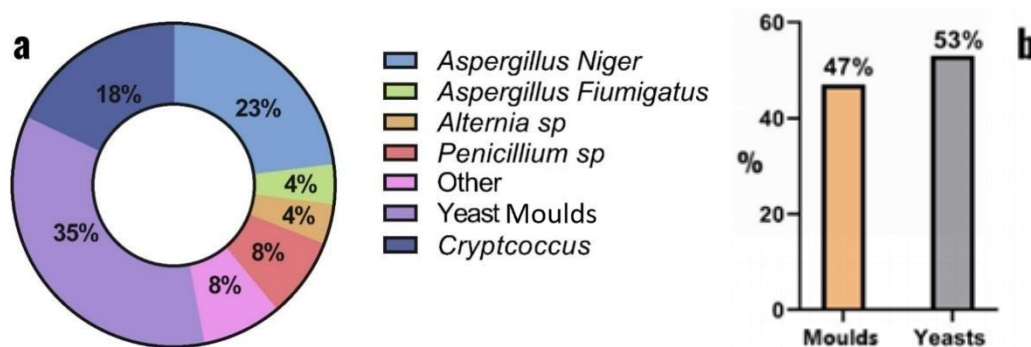


Figure 4. Detailed distribution of fungal species (a) and overall proportions of Moulds and Yeasts (b) based on macroscopic colony examination.

Results of Microscopic Examination with Indian Ink

Of the ten samples processed in our study using India ink microscopy, samples 1, 3, 6, and 10 tested positive. Encapsulated *Cryptococcus* yeasts were observed under an optical microscope at 40× magnification (Figure 5).

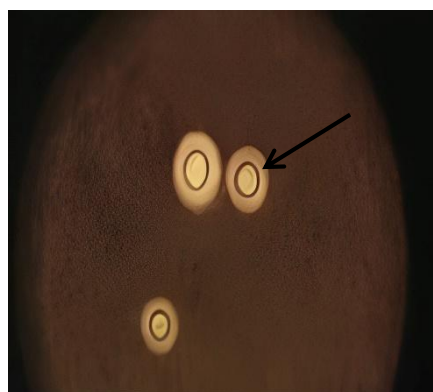


Figure 5. Microscopic observation of *Cryptococcus* (encapsulated yeast) at 40X magnification. The black arrow indicates the capsule.

Urease Test Results

Of the ten samples processed in our study using the urease test, samples 1 and 3 were positive, as indicated by a colour change from yellow to pink (Figure 6).



Figure 6. Urease test results.

Comparative Results

In our study, we found that 20% of the samples were positive for *Cryptococcus*, but 80% were negative.

Table 3. Comparative results of culture, direct examination, and Urease test.

	Examination type		
	Macroscopic examination	Direct examination (India ink)	Urease test
Results	4 out of 10 samples are positive for <i>Cryptococcus spp.</i>	2 out of 10 samples were positive for <i>Cryptococcus spp.</i>	2 out of 10 samples tested positive for <i>Cryptococcus spp.</i>
Mean ±SD	0.4±0.155	0.3±0.145	0.2±0.126

Discussion

Cryptococcosis is a common and serious opportunistic fungal infection affecting various parts of the body in both immunocompromised and immunocompetent individuals [29]. It is mainly caused by two yeasts of the *Cryptococcus* genus: *C. neoformans* and *C. gattii* [30]. Pigeon droppings serve as a natural reservoir for these yeasts, with transmission occurring through the inhalation of dust particles contaminated with *Cryptococcus* [31]. To explore environmental contamination by *Cryptococcus* in Casablanca, we collected samples of pigeon droppings from Mohammed V Square and United Nations Square, areas known for their high abundance of pigeons. Our study isolated 47% moulds (23% *Aspergillus niger*, 4% *Aspergillus fumigatus*, 4% *Alternaria sp.*, 8% *Penicillium sp.*, as well as other moulds) and 53% yeasts (18% *Cryptococcus sp.* and 35% *Candida* and other yeasts).

The presence of moulds in the samples can be attributed to their high abundance in the environment and their rapid growth on Sabouraud agar. As part of our identification efforts, we performed the urease test, which revealed that 2 out of 10 samples tested positive for *Cryptococcus spp.* Interestingly, both positive samples were obtained from pigeon droppings at Mohammed V Square, near the fountain. The samples from United Nations Square were negative. The absence of *Cryptococcus* in the latter can likely be explained by the low number of pigeons in this area, because a regulation prohibits pigeon feeding. An advertising barrier has been put in place to enforce this rule, which has further reduced the presence of pigeons and, consequently, their droppings in the area. Our study showed that the *Cryptococcus sp.* positivity rate was 20% among the samples collected. To our knowledge, this is the first investigation into the isolation of environmental *Cryptococcus* in Casablanca.

In comparison, our results are consistent with those of a 2021 study by Touati *et al.* in Algeria, which examined various environmental samples such as pigeon droppings, leaves,

eucalyptus seeds, citrus leaves, and soil. The overall *Cryptococcus* positivity rate was found to be 16%, with 3 samples identified as *Cryptococcus terreus* (12%) and 1 as *Cryptococcus uniguttulatus* (4%) [28]. Additionally, a 2020 study conducted by Hamroune in Algeria, focusing on pigeon droppings collected across several wilayas, reported a *Cryptococcus* positivity rate of 5.55% from 116 samples. This included 2 *Cryptococcus neoformans* (0.92%), 9 *C. albidus* (4.16%), and 1 *C. laurenti* (0.46%) [32]. Thus, *Cryptococcus* positivity rates in Algeria range from 5.55% to 16% [28,32]. Moreover, a Tunisian study carried out in 2011 by Mseddi *et al.* concerned 950 samples of pigeon droppings and plant matter from various regions of Sfax. In pigeon droppings, *C. neoformans* was found in 26 isolates (10.4%), with a higher prevalence in dry droppings (9.2%) compared to wet droppings (1.2%) [33].

The literature reveals a lack of consistency in the reported positivity rates, with variations observed across studies. This discrepancy may stem from several factors. Firstly, the studies were conducted in different countries (Morocco, Algeria, Tunisia) and regions, each with its unique environmental conditions, such as temperature and humidity, which have a significant impact on the emergence, reproduction, and transmission of *Cryptococcus*. Additionally, the initial state of the collected droppings, whether wet or dry, plays a crucial role. Previous studies have indicated that yeast is more commonly isolated from dry droppings than from wet ones. Dry droppings provide a more favourable substrate, as they contain fewer bacteria, and the lack of moisture limits bacterial growth, reducing competition for resources.

Our study represents a pilot investigation with a relatively small sample size (10) compared to other studies conducted in the Maghreb region. Given this limited sample, it is possible that increasing the sample size could lead to higher positivity rates. The observed high *Cryptococcus* positivity in our study may be attributed to the targeted sampling locations near the fountain, where multiple pigeon droppings were likely pooled into a single sample. Additionally, the isolation of different *Cryptococcus* species, both environmental and of medical significance, may have contributed to the findings. This highlights the importance of using the Api gallery and molecular biology for species identification and assessing the potential health risks posed to the population of Casablanca, particularly those frequenting these areas. *Cryptococcus* is a serious threat to human health, as it can affect multiple organ systems, including the bloodstream and internal organs, and can cause fatal meningoencephalitis if left untreated [34]. This risk is particularly pronounced for immunocompromised individuals, such as those with AIDS, as the HIV envelope protein gp120 inhibits the ability of human alveolar macrophages to phagocytose *Cryptococcus* [35]. Even immunocompetent individuals are at risk, especially if infected with *C. gattii* [36].

Our preliminary results constitute a preventive approach to cryptococcosis, as our isolates were found in pigeon droppings, suggesting a potential public health risk from human exposure

to airborne *Cryptococcus* spp. cells and other fungi at Mohammed V Square "Sahhat el Hmam". These results reinforce the hypothesis that pigeon droppings are an important factor in infection by this yeast in Casablanca, highlighting the relationship between environmental health and public health.

Conclusion

This preliminary study, the first of its kind in the public squares of Casablanca, reveals significant contamination of pigeon droppings by *Cryptococcus* and various other pathogenic fungi, including *Candida* spp., *Aspergillus niger*, *Aspergillus fumigatus*, *Alternaria* spp., and *Penicillium* spp. A comprehensive environmental study is essential to enhance our understanding of the epidemiology of cryptococcosis in Casablanca and throughout the country. To investigate further, it would be beneficial to expand the study to include a larger sample size from diverse *Cryptococcus* habitats and to identify the species and serotypes circulating in these areas using techniques such as the Api gallery and PCR. Additionally, implementing educational and preventive measures in public spaces and educational institutions could play a crucial role in reducing or eliminating the sources of *Cryptococcus* contamination.

Ethics Approvals: Not applicable.

Funding: None.

Data Availability: The data that support the findings of this study are available from the corresponding author upon reasonable request.

Code availability: Not applicable.

Conflict of interest: All the authors declare no conflicts of interest.

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A Comparative Study of the Physicochemical and Nutritional Properties of Moroccan Picholine Olives and Related Varieties: A Pathway to Development and Improvement

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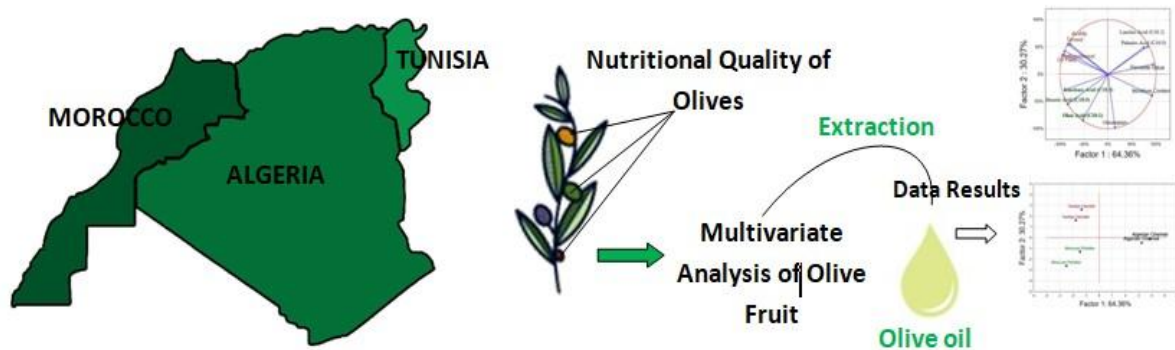
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Graphical Abstract

This research focuses on a comparative study of the nutritional quality of olives in Maghreb countries (Morocco, Algeria, Tunisia). It combines multivariate analysis of olive fruits, olive oil extraction, and data interpretation through advanced statistical tools. The study adopts a regional approach to valorise olives and their derived products, providing valuable insights for improving production processes and enhancing the quality of olive oil.



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Abstract: Understanding the quality of Moroccan Picholine olives is essential for both consumers and producers. This study investigates their physicochemical and nutritional properties, comparing them to neighbouring varieties: Tunisian Chemlali and Algerian Chemlal. Our analysis reveals that Moroccan Picholine olives have a significantly higher oleic acid content (68.47%), associated with cardiovascular health benefits, compared to lower levels in the other varieties. Additionally, they demonstrate superior oleuropein levels (167.81 mg.kg⁻¹), a phenolic compound linked to antioxidant properties, while showing potential for increased hydroxytyrosol content. Quality assessments indicate moderate moisture content (53.66%) and acidity (0.29%), alongside a competitive oil yield (42.32%) and low peroxide value (4.37 meq/kg), suggesting good oil freshness. These findings highlight the nutritional advantages of Moroccan Picholine olives and propose strategies for enhancing cultivation and processing methods to elevate their quality and market position.

Keywords: Moroccan Picholine, Nutritional properties, Market differentiation.

Introduction

The olive tree holds a distinguished position within Mediterranean cultures and territories, to the extent that it is often regarded as the quintessential indicator of the Mediterranean climate zone. Once climatic conditions allow, olive trees are promptly planted and become a prominent feature of the landscape. They define the Mediterranean region in which they thrive. Different olive varieties are valued for their unique characteristics, such as flavour, oil yield, and disease resistance. These attributes influence their suitability for various products, including table olives and olive oil, and play a significant role in their economic impact within the agricultural sector [1].

In Morocco, the olive tree accounts for 65% of the total tree-growing area, covering 1,045,186 ha. Of this, 37% (384,528 ha) is irrigated, while 63% (660,658 ha) is non-irrigated. In terms of production, 65% of olives are used for pressing, 25% for canning, and the remaining 10% is allocated to losses and domestic consumption [2]. Moreover, olive cultivation is a vital socio-economic sector, accounting for up to 5% of the agricultural GDP and 15% of the country's agricultural food exports [3]. It stands out in the Mediterranean olive-growing landscape with its unique varietal profile. Despite having several local varieties, the 'Moroccan Picholine,' locally known as 'Zeitoun' or 'Zeitoun Beldi,' remains the dominant variety [4]. The Ministry of Agriculture's technical services designate this variety as 'Moroccan Picholine' (PM) and estimate that it comprises at least 92% of the olive trees in Moroccan orchards.

Moroccan Picholine olives are celebrated for their distinctive flavour, making them highly sought after in both domestic and international markets. Their unique taste and texture make them ideal for use in table olives as well as in olive oil production [5]. This variety thrives in Morocco's particular climatic and soil conditions, which imparts distinctive physicochemical properties. Attributes such as oil content, acidity, and flavour profile are essential for assessing their quality and determining their suitability for various applications. The nutritional profile, including fatty acid composition and antioxidant content, has implications for health and wellness [6]. Understanding the physicochemical and nutritional properties of Moroccan Picholine in comparison to neighbouring varieties will help producers better position their product in the market based on quality, taste, and health benefits [7]. By evaluating the environmental impact of different olive varieties, such as water use and soil management, it is possible to adopt more sustainable practices. This can help in reducing the environmental footprint of olive cultivation [8].

The physicochemical properties of olive oils and their abundance of bioactive components have a major impact on their nutritional value. Key ingredients that contribute to their health benefits include

fibre, vitamin E, and oleic acid. Oleic acid, a monounsaturated fatty acid, is well-known for its cardio protective properties, as it lowers bad cholesterol and promotes overall heart health [7]. Furthermore, hydroxytyrosol and oleuropein, two phytochemicals found in abundance in olive oils, have anti-inflammatory and antioxidant qualities that can reduce oxidative stress and the risk of developing chronic illnesses. The fibre content of olives adds to their health benefits by fostering satiety and supporting digestive health. Establishing a clear classification system based on these parameters can better highlight the nutritional advantages of olive oils and help consumers make informed choices, ultimately showcasing the potential for particular kinds, such as Moroccan Picholine olives, to stand out in the market [6].

The aim of this study is to comprehensively evaluate and compare Moroccan Picholine olives with selected neighbouring olive varieties across several key dimensions. This includes assessing and contrasting their physicochemical characteristics, such as oil content and acidity, alongside their nutritional profiles, including levels of vitamins, minerals, and antioxidants. The study also seeks to analyse the economic implications and market potential for Moroccan Picholine olives, focusing on production costs, market prices, and consumer preferences. Additionally, the research will explore the agricultural requirements and adaptability of these olives, examining factors such as soil and climate needs, and resistance to diseases and pests. Finally, the study aims to identify and promote sustainable cultivation practices by evaluating resource use efficiency and environmental impact, thereby providing valuable insights for enhancing olive production and market strategies

Materials and Methods

Date and Period of Study

The study was conducted over a period of six months, from november 2022 to june 2023. This timeframe allowed for comprehensive data collection on the physicochemical and nutritional properties of Moroccan Picholine olives, as well as for their comparison with neighbouring varieties, Tunisian Chemlali and Algerian Chemlal. The extended duration was essential to ensure that seasonal variations and environmental factors were adequately accounted for in the analysis

Sample Collection

In this study, the olive varieties selected for comparison include Moroccan Picholine olives, along with several neighbouring varieties, such as Algerian Chemlal and Tunisian Chemlali. Moroccan Picholine olives are celebrated for their unique flavour profile and culinary versatility. Grown predominantly in

the fertile regions of Morocco, these olives are prized not only for their taste but also for their high-quality oil, which is rich in healthy monounsaturated fats. As represented in Figure 1, Moroccan Picholine olives are often harvested at various stages of ripeness, allowing producers to create a range of products, from table olives to premium olive oil. Their firm texture and distinctive green hue make them a popular choice in Mediterranean cuisine, while their nutritional benefits, including antioxidants and healthy fats, contribute to their growing popularity in health-conscious markets.



Figure 1. Moroccan Picholine Olives tree.

Samples were collected from local olive farms in the Fes-Meknes and Gharb regions of Morocco (Figure 2). These locations were selected to reflect the diverse growing conditions and agricultural practices characteristic of the country's olive cultivation. This focus on Moroccan Picholine olives enabled an in-depth analysis of their unique physicochemical and nutritional properties.

Description Sites

The study was conducted in two major olive-growing regions of Morocco: the Fes-Meknes region and the Gharb region.



Figure 2. Geographic regions of high-quality Moroccan Picholine olive production.

Fes-Meknes Region

Located in central Morocco at approximately $34^{\circ} 02' 00''$ N, $5^{\circ} 00' 00''$ W, this region features rolling hills and varied topography, creating diverse microclimates ideal for olive cultivation. With elevations ranging from 400 to 1200 metres above sea level, it benefits from rich soil and ample sunshine, supporting the growth of high-quality Moroccan Picholine olives. Cooler temperatures at higher elevations and warmer, more humid conditions in the valleys further enhance this favourable environment for olive production [7].

Gharb Region

Renowned for its fertile plains, the Gharb region is located in north-western Morocco at approximately $34^{\circ} 15' 00''$ N, $6^{\circ} 35' 00''$ W. This area benefits from alluvial soil enriched by the Oued Bouregreg river, which provides excellent drainage and nutrient availability for olive cultivation. The Mediterranean climate, characterised by mild, wet winters and hot, dry summers, creates optimal conditions for the growth and ripening of olives. Additionally, the region's flatlands facilitate mechanised farming practices, enhancing productivity and making it ideal for cultivating various olive varieties, including Moroccan Picholine [1].

Physicochemical Properties

Oil Extraction

The oil was extracted using the cold pressing method, which preserves the natural flavours and nutritional properties of the olives. This technique involves mechanically crushing the olives at low temperatures to maintain the integrity of the oil's bioactive compounds. The extraction process utilised a hydraulic press, which efficiently crushes the olives, followed by a decanter centrifuge that separates the oil from the solid and aqueous phases. This combination of equipment ensures the production of high-quality extra virgin olive oil [9].

Oil Yield

Olives are first washed, sorted, and then crushed into a paste. The paste is then subjected to mechanical pressing or cold extraction methods to separate the oil from the solid materials. The yield is calculated based on the weight of the extracted oil relative to the initial weight of the olives [9].

Storage Conditions Prior to Analysis

The oil was stored at a temperature of 15-20°C in dark glass bottles. These conditions were selected to protect the oil from exposure to light and air, ensuring its quality and stability prior to analysis.

Acidity

A sample of olive oil is mixed with a solvent (e.g., ethanol or hexane) and titrated with a sodium hydroxide (NaOH) solution. The acidity is determined by the volume of NaOH required to neutralise the free fatty acids in the oil and is expressed as a percentage of oleic acid [10].

Peroxide Value

A sample of olive oil is reacted with an iodine solution, and the peroxide value is determined by titrating the excess iodine with a sodium thiosulphate solution. This measures the concentration of peroxides, which are indicators of oxidation [10].

Phenolic Compounds

Olive oil is prepared and analysed using HPLC to separate and quantify phenolic compounds, which are important for flavour and antioxidant properties [11].

Fatty Acid Composition

The olive oil sample is trans esterified to convert fatty acids into fatty acid methyl esters (FAMES). These FAMES are then analyzed by gas chromatography to determine the fatty acid profile [9].

Moisture Content

Moisture content is determined by oven-drying for 24 h at 105°C [12].

Nutritional Analysis

Table 1. Methods and references for the analysis of physicochemical and nutritional properties of Moroccan Picholine Olives

Parameter	Method	Reference
Vitamin: A, E, k	Vitamins A and K were determined according to the modified AOAC method. Vitamins E by HPLC	[12-13]
Minerals: Ca, Fe, K, Mg	Utilize Inductively Coupled Plasma Mass Spectrometry (ICP-MS) or Atomic Absorption Spectroscopy (AAS)	[14]
Fatty acid	Analyze using Gas Chromatography (GC) to determine the types and quantities of fatty acids	[15]
Antioxidants	DPPH radical scavenging test	[16]
Fibre content	Analyze using enzymatic-gravimetric methods	[17]

Statistical Methods

To compare the physicochemical and nutritional properties of Moroccan Picholine olives with those of neighbouring varieties (Tunisian Chemlali and Algerian Chemlal), several statistical methods were employed [18]. Descriptive statistics, including means and standard deviations, were calculated for each variable to summarise data distribution and variability. A one-way ANOVA was conducted to assess whether significant differences existed among the means of the three olive varieties for each measured parameter. In cases where significant differences were detected, Tukey's HSD (Honestly Significant Difference) test was utilised to identify specific group differences while controlling for Type I errors. Additionally, Pearson correlation coefficients were calculated to examine relationships between different fatty acid profiles, phenolic compounds, and quality parameters, allowing for insights into potential associations. A significance level of $p < 0.05$ was established for all tests to ensure robust findings. These comprehensive statistical approaches ensure that the comparisons made are reliable and meaningful, providing a solid foundation for interpreting the result.

Results and Discussion

Physicochemical Properties

Table 2. Physicochemical properties of Moroccan Picholine Olives and neighbouring varieties.

Parameters	Unit	Moroccan Picholine*	Tunisian Chemlali [19]	Algerian Chemlal [20]
Moisture content	%	53.66 ± 3.11 ^A	49.30 ± 0.24 ^{AC}	61.05 ± 2.50 ^A
Acidity	%	0.29 ± 0.71 ^{AB}	0.33 ± 0.1 ^B	0.2 ± 0.01 ^D
Oil Yield	%	42.32 ± 2.82 ^A	48.24 ± 0.20 ^C	35 ± 2.00 ^{AB}
Peroxide Value	meq kg ⁻¹	4.37 ± 1.41 ^B	4.5 ± 0.50 ^{AB}	6 ± 1.00 ^A

Mean ± standard deviation. Values sharing same letters differ non-significantly ($P > 0.05$). *This study

Moisture Content

Moroccan Picholine olives have a moisture content of $53.66 \pm 3.11\%$, which is slightly higher than Tunisian Chemlali ($49.30 \pm 0.24\%$) but lower than Algerian Chemlal ($61.05 \pm 2.50\%$). A higher moisture content can enhance the juiciness and flavour of the olives, making them more palatable. However, it also raises concerns about shelf stability and spoilage, particularly in warmer climates. The moderate moisture level in Moroccan Picholine olives suggests they may strike a balance between flavour and preservation, appealing to consumers seeking quality products without sacrificing shelf life. Producers may consider employing appropriate storage and handling techniques to maintain quality [21].

Acidity

The acidity of Moroccan Picholine ($0.29 \pm 0.71\%$) is comparable to that of Tunisian Chemlali ($0.33 \pm 0.1\%$), indicating that both are within acceptable limits for quality olive oil. However, Algerian Chemlal shows lower acidity ($0.2 \pm 0.01\%$), which might appeal more to premium markets seeking superior quality. Acidity is a key determinant of olive quality, with lower levels generally indicating better taste and preservation properties. The acidity levels observed in Moroccan Picholine olives suggest a well-rounded flavour profile that can cater to diverse consumer preferences. This balance may enhance its attractiveness to both local and international markets, where flavour complexity is valued [22].

Oil Yield

With an oil yield of $42.32 \pm 2.82\%$, Moroccan Picholine is slightly lower than Tunisian Chemlali ($48.24 \pm 0.20\%$) but higher than Algerian Chemlal ($35.00 \pm 2.00\%$). This suggests that while Moroccan Picholine performs well, there is still potential to enhance oil extraction techniques to improve yield. The lower yield of Moroccan Picholine olives indicates a need for optimisation in cultivation practices or processing techniques to enhance oil extraction efficiency. Focusing on improving yield could not only benefit producers economically but also position Moroccan Picholine olives more competitively in the market [23].

Peroxide Value

The peroxide value for Moroccan Picholine is 4.37 ± 1.41 meq kg^{-1} , which is similar to Tunisian Chemlali (4.50 ± 0.50) and indicates good oxidative stability. However, Algerian Chemlal has a higher peroxide value (6 ± 1.00), suggesting that Moroccan Picholine may have better freshness and shelf life. A lower peroxide value indicates fresher oil with a reduced risk of rancidity, which is critical for consumer acceptance and product quality. This finding underscores the potential of Moroccan Picholine olives in terms of oil quality, suggesting they could be marketed as a premium product with superior freshness. The ability to maintain low peroxide levels can enhance brand reputation and consumer trust [24].

Fatty Acid

Table 3. Fatty acid composition of Moroccan Picholine Olives compared to Neighbouring varieties.

Fatty acids	Units	Moroccan Picholine Our study*	Tunisian Chemlali [25]	Algerian Chemlal [26]
Oleic acid (C18:1)	%	68.47 ± 10.00^B	62.55 ± 0.02^D	62.39 ± 1.87^A
Linoleic acid (C18:2)	%	12.51 ± 3.72^{AC}	13.49 ± 0.02^B	14.11 ± 1.03^{AD}
Palmitic acid (C16:0)	%	16.24 ± 2.16^C	16.61 ± 0.30^{AB}	16.87 ± 0.30^C
Stearic acid (C18:0)	%	3.01 ± 1.58^A	2.18 ± 0.03^C	1.67 ± 0.61^D
Linolenic acid (C18:3)	%	1.2 ± 0.96^B	0.66 ± 0.02^{AC}	0.20 ± 0.56^{AB}

Legend: Mean $\pm$ standard deviation. Values sharing same letters differ non-significantly ($P > 0.05$).

Oleic Acid (C18:1)

The Moroccan Picholine olives have a significantly higher oleic acid content (68.47%) compared to Tunisian Chemlali (62.55%) and Algerian Chemlal (62.39%). The higher oleic acid content in Moroccan Picholine olives offers significant health benefits, particularly for cardiovascular health. Oleic acid, a monounsaturated fatty acid, helps reduce LDL (bad) cholesterol levels while increasing HDL (good) cholesterol, thereby lowering the risk of atherosclerosis and other heart diseases [27]. Additionally, its anti-inflammatory properties can help mitigate the risk of chronic conditions such as arthritis, diabetes, and certain cancers by reducing inflammatory markers in the body [28]. Research also suggests that diets rich in oleic acid may improve insulin sensitivity, which is crucial for maintaining healthy blood sugar levels. Furthermore, olive oil, known for its culinary versatility, remains stable at higher temperatures, making it a healthier alternative to saturated and trans fats. Overall, the elevated oleic acid levels in Moroccan Picholine olives highlight their potential as a functional food that supports heart health and enhances overall nutritional intake [29].

Linoleic Acid (C18:2)

Moroccan Picholine olives show a lower linoleic acid percentage (12.51%) than both the Tunisian (13.49%) and Algerian (14.11%) varieties.

Palmitic Acid (C16:0)

The levels of palmitic acid in Moroccan Picholine (16.24%) are comparable to those in the other varieties (Tunisian Chemlali at 16.61% and Algerian Chemlal at 16.87%).

Palmitic acid, a saturated fatty acid found in various oils, including olive oil, has a complex role in health that often sparks debate. While saturated fats, including palmitic acid, can raise LDL cholesterol levels, which is linked to increased cardiovascular risk, the overall impact of palmitic acid on health is nuanced [30]. Some studies suggest that the effect of palmitic acid may vary based on its source and the overall dietary context in which it is consumed. In the case of olive oil, which is rich in monounsaturated fats and antioxidants, the presence of palmitic acid is often outweighed by its beneficial components [31]. For instance, the health benefits of olive oil, such as its ability to reduce inflammation and improve heart health, can mitigate some of the potential negative effects associated with palmitic acid. Furthermore, emerging research indicates that the impact of saturated fats on heart health may not be as straightforward as previously thought, suggesting that the focus should be on the overall dietary pattern rather than individual fatty acids. Thus, while palmitic acid is indeed a saturated

fat present in Moroccan Picholine olives, its health implications should be viewed within the broader context of a balanced diet rich in diverse nutrients and healthy fats [32].

Stearic Acid (C18:0)

Moroccan Picholine has a higher stearic acid content (3.01%) compared to Tunisian Chemlali (2.18%) and Algerian Chemlal (1.67%).

Stearic acid is a saturated fatty acid that stands out due to its unique effect on cholesterol levels. Unlike many other saturated fats, which can raise LDL cholesterol (the "bad" cholesterol) [33], stearic acid has been shown to have a neutral effect on blood cholesterol levels. This characteristic makes stearic acid a favourable component in dietary fats [34]. In the context of Moroccan Picholine olives, which contain a higher percentage of stearic acid compared to some other olive varieties, this can be a significant positive aspect. The presence of stearic acid, combined with the high levels of beneficial monounsaturated fats and antioxidants typically found in olive oil, contributes to a heart-healthy profile. This property can be particularly appealing to health-conscious consumers who are wary of saturated fats. By highlighting the favourable fatty acid composition of Moroccan Picholine oil, producers can position it as a heart-healthy choice that provides the richness of olive oil without the potential drawbacks associated with other saturated fats [35].

Linolenic Acid (C18:3)

The Moroccan Picholine has a higher linolenic acid content (1.2%) than both the Tunisian (0.66%) and Algerian (0.20%) varieties.

Alpha-linolenic acid (ALA), a type of omega-3 fatty acid found in Moroccan Picholine olives, plays a significant role in human health due to its numerous potential benefits. ALA is an essential fatty acid that the body cannot synthesise, necessitating its intake through diet. One of its primary advantages is its anti-inflammatory properties, which can help mitigate chronic inflammatory conditions such as arthritis and inflammatory bowel disease by competing with omega-6 fatty acids and balancing the inflammatory response [36]. Furthermore, research indicates that higher intakes of omega-3 fatty acids, including ALA, are associated with a reduced risk of heart disease, as ALA contributes to lowering blood pressure, reducing triglyceride levels, and improving endothelial function [37]. Additionally, omega-3 fatty acids are crucial for brain health, supporting cognitive function and potentially lowering the risk of neurodegenerative diseases [38]. Some studies also suggest that linolenic acid may improve insulin sensitivity, thereby reducing the risk of type 2 diabetes. The presence of ALA in Moroccan Picholine olives not only enhances their nutritional profile but also positions them as an appealing

option for health-conscious consumers seeking to incorporate beneficial omega-3 fatty acids into their diet.

Table 4. Phenolic Compound Content in Moroccan Picholine Olives Compared to Neighbouring Varieties.

Phenolic compounds	Units	Moroccan Picholine Our study*	Tunisian Chemlali [21]	Algerian Chemlal [23]
Oleuropein	mg kg ⁻¹	167.81 ±15.50 ^B	45.50 ± 0.73 ^C	133±0.01 ^D
Hydroxytyrosol	mg kg ⁻¹	58.31 ±10.00 ^{AB}	83.34 ± 5.8 ^{AC}	3 ± 0.08 ^A
Tyrosol	mg kg ⁻¹	31.14 ± 5.24 ^A	56.60 ± 2.7 ^B	3 ± 0.02 ^{AB}

Legend: Mean ± standard deviation. Values sharing same letters differ non-significantly ($P > 0.05$).

Oleuropein Content

The Moroccan Picholine variety demonstrates a remarkably high oleuropein concentration (167.81 mg kg⁻¹) compared to the Tunisian Chemlali (45.50 mg/kg) and the Algerian Chemlal (133 mg kg⁻¹). Oleuropein is well known for its antioxidant and anti-inflammatory properties, which play a crucial role in promoting cardiovascular health and protecting against oxidative stress. The substantial oleuropein content in Moroccan Picholine not only highlights its potential health benefits but also suggests that this variety may be more effective in supporting heart health and preventing chronic diseases than its neighbours. This characteristic could serve as a pivotal selling point for producers, particularly in markets increasingly focused on functional foods [39].

Hydroxytyrosol Levels

With a hydroxytyrosol content of 58.31 mg kg⁻¹, Moroccan Picholine olives are lower than the Tunisian Chemlali (83.34 mg kg⁻¹) but much higher than the Algerian Chemlal (3 mg kg⁻¹). Hydroxytyrosol is recognised for its potent antioxidant effects, including the ability to lower blood pressure and provide neuroprotective benefits. While the Moroccan variety may not lead in hydroxytyrosol content, it still offers significant health advantages, suggesting that it could be marketed effectively to consumers seeking antioxidants. Additionally, strategies to enhance hydroxytyrosol content during cultivation or processing could further increase the appeal of Moroccan Picholine olives [39].

Tyrosol Content

The tyrosol levels in Moroccan Picholine olives (31.14 mg kg^{-1}) position them between the Tunisian Chemlali (56.60 mg kg^{-1}) and the Algerian Chemlal (3 mg kg^{-1}). Tyrosol possesses anti-inflammatory and antioxidant properties, making it another beneficial component for health-conscious consumers. While the Moroccan variety is slightly lower than the Tunisian, it still provides a healthful option. This information can be leveraged to market Moroccan Picholine olives as a wholesome choice, emphasising their comprehensive phytochemical profile [40].

Comparisons between Moroccan Picholine and Neighboring Olive Varieties

Quality Parameters

In terms of quality, Moroccan Picholine has a moisture content of 53.66%, higher than Tunisian Chemlali (49.30%) but lower than Algerian Chemlal (61.05%). This balance may provide a favourable flavour without significantly compromising shelf life. The acidity level of 0.29% is comparable to Tunisian Chemlali (0.33%), indicating a similar flavour profile, while the lower acidity of Algerian Chemlal (0.20%) suggests a potentially sweeter taste. The oil yield of Moroccan Picholine (42.32%) is lower than that of Tunisian Chemlali (48.24%), indicating a need for improvement in oil extraction efficiency, though it is higher than Algerian Chemlal (35%). Lastly, the peroxide value of Moroccan Picholine (4.37 meq kg^{-1}) is similar to that of Tunisian Chemlali (4.5 meq kg^{-1}) but significantly lower than Algerian Chemlal (6 meq kg^{-1}), suggesting better oil freshness and quality.

Fatty Acid Composition

Moroccan Picholine olives exhibit a notably higher oleic acid content (68.47%) compared to Tunisian Chemlali (62.55%) and Algerian Chemlal (62.39%). This higher oleic acid level is beneficial for cardiovascular health, highlighting Moroccan Picholine's potential as a healthier choice. However, it contains lower linoleic acid (12.51%) than both neighboring varieties, which could influence consumer choice based on dietary preferences.

Phenolic Compound Content

The phenolic profile of Moroccan Picholine is impressive, especially its oleuropein content ($167.81 \text{ mg kg}^{-1}$), significantly higher than Tunisian Chemlali (45.50 mg kg^{-1}) and competitive with Algerian Chemlal (133 mg kg^{-1}). Oleuropein is associated with various health benefits, including antioxidant and anti-inflammatory effects. In contrast, hydroxytyrosol (58.31 mg kg^{-1}) is lower than in Tunisian Chemlali (83.34 mg kg^{-1}), suggesting that while Moroccan Picholine excels in oleuropein, it could enhance its hydroxytyrosol levels to boost its overall health benefits.

Interpretation of Results and their Implications

The findings regarding Moroccan Picholine olives reveal a promising profile in terms of fatty acid composition, phenolic compounds, and quality parameters. The high oleic acid content (68.47%) suggests significant cardiovascular benefits, aligning with literature that associates oleic acid with a reduced risk of heart disease Sofi *et al.* [41]. The elevated oleuropein levels (167.81 mg kg⁻¹) highlight the variety's antioxidant potential, reinforcing its appeal as a functional food. However, the relatively lower linoleic acid content and hydroxytyrosol levels indicate areas for improvement that could impact consumer choice, especially among those prioritising balanced fatty acid profiles in their diets.

In comparison to existing literature, Moroccan Picholine's oleic acid levels align well with studies indicating that high oleic acid content in olives enhances nutritional value Cicerale *et al.* [42]. The oleuropein concentration also supports findings from previous studies that emphasise the health benefits of phenolic compounds in olive oils Servili & Montedoro [43]. However, the lower hydroxytyrosol content compared to Tunisian Chemlali suggests that Moroccan Picholine could benefit from enhanced cultivation or processing methods aimed at increasing this compound, as hydroxytyrosol has been associated with various health benefits, including anti-inflammatory effects.

Comparative Analysis of Nutritional and Physicochemical Properties of Moroccan Picholine, Tunisian Chemlali, and Algerian Chemlal Olives.

Principal Component Analysis (PCA)

Principal Component Analysis (PCA) was employed to identify patterns in the physicochemical and nutritional properties of Moroccan Picholine olives compared to Tunisian Chemlali and Algerian Chemlal varieties. The PCA results shown in Figure 3a revealed that the first two principal components explained a significant portion of the variance in the dataset, effectively differentiating the olive varieties based on key attributes such as oleic acid content, oleuropein levels, and moisture percentage. Specifically, Moroccan Picholine olives clustered distinctly, indicating their superior nutritional profile, characterised by high oleic acid and oleuropein concentrations. This clustering suggests potential health benefits associated with their consumption, particularly in relation to cardiovascular health. The visualisation of the PCA biplot in Figure 3a further highlighted the contribution of each variable, underscoring the importance of oleic acid and phenolic compounds in distinguishing Moroccan Picholine olives from their neighbours. These findings not only enhance our understanding of the unique qualities of Moroccan Picholine olives but also point to avenues for targeted improvement in cultivation and processing practices.

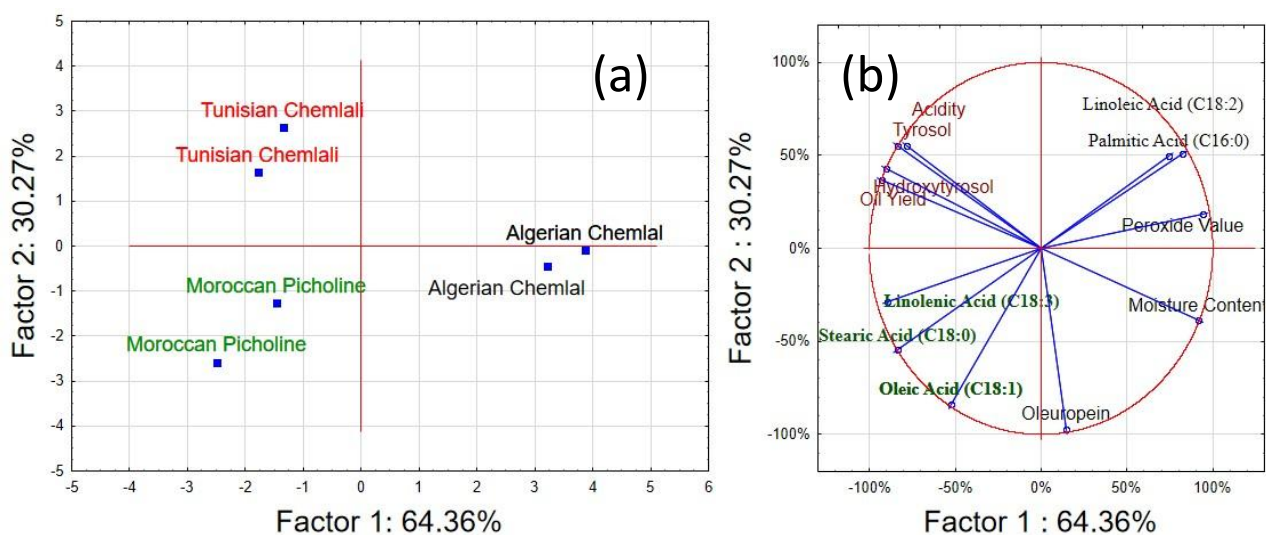


Figure 3. Biplot analysis of nutritional and physicochemical properties of olive varieties (a); correlation circle of key nutritional components in Moroccan Picholine Olives (b).

Based on the provided data, Moroccan Picholine olives consistently exhibit higher oleic acid content, averaging 68.47%, compared to Tunisian Chemlali and Algerian Chemlali, which have lower values of 62.55% and 62.39%, respectively. This elevated oleic acid level suggests greater cardiovascular health benefits associated with Moroccan Picholine. Additionally, the oleuropein content stands out significantly at 167.81 mg kg⁻¹, in contrast to the 45.50 mg kg⁻¹ and 133.00 mg kg⁻¹ found in the neighbouring varieties, indicating superior antioxidant properties. While moisture content is moderate at 53.66%, the oil yield of 42.32% remains competitive, reflecting quality. The lower acidity (0.29%) and peroxide value (4.37 meq kg⁻¹) further emphasise the freshness of Moroccan Picholine olives. Overall, these metrics highlight the unique nutritional profile of Moroccan Picholine, reinforcing its potential for market differentiation and health promotion.

Potential for development and improvement in cultivation or processing

To further capitalize on the strengths of Moroccan Picholine olives, targeted improvements in cultivation and processing practices could enhance both yield and health benefits:

Cultivation Practices

Soil Management: Implementing soil amendments and optimising irrigation could improve the overall health of olive trees, potentially leading to higher oleic and linoleic acid yields. Adopting sustainable agricultural techniques, such as organic farming and integrated pest management, can enhance the health of olive trees and increase yields. Optimising irrigation methods to ensure consistent moisture levels, particularly in arid regions, will improve fruit quality [44].

Varietal Selection: Exploring cross-breeding or selection techniques could introduce traits from neighbouring varieties, such as higher hydroxytyrosol content, without compromising the unique characteristics of Moroccan Picholine.

Processing Enhancements

Invest in modern processing equipment to minimize the time between harvesting and oil extraction, preserving the olives' nutritional properties and freshness. Implement best practices in oil extraction and storage to maintain quality, such as using cold-press methods and minimizing exposure to light and air [45].

Improving Hydroxytyrosol Content

Focus on breeding programmes aimed at increasing hydroxytyrosol levels, which are beneficial for their antioxidant properties. Research into genetic variations could lead to the development of higher-yielding varieties. Educate farmers on harvesting techniques that maximise the retention of phenolic compounds, which can be affected by timing and handling [46].

Collaboration with Farmers and Stakeholders

Foster partnerships with local farmers to share knowledge and resources, ensuring that best practices in cultivation and processing are widely adopted. Collaborate with industry stakeholders, such as researchers and agricultural organisations, to promote research and development initiatives aimed at enhancing olive quality and sustainability [47].

Processing Techniques

Cold Extraction Methods: Utilising advanced extraction methods can enhance oil quality and yield, reducing oxidation and increasing the retention of beneficial compounds. **Timing of Harvest:** Harvesting at the optimal ripeness can maximise oil yield and enhance the phenolic profile, particularly oleuropein and hydroxytyrosol [48].

Enzymatic Treatments: The application of specific enzymes during the oil extraction process can enhance oil yield by breaking down cell walls in the fruit, allowing for better oil release. Exploring enzymatic pre-treatments prior to pressing could lead to significant yield improvements [48].

Varietal Crossbreeding

Research into higher-yield varieties: Ongoing research into crossbreeding Moroccan Picholine with higher-yield varieties presents an opportunity for genetic improvement. By leveraging the genetic traits of other olive cultivars known for their superior oil yield, it may be possible to develop new strains that maintain the unique flavour and quality characteristics of Picholine olives while improving overall productivity [50].

Comparative Analysis of Cultivation Methods

Traditional Versus Modern Farming

In the cultivation of olives, traditional and modern farming practices coexist and evolve across regions. Moroccan Picholine olives are traditionally grown using organic methods with minimal intervention, fostering a deep connection to sustainable agriculture. Recently, however, there has been a gradual shift towards modern techniques, including the implementation of drip irrigation and integrated pest management, which aim to enhance both yields and fruit quality [51]. In Tunisia, Chemlali olives thrive in traditional groves that benefit from a rich heritage of cultivation techniques. The recent adoption of modern practices, such as soil health management and mechanised harvesting, seeks to improve efficiency and increase oil yield [51]. Similarly, Algeria blends traditional and modern approaches, utilising local varieties while incorporating improved agricultural methods like crop rotation and organic fertilisation to boost productivity [50]. This integration of practices across these regions highlights the ongoing evolution of olive cultivation in response to both environmental and market demands.

Conclusion

This study highlights the distinct physicochemical and nutritional properties of Moroccan Picholine olives compared to neighbouring varieties, Tunisian Chemlali and Algerian Chemlal. The results indicate that Moroccan Picholine possesses a significantly higher oleic acid content, which is beneficial for cardiovascular health, alongside impressive levels of oleuropein, a phenolic compound known for its antioxidant properties. However, there is potential for improvement in hydroxytyrosol content and oil yield, which could enhance its marketability and health benefits. Statistical analyses confirmed significant differences among the varieties, providing a robust foundation for the findings. The insights gained from this research underscore the importance of targeted cultivation and processing practices to further enhance the quality and nutritional profile of Moroccan Picholine olives. By focusing on these improvements, producers can position Moroccan Picholine olives as a

premium product in the global olive oil market, emphasising their unique health benefits and flavour characteristics. Future research should explore innovative agricultural techniques and processing methods to maximise the potential of this valuable variety, contributing to the economic development of the olive industry in Morocco.

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Data Availability: The data that support the findings of this study are available from the corresponding author upon reasonable request.

Code availability: Not applicable

Conflict of interest: All authors declare no conflicts of interest

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Biofilms in the Food Industry: Adhesion Mechanisms, Control Strategies, and Mastery

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Graphical Abstract

This article reviews the formation of biofilms in the agri-food industry, exploring the mechanisms and factors involved in the adhesion of micro-organisms, the different methods used to study their development, and new strategies and advances to combat this phenomenon.



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Abstract: Biofilms are microbial communities protected by a self-secreting extracellular polymeric substance. Although their presence can be beneficial in certain industrial contexts, the adhesion of pathogenic microorganisms to the surfaces of materials and equipment represents a major challenge in the food industry. The aim of this article is to review recent advances in biofilm formation in the food industry and to summarise and discuss the different factors that promote adhesion. It also focuses on the different methodologies and techniques used in research studies for a better understanding of biofilm formation, as well as new approaches and recent advances in their prevention and elimination. Further research is needed to identify the complex mechanisms of biofilms in the food sector in order to improve product quality while minimizing health risks and financial losses.

Keywords: *Biofilms, Adhesion, Food Industry, Food Safety*

Introduction

Contemporary food hygiene requires scrutiny of all matters connected with manufacture, sale and consumption to ensure quality and safety for consumers. The adhesion of microorganisms to surfaces and equipment is a major problem. This adhesion is generally followed by the development of a biofilm, which is characterized by high resistance to antimicrobial agents. This resistance causes the quality of the product to deteriorate. Food safety problems result, with risks to human health. It is estimated that about 60% of food-borne epidemics are caused by biofilms [1]. The resistance of biofilms to biocides in industrial processes often results in treatment failures, as stated by Wille and Coenye [2], and hence poses a risk to food safety in industrial settings.

Hall and Mah [3] state that the concentration of antibiotics needed to eradicate biofilms can be as high as 1,000 times more than required to eliminate planktonic cells. Biofilm-related problems may be current in most sectors linked to the food industry: juice and beverages, dairy industries, meat treatment, and fishing industries [4,5]. Food processing chains provide a convenient environment for developing biofilms on equipment surfaces [6]. Factors such as the components of the food matrix in processing environments, the properties of food contact surfaces, and the specific characteristics of bacterial strains play a crucial role in microbial adhesion [6,7].

Biofilms are not always harmful. In some contexts, these biofilms composed of non-pathogenic bacteria have considerable advantages; for example, in food production, in wastewater treatment, and agricultural processes. It is necessary to understand the mechanisms of biofilm formation, the conditions that favour its development, its negative impact on risk management, and the advantages of its industrial use.

The goal of this review is to highlight present knowledge on food industry biofilm formation, those factors affecting such a development process, and its detrimental impact within the different agro-food chains. Furthermore, this paper discusses predictive and experimental approaches taken in the study of this complicated phenomenon, which mainly focus on observation and analytical methodologies as well as recent advances in the fight against biofilm.

General Information on Biofilms

Definition of Biofilm

Several definitions of biofilms have been proposed. Costerton *et al.* [8] described biofilms as "a microbial population enclosed in a matrix, adhering to each other and/or to surfaces or interfaces". The International Union of Pure and Applied Chemistry (IUPAC) defined biofilm in 2012 as an aggregate of microorganisms, with cells often encased in a self-produced matrix of extracellular polymeric substance (EPS) adhering to each other and/or a surface [9]. The IUPAC highlighted that "a biofilm is a fixed system adaptable to environmental conditions by its inhabitants," and that the self-produced EPS matrix, or 'slime', is composed of extracellular biopolymers in various structural forms [9].

History

Biofilms have been observed for centuries, though their structure was not fully understood. For much of microbiology's history, microorganisms were mainly seen as planktonic cells in suspension, identified by their growth in nutrient-rich media [10]. Between 1683 and 1708, Antoni van Leeuwenhoek first observed biofilms in "dental plaque" and "particles scraped from his tongue" using a primitive microscope [10]. As early as 1923, Angst demonstrated the existence of bacterial biofilms on the hulls of ships, indicating that the bacteria in the biofilm were much more numerous than the floating cells. In 1933, Arthur Henrici observed biofilms on aquarium slides, and in 1934, Claude Zobell accumulated data proving the predominance of bacterial cells on surfaces compared to free water. This suggested solid surfaces concentrated nutrients, promoting bacterial adhesion. In the 1980s, studies expanded to show microorganisms attaching to surfaces in diverse environments, including medical devices, wastewater systems, and food production materials like vinegar [11].

Stages in Biofilm Formation

Biofilm formation follows a sequence of events, starting with the transport and attachment of cells to a substrate, forming a conditioning film. According to Stoodley *et al.* [12], biofilm formation consists of five stages:

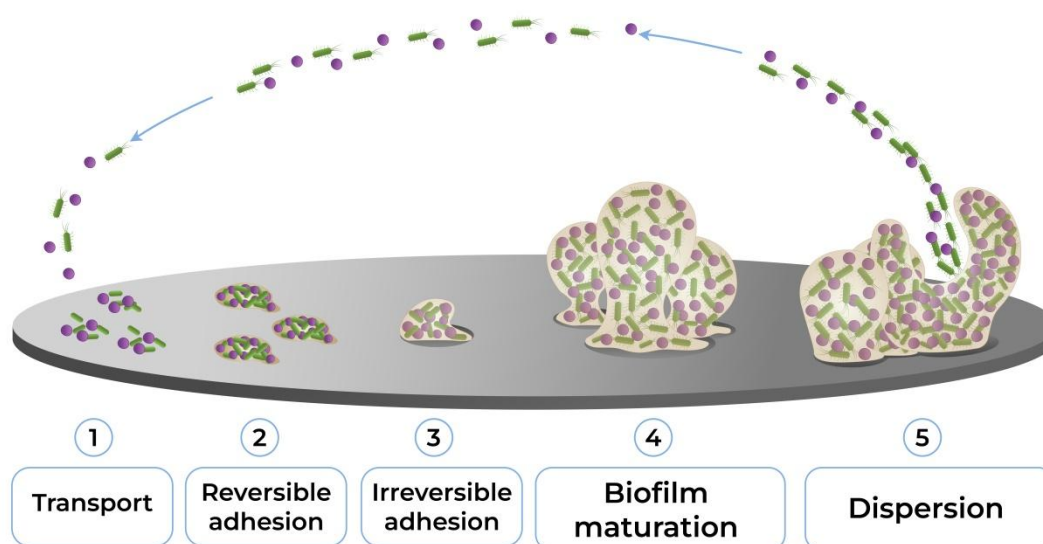


Figure 1. Schematic representation of biofilm formation.

1. Transport of microorganisms.
2. Reversible adhesion of cells.
3. Irreversible adhesion and secretion of extracellular polymeric substances, forming a matrix that consolidates attachment and shifts cells from a free-living to surface-attached state.
4. Biofilm maturation, leading to increased biomass, microorganism count, and biofilm thickness.
5. Microbial detachment and dispersion of biofilm colonies in response to hydrodynamic conditions.

Biofilm formation is a multi-stage process; firstly, rapid building up of a conditioning film modifies surface properties and creates an appropriate microenvironment for bacterial adhesion. Passive transport refers to the uptake of microorganisms via physical mechanisms such as convection and sedimentation or other active mechanisms using bacterial appendages such as flagella and chemotaxis. The initial attachment is reversible and influenced by various factors like fluid flow and physicochemical interactions. This is followed by the irreversible stage when the cells adhere via structures like curli and fimbriae and through the secretion of an exopolysaccharide matrix that anchors them and offers protection. Biofilm growth includes cell division, increased biomass, and thickening of the structure by additional matrix formation, all of which are coordinated by quorum sensing that, in turn, regulates biofilm structure and virulence factors. The final stage of biofilm development occurs when the cells switch from a sessile state back to primary planktonic cells. Usually, this is a response mechanism when there is depletion of nutrients or exposure to other forms of stress. Dispersion could take place passively or actively, caused, e.g., by environmental stresses or genetic changes that affect motility or attachment. Once detached, the microorganisms or colonies migrate as planktonic cells

with the potential to colonize a new surface. Such knowledge about the stages is important when it comes to controlling biofilm formation and development in many sectors [13].

Nature of Biofilms

Biofilms can be homogeneous, consisting of a single microbial species, or heterogeneous, composed of several species. The heterogeneity of microbial cells ensures protection and resistance to environmental stress, guaranteeing the sustainability of the microbial community compared to homogeneous populations [14]. Environmental conditions determine the nature of the species present in a biofilm. For example, phototrophic micro-organisms present in light environments use light energy to synthesise nutrients [15]. Geesey *et al.* [16] described phototrophic biofilms as complex aggregates of bacteria, algae, protozoa, fungi and meiobenthos. Exposure to dynamic water flows leads to the formation of complex biofilms, which are crucial in aquatic ecosystems. In light-deprived environments, diatom biofilms are heterotrophic [17]. Biofilms can also transform into films, microcolonies, macrocolonies and other structures in response to various environmental conditions [18].

Biofilm Structure

After biofilm formation and secretion of the exopolysaccharide matrix, the structure becomes highly viscoelastic and can quickly adapt to changes in the substrate [19]. Scanning electron microscopy and confocal laser scanning microscopy, have clarified biofilm structure and its significance [20]. Biofilms are complex communities of surface-bound cells in a polymer matrix, not homogeneous deposits, with open water passages. Environmental factors are one factor that influences biofilm structure; e.g., under oxic conditions, *Pseudomonas aeruginosa* forms a fungal structure with channels between rod-shaped cells; in anoxic conditions, it develops a mesh-like structure with filamentous cells [21]. Temperature changes can alter cell thickness and density, and shear forces affect biofilm strength, with low shear leading to low tensile strength and high shear making biofilms more resistant to mechanical breakage [22].

Biofilm Constituents

Biofilms are made up of microbial cells and a self-produced exopolysaccharide matrix (EPS) that promotes communication, protects against damage and encourages adhesion to the surface and biofilm formation [23,24]. Microorganisms make up 10 to 25% of the material, and the extracellular matrix constitutes 75 to 90% of the organic carbon mass [22,23]. The matrix is highly hydrated - up to 97%

water- and comprises nucleic acids, that is, eDNA and eRNA, proteins, polysaccharides, surfactants, lipids, glycolipids, and cations [20]. These EPSs from bacteria consist of 20 to 50% uronic acids and 75 to 90% proteins, carbohydrates, including glycoproteins and lipoproteins, respectively [25]. High moisture content and hydroxyl groups present in the polysaccharides increased mechanical strength and prevented drying in nature [26]. The composition and structure of EPS depend on the type of microorganism and the conditions of its growth.

Mechanisms Regulating Biofilm Formation

A mechanism called Quorum Sensing (QS) regulates biofilm formation. This takes place within bacterial cells, expressing intercellular signals linked to the quantity of cells [27]. This process is governed by autoinducers (AIs) which are tiny molecule capable of spreading and aggregating in the environment, triggering a reaction when their concentration crosses a certain threshold. When specific receptors detect a signal, they trigger the activation of certain genes, altering gene expression. The key quorum sensing (QS) systems include the peptide autoinducer (AIP) system in Gram-positive bacteria, acyl homoserine lactones (AHLs) in Gram-negative bacteria, and autoinducer-2 (AI-2), which is found in both [28].

AIPs are modified oligopeptides (5–17 amino acids) that bind to histidine kinase sensors, triggering a phosphorylation cascade [29]. AHLs, produced by Gram-negative bacteria, enhance bacterial attachment and biofilm formation since their accumulation leads to an increase in adhesins and a decrease in antibiofilm substances [30]. AI-2, a nonspecific signal produced by Gram-positive and Gram-negative bacteria, is synthesized by LuxS in the active methyl cycle and is active in intra- and inter-species communication that influences biofilm formation, virulence, growth, and metabolism [31,32]. QS coordinates microbial behaviour in biofilm formation, bioluminescence, secondary metabolite production, and virulence regulation [33].

Biofilms in the Food Industry

The Industrial Biofilm Model

Biofilms formed in the food industry are exposed to environmental conditions where nutrients are abundant, unlike biofilms found in natural environments which are known to form in response to environmental stresses. The micro-organisms present in biofilms vary with the food processing environment [34]. Therefore, understanding the factors influencing microbial adhesion under food industry conditions is important to develop not only preventive strategies to avoid the formation of negative biofilms, but also to optimize conditions favouring beneficial biofilms.

In the dairy industry

Milk and dairy products can be easily contaminated by poorly cleaned and disinfected equipment because of their nutrient-rich composition [7]. The presence of traces of milk from the production process may favour the formation of biofilms on surfaces of food processing materials such as stainless steel (SS) surfaces, heat exchangers, evaporators, tubes and tanks [35,36]. The presence of a protein and/or lipid conditioning film can improve bacterial attachment and subsequently influence biofilm formation in a dairy plant [37]. Various microorganisms have been found in dairy products, e.g., *Staphylococcus*, *Enterobacter*, *Bacillus*, *Pseudomonas*, *Listeria*, *Streptococcus*, *Coronobacter sakazakii*, and *Salmonella Typhimurium* [7,38]. Much research has been conducted on the different bacteria present in dairy plants. *Bacillus cereus* is one of the species posing a health risk to consumers because it can grow in industrial dairy piping and storage systems, putting the food production environment at risk of contamination [39]. *Listeria monocytogenes* is a foodborne pathogen that has also been detected in dairy products and can survive in various adverse conditions, withstanding low temperatures, low pH and even high salt concentrations [38,40]. Despite the application of bactericidal treatments, *L. monocytogenes* can persist for months and years on processing surfaces in factories and industrial environments [41].

In the juice and drinks industry

Biofilms are a major source of contamination in juice industries, leading to economic losses and health problems. Tarifa *et al.* [4] demonstrated that in juice production facilities, biofilms of yeast species can survive and exhibit resistance to the cleaning and disinfection process. Other studies have also confirmed that yeasts belonging to the *Candida* and *Rhodotorula* genera can persist over time on surfaces used in the juice and beverage industry such as stainless steel surfaces used in processing facilities and ultrafiltration membranes used in clarification processes [42,43]. *Salmonella enterica* and *Escherichia coli* O157:H7 have been found in apple and citrus fruit juice and are responsible for food-borne epidemics of *Salmonella* and *L. monocytogenes* have been associated with the consumption of mango, papaya and cantaloupe [44]. Other species responsible for most of the deterioration in the quality of fruit juices and drinks include *Alicyclobacillus acidoterrestri* [45].

In the meat industry

In the meat industry, the transfer of pathogens during slaughtering, curing, processing and storage can lead to cross-contamination of meat and meat products. One of the main sources of contamination of carcasses during processing in the meat sector is the presence of micro-organisms on animal skin [46]. Wagner *et al.* [47] showed that under meat processing conditions, the *P. fragi* strain produces mono-

species biofilms on stainless steel. Other studies have also shown the ability of *P. fragi* to form biofilms on stainless steel material at a temperature of 30°C, and on polystyrene and meat at 10°C [48]. Other biofilm-forming strains with high resistance to typical disinfectants, such as *Salmonella* strains, have been collected from various commercial meat processing facilities [46]. Components of meat residues and meat exudates affect biofilm formation. This was verified in a study which showed that samples taken from the cutting table and drain surface in the deboning and trimming plant had the highest diversity of biofilm population and contained meat residues within the biofilm matrix [5].

In the fish and seafood processing industry

Seafood products are characterized by their high nutrient content, which makes them susceptible to bacterial contamination and enzymatic oxidation, altering their physical, chemical and organoleptic properties [49]. According to WHO, 66% of human diseases are associated with biofilms formed by pathogenic bacteria, of which 31 pathogens have been identified as responsible for seafood-borne diseases [50]. *Vibrio cholerae*, *Vibrio parahaemolyticus*, *Vibrio vulnificus* and *Vibrio alginolyticus*, *L. monocytogenes*, *Salmonella* spp., *Bacillus* spp., *Aeromonas* and *Pseudomonas* spp. are among the pathogens known to form biofilms in fish and seafood processing [38]. In the aquaculture industry, water plays an important role in the transfer of microorganisms from one operation to another. Various authors have stated that the loss of quality of water used in the fish and seafood industry follows when biofilms break loose in the distribution system. For economic reasons, the fresh water used in this industry is sometimes replaced by treated seawater [38]. Seawater treatment sometimes seems ineffective because of the formation of resistant biofilms in distribution systems. Shikongo-Nambabi et al. [51] found that even when seawater was treated with a combined system of chlorination and ultraviolet (UV) radiation, the water was contaminated with *Vibrio* spp. Water storage tanks, polyethylene water pipes and the surfaces of larval tanks are susceptible to biofilm formation [38].

Negative Impact of Biofilms

In the food industry, biofilm formation causes biofouling in production equipment, pipes, cooling towers and heat exchangers. Biofouling, a widespread problem, limits the operation of some membranes, leading to concentration polarisation, reduced permeate flux, and increased feed channel pressure drop; this polarisation further raises operating and maintenance costs [52]. The formation of biofilms on raw materials or processing surfaces can lead to contamination of food products and food spoilage. Several studies have shown that the presence of biofilm can alter the organoleptic characteristics of products. For example, *B. cereus* is one of the most common contaminating bacteria in the dairy sector capable of forming biofilms on stainless steel pipes, conveyor belts and storage tanks and secreting metabolites, surfactants, bacteriocins and enzymes such as lipases and proteases

that affect the sensory qualities of foods [53,54]. The presence of contaminants and changes in the organoleptic characteristics of products, such as texture, taste and odour, can reduce the perception of food quality, which can reduce their market value. Indeed, the formation of biofilms in industry results in annual expenditure of billions of US dollars for nations in terms of equipment and machinery maintenance, product contamination and energy loss [55]. For example, the cost associated with biofouling results from a number of factors, including costs associated with the drop in equipment performance, the need for thorough cleaning and disinfection processes, the use of biocides, the search for additional effective procedures, the labour costs required to clean and replace membranes as well as economic losses associated with plant idleness. According to the FAO, an estimated 1.3 billion tonnes of food are lost or wasted worldwide each year [56]. In addition to the economic impacts, the formation of biofilms on agri-food surfaces contributes to cross-contamination of food, leading to the occurrence of foodborne infections and food poisoning, affecting many people. Globally, foodborne diseases affect 600 million people and cause approximately 420,000 deaths each year [46]. In the United States, 842 outbreaks occur each year, resulting in 14,237 illnesses [34]. Another American study of food-borne illness between 1998 and 2008 reported that 117 epidemics were linked to several food poisoning outbreaks caused by the ingestion of rice-based dishes contaminated by the *B. cereus* bacterium [57]. For example, outbreaks and deaths have been caused by certain foods contaminated with Shiga toxin-producing *Escherichia coli* (Stx) (STEC) such as soups, cooked chicken, sauces, minced beef, salads and other fresh produce causing enterohaemorrhagic gastroenteritis and, in some cases, haemolytic uraemic syndromes with acute kidney injury [58,59]. The hazardous presence of biofilms in the food industry makes it necessary to know much more about them.

Factors Influencing the Formation of Biofilms on Food Processing Materials

Factors Related to the Properties of Food Processing Materials

Depending on the different applications, numerous materials are used in equipment and packaging intended for contact with food. According to the framework regulation (EC) no. 1935/2004 required by EU regulations, 17 types of materials and objects likely to be subject to specific measures may come into contact with food: Plastics, Paper and cardboard, Active and intelligent materials and articles, Glues, Ceramics, Cork, Rubber, Glass, Regenerated cellulose, Silicones, Wood, Textiles, Varnishes and coatings, Waxes, Printing inks, Ion exchange resins, Metals and alloys. Numerous studies have described the influence of the physico-chemical properties (hydrophobicity, roughness, surface energy) of materials used for food contact surfaces on bacterial adhesion, biofilm formation and organisation. The variation in adhesion capacity on different materials can be explained by the

difference in surface physicochemical properties. Table 1 summarizes the general effect of these factors on biofilm formation.

Table 1. Effects of the physicochemical properties of materials on the formation of biofilms in the food industry.

Factors	Effect on biofilm formation	Reference
Surface charge	Positively charged surfaces favour the adhesion and formation of <i>Geobacter</i> biofilms.	[60]
	Negatively charged material surfaces lead to a decrease in bacterial adhesion.	[61]
	Positively charged materials exhibit an antibacterial effect on Gram-negative bacteria.	[61]
	Positively charged surfaces show antibacterial and antibiofilm activity against <i>Staphylococcus aureus</i> and <i>E. coli</i> .	[62]
Surface roughness	Surfaces with large pores promote bacterial adhesion.	[63]
	Reduced surface roughness decreases <i>S. aureus</i> adhesion.	[64]
	Increased roughness of AISI 316L stainless steel enhances <i>E. coli</i> adhesion.	[65]
Surface hydrophobicity and hydrophilicity	Hydrophobic surfaces promote microorganism adhesion.	[66]
	Hydrophobic polypropylene (PP) surfaces enhance the formation of <i>S. aureus</i> biofilms.	[67]

Effect of Solid Surface Charge

With regard to surface charge, it is demonstrated that positively charged materials favour the adhesion of negatively charged bacteria, whereas negatively charged materials can generate electrostatic repulsion preventing adhesion [61]. Guo *et al.* [60] found that positively charged surfaces favoured the adhesion and formation of biofilms by *Geobacter* bacteria. Similarly, Scholl *et al.* [68] studied the adhesion of indigenous groundwater bacteria to mineral surfaces: fragments of quartz, muscovite, limestone and quartz and muscovite surfaces coated with iron hydroxide. These authors observed that adhesion of negatively charged bacteria was more favoured on surfaces coated with positively charged iron hydroxide than on clean surfaces with a negative charge. Gottenbos *et al.* [61] noted a decrease in bacterial adhesion on the negatively charged surface. But, the positively charged surface was able to promote bacterial adhesion, with the exception of Gram-negative bacteria. This suggests that materials with a positive charge have an antibacterial effect on Gram-negative bacteria. In another study, it was shown that modifying the surface charge of magnetic iron oxide nanoparticles by functionalisation with different chemical groups had an effect on biofilm formation [62]. It would even

appear that the positively charged surface, characterised by hydrophobicity, exerted antibacterial and antibiofilm activity against *S. aureus* and *E. coli*.

Effect of Surface Roughness

Surface roughness is an important factor in the adhesion process. It results in irregularities on the surface of the material that can include micrometric and nanometric scales allowing a reduction in the physicochemical potential barrier and a decrease in the repulsive forces between the microorganism and the surface, which leads to preferential colonization on rough surfaces [69]. Cheng *et al.* [63] mentioned that surfaces that include large pores present a lower energy barrier which leads to bacterial adhesion; but these microorganisms have weaker contact with surfaces where the pores are small. For example, the adhesion of *S. aureus* (ATCC 25923) to a bioceramic surface was affected by the surface texture and the anchor points for bacterial adhesion are reduced when the surface roughness is decreased [64]. In a recent study on the effect of structural and physicochemical properties of plastic surfaces on biofilm formation, it was shown that among 4 plastic surfaces, ranked in order of roughness, biofilm formation was more favourable on PLA (polylactic acid) plastic, which had the highest surface roughness [70]. But, in another study, the adhesion and biofilm formation of 4 microorganisms: *S. epidermidis* (ATCC 35984); *S. aureus* (ATCC29213); *E. faecalis* (ATCC 29212) and *C. albicans* (ATCC 90028) were not influenced by the variation in the roughness of the acrylic resin, suggesting that roughness is not the only factor in microbial adhesion [71].

Effect of Surface Hydrophobicity and Hydrophilicity

Surface hydrophobicity influences bacterial adhesion and biofilm formation on food processing materials. It is measured using the contact angle technique. Microorganisms can more often adhere to hydrophobic surfaces than hydrophilic surfaces. Faille *et al.* [66], examining the adhesion of *E. coli* to six materials (glass, stainless steel, high density polyethylene (HDPE), polyamide-6, polyvinyl chloride and Teflon), showed that hydrophobic materials (HDPE and Teflon) favoured the adhesion of a large number of bacterial cells compared to other hydrophilic surfaces. Pagedar *et al.* [67] also noted that the formation of *S. aureus* biofilms isolated from dairy industries was more favoured on hydrophobic polypropylene (PP) surfaces than on stainless steel with a hydrophilic character. This is at odds with the results of a recent study by Omari *et al.* [72] who observed a decrease in bacterial adhesion to a new formulation of drinking water pipes incorporating natural oils and characterized by superior hydrophobicity. This contradiction shows that the phenomenon of adhesion and biofilm formation remains a complex phenomenon with several parameters and cannot be explained solely by the hydrophobicity properties of materials.

Factors Related to Bacterial Surface Properties

The various characteristics of bacterial strains can lead to an increase or decrease in bacterial adhesion potential to a given surface. Therefore, understanding the mechanisms of adhesion and biofilm formation requires a good knowledge of these characteristics, such as the effect of the physicochemical properties of the cell wall, structures anchored to the bacterial wall, and the production of exopolysaccharides.

- Effect of the Physicochemical Properties of the Cell Wall

The adhesion of microorganisms to surfaces may be influenced by various physicochemical properties of the cell wall, such as charge, hydrophobicity/hydrophilicity, and acidic or basic character. Physicochemical properties vary among species and strains and even within the same strain under different growth conditions [73]. Various studies showed that hydrophobic bacteria can adhere better than hydrophilic bacteria. For example, Boutaleb *et al.* [74] stated that hydrophobic *P. aeruginosa* exhibited adhesion capacity much higher than that of hydrophilic *E. coli* strain. Bruinsma *et al.* [75] showed that *P. aeruginosa* being hydrophobic showed greater efficiency than hydrophilic *S. aureus*. Hydrophilicity of the cell surfaces is also influenced by its electrostatic charges. The surface of bacteria, which is usually negatively charged owing to the presence of phosphate ions, could therefore lead to electrostatic repulsion when the surface of a material is also negatively charged, thus preventing adhesion [76].

-Effect of Structures Anchored to the Bacterial Wall

Flagella, fimbriae, or pili are among the structures anchored to the bacterial wall that are crucial in adhesion to surfaces and in the stabilization of the extracellular matrix. Chakroun *et al.* [77] showed that *S. typhimurium* uses flagella, fimbriae, adhesins, and invasins to ensure motility, which leads to biofilm formation. The presence of flagella ensures that bacteria have mobility and can adhere to a surface by counteracting repulsive forces [78]. They allow for the localisation and initiation of microbial attachment. The flagella of *V. cholerae* are essential for the initial stage of biofilm formation, and it was also suggested that reduced motility, caused by flagella affected by an antimicrobial peptide (polymyxin B (PmB)), led to a decrease in biofilm formation capacity [79]. Besides, the ability of bacterial adhesion may be changed by the presence of different types of fimbriae, which are very important for biofilm-forming ability in some bacteria. McLay *et al.* [80] mentioned that increased expression of fimbriae enhanced the ability of the cells to adhere to solid surfaces. Other authors have also observed that the other adhesive organelles involved in the biofilm formation process are pili. Electrostatic adsorption may be generated through pili and pilus, which consequently ensures bacterial adhesion to surfaces [81].

-Effect of EPS Production

The EPS matrix accounts for more than 90% of the total dry mass of biofilms and consists of various extracellular polymeric substances such as polysaccharides, proteins, lipids, nucleic acids, and other biopolymers [21]. It is essential in the initial attachment to the surface and in the transition to irreversible adhesion [82]. Saravanan *et al.* [83] demonstrated a correlation between biofilm formation potential and EPS production. Parkar *et al.* [84] observed that the exopolysaccharide layer in *Bacillus* cells played an important role in their adhesion to stainless steel surfaces, and the removal of this matrix using trichloroacetic acid or lysozyme solutions led to a reduction in their attachment. Some EPS polymers are hydrophilic. This maintains and stabilizes the biofilm structure by protecting cells from dehydration by creating a hydrated gel around the cells [85]. Tamaru *et al.* [86] have illustrated that during desiccation, isolated cells of *Nostoc commune* presented a loss of cellular viability and photosynthetic activity, whereas biofilm cells were much more resistant to desiccation. Similarly, Liu *et al.* [87] also illustrated the significantly enhanced survival capability of *Pseudoalteromonas* sp. owing to EPS production in surviving freeze-thaw cycles.

Environmental Factors

- Effect of Medium Composition

Bacteria on the surface of food materials are likely to come into contact with the food and its components. Therefore, the composition of the medium plays an important role in the morphology, thickness, biomass, activity, and development of the biofilm, depending on the availability of nutrients. One such study shows the nutrient-dependent features of biofilm produced by the *L. monocytogenes* bacteria. Nutrient deficiency promoted biofilm formation by this bacterium, as demonstrated in the study [88]. In conditions where nutrition levels are minimal, a well structured net-like *L. monocytogenes* cell population could be noticed in their biofilms. Kadam *et al.* [89] found that nutritional limitations can act as a factor promoting biofilm formation, leading to the stimulation of exopolymer secretion, essential for the initial adhesion of microorganisms. Soanen *et al.* [90] found that nitrogen deficiency resulted in higher exopolysaccharide secretion in *Porphyridium marinum* [90]. Other research suggests that biofilm formation is favoured when nutrient availability is high. For example, in *Enterococcus faecalis*, media rich in glucose and vitamin K, containing high concentrations of nutrients, promoted better biofilm formation [90].

-Effect of Hydrodynamic Forces

Hydrodynamic conditions can control biofilm development. They can influence biofilm thickness, the amount of biomass adhered, the nature and quantity of EPS produced by the biofilm, by altering

nutrient and oxygen supply and applying shear forces [92]. Indeed, a study on the effects of various hydrodynamic conditions on the biofilm architecture and EPS composition showed that laminar flow resulted in thick but relatively loose biofilms, but under turbulent flow conditions, the biofilms were thin but dense, with a higher total EPS content [93]. Another study demonstrated that under a laminar flow regime, biofilm growth of *E. coli* was enhanced after 72 h, but a turbulent flow regime resulted in a reduction of biomass after 48 h [95]. Hydrodynamic forces may also cause biofilm dispersion. Fink *et al.* [95] found that high shear caused by a high liquid flow rate facilitates dispersion and hence inhibits biofilm formation. But, Fanesi *et al.* [96] found that the cohesion of the *Chlorella vulgaris* microalgal biofilm and biomass production was improved under high shear; a low shear provided heterogeneous cohesion.

-Effect of Temperature

Research has demonstrated that temperature plays a crucial role in biofilm formation. For example, Song *et al.* [97] showed that 25 °C is the optimal temperature for *Vibrio parahaemolyticus* biofilm formation. Biofilm increases with increasing temperature. *S. enterica* formed more robust biofilms at higher temperatures (25–42°C), whereas lower temperatures (4–10°C) significantly reduced biofilm formation [98]. In the case of *Clostridium perfringens*, temperature also influenced adhesion: cells adhered effectively at 37°C but not at 25°C [99]. Da Silva *et al.* [100] found that there was an increase in adhesion of *S. aureus* to polyethylene and stainless steel surfaces at 20°C incubation temperature, used comparatively to 7, 10, 12, and 15°C incubation temperatures. Temperature can alter the surface characteristics of bacterial cells, enhancing or reducing biofilm formation on specific surfaces. For instance, the hydrophobicity of the surfaces of *S. aureus* and *L. monocytogenes* is augmented by increased temperature which translates into increased biofilm formation [100]. Temperature has an impact on the adhesive structures of bacteria such as flagella, fimbriae and other parts. In aquatic *Pseudomonas*, lower operational temperatures have been linked to reduced adhesive properties [101].

-Effect of pH

Microbial development and biofilm formation can cease if the pH of the surrounding medium deviates from the optimal growth pH [101]. The pH can affect changes in the charge of the surfaces of microorganisms and solid supports, leading to either more or less adhesion. Nostro *et al.* [102] maintain that alkaline pH can alter the conformations of proteins that are supposed to govern the hydrophobicity of the bacterial surface. In *S. aureus* and *Staphylococcus epidermidis*, higher pH had an inhibitory effect on adhesion. Mathlouthi *et al.* [103] demonstrated that there was a correlation between biofilm formation in *E. coli* and the surrounding pH. Bacterial cultures were grown in Luria Bertani (LB) medium under different pH conditions: neutral pH 7.4 and acidic pH 5.5 (at 37°C and 25°C). It was

concluded that acidic pH and a temperature of 37°C were more conducive to biofilm formation. The results of the study showed that *E. coli* biofilm formation is influenced by both pH and temperature. In contrast, in *Lactiplantibacillus plantarum*, increasing pH promoted increased biofilm formation [104].

Materials and Methods Used to Study Biofilms

Theoretical Approach

In thermodynamics, the DLVO and XDLVO theories present two predictive models for bacterial adhesion [75]. The classical DLVO theory proposed by Derjaguin, Landau, Verwey, and Overbeek accounted for adhesion as a result of two oppositional forces; attractive Lifshitz van der Waals forces (GLW) and electrostatic interactions (GEL). This, however, did not account for Lewis acid-base interactions. It takes into consideration that the bacteria (0.5-2 µm) are colloidal particles in neutral medium interacting with charged surfaces; adhesion is influenced by ionic concentration-low concentrations which provoke repulsive barriers; moderate concentrations allow reversible adhesion but high concentrations provoke irreversible adhesion [105].

DLVO has some deficiencies, as it overlooks short-distance interactions and the assumption of homogeneity of bacterial surfaces does not take into account the adaptive behaviour of microorganisms when subjected to different environmental conditions [106]. The XDLVO theory of van Oss works by further inputting the acid-base interaction (ΔGAB) along what is accounted for in DLVO; i.e., for Lifshitz-van der-Waals (ΔGLW) and Electrostatic (ΔGEL) interactions and total interaction energy is expressed as $\Delta GTOT = \Delta GLW + \Delta GEL + \Delta GAB$. Adhesion is favourable according to thermodynamics if $\Delta GTOT$ is negative, but if positive, repulsive [107].

The free energy of interaction ($\Delta GMSL$) between a microorganism (M) and a surface (S) in liquid (L) using the Dupré equation is $\Delta GMSL = \gamma_{MS} - \gamma_{ML} - \gamma_{SL}$ (where γ denotes surface free energy). By measuring the contact angle, one can measure microbial and surface energy values with acid-base interactions very often dominating, making XDLVO more successful than DLVO in explaining bacterial adhesion, e.g., *Acinetobacter calcoaceticus* on polystyrene [108]. As congruent as it is strong, XDLVO sees bacteria just as inert and uniform colloidal particles, thereby wrongfully skipping over exopolymers and adhesins, thus limiting predictions on some results of experiments.

Experimental Approach (Study of Biofilm Formation in the Laboratory)

Static Mode

Static mode adhesion tests are tests carried out in the laboratory without any agitation or circulation to assess the ability of a particular microorganism to adhere to a given support under different conditions [75]. The supports to carry out these tests must be sterilized and disinfected and then immersed in a tube containing the cell suspension. A gentle rinse is then carried out to remove any cells not adhering to the surface [75]. Counting and observation of the surfaces can be carried out using different types of microscope. The choice of microscope will depend on the objective of the study and the type of surface to be examined. The ability of hygiene indicator bacteria to adhere to the surfaces of food processing materials has been studied by different authors using adhesion tests. For example, Elfazazi *et al.* [74] examined the adhesion of twelve *E. coli* strains isolated from food to the glass surface using adhesion tests.

Plate Systems (Microtitration)

The microtiter plate method is a study method used to assess the ability of pathogenic strains to form biofilm on abiotic [109]. This test, also known as a 96-well plate test, uses dyes such as crystal violet to stain adherent biofilms [109,110]. An absorption microtiter plate reader quantifies and measures optical densities (OD). Various adhesion substrates such as glass beads, titanium discs and hydroxyapatite discs can be added to the bottom of the plate and be used for imaging [110]. Evaluation of early biofilm formation of various microorganisms such as *Pseudomonads*, *Staphylococci*, *Enterococci*, *Mycobacteria*, *V. cholerae*, *E. coli*, and fungi has been studied using this microtiter assay [111]. Despite the simplicity of the tests and their ease of use, their static mode limits their operation as the biofilms are not exposed to the shear forces that can be present in industrial environments.

Dynamic Mode

-Continuous Flow Culture Systems

The continuous flow chamber system (or basic Flow Cell) is a more advanced system that allows biofilm formation and growth in small chambers with continuous availability of nutrients under hydrodynamic conditions [110]. One advantage of this model is that it is more representative of real conditions and makes it possible to monitor the growth of microorganisms in real time using confocal microscopy, thanks to biofilm cells that can be labelled with fluorescent probes or proteins compatible with the analysis [110,112]. In these flow chambers, the flow is mainly laminar, which facilitates the formation of biofilms under controlled conditions and allows changes to be made to the growth

medium efficiently at a specific time. The Flow cell system has been used to study the biofilm development of various microorganisms such as *Candida albicans* and *Staphylococcus epidermidis* [113,114].

-Bioreactors

Biofilm reactors for studying the phenomenon of biofilm formation or its effects include: continuous stirred tank reactors, packed bed reactor, filter bed reactor, moving bed biofilm reactor (MBBR), batch sequential biofilm reactor (SBBR), rotating disc reactor, MBR membrane bioreactor, pulsed plate reactor, continuous stirred tank reactors, [115]. In most of these, the microorganisms attach themselves to organic or inorganic supports, forming a biofilm and thus catalysing reactions within the bioreactor, before being eliminated or inactivated once their function has been accomplished [116]. Fluid dynamics is one of the defining characteristics that allow a researcher to choose the best reactor to use for the study [117]. For example, the CDC is a continuously stirred tank biofilm reactor that was designed by CDC researchers to study different biofilms. Within this reactor, nutrients are introduced at a constant rate and by-products are discharged at the same rate which avoids any net accumulation of liquid [117].

-Microfluidic Systems

In recent years, microfluidic systems have been used for the experimental modelling and analysis of biofilms because of their low cost, rapid analysis, small sample volumes, and controllable conditions [110,118]. Microfluidic devices manipulate fluids using small channels, typically just a few microns thick, with a constant and low fluid flow rate that facilitates submillimetric scale experiments [119]. Among the microfluidic devices that provide a promising platform for studying biofilms are the Bioflux and BiofilmChip. The Bioflux system allows for the control of biofilm growth at different temperatures using a heated plate, varying airflow rates depending on the applied pressure, and varying oxygen requirements by adding gas reservoirs [110]. It also enables switching between different growth media via its dual input system. The BiofilmChip system is similar to the Bioflux but does not use the same plate technology. It allows for dynamic growth analysis without the need for a confocal microscope by using electrochemical impedance spectroscopy (EIS) technology. The use of probes and stains, as well as confocal microscopy in microfluidic systems, offers the possibility of imaging the biofilm directly in situ in real-time [120].

Determination of Surface Properties

The determination of the energy components of bacterial surfaces and materials has been studied by various authors in order to compare the results of predictive and experimental approaches and validate

the usefulness of the thermodynamic approach (ΔG iwi) to explain the bacterial adhesion capacity on a given surface. The goniometer is one of the instruments used to determine the physicochemical properties of bacterial surfaces and substrates using the sessile drop method [121]. The contact angle of three reference solvents: water, ethylene glycol, and diiodomethane is often measured to determine the surface energies of bacteria and substrate surfaces. Table 2 presents examples of contact angle measurements and surface energy components (± 1 mJ/m²) of different bacterial surfaces. Table 3 provides examples of surface properties of construction materials used in the food industry.

Table 2. Contact angle measurements and surface energy components (± 1 mJ/m²) of different bacterial surfaces (γ_{LW} : dispersive component; γ_{AB} : polar component; γ_+ : electron acceptor component; γ_- : electron donor component).

Substrates	Contact angle (°)				Ref
	D	F	EG	W	
<i>B. cereus</i>	89 (± 2.9)	50.2 (± 4.9)	ND -	52.6 (± 1.9)	[122]
<i>E. coli</i>	61.40 (± 3.1)	ND -	36.7 (± 5.3)	17.5 (± 2.1)	[123]
<i>S. aureus</i>	51.10 (± 0.61)	ND -	44.20 (± 1.58)	42.70 (± 1.36)	[124]
<i>P. aeruginosa</i>	79.4 (± 2.5)	ND -	53.2 (± 2.6)	50.3 (3.3)	[123]
<i>P. savastanoi B97</i>	47.83 (± 0.34)	43.40 (± 0.29)	ND -	31.80 (± 0.65)	[125]
Substrates	Surface tension (mJ m ⁻²)				Ref
	γ_{LW}	γ_{AB}	γ_+	γ_-	
<i>B. cereus</i>	13.1 -	33.54 -	8.9 -	31.6 -	[122]
<i>E. coli</i>	22.2(± 5.3)	ND	6.3 (± 1.1)	50.9 (± 3.2)	[123]
<i>S. aureus</i>	37.50(± 0.11)	ND -	0.40 (± 0.01)	55.70 (± 1.43)	[124]
<i>P. aeruginosa</i>	17.7(± 1.2)	ND -	0.2 (± 0.0)	35.4 (± 2.0)	[123]
<i>P. savastanoi B97</i>	37.60 -	ND -	0.57 -	29.23 -	[125]

Table 3. Contact angle measurements and surface energy components (± 1 mJ/m²) of different materials used in the food industry (γ_{LW} : dispersive component; γ_{AB} : polar component; γ_{+} : electron acceptor component; γ_{-} : electron donor component).

Substrates	Contact angle (°)			Surface tension (mJ m ⁻²)				Ref
	D	F	W	γ_{LW}	γ_{AB}	γ_{+}	γ_{-}	
Glass	46.40 (1.40)	45.80 (3.30)	36.50 (3.10)	36.30	ND	40.36	1.54	[121]
	59.52 (2.15)	46.28 (1.53)	36.14 (3.20)	28.79	ND	0.73	54.90	[74]
PVC	91.80 (0.70)	76.20 (1.40)	80.60 (2.50)	41.20	ND	1.01	6.81	[121]
	39.00 (-)	76.00 (-)	92.00	40.00	5.00	1.30	4.60	[75]
SS	60.00 (5.00)	68.00 (1.80)	64.00 (2.00)	28.10	3.16	0.10	32.50	[126]
PEX	35.00 (-)	55.00 (-)	71.00 (-)	42.00	0.30	0.00	12.00	[75]
Si	61.40 (1.65)	63.90 (6.15)	99.30 (2.95)	27.80	0.93	0.10	2.20	[122]
HDPE	43.00 (-)	67.00 (-)	73.00 (-)	38.00	6.00	0.60	19.00	[75]
PP	57.60 (-)	50.10 (-)	77.80 (-)	30.00	6.65	2.70	4.10	[127]
PET	36.80 (-)	57.30 (-)	80.90 (-)	41.20	0.00	0.00	4.90	[127]

ND: Not determined; D: Diiodomethane; F: Formamide; EG: Ethylene glycol; W: Water ; Glass (Glass), Polyvinyl chloride (PVC), Stainless steel (SS), Cross-linked polyethylene (PEX), Silicone (Si), High-density polyethylene (HDPE), Polypropylene (PP), Polyethylene terephthalate (PET).

Some studies have shown that measuring the contact angle, along with the data obtained from the predictive approach, constitutes a valuable tool for predicting bioadhesion. Other studies have demonstrated that there is not always a correlation between surface properties and microorganism adhesion, and that *inter al.* environmental conditions govern this process.

Microscopy Methods for Biofilm Observation

Optical Microscopy

Optical microscopy is a basic technique for visually identifying biofilm formation. It provides general information about the presence of the biofilm, as well as its appearance, and organization on the studied surface [128]. Cost-effective staining methods, such as haematoxylin and eosin (H&E), periodic acid-Schiff (PAS), and Gram Brown and Brenn, are commonly used to assess biofilm biomass [129]. For observation under an optical microscope, microorganisms are fixed on a flat, transparent surface [130].

The use of optical microscopy does not allow for a detailed description of the biofilm cell morphology and the architecture of the exopolysaccharide matrix, as its resolution is limited by the wavelength of light [129]. Combining optical microscopy with transmission electron microscopy (TEM) or scanning electron microscopy (SEM) is optimal for studying biofilm formation [129].

Confocal Laser Scanning Microscopy (CLSM)

Confocal Laser Scanning Microscopy (CLSM) is a non-invasive technique for examining fully hydrated living biofilms using an optical microscope with a laser beam [6]. It enables visualisation of biofilm 3D architecture and real-time (4D) monitoring of structural changes [131]. Samples are stained with specific fluorescent dyes, such as SYTO-9 and SYBR-Green, which are used as microbial cell markers [129]. Live/dead staining has the ability to determine cell viability in biofilms through this technique [132]. CLSM cannot view matrix components because of its resolution limitations and possible interference with signals by the intrinsic fluorescence of biofilms [131].

Atomic Force Microscopy (AFM)

Atomic Force Microscopy (AFM) is an effective method for examining how biofilms interact with substrates and for observing individual cells in real-life conditions [133,134]. It provides resolution at the nanometer or even sub-nanometer level, setting it apart from both optical and electron microscopy [133]. Compared to optical microscopy, limited by the wavelength resolution, AFM offers much higher resolution during scanning, which is affected by the radius of curvature of the AFM tip, typically between 2 and 5 nm [135]. AFM can also monitor living cells in real-time in liquid environments [134]. It has been used to study biofilms on material surfaces, such as freshwater biofilms on copper [136]. AFM also allows observation of EPS distribution alongside cells [133].

Scanning Electron Microscopy (SEM)

SEM is a highly effective method for describing biofilm morphology, providing high magnification (20 to 30,000×) and resolution (50 to 100 nm) [131]. It also allows imaging of samples ranging from a few microns to tens of centimetres in diameter [137]. Numerous studies have published images of biofilms adhered to the surfaces of various food industry materials using scanning electron microscopy. Boutaleb et al. [75] captured SEM images of *E. coli* and *P. aeruginosa* biofilms on materials in drinking water pipelines. SEM has been used to confirm the anti-adhesion properties of PVC pipes incorporating natural oils [73]. Local elemental analysis with Energy-Dispersive X-ray Spectroscopy (EDS) is possible under vacuum conditions (10^{-5} to 10^{-6} mbar) and requires sample fixation with formaldehyde-based fixatives like paraformaldehyde or glutaraldehyde, causing protein

cross-linking [132,137]. Alternatives to conventional SEM include Variable Pressure SEM (VP-SEM), Cryo-SEM, Environmental SEM (ESEM), and Field Emission SEM (FE-SEM).

Transmission Electron Microscopy (TEM)

Like SEM, TEM uses an electron beam and operates under vacuum [132]. TEM is less commonly used for biofilm analysis because it needs very thin sections [110,138]. The chief distinction is the source of the electrons used – in TEM, the electrons are emitted from the sample, resulting in higher spatial resolution compared to the other types of electron microscopes [132,138]. Microbial cells, cell walls, and biofilm components can be imaged with high resolution and no markers using TEM. But, the information is ‘static’ only - because of the fixation of the specimen, limiting dynamic studies [138]. Nevertheless, MET is preferred for in-depth studies of biological nanostructures. Several microscopy methods are available for biofilm analysis and visualization, but each method has unique advantages and disadvantages depending on the goal of the study. Different types of microscopes can also be used to capture the same object. Figure 2 shows comparative images of *E. coli* that were taken by other microscopes to illustrate.

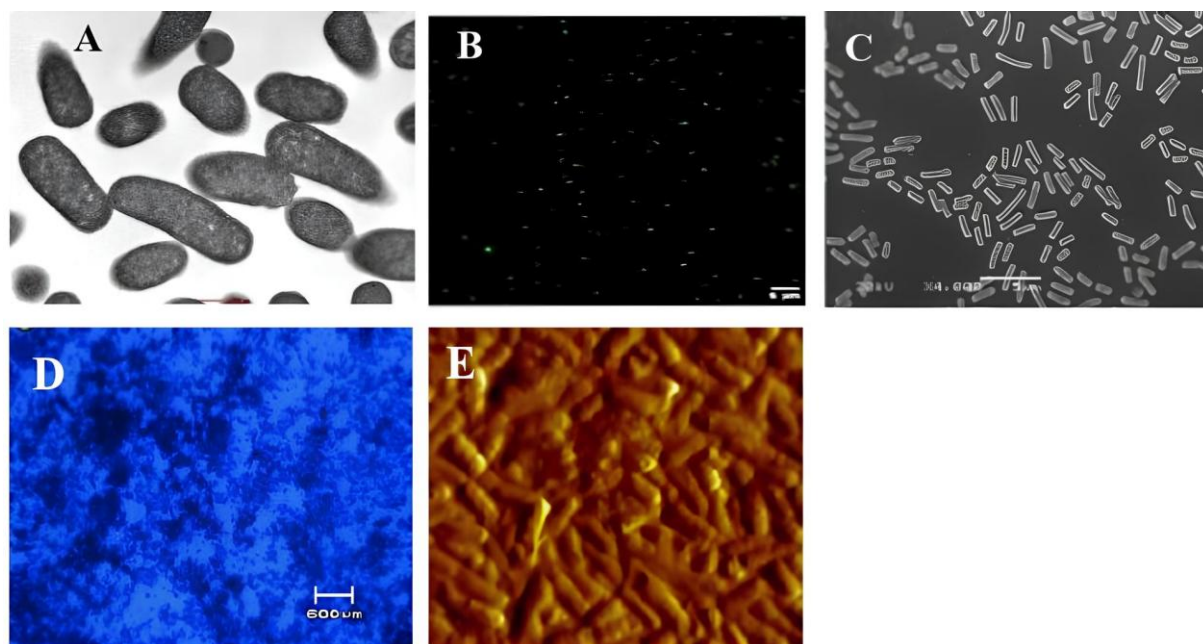


Figure 2. Comparative images of *Escherichia coli* biofilms observed using different microscopic techniques. (A) Transmission Electron Microscopy (TEM) image [139]; (B) Confocal Laser Scanning Microscopy (CLSM) image [140]; (C) Scanning Electron Microscopy (SEM) image [141]; (D) Optical Microscopy image stained with crystal violet dye [142]; (E) Atomic Force Microscopy (AFM) image [143].

Recent Advances in the Fight Against Biofilms

Disinfection and Cleaning Methods

Numerous disinfection strategies have been adopted in the food industry to inhibit biofilm formation and reduce microbial loads. Biocide cleaning is one treatment used to disinfect material and equipment surfaces. The wide variety of biocides includes quaternary ammonium compounds (QACs), hypochlorites, halogen-releasing compounds, peroxygen compounds, chlorine dioxide, alkylamines, and alcohol-based compounds [144,7]. There are also other innovative approaches based on physics for surface disinfection and biofilm eradication, such as the application of ultrasonication, ultraviolet irradiation, and cold plasma treatment. Direct contact with ultrasound is a non-chemical and environment-friendly approach used in the food sector, with a significant inhibitory effect on biofilms of various bacterial species such as *S. aureus*, *Salmonella enteritidis*, *L. pneumophila*, and *A. castellani* [7]. Another non-chemical application used in the food industry is ultraviolet irradiation, which can remove biofilms. The UV rays of 200 to 300 nm, especially near 260 nm, can cause damage to nucleic acid molecules [7, 145]. Cold plasma treatment of materials is another option. It aims to improve the quality of finished products. The active components of cold plasma exert antimicrobial effects through disintegration of intracellular components and interference with the metabolic pathways in the pathogens according to Zhu et al. [146].

Essential Oils

Essential oils can be used as biocides to disinfect and clean food contact surfaces or as additives in films and coatings for active food packaging. Essential oils include various bioactive compounds that are characterised by antioxidant, anti-inflammatory, antimicrobial, and anti-biofilm activities [147]. Bazargani and Rohloff [148] demonstrated that essential oils of coriander, anise, and peppermint inhibit the adhesion of *E. coli* and *S. aureus* bacteria and the formation of their biofilms. Essential oils can inhibit biofilm formation in various ways, either by inhibiting microorganism motility, modifying the expression of genes related to quorum sensing, affecting the formation of extracellular polymeric substances, or causing disruption of the cell membrane [149]. Leesombun et al. [150] showed that the essential oil of *Coleus amboinicus* exhibited anti-biofilm activity against *S. Typhimurium* through downregulation of genes active in motility, curli fimbriae production, and suppression of the invasion gene expression. Guillín et al. [151] investigated the anti-biofilm activity of the essential oil of *Lippia origanoides* against *S. enterica*. The results showed that *Lippia origanoides* essential oil modified the expression of QS-related genes, inhibition of curli genes, genes active in cell communication, and negative regulation of motility genes.

Antimicrobial Peptides

Antimicrobial peptides (AMPs) are a class of small peptides naturally produced by various organisms (mammals, amphibians, insects, microorganisms) as part of their defence system [152]. The positive charge of the peptides allows them to interact with the negatively charged bacterial cell membranes and the biofilm surfaces, thereby disrupting the membrane structure [153]. AMPs act through various mechanisms to affect biofilm formation, such as inhibiting microorganism adhesion, disrupting the quorum sensing system, and destroying pre-formed biofilms [153]. For example, the biofilm of *P. aeruginosa* could be inhibited by AMPs by preventing the expression of genes encoding proteins active in quorum detection, pili IV, rhamnolipid synthesis, and flagella production [7]. The work of Woraprayote et al. [154] demonstrated that coating a food packaging film with bacteriocin peptides resulted in a decrease in biofilms of *E. coli*, *A. hydrophila*, *L. monocytogenes*, and *S. aureus*. Additionally, coating a sodium caseinate film intended for cheese storage with nisin peptide led to a significant reduction in *L. innocua* biofilms [155].

Nanoparticules

Nanoparticles are materials characterized by their size, ranging from 1 to 100 nm, which enables them to cross biological barriers [156,157]. The high reactivity of nanoparticles may justify their inhibition capacity [158]. Nanomaterials have been used in various applications in the food industry, such as food packaging, design of food contact surfaces and food additives [159]. Among the main metallic nanoparticles of interest are silver, gold, copper, titanium, iron and zinc [53]. For example, the effect of different concentrations of silver nanoparticles on the bacterial biofilm of *K. pneumoniae* was examined and the results revealed a correlation between increasing concentrations of AgNP and a reduction in the biofilm owing to the destruction of the EPS matrix [160]. Silver nanoparticles exhibit growth inhibitory activity against various food-borne pathogenic microorganisms such as *Salmonella*, *E. coli* and *L. monocytogenes* [156]. In addition, it has been reported that glass coatings with zinc oxide nanoparticles exhibit antibacterial effects, leading to a reduction in *E. coli* and *S. aureus* biofilms [161].

Use of Bacteriophages

Bacteriophages are host-specific viruses capable of infecting bacteria [162]. They can eliminate biofilms either by integrating into the bacterial genome, thereby affecting biofilm formation, or by inducing cell lysis through the coding of lyases, or by expressing certain enzymes capable of degrading extracellular polymers and thus destroying the polysaccharide matrix [163]. Bacteriophages can eliminate biofilms of *S. Enteritidis*, *Salmonella Heidelberg*, *Salmonella Kentucky*, *Salmonella Senftenberg*, and *S. Typhimurium* on surfaces found in chicken slaughterhouses [164]. The treatment of polyethylene, rubber, and stainless steel surfaces with phage JK004 appears to decrease the

adherence of *Cronobacter sakazakii* [165]. There are constraints on phage use because of the rise of resistant mutants [162]. Thus, the application of phage mixtures (phage cocktails) has been implemented to reduce the emergence of phage resistance and cope with bacterial regrowth [163].

Probiotics

Probiotics are live microorganisms that are beneficial to the host, capable of producing bacteriostatic agents and competing with pathogenic microorganisms for binding sites and nutrients, thus preventing their growth and adhesion to surfaces [166]. Probiotics can be used for biological food preservation, active food packaging and the production of biosurfactants [170]. *Lactobacillus* is an example of lactic acid bacteria used as probiotics, releasing metabolites that inhibit the formation of biofilms on surfaces [166,167]. A biosurfactant produced by the strain *Lactobacillus acidophilus* exhibited antibiofilm activity, significantly inhibiting the quorum sensing-regulated virulence factors of Gram-negative pathogenic bacteria, thus reducing swimming motility and the production of exopolysaccharides (EPS) [168]. The incorporation of probiotics into biopolymer matrices is also a recent trend for controlling and preventing biofilm formation. Several studies have proven that integrating probiotics into edible packaging improves the shelf life of food products by inhibiting the growth of pathogenic agents [169].

Surface Modification - Anti-Adhesion Coating (Antibacterial Surface)

Coating surfaces to prevent the initial stages of biofilm formation is a strategy that has attracted the attention of various researchers. In addition to those antibiofilm coatings mentioned in previous paragraphs, such as nanoparticulate coatings and peptide-based coatings, other notable examples include antibacterial polymer coatings functionalised with antibacterial enzymes, coatings with integrated antibiotics and other antibiofilm agents, and essential oil-based coatings. For instance, Louanjli et al. [123] demonstrated that the incorporation of bioactive oils, such as cade, turmeric, and cumin oils, into the industrial formulation of plastic films could inhibit the bacterial adhesion of *E. coli* and *P. aeruginosa*, with anti-adhesion properties of up to 80%. Modifying a silicon surface with a zwitterionic surface containing positively charged quaternary ammonium, hydrophobic benzyl, and hydrophilic lysine methacrylamide groups reduces bacterial adhesion [56,123,170]. Furthermore, it has been demonstrated that grafting acrylic acid onto polyethylene terephthalate films by copolymerisation with gamma radiation alters their physicochemical properties by increasing their hydrophilicity, consequently leading to a reduction in bacterial adhesion [56,123,170].

Conclusion

The main problems of the agri-food industry are the economic losses and serious public health risks caused by biofilm formation. Depending on the microbial surface properties, the nature of the agri-food materials, and the environmental conditions, a large number of microorganisms can attach to agri-food surfaces and develop robust biofilms. Therefore, researchers are engaged in active study regarding biofilm formation and the promoting factors of adhesion to develop effective strategies of prevention and cure. Such efforts will lead to an improvement in food production processes and reduce the possibility of surface contamination. This review has surveyed biofilm development in the agri-food area, starting with factors affecting microbial adhesion, predictive and experimental methodology for biofilm study, techniques of visualization, and techniques of control and eradication. There are many difficulties in applying the valuable data at the industrial level.

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Iraq's Desertification: The Complication of Environmental Security Issues

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Graphical Abstract

Iraq's rapid desertification of its arable land poses an existential threat to its population and environment. It manifests many other environmental problems and adds to an already deteriorating situation. This research addresses the causes of desertification in Iraq and assesses the green belt project, focusing on its challenges. It also derives lessons from other countries with similar experiences and suggests solutions from a security standpoint.



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Abstract: Iraq's numerous environmental problems are interconnected, with one issue leading to another and complicating matters further. Natural and human-induced climate change, a significant decline in rainfall, surface and groundwater depletion, reduced vegetation, deforestation, increasing dust and sandstorms, soil erosion, loss of fertile land and green cover, and the shrinkage of marshes are all intertwined and affecting one another. Their most prominent manifestation is perhaps the transformation of Iraq's fertile lands into deserts, a phenomenon known as desertification. This paper examines the government's attempts to combat the problem and the obstacles encountered. It contrasts the efforts of China and Africa against desertification and draws lessons for Iraq. It argues that desertification, along with other environmental issues, poses a genuine threat to the security of the populace and the future of the country.

Keywords: Desertification, Iraq, Green belt, Community involvement.

Introduction

Environmental problems gained prominence from the second half of the twentieth century as a matter directly related to the sustainability of modern life [1,2]. Since the turn of the century, they began to be seen in a new light, equating them with traditional security matters involving military threats from other states and, by default, justifying the allocation of the greatest financial and human resources aimed at repelling those threats [3-5]. The intention was that environmental issues must be treated with urgency and with all available means because the threats posed by environmental problems are existential; they represent a matter of 'to be or not to be,' or 'the point of no return,' [6].

Iraq's most formidable and increasingly urgent environmental issue is the desertification of its arable land. Iraq was known in the past for its abundance of water, heavy snowfall in the north during winter, and excessive rainfall at times in autumn and mainly in spring in the north, as well as in other parts of Iraq. Unobstructed water flow from Türkiye and Iran through the Tigris and Euphrates Rivers often filled all the rivers and canals and occasionally caused floods. The presence of large marshes and many oases in the south, cold weather in winter, and moderate to hot weather in summer characterized Iraq; but, all these characteristics have changed. Iraq now faces significant environmental problems. The country's climate is radically changing, resulting in a critical water shortage in many areas, sharply rising temperatures, degradation and rapid loss of fertile land owing to desertification.

Iraq is one of those countries with a severe desertification problem. Vast deserts in the west and southwest are expanding toward the east and northeast, rendering lands infertile and forcing inhabitants to seek other places to live. Desertification is also occurring in enclaved areas, not just as an extension of the desert. It is caused by many factors, such as the increasing occurrence of dust and sandstorms, the decline in annual precipitation, the reduction of Iraq's share of waters from neighbouring states, increasing salinity, and a rise in pollutants in surface and groundwater, along with a lack of proper drainage systems, deforestation, urbanisation, and woodfires. The problem is worsened by the government's insufficient action.

Iraq's soil and its various climatic systems, from north to south, are significant for agricultural productivity and animal husbandry. The country was often referred to as the 'black land' to indicate the richness of its soil. This name can no longer be upheld. This paper examines the increasing threat of desertification in Iraq and suggests solutions in a security perspective. It reviews the concept, demonstrates the scale of the problem in Iraq, examines the factors behind it, and discusses Iraq's attempts to confront it, along with the obstacles faced. Finally, the paper analyses two examples of combating desertification in other regions of the world, draws a conclusion, and makes suggestions.

Desertification

Desertification is the process by which fertile land becomes deserts. Deserts are typically defined as regions ‘whose soils have been degraded to pebbly and rocky stretches or to dunes and other unproductive strata,’ regions with little or no rain, or those that experience excessive evapotranspiration. Consequently, they do not support vegetation or agriculture [7]. Desertification converts fertile lands into deserts; the lands acquire desert-like features in terms of productivity, either because of climate change or human activities [8]. It is defined as land degradation or land deterioration, “the prevalence of desert conditions”, as well as ‘the encroachment of the desert’, ‘high rates of drought’, or ‘poor ability of the land to produce’, in arid, semi-arid, and dry subhumid areas resulting from various factors, including climatic variations and human influences. Thus, desertification means that lands become barren and unstable; they become unproductive and undermine livelihoods [9,10].

Desertification was first noticed as an environmental problem in the 1930s when parts of the United States became ‘the dust bowl’ - transformed into deserts through drought and poor farming practices [11]. The term ‘desertification’ is not ancient, but it is not the only one used to describe the situation. Scholars generally use the term ‘desertification,’ which can describe the phenomenon more accurately. Other terms have also been used: ‘desiccation,’ and ‘desertization’ [12,13]. We will adhere to ‘desertification’ since it is the dominant term in academe and the media [14]. The essence is that the land dries, does not support food production, leads to poverty or displacement of any settlers, and therefore poses unexpected socio-economic problems.

The 1994 UN Convention to Combat Desertification (UNCCD) defines desertification as ‘land degradation in arid, semi-arid and dry sub-humid areas resulting from various factors such as drought, deforestation, overgrazing, overcultivation, and inappropriate agriculture’ [15]. It is the ‘diminution or destruction of the biological potential of the land’, which can ultimately lead to desert-like conditions [16].

Desertification is attributed to climate change and the various weather patterns throughout the earth’s history. What is now uncultivated land in northern Africa was once humid and used to grow grain for supply to Europe during Roman times [7]. The process, however, is increasingly and largely ascribed to human activity [17]. It occurs because of ‘man’s excessive economic activities that destroy the ecological balance of arid and semi-arid areas, resulting in blown sand and dunes extending into these regions’ [18]. The United Nations Environment Programme maintains that desertification can be attributed solely to human activity. It results from ‘adverse human impact’ in arid, semi-arid, and dry sub-humid areas, with the outcome being ‘land degradation’ [19].

Many scholars agree on the combination of both climatic and human factors. They refer to population growth and increasing pressure on water resources, depletion of groundwater caused by over-extraction, salinization, overgrazing, deforestation, improper irrigation and farming methods, climate changes such as rising temperatures, an increase in dust and sandstorms, deterioration of air quality, heightened industrial activities and mining, and reduced precipitation as significant contributors to desertification in arid and semi-arid regions, aka dryland areas, worldwide [20-22]. Whether caused by human beings, nature, or a combination of both factors, the outcome is that large areas become unproductive and unsupportive of vegetation, with numerous consequences for life and human wellbeing. Dryland areas cover over 45% of the Earth's land mass and are home to approximately 3 billion people. This figure is anticipated to rise to 4 billion by 2050 [23-25].

Desertification is not exclusive to Africa, as was previously thought [15]. In fact, it is a growing problem worldwide: in Africa, Asia, the Americas, the Middle East, and even Europe [26]; it is certainly a formidable existential threat in Iraq. According to UNCCD, 'around 12m hectares of productive land are lost to desertification and drought each year'. The same source estimates that this area *could produce* 20 million tonnes of grain annually [27].

Desertification causes numerous problems for people and the environment. It leads to the deterioration of the life-support system in the affected area, thus forcing people to move to other areas; reduces the planet's capacity to produce food; destroys vegetation and grazing lands; diminishes plant and animal populations; increases atmospheric dust; and creates many other complications [15].

Desertification Problem in Iraq

Iraq's desertification problem is growing fast. The vast deserts in the Midwest and Southwest, which constitute over 30% of Iraq's land, are expanding, while the cultivable land and green covers in the south, centre, and north are shrinking at an accelerating rate, threatening the inhabitants in those regions and forcing them to leave [28,29]. The vegetation cover in many areas, such as between the Tigris and Euphrates rivers, is rapidly decreasing [30], and many fertile lands have already been lost to desertification, including the marshlands in the southern areas of Iraq, 90% of which have dried up [31,32]. The Environment Directorate in the city of Najaf, in southern Iraq, reported in 2023 that desertification has increased by 5% in the governorate compared to the previous year, and that desertification has affected about 70% of the area [33]. Other reports suggest that 90% of Iraq's total land is under the threat of desertification, and 45% of agricultural land faces drought and desertification [34,35].

Iraq is among the countries least prepared for climate change. Iraq's prolonged wars and conflicts, both internally and with neighbouring states, its long-standing dictatorial regime under Saddam Hussein until 2003, and problems with terrorism, along with numerous ethnic and religious conflicts since then, have resulted in weak state institutions and massive corruption at all levels. This combination makes Iraq highly vulnerable to the impacts of climate change, which have been largely manifested in intense and prolonged heat waves, higher temperatures, frequent occurrences of dust and sandstorms, and water scarcity caused by increasing demand and decreasing precipitation. Unfortunately for Iraq, climate change affects some regions more than others worldwide, and Iraq is among the most affected areas [36]. In the words of its Prime Minister at a climate conference in 2023, climate change has affected more than seven million Iraqis and displaced hundreds of thousands of people [37], including 3 thousand families in 2022 [38].

The Iraqi climate is characterized as dry or semi-dry; 90% of the total area of Iraq's land lies within the arid and semi-arid climate zone, with an average of 150 mm of rainfall per year and high evaporation [10]. Rainfall varies considerably between different areas, with the northeast receiving the most rainfall – up to 1,000 mm in some narrow mountainous areas – and the southwest receiving less than 100 mm. Under the impact of climate change, the rain line continuously shifts further north, resulting in more areas receiving progressively less rain [39].

Iraq's surface water, which largely comes from neighbouring countries, with 80% sourced from Türkiye, faces a significant decline because of holding water in reservoirs in those countries and droughts. Combined with water mismanagement, this deepens the already dangerous situation of desertification and increasingly renders land uncultivated because of water shortages. Studies show that the cultivated lands in Iraq dropped from 12.2 % to 8.3% during the period 1970-2010, and over half of the country's land is under threat of desertification [40,41].

Water shortages combined with water pollution worsen another problem: the increase in soil salinity, which leads to land degradation and desertification. Soil salinity has always been an issue in Iraq, particularly in the central and southern regions. Several factors contribute to rising salinity in the soil: increased salinity in surface water caused by a significant decrease in water levels in rivers and canals; pollution of surface water and groundwater caused by municipal, industrial, and agricultural activities; excessive irrigation combined with inadequate or poorly functioning drainage systems; and overgrazing of pasture lands, which leads to greater exposure of soils to sunlight. In large areas of Basra, soil salinity has an additional factor: the significant drop in the water level of the Shatt al-Arab allows the tidal waves of the Arabian Gulf, one of the most saline bodies of water in the world, to move northward and salinize the farmlands. Studies show that 'soil salinity has robbed the production

potential of 70% of the total irrigated area of Iraq, with up to 30% completely out of production' [42-46].

Iraq has significantly lost its capacity to produce food to the extent that an official from Iraq's Ministry of Agriculture was quoted as saying, 'Agriculture in Iraq is almost finished because high temperatures, desertification, drought, and other factors have killed all the spirit of the earth' [34]. A survey in 2021 found that '7% of wheat farmers and 30% of barley farmers suffered crop failure' owing to 'water scarcity, the removal of topsoil, and decline in land productivity' [38]. 'Iraq loses around 100 square kilometres annually from its arable lands as a consequence of desertification' [47]. The country, known as the land of the two rivers – the Tigris and Euphrates – or the country of black soil, referring to the high fertility of its land, is one of the five countries in the world most vulnerable to climate change [48], which also means losing its capacity to produce food. Every ten year, Iraq loses half its total capacity to produce food. It currently imports 70% of its food needs [10].

The rise in temperature and the increase in the number of hot and dry days in Iraq are leading factors of desertification. Summer now extends to over seven months in large parts of Iraq. It is getting longer, hotter, and drier. The rise in global temperature is felt more acutely in Iraq than in other areas of the world. In the words of the UN High Commissioner for Human Rights, 'the global boiling has indeed begun' in Iraq [49]. Baghdad, for example, experienced more than 26 days with temperatures exceeding 50 degrees in the summer of 2021. The effects of the prolonged heat were very apparent on the water supply, which became short and interrupted for households as well as for the farmlands [50].

Iraq has continuously relied on groundwater for domestic, irrigation, and industrial purposes. Surface water has seen a sharp decline owing to decreased precipitation in the area and reduced water in the Euphrates and Tigris rivers and their tributaries. As a result, groundwater is under massive pressure. This is because withdrawing groundwater, although justified by the increasing need for water from a fast-growing population, has not occurred scientifically or according to a well-studied plan. The governments, both central in Baghdad and regional in Erbil, are generally weak and cannot oversee and implement plans and strategies regarding water withdrawal. Therefore, the country's aquifers suffer greatly from over-withdrawal, which leads to depletion [51].

Iraq's surface water mainly comes from two upstream neighbours: Türkiye and Iran, through shared rivers. Climate change has reduced the amount of rainfall in the area; a growing population has increased demand for an expanded agriculture and industry; and the construction of many large and small dams in upstream states have all contributed to the diminishing water reaching Iraq, and so rivers have dried up rapidly, preventing spring floods that help restore soil fertility with the silt they

carry and revive green cover in the valleys and plains, hence accelerating the process of soil erosion and desertification [52,53].

Population growth increasingly puts pressure on the demand for freshwater and other national resources, accelerating the process of desertification, particularly as Iraq lacks effective state organization, suffers from mismanagement, and has weak administration. This population growth is despite many wars that have caused millions of deaths. A population estimated to have been about 7 million in the 1960s, has jumped to over 45 million in 2024, according to a nationwide census [54].

Iraq's many wars in the past decades have left huge marks on the country's landscape. The 1980-1988 Iran-Iraq War, the 1990-1991 First Gulf War, the 2003 US invasion and its catastrophic aftermath, the 2014-2017 war against the Islamic State of Iraq and the Levant, and many other smaller wars and military conflicts have all caused great damage to the country's green areas. Date palms have been the main victims. Date palms are a national symbol of Iraq and dates are its second-largest export after oil. Iraq was known as the King of Dates and led the world in date production. Through decades of war, millions of palm trees were lost; they were burned, cut down, or became unproductive after wounds from mortar and artillery shrapnel. Their population has been greatly reduced, and now Iraq's share of the world's date market stands at just 5% [55-57].

Along with losing date palms, Iraq has quickly lost its forests to drought, fire, illegal logging, neglect, and military conflicts. Most of Iraq's forests are located in the northeast. Over the last few decades, high summer temperatures combined with dry weather and wind, drought in large parts of the Kurdistan Region of Iraq (KRI), deliberate or accidental wildfires, illegal logging by villagers for housing, heating, and farming purposes, and by dealers for selling in the black market, diseases among the trees and shrubs resulting from negligence and abandonment, lack of appropriate firefighting technology, and military conflicts involving armed groups based in the mountainous areas against Türkiye, Iran, and Iraq, have all contributed to the massive loss of Iraq's natural trees. The forest area in Iraq was estimated to be 1.4 million ha at the beginning of the twentieth century. The area had shrunk to an estimated 10 thousand ha by the beginning of the twenty-first century [58].

Poverty is cited as a factor causing desertification [59]. Poverty in Iraq contributes to desertification in two ways: cities are expanding rapidly because of the immigration of rural people seeking jobs, which increases pressure on food, water, and other national resources. The cities already suffer from weak infrastructure and insufficient job opportunities. Another factor is that farmers and villagers lack the knowledge, expertise, or sufficient resources to use their land for agriculture in an environmentally friendly manner, nor do their animals graze sustainably.

Another factor is the oil industry in Iraq, which contributes to desertification by polluting the air and contaminating the land with residue [60]. Iraq's large oil sector occupies vast areas in several provinces from north to south polluting the air and spreading particles. This will in turn affect the quality of the soil and degrade it. Iraq also has hundreds of oil refineries producing largely for domestic consumption. The oil refineries contaminate the land with their chemical effluents and the gases they release into the air, which are sometimes combined with dust and sandstorms. The soil in the adjacent areas provides a good sample of how the soil is degraded and rendered unsuitable for agriculture. The continuous degradation of soil adds to the increasing severity of desertification.

Dust and sandstorms are generally increasing in Iraq, with some variations from year to year, threatening Iraq's environment. Dust and sandstorms reduce the chance of rainfall [61]. They are considered the most common active factors causing land degradation and soil erosion in Iraq, according to the Iraq Ministry of Agriculture report [62]. Most dust and sandstorms occur in the middle and south of Iraq during the spring and summer seasons; but, the north and northeast regions are also affected. According to one study, the Mosul province in the northwest had 2.2% of its territory transformed into 'desert... between 1992 and 2017' as a result of increasing dust and sandstorms in the area [63]. Many dust storms have occurred in the past several years. The issue was so intense during the spring season of 2022 that the author of this article coined the term 'soilfall' to describe the phenomenon as a detrimental change from 'dustfall' [64].

According to one study, there are mainly two types of dust and sandstorms in Iraq. One of them is called Haboob [the term is Arabic and means grain or cereals], which moves quickly in the form of a large wall of dust. It turns daylight into complete darkness in just a few minutes. The other type is called Shamal, which occurs rather slowly, but it can last for several days, covering the ground with a thick layer of dust. KRI mainly experiences Shamal because of its geographical position [63].

Desertification impacts the land and its inhabitants. Many people are forced to leave their homes and seek other places to live. They abandon their traditional lifestyles and often move to urban centres to find employment. This strains the cities' infrastructure and public services. It also signifies a loss of productivity in the affected areas. Millions of people in Iraq and many other regions of the world have been displaced by desertification or are 'at risk of being displaced by desertification' [65].

Government's Response to the Crisis

The response of the Iraqi government to the desertification crisis has not been adequate, nor has it been swift. The country has endured many wars and economic sanctions, and the Iraqi governments established following the 2003 invasion, which toppled the Ba'ath dictatorship under Saddam Hussein, have all faced numerous internal and external security challenges, along with many social

and economic problems, all of which have established different priorities for the Iraqi governments. Nevertheless, the increasing threat of desertification to environmental security and the safety of people must be recognized, necessitating a change in the course of action for Iraq regarding prioritisation on the government agenda and the implementation of urgent and appropriate responses [66-74].

Iraq is one of the 131 countries which have pledged to arrest land degradation by 2030, by adhering to the UN's Convention to Combat Desertification (UNCCD) and so achieve Land Degradation Neutrality (LDN). The purpose, according to Iraq's Ministry of Agriculture, is to meet Goal 15 of the 2030 Sustainable Development Goals (SDGs), adopted by the United Nations General Assembly in 2015, which urges countries to 'Protect, restore and promote sustainable use of terrestrial ecosystems, sustainably manage forests, combat desertification, and halt and reverse land degradation and halt biodiversity loss' by 2030 [75]. The LDN programme in Iraq aims to minimize the effects of global and local drivers of land degradation and improve land productivity; recognize salinity, sandstorms, and sand dune movements as very serious factors affecting land in Iraq; monitor land degradation; and select and monitor the degraded areas [62].

There have been several developments regarding the targets of the LND programme, according to the 2017 report by Iraq's Ministry of Agriculture: many hectares of land have been converted from sandy terrain to agricultural fields, several hundred thousand of which are now equipped with modern irrigation systems; numerous water storage projects have been built to enhance water management; and there are plans for the forests, which have considerably shrunk [62]; and plans for creating greenbelts to act as windbreaks against dust and sandstorms [37].

Iraq's National Green Belt (NGB) is a major part of the above anti-desertification programme. It is set to be established on the edge of the desert in the western areas of Iraq, stretching from the north of Mosul to the south of Basra. The NGB encompasses 'more than 200 million trees...palm trees, plants, herbage and grasses that are resistant to the severe desert-like conditions...to be planted over an area of at least 3,000 sq. km, irrigated by groundwater through closed irrigation systems powered by solar energy.' The project requires the construction of numerous townships to accommodate workers, engineers, scientists, and researchers, and a network of roads must be built to facilitate the movement of people and materials necessary for the implementation of the NGB. Many Iraqi politicians have voiced their support for this initiative [76].

The NGB was initiated in 2023 by Iraq's State Board of Combating Desertification. The initiative also includes creating plant nurseries and training local farmers and citizens. Several campaigns have been launched thus far, with volunteer participation and coordination by government ministries,

resulting in the planting of a large number of saplings [77]. Tree planting is a practice worldwide to combat desertification. Trees reduce soil erosion, improve soil fertility, absorb carbon dioxide from the atmosphere, which can help mitigate the impacts of climate change, and increase water availability [78]. According to one study, Amazon forests generate half of their rain, which means that clearing forests and widespread logging, as happened in Iraq because of war and poverty, causes the local climate to dry out and become drier [79].

One challenge to tree planting in Iraq is illegal logging, which has become a habit for many people, both rural and urban dwellers, for domestic, commercial, and economic purposes. In the 1990s, during the international economic embargoes on Iraq, many people resorted to the forests in the KRI mountains to log trees and burn them as fuel for heating in winter. This resulted in the loss of numerous indigenous trees and left vast areas barren. As the economic situation improved and the government's ability to uphold the law increased, the Kurdistan Regional Government took action to stop illegal logging through legislation, although it was not very effective.

The KRI Parliament issued a 'Forest Law' in 2012 for the preservation of the forests and scattered trees in the mountainous areas, as well as in other regions of KRI. Law No. 10 of 2012 bans cutting trees or any other act that might harm a tree and establishes heavy punishments for offenders. The law aims to conserve the natural forests and trees, helping them expand and increase their area; maintaining the balance and diversity of nature; mitigating the effects of climate change; and encouraging tourism [80]. KRI is geographically positioned to confront desertification if its trees are increased and protected, and wild bushes and shrubs are preserved from harm. But, the law is often breached; and the offenders escape punishment.

A special police force, called the 'Forest Police', is tasked with enforcing forest laws and other environmental regulations, including the protection of trees and bushes in the mountains and other public areas, as well as wild animals, birds, and more. The force is under-resourced; they do not have enough personnel to cover all areas, nor do they have the necessary equipment [81]. Consequently, they often report large-scale attacks on trees and wildlife. Many trees are chopped down every year and sold as logs, charcoal, and so on [82]. Some impoverished individuals also resort to the forests for logs, as they cannot afford kerosene or gas to heat their homes.

Apart from tree planting, Iraq's Board for Combating Desertification has implemented several other projects, such as building about 50 oases in the country's western desert, about 10 artificial forests, and 23 pastures [35]. Many of these projects have declined and deteriorated since their establishment because of the worsening security situation in those areas, lack of state funding for maintenance of the projects, lack of care, logistical support, and so on. Implementing an anti-desertification project

is important, but equally crucial is caring for and nurturing it; otherwise, all the money and efforts will be wasted.

Lessons from Other Countries

There are efforts in many countries aligned to UNCCD. Some countries have begun taking serious action. It is important for Iraq to understand what other countries do regarding this issue and what lessons they have for others. It is equally important to examine the hindrances and find ways to overcome them.

A common practice is planting trees in dry areas and creating green walls around cities. We have the examples of Boumedienne's green barrier in Algeria and the Great Green Wall in China as two megaprojects to minimize the impacts of desertification and restore the natural balance.

In 2007, the African Union announced the project of the Great Green Wall in the Sahel region to reduce the process of desertification by planting millions of trees across the entire width of the continent, covering 8,000 km of land. It was promoted by UNCCD. The Sahel region in Africa is severely affected by climate change, which has led to continuous degradation of its land. The project aimed to reduce desertification in the region by planting trees and restoring 100 million ha of land across 20 countries in Africa by 2030. Trees are proven to be effective in moderating temperature, wind patterns, and soil erosion while increasing humidity for agriculture [83,17].

Unfortunately, according to a UN Status Report, only 4% of the project was achieved by 2020 – thirteen years after its launch. Two main factors are cited for the slow implementation or partial failure of the project: one is the lack of funding and technical support, and the other is poor monitoring and oversight. The countries involved and tasked with implementing the project complained about not receiving enough funds from contributors, namely the European Union, the World Bank, and the United Nations. Underfunded and also suffering from poor governance, those countries could not supervise, monitor, and coordinate with relevant actors and, more importantly, with the indigenous population to implement the project [83,84].

Monitoring and nurturing newly planted seedlings or saplings, along with coordinating with the state administrative units, farmers, rural populations, and local communities in general, is the most important factor for the success of any green walls or tree planting projects anywhere, especially in Iraq since all farms are managed by individual households. Besides, the government authority in Iraq is weak outside the cities, and people often take matters into their own hands. They can sabotage government plans if they feel the need to or if they perceive that their property rights have been violated – for example, if their lands have been used for the tree planting project without their

permission. Without the full involvement of local people, especially the landowners and the tribal leaders in control of the areas, the Iraqi tree-planting project is destined to fail.

China is another country facing severe desertification and has tree-planting projects to tackle the problem. One-fourth of China's landmass has undergone desertification, and it is expanding. The issue has affected more than 400 million people. The Great Green Wall of China has somewhat slowed the desertification process. It is the largest of its kind in human history, planning to use 88 million acres of land across approximately 4,800 km at a cost of hundreds of millions of US dollars. Billions of trees will be planted in areas where there were previously none, a gigantic afforestation project. It aims to halt the advance of sandstorms and restore the lands that have been lost thus far. It is expected to be completed by 2050 [85,86].

China's Great Green Wall project reports some early success. The targeted areas that once appeared barren now show greenery with rows upon rows of trees, and clearer, cleaner air is felt throughout those regions. One key factor contributing to the success of this megaproject is that it engages local communities, listens to their needs, and respects their knowledge about tree planting, etc. But, critics argue that many trees die from inadequate care, and the planted trees are not native to the area, absorbing too much water. The region already suffers from a water shortage [87].

Alongside caring for the plants with the support of local people, as we have explained, Iraq's climate must be considered when deciding on tree planting. Suitable trees for Iraq are those which can withstand extreme heat, require little water, and can grow in the soil while adapting to their environment: quality matters. A consultant at Iraq's Ministry of Agriculture has criticised his government's NGB project on the grounds that Iraq already has a significant water deficit problem and that such a project is unsustainable [77]. A drip irrigation system in the targeted areas could produce the desired result with water efficiency.

Conclusion

Iraq's approach to its numerous environmental problems has been poor and significantly lagging behind its strategy for traditional threats. For example, the Iraqi government allocated 1.63 billion US dollars of its annual budget in 2018 to Hashd al-Shaabi (translated into English as Popular Mobilisation Forces; an umbrella organization that oversees irregular armed groups in Iraq) and many more billions to its other armed and security forces [88], while farmers in Basra were suffering from water salinization and a shortage of water to keep their farms green and productive. Many farmers reported they did not receive any government support, and as a result, their palm trees, and other fruit

trees and vegetables had all died [42]. Iraq's desertification is a serious environmental problem that requires urgent action. The country's land is degrading, and traditional lifestyles are changing. The soil in vast areas increasingly loses its capacity to support agriculture or produce grassland for pasturage. This means that the livelihoods of many rural people are at risk, as they are compelled to leave their lands and seek jobs in urban areas. This is a dangerous development. It affects Iraq as a whole and particularly impacts those in the troubled areas.

Desertification works like rolling a snowball; it creates more problems as it rolls. As desertification expands and occupies more land, it generates many other environmental issues, such as an increase in dust and sandstorms and a decrease in precipitation. It is best to prevent it from occurring: 'prevention is better than cure.' However, once it occurs, it must be addressed quickly with the best possible solution. In other words, the quicker the problem is minimized and the degraded land is rehabilitated or restored, the better.

Iraq needs to take appropriate and multiple actions on its desertification problem, tackling all possible causes. This requires prioritising the issue and allocating sufficient human and financial resources, raising awareness about the danger, and involving local people, especially tribal leaders and heads of large families who control their community's matters and can guide them on what they should and should not do, in the projects designed to reverse desertification; top-down interventions are ineffective. Iraq's only solution so far, which is planting trees and creating green walls around residential areas, needs to consider success factors and continually observe and monitor its implementation. Tree planting is recognized as the most common and, so far, successful way to combat desertification. Plants that can survive the dry and hot conditions of arid lands must be introduced. The present and future of Iraq are tied to preventing land degradation and to restoring the degraded lands. The causes of desertification – climate change, drought, dust and sandstorms, deforestation, over-cultivation, and overgrazing – can be ameliorated and addressed through long-term state-adopted plans. Iraq can and should learn from the experiences of other countries in combating desertification, such as China, and send its experts overseas for additional knowledge and training. Coordination with the Kurdistan Regional Government is also a necessary step to combat desertification in Iraq.

Iraq can also collaborate with neighbouring states to combat a common enemy. Saudi Arabia, Kuwait, Jordan, Syria, and Iran all have growing deserts. It is in their interest to reduce these and mitigate the damages. The neighbouring states can enhance anti-desertification projects with their capital and expertise. There will be a better outcome if they all work together and share information about the issue. Solutions take time. It is crucial for everyone to start promptly and act decisively.

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The Kakhovka Sea Before and After the Destruction of the Kakhovka Dam

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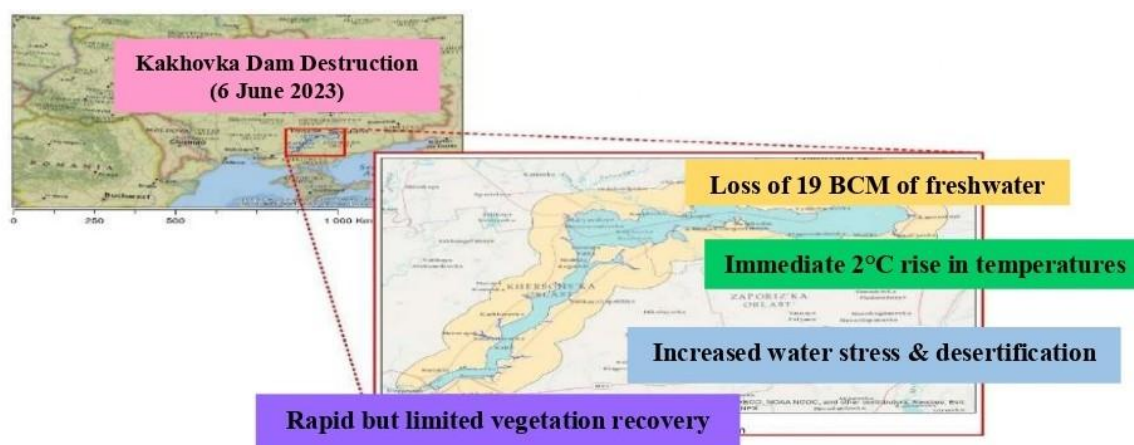
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Graphical Abstract

The destruction of the Kakhovka Dam on 6 June 2023 resulted in the loss of 19 billion cubic metres of freshwater, exacerbating drought conditions and increasing water stress in the affected region. This disaster triggered an immediate 2°C rise in local temperatures, accelerating desertification and limiting vegetation recovery. The environmental impact highlights the vulnerability of ecosystems to human-induced disruptions and underscores the urgent need for sustainable adaptation strategies.



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Abstract: Sabotage of the Kakhovka dam on the Dnipro River eliminated one third of Ukraine's fresh water reserves, clean drinking water for 6 million people and domestic supply for more than 13 million. Opinions differ on whether and how to replace it. Fieldwork is constrained by fighting along the course of the lower Dnipro but we have used remote sensing to examine the natural-climatic transformation of the reservoir and dependent areas, which experienced an almost immediate increase of 2.0°C in the land surface temperature, two-fold acceleration of evapotranspiration, and exacerbation of water scarcity.

Keywords: Kakhovka Reservoir, Hydrology, Temperature, Evapotranspiration, Vegetation health.

Introduction

Russian aggression has claimed the lives of hundreds of thousands of Ukrainians and driven millions from their homes; destroyed natural landscapes, flora and fauna; polluted soil and water with petroleum products and heavy metals from fuel spills, munitions, industrial plant and storage facilities, and untreated sewage from disrupted water infrastructure [1-3]. On 6th June 2023, sabotage of the Kakhovka dam on the lower Dnipro River eliminated one third of the country's fresh water resources – some 19 billion cubic metres (BCM) worth more than \$US18 billion [1]. As a result of Russian military action across the country, six million people in Ukraine do not have safe drinking water and domestic supplies are restricted for more than 13 million and damage to the environment already exceeds \$60billion [4]. Repair and reconstruction require thorough investigation to find effective ways to restore ecosystems and **optimize** the management of natural resources.

Material and Methods

Fieldwork is restricted by active conflict along the course of the lower Dnipro but we have used satellite imagery to establish the status of the Kakhovka Reservoir before and after destruction of the retaining dam (Fig. 1). Data on the flow of the lower Dnipro River over 205 years were taken from Lotsmanska Kamianka within the city of Dnipro up to 1975 [5] and, subsequently, observations of the Kherson hydro-biological station. Values of near-surface air temperature, precipitation, and reference evapotranspiration were provided by the University of East Anglia Climatic Research Unit (<https://crudata.uea.ac.uk/cru/data/hrg/>) and NASA POWER (<https://power.larc.nasa.gov/data-access-viewer/>). Sentinel and Landsat satellite imagery were abstracted from the Copernicus Browser at EO Browser.

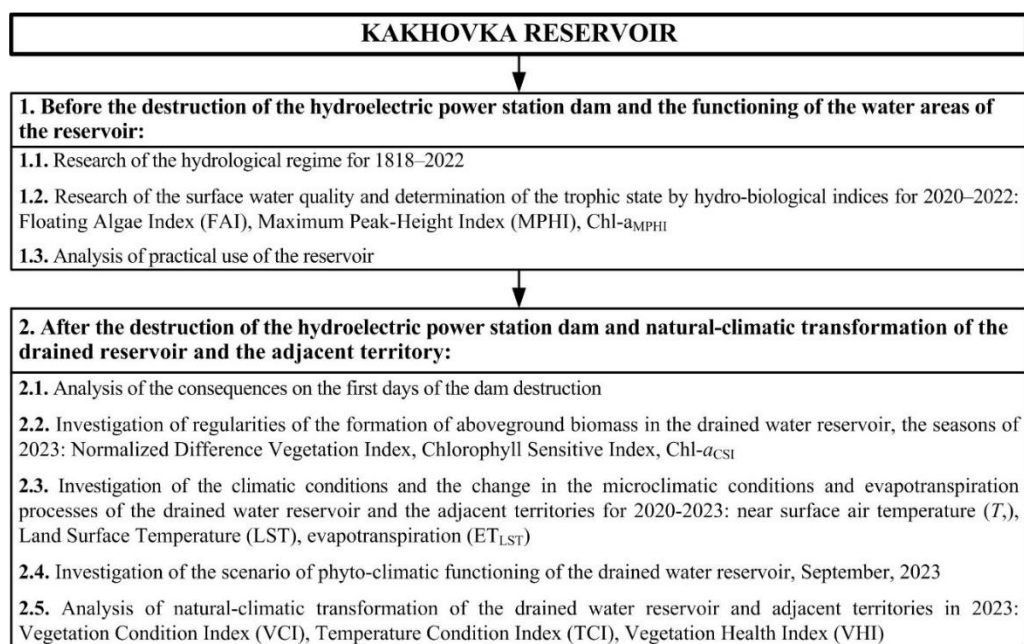


Figure 1. Scheme of research.

Study Area

Historically, the territory of the reservoir was the Velykyi Luh, the Great Meadow, that hosted the fortified Cossack encampments of Zaporizka Sich (1564-93), Tomakivska Sich (1593-1630), Bazavlutska Sich (1630-52), Mykytynska Sich (1652-1709), Chortomlytska Sich (1734-1775), and Nova (Pidpilnetska) Sich. Construction of the Kakhovka dam between 1955 and 1958 (Fig. 2) flooded 2,800 ha of arable land, 36,000 ha of pasture, and more than 90 settlements, including parts of the cities of Nikopol and Kamianka-Dniprovska, to create a reservoir with a total capacity of 18.2 BCM and an active capacity of 6.8 BCM, a normal storage level of 16.0 m and a dead storage elevation of 12.7 m, a maximum depth of 32.0 m and an average depth of 8.5 m, a maximum length of 230 km, a maximum width of 25 km, and an average width of 9.3 km, with a shoreline of 869 km [6]. This transformation significantly altered the social and economic conditions of the local population, affecting settlements, livelihoods, and water management systems in the region [7]. Known locally as the Kakhovka Sea, its area of 2,155 km² was 13 times the size of the Sea of Galilee and one-thirtieth of the Aral Sea at that time. Its hydroelectric power station had a capacity of 329 MW – only one-twelfth of the whole Dnipro cascade. However, power was not its main purpose; rather, it was intended primarily for water supply to the cities and industries of the southern steppes and, in particular, for irrigation.

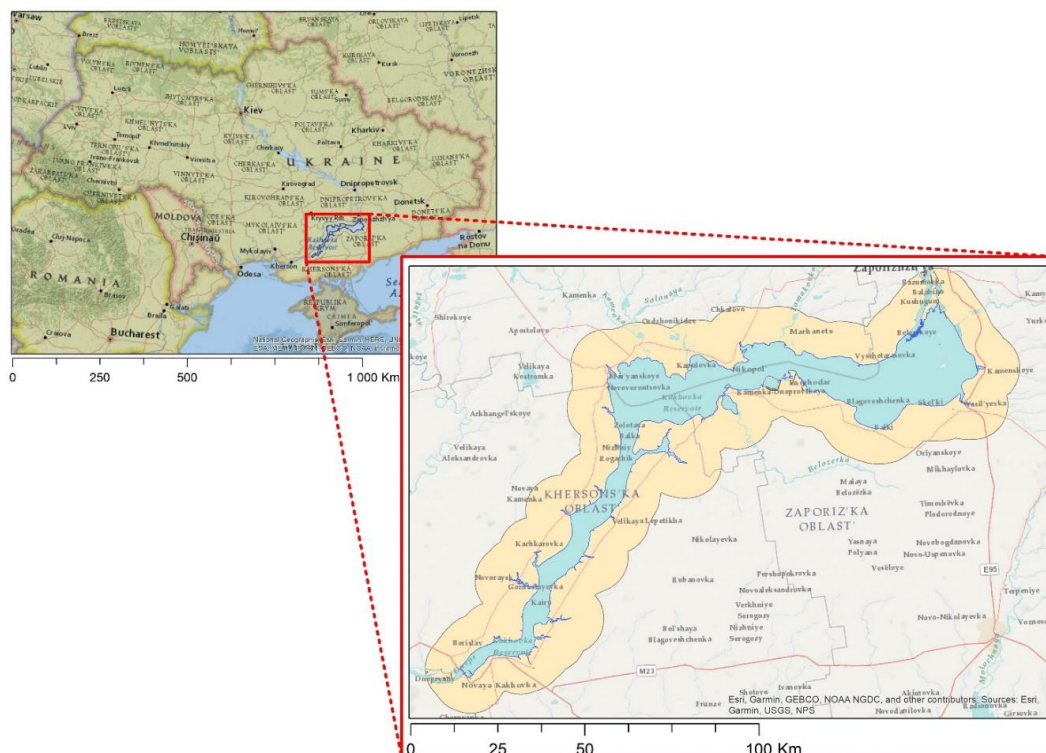


Figure 2. Location: Kakhovka Reservoir (blue) and the 10km buffer zone (cream).

Research Methods

The trophic state of the reservoir and its water quality over the period 2020-2022 was assessed by the activity of algal (cyanobacteria) blooms and the intensity of their saturation with chlorophyll-a, measured by the Floating Algae Index [8]:

$$FAI = B6 - B4 - \frac{(B8 - B4) \times (740 - 665)}{842 - 665} \quad (1)$$

where B4, B6, and B8 are the reflectance of $\lambda B4 = 665$ nm, $\lambda B6 = 740$ nm, and $\lambda B8 = 842$ nm, the central wavelengths of the corresponding bands of Sentinel 2 L2A imagery.

FAI varies between -1.0 and 1.0, where a value greater -0.004 indicates a bloom. Saturation with chlorophyll-a is calculated by the Maximum Peak Height Index (MPHI) [9]:

$$MPHI = B5 - B4 - \frac{(B8 - B4) \times (705 - 665)}{842 - 665} \quad (2)$$

where B4, B5, and B8 are the reflectance, and $\lambda B4$ (665nm), $\lambda B5$ (705nm), and $\lambda B8$ (842 nm) are the central wavelengths of the corresponding bands of Sentinel 2 L2A.

MPHI value varies between -1.0 and 1.0. A value greater than 0.004 indicates presence of Chl-a in the cyanobacteria of the surface water. Chlorophyll content ($Chl-a_{MPHI}$, $\mu g/dm^3$) was calculated by the formula:

$$Chl-a_{MPHI} = 2223.18MPHI + 24.03 \quad (3)$$

Surface water quality was determined according to Chl-a content (Table 1) [10].

Table 1. Ecological classification of surface water quality.

Water Quality Class	I	II		III		IV	V
Water Quality Category	1	2	3	4	5	6	7
Chl-a, $\mu g/dm^3$	< 2	2–4	5–10	11–30	31–50	51–150	>150
Water quality according to cleanliness (pollution)	Very Clean	Clean		Polluted		Dirty	Very Dirty
	Very Clean	Clean	Clean Enough	Slightly Polluted	Moderately Polluted	Dirty	Very Dirty

Trophic class	Oligotrophic	Mesotrophic	Eutrophic	Polytrophic	Hypertrophic
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Changes in the structure of the exposed bed of the reservoir and seasonal primary productivity in 2023 were measured by the normalized difference vegetation index (NDVI) and the intensity of plant saturation with chlorophyll-a:

$$NDVI = \frac{B8 - B4}{B8 + B4} \quad (4)$$

where B4 and B8 are the of the corresponding bands of Sentinel-2 L2A.

NDVI ranges from -1.0 to 1.0. Negative values reflect cloud, water and snow; values close to zero (0.05-0.15) indicate bare ground; higher values vegetation. The intensity of plant saturation with Chl-a was calculated by the Chlorophyll Sensitive Index (CSI) [11]:

$$CSI = 2.5 \times \frac{B8 - B5}{B8 + B5} \times \frac{B2}{B5} \quad (5)$$

where B2, B5 and B8 are the of the corresponding bands of Sentinel 2 L2A.

CSI is dimensionless: negative values indicate cloud, water and snow; values from 0.0 to 0.2 bare ground; $CSI > 0.2$ indicates vegetation. Chl-a content in plant leaves ($Chl-a_{CSI}$, $\mu g/m^2$) was calculated by the formula:

$$Chl-a_{CSI} = 130.34CSI - 25.37 \quad (6)$$

Spatio-temporal changes in microclimate caused by drainage of the reservoir were established on the basis of changes in near-surface air temperature (T , °C), reference evapotranspiration (ET_o , mm/day) for 2020-22, and land surface temperature (LST, °C) for 2020-23 using Landsat 8-9 and Sentinel 3 SLSTR L1B imagery. ET_o was determined according to the Penman-Monteith method [12]:

$$ET_0 = \frac{0.408\Delta(R_n - G) + \gamma \frac{900}{T + 273} u_2 (e_s - e_a)}{\Delta + \gamma(1 + 0.34u_2)} \quad (7)$$

where, ET_o is reference evapotranspiration, mm/day; R_n is net radiation at the crop surface, $MJ/m^2/day$; G is the soil heat flux, $MJ/m^2/day$; T is air temperature at 2m, °C; u_2 is wind speed at 2m, m/s; e_s is saturation vapour pressure, kPa; e_a is actual vapour pressure, kPa; $e_s - e_a$ is the saturation vapour pressure deficit, kPa; Δ is the slope of the vapour pressure curve, $kPa/^\circ C$; and γ is the psychrometric constant, $kPa/^\circ C$.

LST relates to the surface energy and water balance of the earth-atmosphere system [13]. Raster values for 2020-23 were retrieved from the [Copernicus Browser](#) from the Sea and Land Surface Temperature Instrument of Sentinel 3 which measures LST with a central wavelength of 10854nm (thermal infrared) at a resolution of 270×270m. Seasonal patterns of LST across the reservoir bed and adjacent territories in 2023 were investigated using raster data from the Landsat 8-9 thermal channel with the central wavelength of 10895nm at a resolution of 75×75 m retrieved from the [EO Browser](#).

Natural-climatic transformation of the drained reservoir bed was characterized by the Vegetation Health Index (VHI) [14]:

$$\left. \begin{aligned} \text{VCI} &= \frac{\text{NDVI} - \text{NDVI}_{\min}}{\text{NDVI}_{\max} - \text{NDVI}_{\min}} \\ \text{TCI} &= \frac{\text{LST}_{\max} - \text{LST}}{\text{LST}_{\max} - \text{LST}_{\min}} \end{aligned} \right\} \rightarrow \text{VHI} = \alpha \times \text{VCI} + (1 - \alpha) \times \text{TCI} \quad (8)$$

where, VCI (Vegetation Condition Index) represents the spatial variability of NDVI.

TCI (Temperature Condition Index) represents the spatial variability of LST, determining plant stress related to high temperatures. High temperatures throughout the growing season correspond with the likelihood of drought.

α – a coefficient determining contribution of VCI and TCI to the general state of vegetation.

VHI evaluates cumulative temperature and moisture for plant development, ranging from 0 (extreme stress) to 1.0 (the most favourable condition). For the drained territory of the reservoir, values lower than 0.50 indicate extreme drought and bare ground; 0.50-0.58, high surface heat, water stress with patches of stunted plants; 0.58-0.63, moisture deficit with low plant biomass and considerable heat stress; 0.63-0.73, satisfactory moisture and plant biomass; greater than 0.73, a high level of moisture, low surface heat, visible vegetation. Coefficient α should be calculated [15] on the basis of the Aridity Index, AI [16]:

$$\text{AI} = P_y / \text{ET}_{y0} \rightarrow \alpha = 0.5 \text{AI} \quad (9)$$

where, P_y is annual precipitation, mm; ET_{y0} is annual reference evapotranspiration, mm; 0.5 is the equilibrium value of the balance of the combination of chlorophyll and moisture content in plants and thermal conditions on the surface.

AI considers both physical phenomena (precipitation and evaporation) and biological processes (transpiration). An AI value lower than 0.5 indicates arid or semi-arid conditions, values greater than 0.65 humid conditions.

Image processing, mapping and spatio-temporal analysis were performed using ArcGis 10.6.

Results

Prior Hydrological Regime

Major engineering over the period 1934-1976 transformed the lower Dnipro to a cascade of reservoirs that multiplied Ukraine's accessible water resources but restricted their circulation – leading to stagnation, eutrophication, and a raised water table [17]. Figure 3 shows a decrease in the average annual flow in the lower Dnipro by $2.0\text{m}^3/\text{s}$ per year; over 205 years of observations:

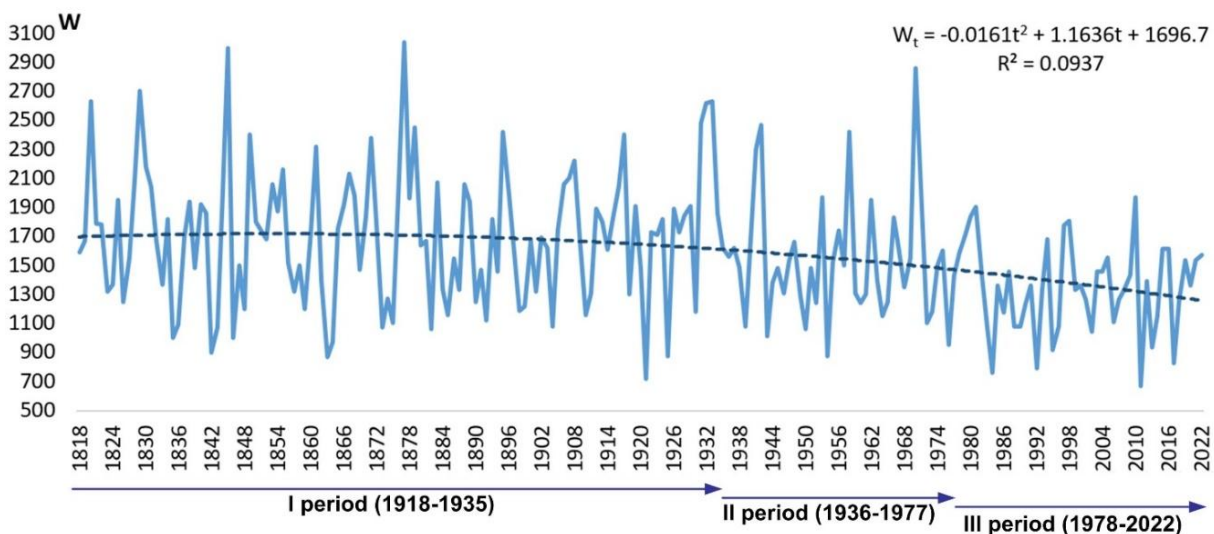


Figure 3. Average annual flow (m^3/s) in the lower Dnipro River, 1818-2022.

Mean annual flow diminished from 1670 to $1258\text{m}^3/\text{s}$. In high-water years, the maximum value ranged from 1970 to $3,040\text{m}^3/\text{s}$, in low-water years from 670 to $1,050\text{m}^3/\text{s}$. The multiyear norm was $158,9\text{m}^3/\text{s}$ with the variance of 28 per cent.

We can recognize three periods: I (1818-1935) more-or-less-natural flows that ranged from 1328 to $1,965\text{m}^3/\text{s}$ in 90% of cases (Fig. 4a), the mean was $1,704\text{m}^3/\text{s}$, variance 27%; II (1936-1977) the creation of the reservoir cascade that dampened the average annual flow from 1,704 to $1,530\text{m}^3/\text{s}$, varying between 1,248 and $1,665\text{m}^3/\text{s}$; III (1978-2022) its functional operation during which the average annual value of the flow fell to $1,344\text{m}^3/\text{s}$, varying between 1,093 and $1,562\text{m}^3/\text{s}$.

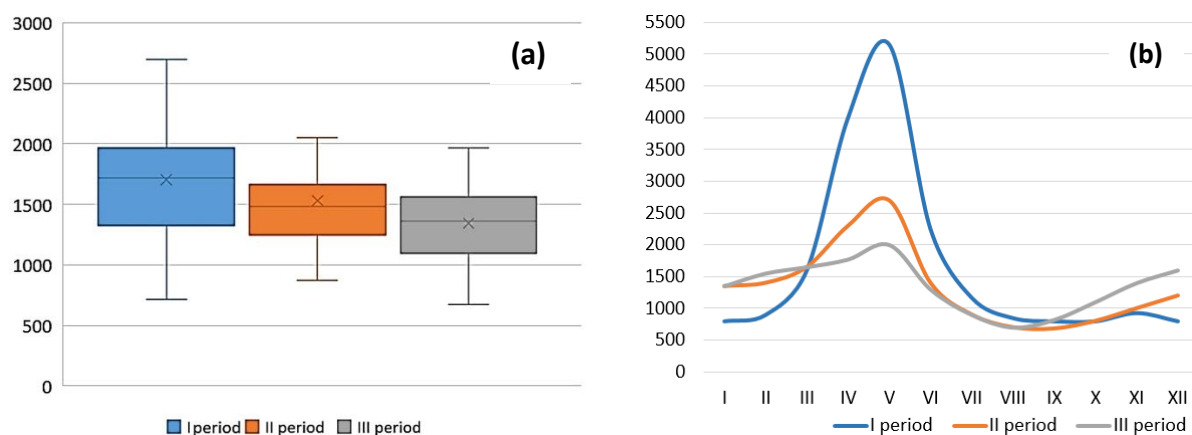


Figure 4. Periodic change in the flow (W , m^3/s) in the lower Dnipro lower course 1818-2022: (a), mean annual values; (b), mean monthly values.

Spring floods were drastically reduced by construction of the six reservoirs – upstream to downstream: Kyiv (filled 1964-6, area 922 km^2) → Kaniv (filled 1974-6, 675 km^2) → Kremenchuk (filled 1959-61, $2,252 \text{ km}^2$) → Kamianske (filled 1964-5, $5,567 \text{ km}^2$) → Dnipro (constructed 1932, reconstructed after World War II in 1948, 410 km^2) → and Kakhovka (filled 1955-8, $2,155 \text{ km}^2$). In Period III, mean annual flow amounted to $1,344 \text{ m}^3/\text{s}$, characterized by low spring floods ($1,680 \text{ m}^3/\text{s}$ on average) and a summer-autumn low water season ($1,090 \text{ m}^3/\text{s}$ on average). At the same time, water exchange was reduced by 24 times.

Trophic State of the Reservoir

Restriction of water exchange led to eutrophication, manifest in algal blooms and a considerable reduction in water clarity in well-heated and well-lit, shallow, stagnant zones. The Floating Algae Index in the period 2020-22 exceeded -0.004 across almost the entire water area in July (Fig. 5a). The greatest intensity was observed in the middle part of the reservoir where FAI reached 0.27 (Fig. 6b) on account of a concentration of living material and metabolites in the surface waters, high temperatures, and a low hydrological capacity for self-treatment.

There is a direct correlation between biogenic pollution and the quantity of phytoplankton in water bodies, so Chl-a concentration is a good indicator of eutrophication. Its concentration increased to more than $270 \mu\text{g}/\text{dm}^3$ in July (Fig. 5b, 6b) and, water quality ranged from moderately polluted to very dirty in 82-87% of the reservoir area over the entire warm spring-summer season (Table 2).

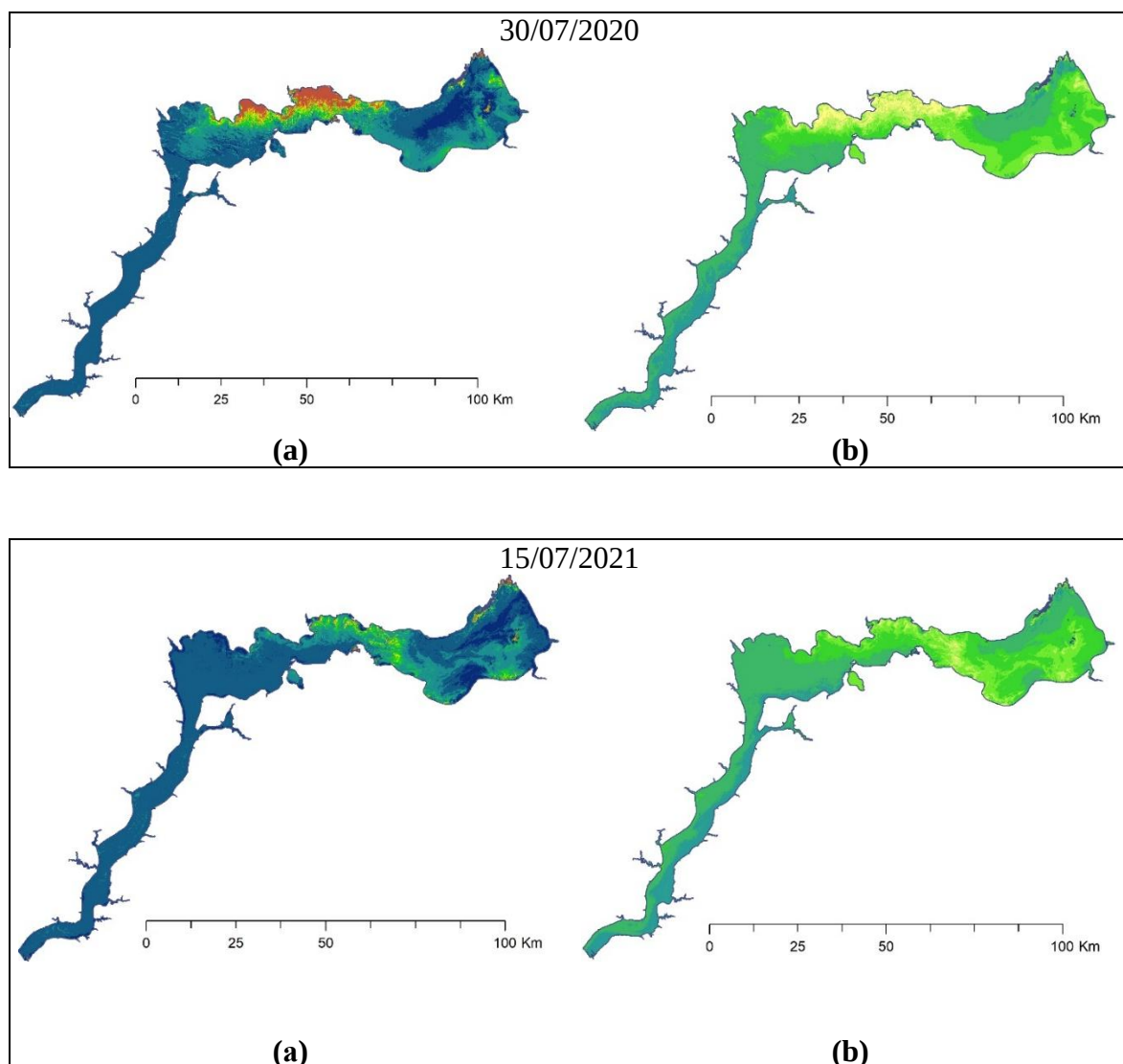
Table 2. Surface water quality in the water area of the Kakhovka Reservoir

Trophic level	Water quality categories	Chl-a, $\mu\text{g}/\text{dm}^3$	30/07/2020	15/07/2021	15/07/2022
Oligotrophic	Very clean	<2	0.36	0.43	0.48

Mesotrophic	Clean	2-4	0.01	0.01	0.01
	Clean enough	5-10	0.05	0.05	0.05
Eutrophic	Slightly polluted	10-30	12.37	12.72	17.57
	Moderately polluted	30-50	33.59	42.21	19.41
Polytrophic	Dirty	50-150	41.43	40.49	47.93
Hypertrophic	Very dirty	> 150	12.19	4.09	14.54

Water Use

The water balance of reservoir was affected by changes in the inflow of water from the upper and middle Dnipro and abstraction from the lower course. Data from the State Agency of Water Resources of Ukraine show that the input of water from the dam of the Dnipro Reservoir to the dam of the Kakhovka Reservoir fell from 48.9 to 26.3BCM in 1989-2021. At the same time, the output to the input ratio fell from 78 to 55% thanks to lesser water consumption by industry and irrigation, notably following Russian occupation of Crimea in 2014 (Table 3).



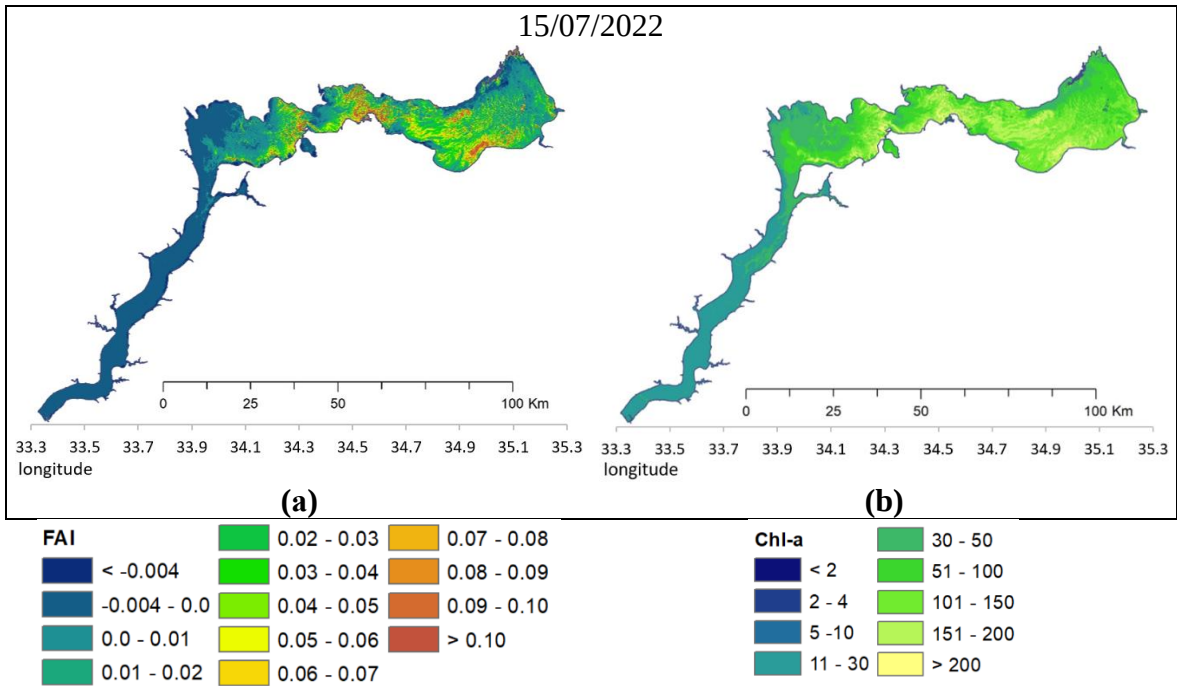


Figure 5. Floating Algae Index (a) and Chl-a (b) – $\mu\text{g}/\text{dm}^3$ in the Kakhovka Reservoir in July, 2020–22 .

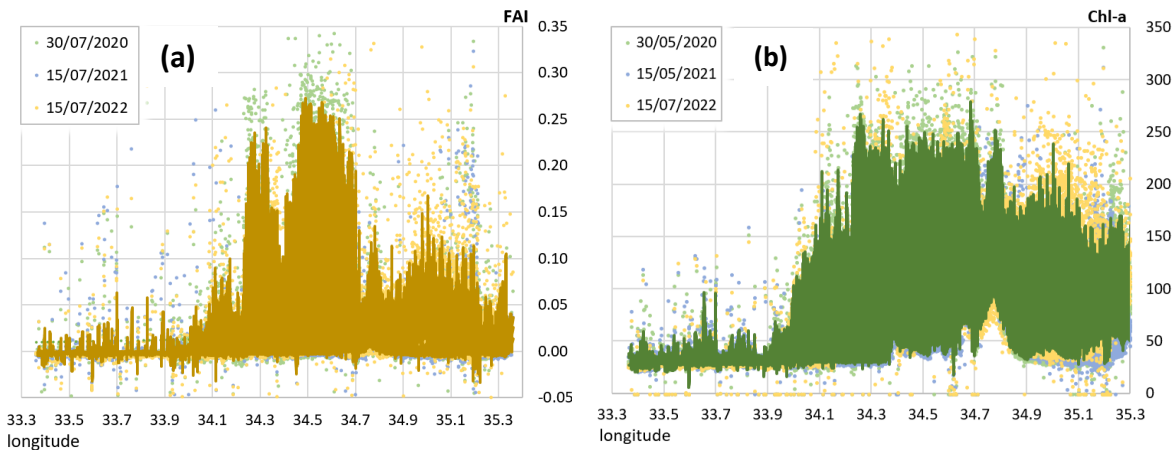


Figure 6. Trophic state of the Kakhovka Reservoir in July, 2020–2022: (a), Floating Algae Index (FAI); (b), Chlorophyll-a content (Chl-a, $\mu\text{g}/\text{dm}^3$).

Table 3. Mean monthly water balance in the lower Dnipro River 2020-21, mln m³.

Components of water balance	Month											
	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII
Input	2517.4	2525.7	2942.0	2958.9	2315.0	2242.8	1569.7	1366.7	1303.1	1799.7	2224.7	2562.6
Output	996.8	2364.584	1145.0	742.9	769.8	682.2	871.0	966.6	1064.3	1137.6	1448.0	2329.6
Including												
Evaporation	0.0	0.0	4.7	4.7	4.7	4.7	4.7	4.7	4.7	4.7	4.7	0.0
Seepage	30.9	30.9	30.9	30.9	30.9	30.9	30.9	30.9	30.9	30.9	30.9	30.9
Groundwater recharge	0.8	0.8	0.9	0.9	1.0	1.0	1.0	1.0	1.0	0.9	0.9	0.8
Transfer of part of the flow	12.6	12.6	12.6	12.6	12.6	12.6	12.6	12.6	12.6	12.6	12.6	12.6
Intake of surface water	74.0	72.9	80.8	89.9	142.1	114.7	122.8	138.0	120.9	97.6	84.8	75.7
Minimum environmental flow in the outlet section	878.5	2247.4	1015.1	603.9	578.5	518.4	699.1	779.4	894.2	991.0	1314.1	2209.7
Balance												
Reserve of water resources	1520.6	161.2	1797.0	2216.0	1545.2	1560.6	698.7	400.1	238.8	662.1	776.8	233.0
Transit flow	2399.2	2408.6	2812.1	2819.9	2123.7	2079.0	1397.8	1179.5	1133.0	1653.1	2090.9	2442.7

The output comprised 12.7 BCM (88%) environmental flow at the outlet of the reservoir; 1.2 BCM (8%) abstracted for domestic and economic needs; about 0.4 BCM (3%) consumed by evaporation, seepage and groundwater recharge, and 0.15 BCM (1.0%) otherwise redirected. The average annual water reserve in the reservoir was 11.8 BCM in 2020-21, and the transit flow to the lower part of the Dnipro, given the minimum environmental flow in the outlet section of the reservoir was 24.5 BCM.

Natural-Climatic Transformation of the Drained Kakhovka Reservoir and its Environs

Consequences of the Destruction of the Kakhovka dam

Everything changed on June 6, 2023 when Russian forces destroyed the Kakhovka Dam, bringing human, economic and environmental disaster: catastrophic flooding of more than 600km² of the lower part of the Dnipro, scouring the channel and flushing pollutants through the Dnipro-Buh estuary to the Black Sea; and drainage of the reservoir and, thereby loss of water supplies to several municipalities and 800,000 ha of irrigated land. According to the Ministry of Foreign Affairs of Ukraine, 180 settlements with the population of more than 900,000 people were included in the emergency zone and, according to the data of Kherson Regional Military Administration, 118 historical monuments were flooded.

By June 10 2023, polluted runoff had reached along the Black Sea coast to the mouth of the Dniester, the polluted area exceeding 7,300 km². On June 14 in coastal waters off Odesa, salinity was reduced from 11.0 to 4.2 g/l; there was an increased concentration of biogenic substances and phytoplankton, and exceedance of the maximum allowable concentration (MAC) of copper by 895-fold, MAC = 0.02 µg/l), zinc 45-fold (MAC=1.0 µg/l), arsenic 3-fold (MAC = 0.6 µg/l), and oil products 2-fold (MAC= 0.05 µg/l) [1]. One hundred and fifty official cases of the death of dolphins were registered in the Black Sea.

According to the Institute of Hydrobiology of the National Academy of Agrarian Sciences of Ukraine, in the first three days, floodwaters carried away 25-50 thousand tonnes of algae that increased algal biomass to 30 g/m³ in the Dnipro-Buh estuary; losses of bottom-living invertebrates reached more than 10 000 tonnes; more than 70 fish species, including 18 species from the UCN Red List perished in the lower part and the delta lake system of the Dnipro, and 42 fish species in the Kakhovka Reservoir – a loss of the commercial catches of about 2,585 tonnes/year forfeiting \$US 5.4 million/y; losses of the fisheries infrastructure amounting to \$270 million [18]. These are underestimates as they include data only from official sources. According to the calculations of Pavel Kutishchev (expert on aquatic bioresources and Director of the S.T. Artyuschyk Production and

Experimental Dnipro Stout Fish Breeding Plant), in the drained area of the reservoir with an area of only 9,313 ha, losses to the fishery from the death of feed organisms amounted to 2.42 thousand tonnes, from the loss of spawning grounds and future offspring of industrial fish species amounted to 1.07 thousand tonnes. In relation to 1 ha drained shallow waters (≤ 2.0 m), losses of feed organisms reached about 260 kg, fish resources 115 kg. For the drained deep-water areas, which occupied 90% of the water area of the reservoir, these costs are much higher.

Over 50 territories of the Nature Reserve Fund, amounting to 80,000 ha, suffered. The floods drowned most terrestrial invertebrates and vertebrates. In particular, 70% the world population of Nordmann's birch mouse, up to 50% of blind hare and populations of common copperhead, steppe viper, yellow-red snake and Sarmatian snake, and other species from the IUCN Red List.

Colonisation by Vegetation

The exposed bottom sediments of the reservoir were colonized by terrestrial vegetation (Fig. 7). New vegetation was observed at the beginning of June, 2023, the maximum NDVI value being 0.2 (Fig. 8a) with sparse vegetation emerging mainly in the upper part of the reservoir and watercourses. NDVI values in the period July-October, 2023 varied between 0.09 and 0.37 (Fig. 8b), maximum values of vegetated areas varied between 0.2 and 1.0 with the highest values along the course of the Dnipro River (Fig. 8c). Over September-October, the landscape comprised (Fig. 8d): water bodies (NDVI < 0.05), *i.e.* the main course of the river and isolated shallows comprised 7-10%; bare ground (NDVI 0.05-0.15) 14-17%; sparse or stunted vegetation (NDVI 0.15-0.40) 31-38%; areas with NDVI > 0.40 with good and very good vegetation including well-developed tall plants and shrubs varied between 44 and 46% in the period of maximum vegetation (Fig. 8e).

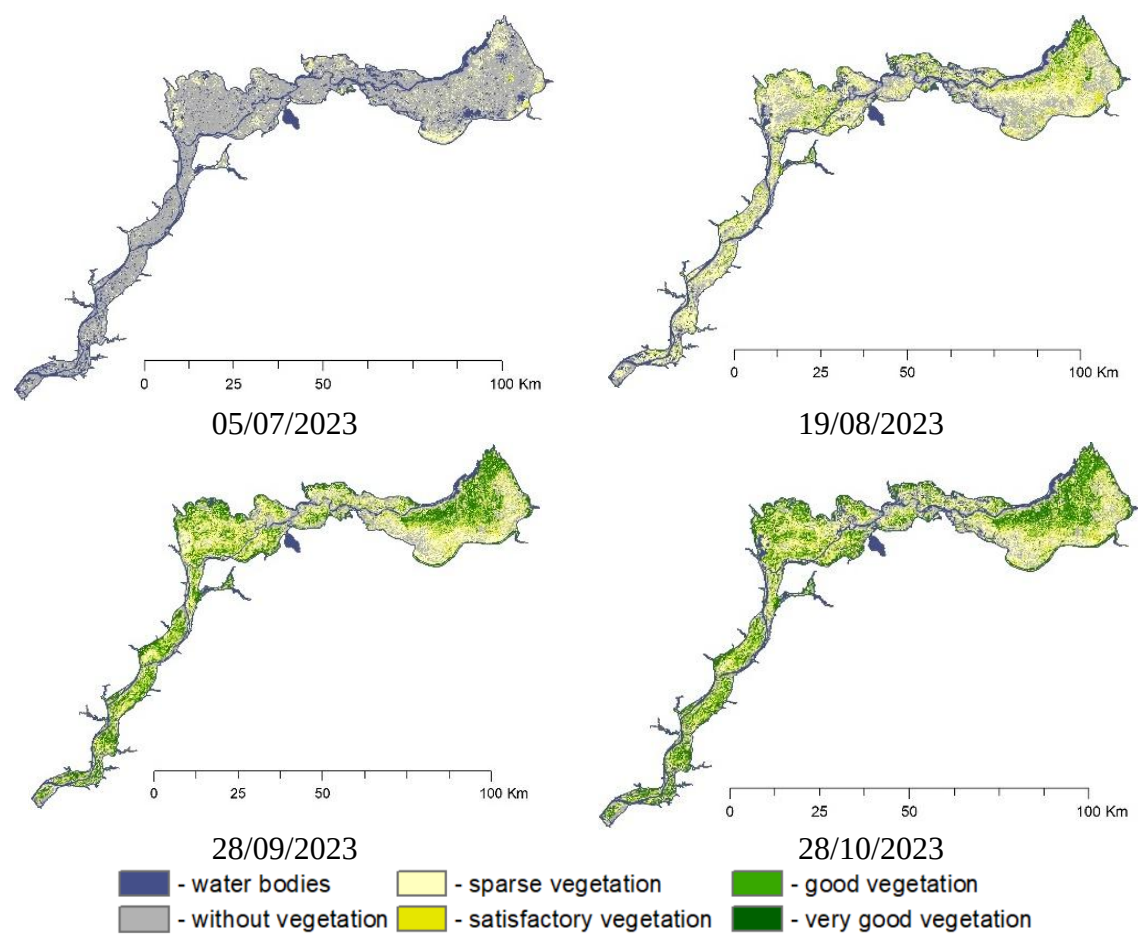
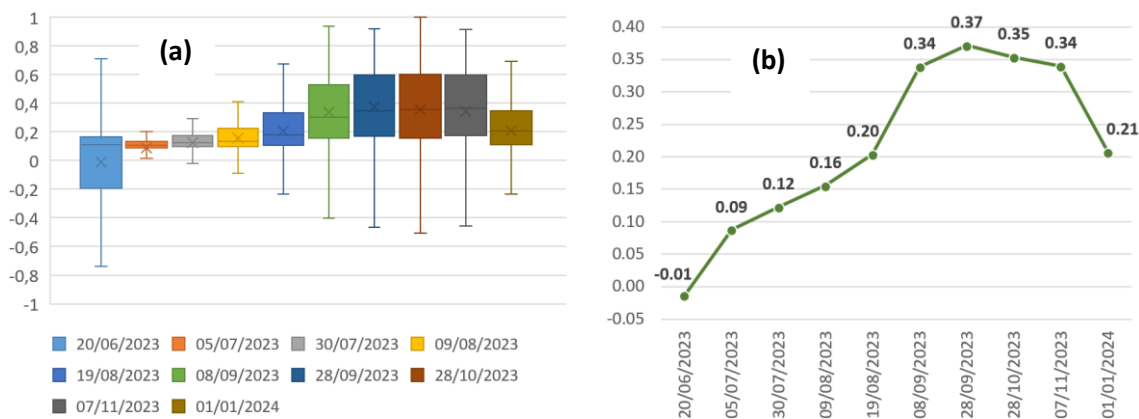


Figure 7. Plant cover in the territory of the Kakhovka reservoir revealed by NDVI.



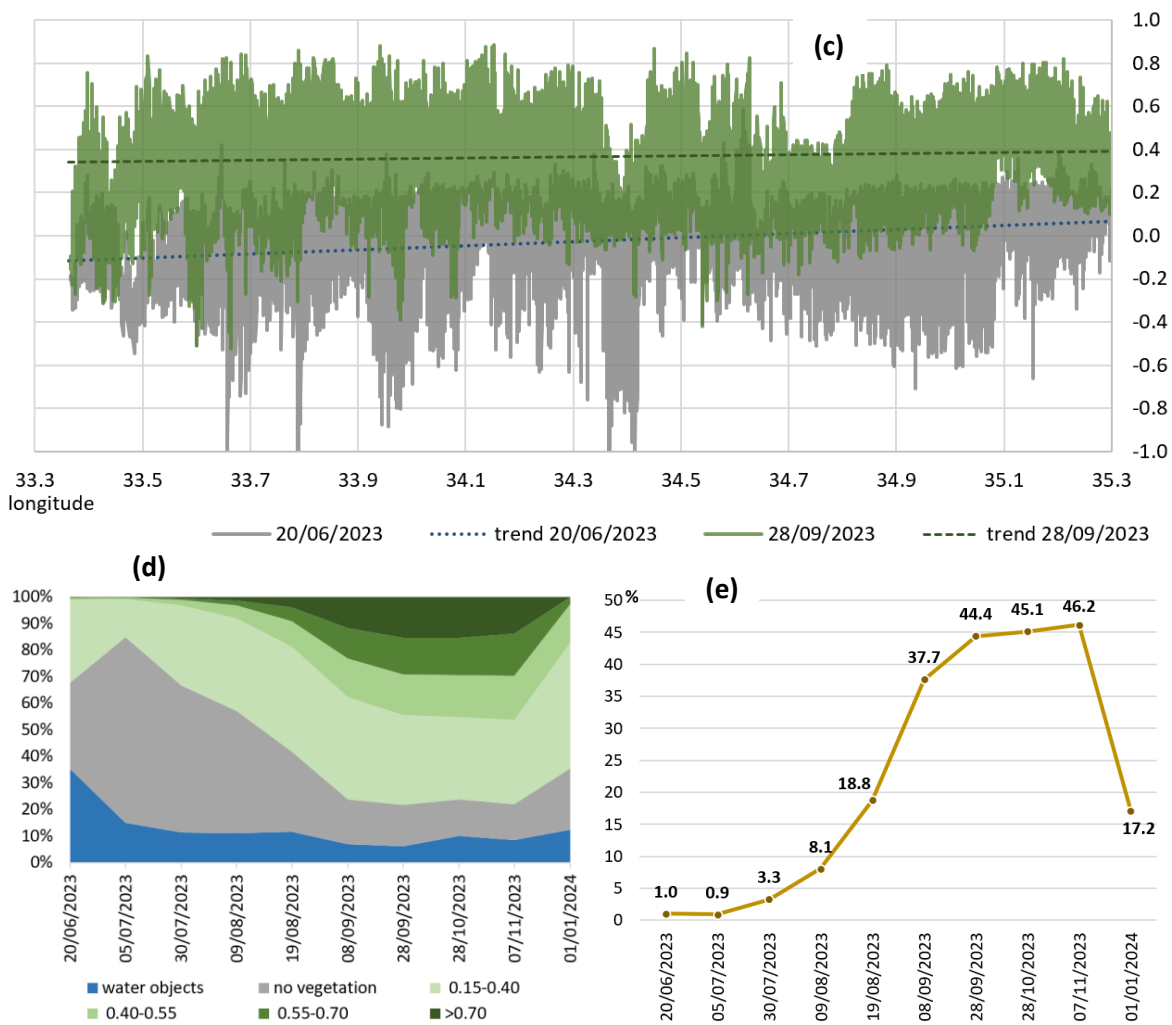


Figure 8. Structure of the drained territory of the Kakhovka Reservoir (a), differentiation of NDVI values; (b), average NDVI values; (c), spatial trends on 20/06/2023 and 28/09/2023; (d), dynamics of the distribution of the reservoir area, %; (e), share of the area covered with plants (NDVI>0.4).

Chlorophyll (Chl-*a*), the key indicator of the plant physiology, may be measured by red-edge wavelength reflection (680 ~ 750nm), which rises sharply from maximum absorption in the red band to the near-infrared. More than ten indices have been proposed for calculating Chl-*a* content [11], amongst which the Chlorophyll Sensitive Index (CSI) has the advantage of high sensitivity to Chl-*a* content in plants, a decrease in the blue spectrum reflection that ensures a reduction in the index sensitivity to the leaf area index and optical properties of the soil background, and a mathematical function (Formula 6) for calculating Chl-*a* content on the basis of a correlation between CSI and field measurements ($R^2=0.88$).

Satellite imagery reveals a seasonal pattern of Chl-*a*_{CSI} accumulation in the aboveground biomass (Fig. 9). At the end of June, after the water receded, Chl-*a*_{CSI} was registered across almost 20 per cent of the drained bed in the range 5-30 $\mu\text{g}/\text{m}^2$, a weighted average of 5.6 $\mu\text{g}/\text{m}^2$, on account of phytoplankton residues (Fig. 10a). These dried at the beginning of July and their chlorophyll was

lost. Aboveground vegetation appeared at the end of July, a range of $5\text{--}30\ \mu\text{g}/\text{m}^2$ (weighted average of $6.7\ \mu\text{g}/\text{m}^2$) reflecting sparse or stunted vegetation. At the September peak (Fig. 10c), Chl- a_{CSI} accumulation varied between $22\text{--}68\ \mu\text{g}/\text{m}^2$ (weighted average $44\ \mu\text{g}/\text{m}^2$) with the local peak of over $120\ \mu\text{g}/\text{m}^2$ representing tall plants and shrubs.

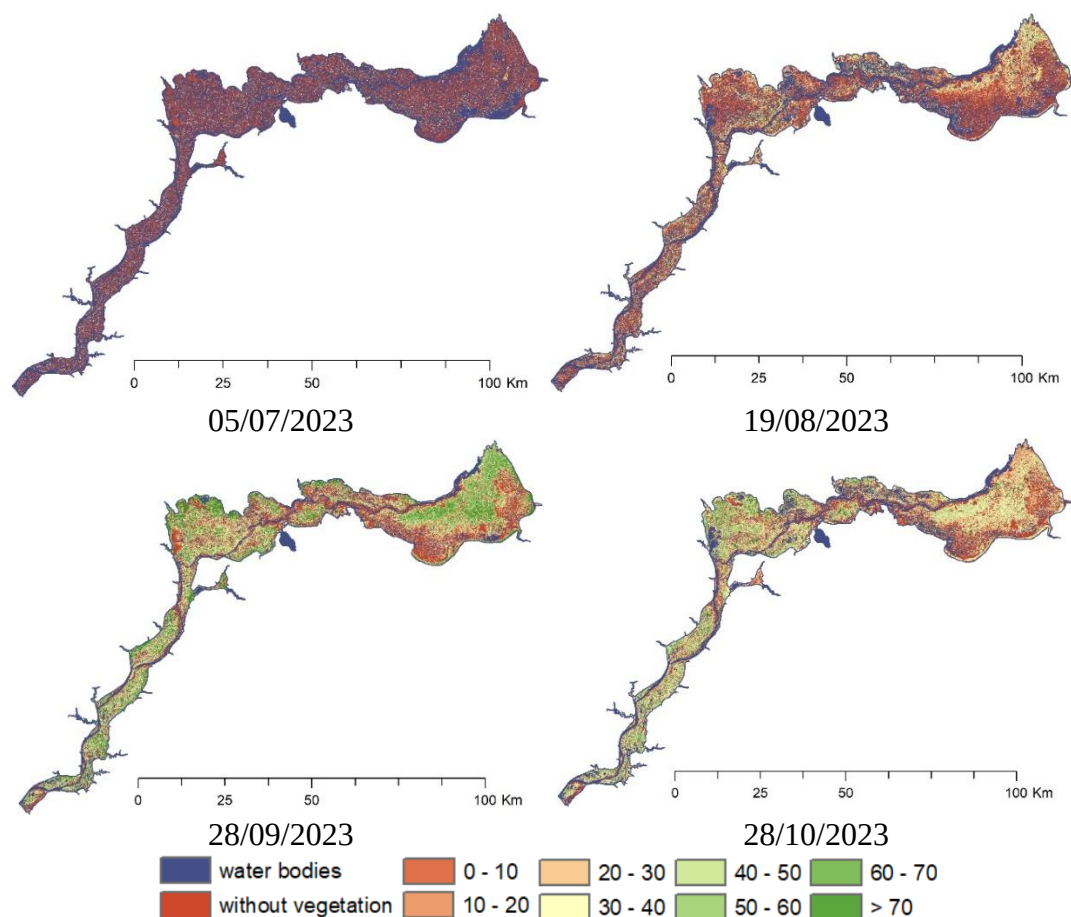
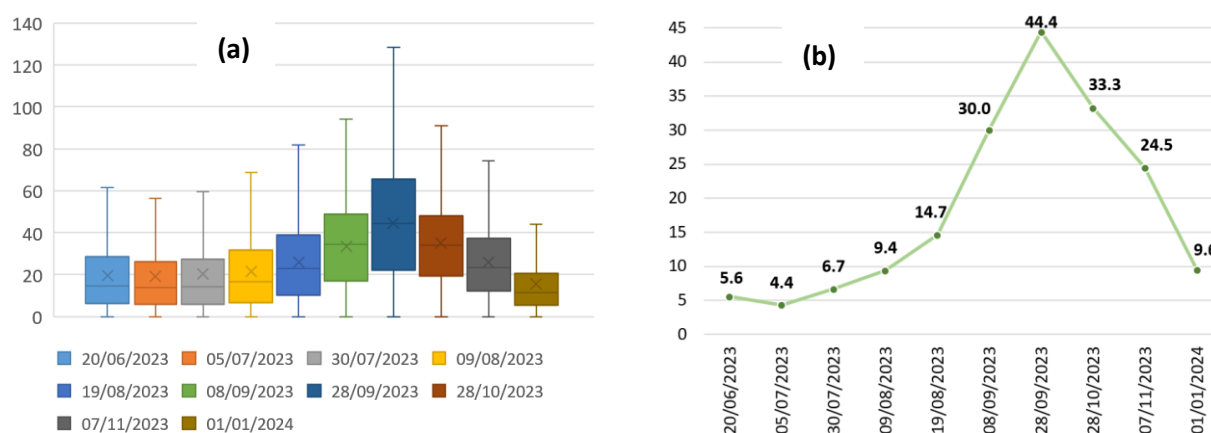


Figure 9. Chlorophyll (Chl- a_{CSI} , $\mu\text{g cm}^{-2}$) in the plant cover of the drained reservoir.



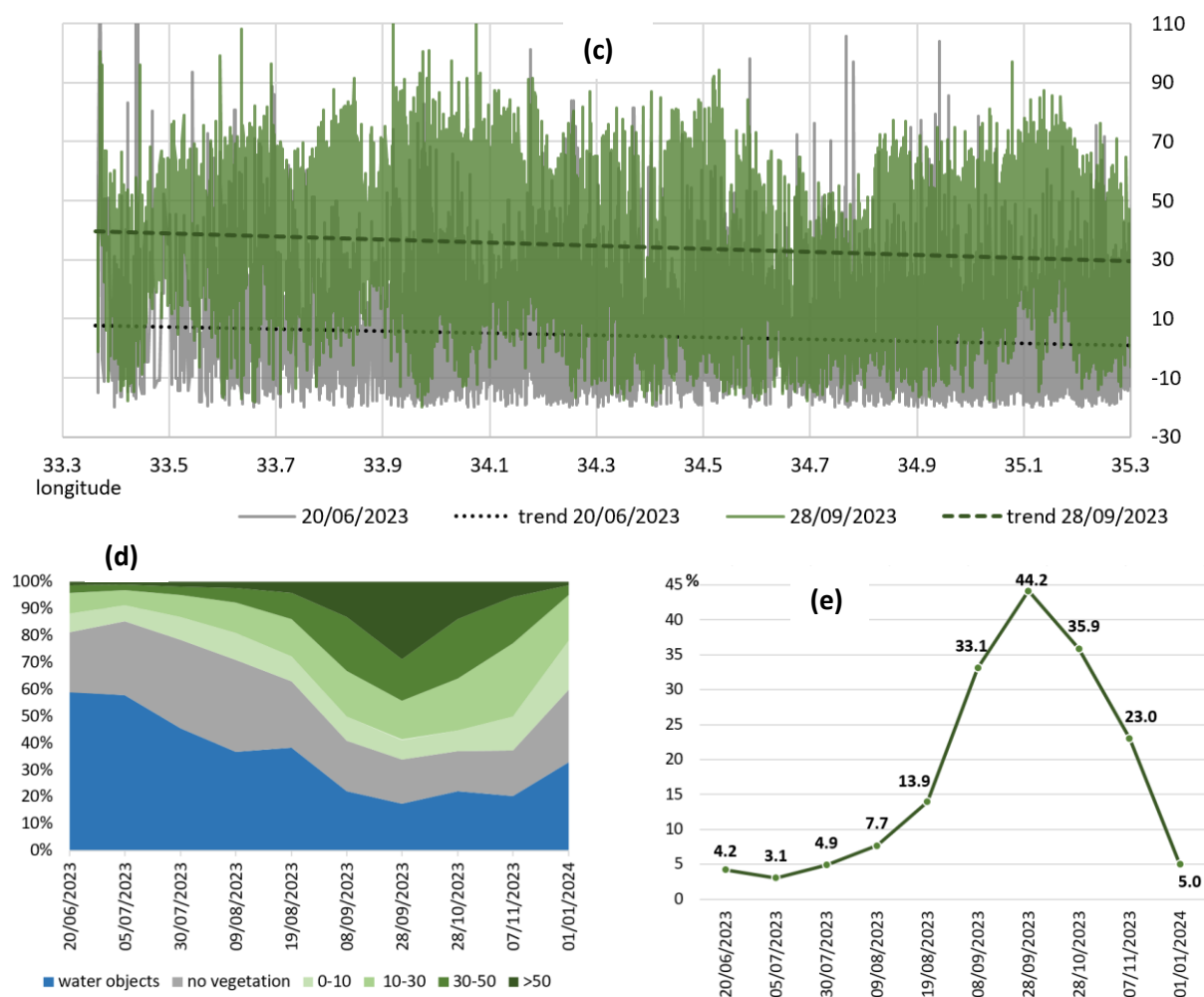


Figure 10. Structure of the drained territory of the Kakhovka Reservoir by Chl-aCSI ($\mu\text{g}/\text{cm}^2$): (a), differentiation of Chl-aCSI values in plant leaves; (b), weighted average of Chl-aCSI against the maximum overgrown area (28/09/2023); (c), comparison of Chl-aCSI values on 20/06/2023 and 28/09/2023; (d), distribution of the reservoir areas, %; (e), vegetated area, Chl-aCSI >30 $\mu\text{g}/\text{cm}^2$.

At the end of the growing season, water bodies occupied 17.5-22% of the former reservoir, bare ground 16-19%, sparse or stunted vegetation 22-27%, good vegetation 15-22%, and very good vegetation 14-29%.

Climate, Microclimatic Conditions and Evapotranspiration

Across the Ukrainian steppe, the period 1945-2019 saw mean annual temperatures rise by 3.5°C . Annual precipitation ranged from 186 to 778 mm with 27% variation but decreased by 40% over the last 20 years to 500-300 mm, although with more frequent heavy rain in the spring-summer period. Overall, mean annual air temperature increased by 0.06°C per year and mean annual precipitation decreased by 62 mm per year [19]. There has been an 18.7 per cent rise in the solar radiation reaching the earth surface and a 62% decrease in vegetation productivity that is likely to decrease by a further

20%. Over the past 20 years, the natural moisture coefficient has fallen by 66% and will likely decrease by a further 20%.

Against this backdrop, the reservoir created welcome sea breezes felt tens of km from the water area and its loss has diminished the moisture supply.

Land Surface Temperature (LST, °C) is a radiation temperature of the earth surface. It depends on the ratio of plant cover and soil moisture and, also, landform; it also determines the near-surface air temperature [20,21]. Destruction of the reservoir brought about an almost immediate 2.0°C increase in the LST of the drained territory of 2,155 km² and the 10 km buffer zone of

5,678 km² (Fig. 11) calculated on the basis of a difference in raster LST surfaces of summer 2023 to the average value of the corresponding period in 2020-2022. Drainage of reservoir increased the surface heating of the drained bed (Fig. 12a) and seasonal heating across 48-83% of the adjacent territory (Fig. 12b).

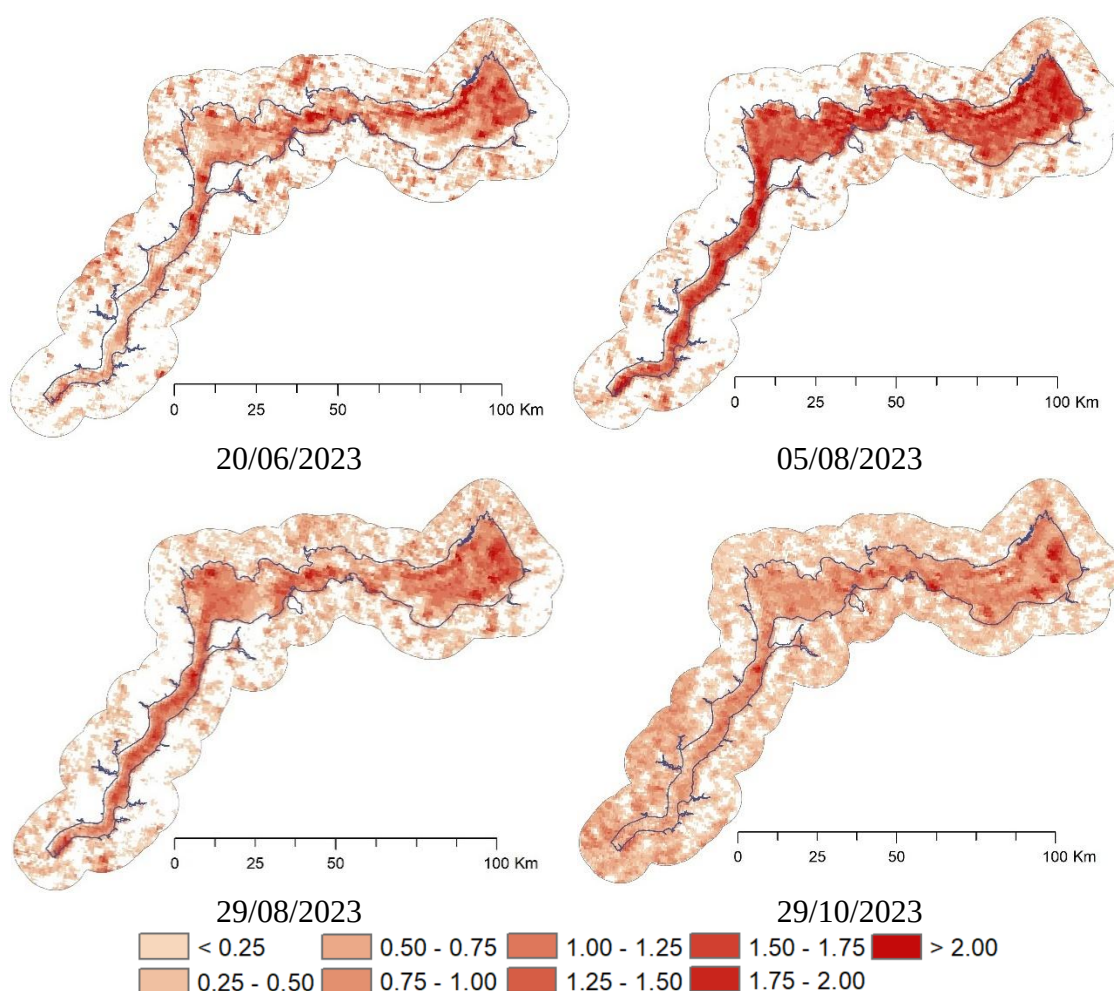


Figure 11. Land surface temperature (LST, °C) of the drained Kakhovka Reservoir and the adjacent territories.

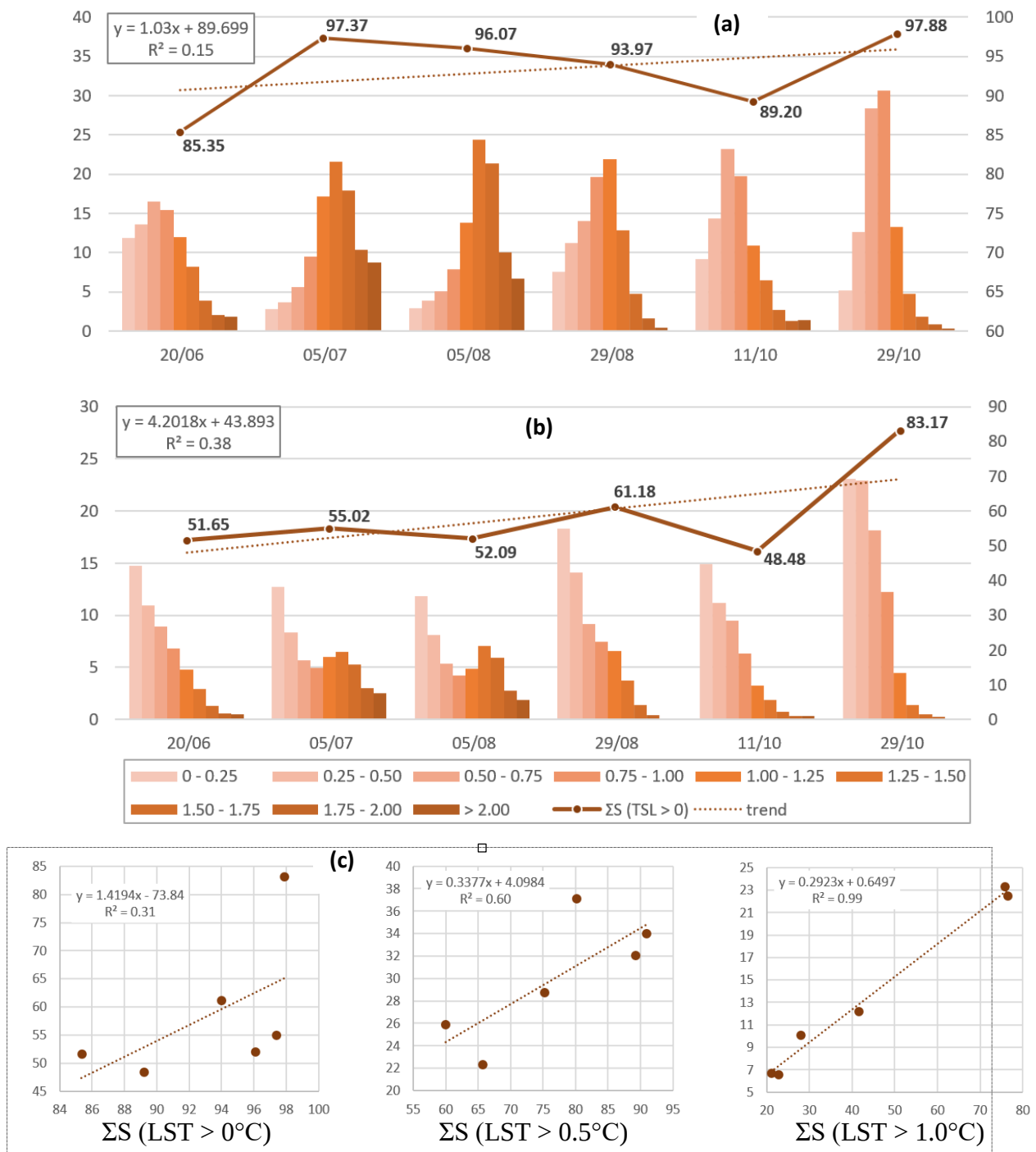


Figure 12. Distribution of the areas (%) with different levels of increase in the land surface temperature (LST, °C): (a), territory of the drained Kakhovka Reservoir; (b), adjacent territories; (c), dependence of the temperature change in the landscape structure of the water reservoir and the adjacent territories.

Moreover, the developing vegetation did not maintain the same level of humidity as the former open water, so evapotranspiration increased, creating a soil water deficit (Fig. 13). In the summer-autumn period, ET_{LST} rose to a record level of more than 4.0 mm/day (Fig. 14); there was an acceleration in

evapotranspiration of up to 10% across 1,070 km² (19%), from 10-20% across 642 km² (11%), 20-30% across 356 km² (6%), 30-40% across 204 km² (4%), 40-50% across 114 km² (2.0%), and more than 50% across 190 km² (3%).

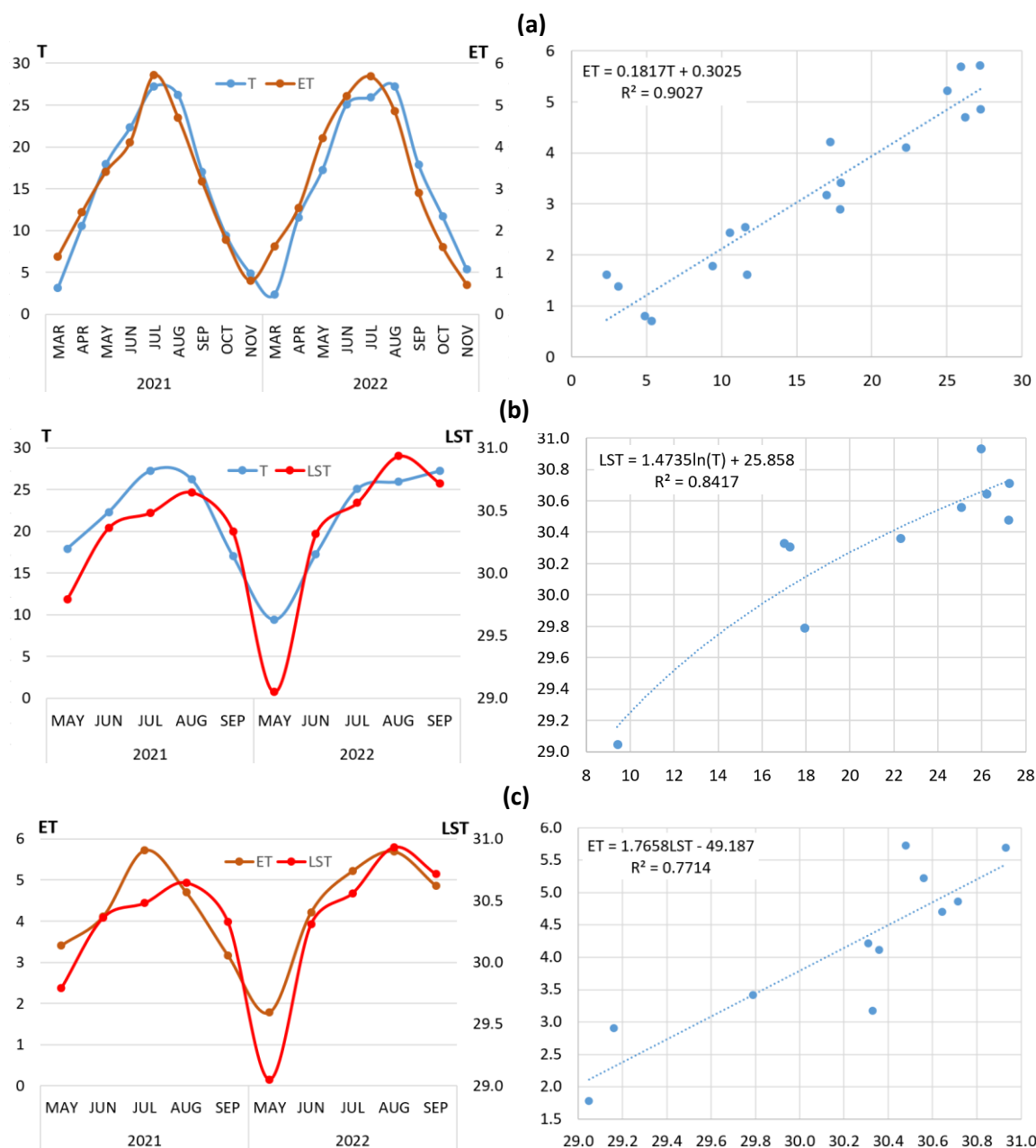


Figure 13. Monthly change in climate-landscape characteristics of the drained Kakhovka Reservoir and the adjacent territories in the warm months of 2021-2022: (a), dependence of reference evapotranspiration (ET, mm/day) and the air temperature (T, °C); (b), dependence of the Land Surface Temperature (LST, °C) and the air temperature (T, °C); (c), dependence of reference evapotranspiration (ET, mm/day) and Land Surface Temperature (LST, °C)

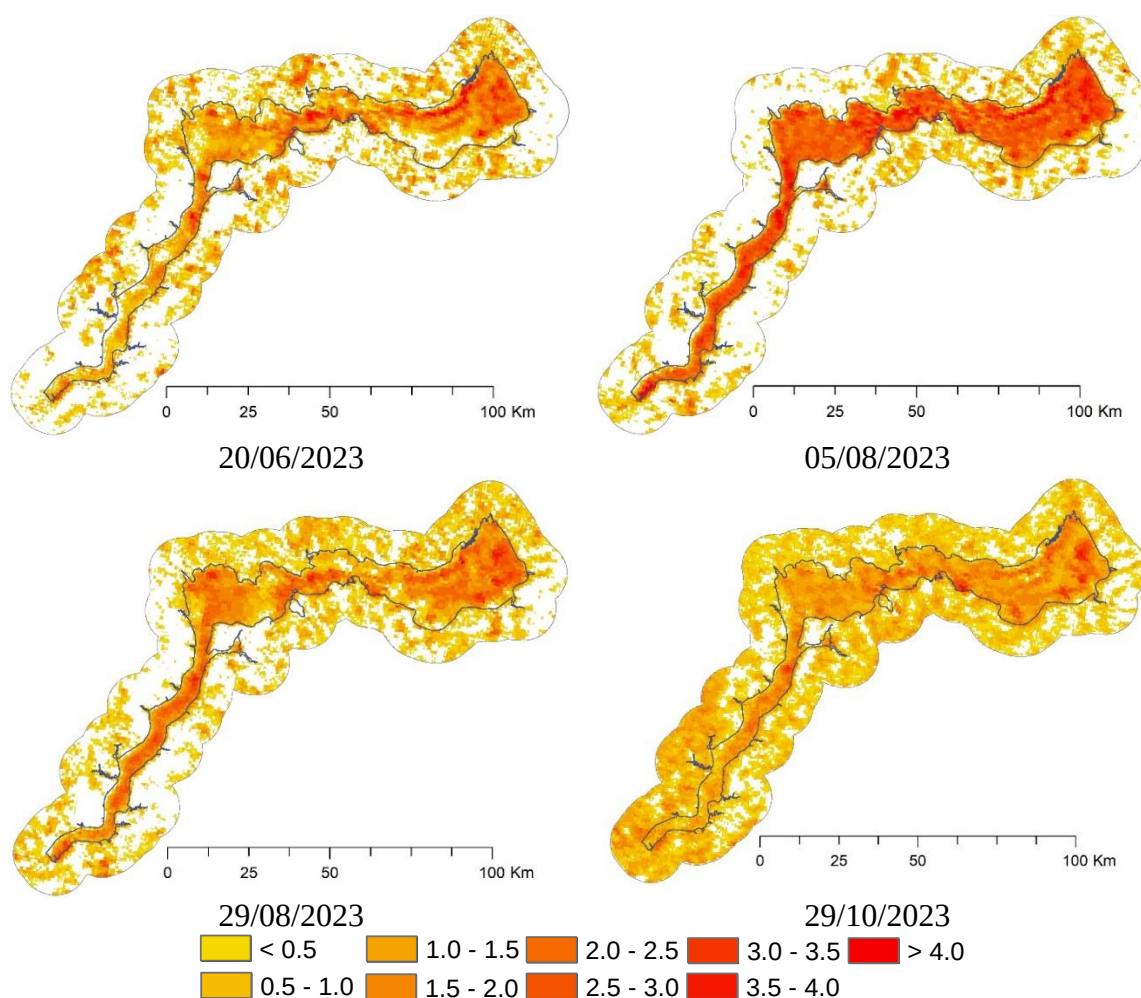


Figure 14. Increase of ET_{LST} on the bed of the Kakhovka Reservoir and adjacent territories, calculated as the difference in the ET_{LST} values in 2023 to the average ET_{LST} values in 2020-2022.

The new world of the drained reservoir

Severe environmental and socio-economic consequences have already been foreseen by Mulvey P&F, Dmytruk, et al. [22]. Our scenario was calculated for September 2023 which was characterized by typical steppe conditions and maximum vegetation cover. Comparison of microclimatic conditions to those for September 2020 showed increased solar radiation reaching the ground surface but no significant difference in rainfall. LST in 2020 was $29.83 \pm 0.52^\circ\text{C}$; in 2023, $29.96 \pm 0.30^\circ\text{C}$. But in 2020 the reservoir moderated evaporation from the water surface: ET_{LST} across the water area was $2.24 \pm 0.19 \text{ mm/day}$ in 2020, and $3.20 \pm 0.46 \text{ mm/day}$ in the drained territory. In 2023, loss of the water body more than doubled the cases of high evapotranspiration values from 3.5 to 4.0 mm/day in the adjacent territory. At the end of September, drainage of the reservoir had caused an increase in the LST values by more than 1.0°C (Fig. 15a) and in the ET_{LST} values by more than 1.5 mm/day (Fig. 15b), exacerbating water scarcity by 58% in the research territory.

According to the State Agency of Water resources of Ukraine, monthly evaporation from the open waters of the lower Dnipro basin during the warm season amounts to 4.72 million m³ (Table 3). By our calculations (Table 4), total evaporation from the water surface was 5.94 million m³ in September 2020; in 2023 the potential evaporation from the dry bed of the reservoir was 12.13 million m³ – more than double. Potential evaporation from the adjacent territories was 28.56 million m³ in 2020, in 2023 this figure rose by 1.4 times. In 2020, potential evaporation from the entire research area (7,812.8 km²) was 34.50 million m³; in 2023, 52.36 million m³. Over the research period, vegetation cover across the buffer zone was comparable with an average NDVI value of 0.37 ± 0.17 in the third decade of September in 2020 and 2023. All of which suggests that the increased land surface temperature, greater water stress, and increased evapotranspiration will prevent regeneration of the historical Great Meadow.

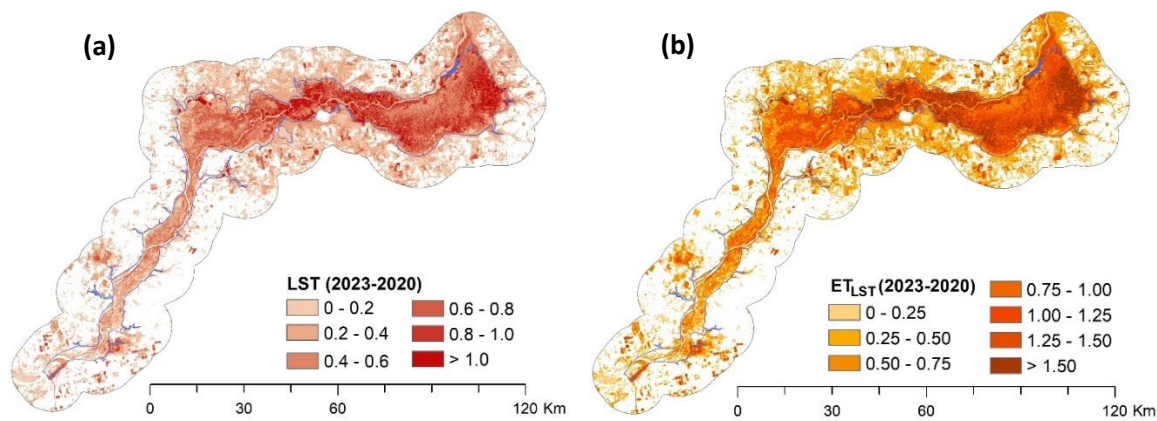


Figure 15. Changes in land surface temperature (LST, °C) and evapotranspiration (ET_{LST}, mm/day) across the drained Kakhovka reservoir and the adjacent territories, September 2020 and 2023: (a), increase in LST values in 2023; (b), increase in ET_{LST} volumes from the land surface.

Table 4. Evapotranspiration (ET_{LST} , mm/day) from the surface of the drained Kakhovka Reservoir and the adjacent territories before and after the destruction of the retaining dam.

Statistic	Data		
	09/2020	09/2023	2023÷2020
Territory of the Kakhovka Reservoir			
Min	1.29	1.80	1.4
Max	4.07	5.08	1.3
Mean	2.24	3.20	1.4
Standard deviation	0.19	0.46	2.4
Variance, %	8.48	14.42	1.7
Sum1 per month	593 592	1 212 512	2.0
Adjacent territory (the buffer zone of 10 km)			
Min	1.49	1.67	1.1
Max	6.77	7.35	1.1
Mean	3.97	4.35	1.1
Standard deviation	0.57	0.42	0.7
Variance, %	14.36	9.66	0.7
Sum2 per month	2 856 164	4 023 688	1.4
Total per month (Sum1 + Sum2)	3 449 756	5 236 200	1.5

Natural-Climatic Transformation

Natural-climatic transformation of the drained territory of the Kakhovka Reservoir was calculated for September 2023 on the basis of the Vegetation Health Index (VHI) which combines the Vegetation Condition Index (VCI) and the Temperature Condition Index (TCI) of the landscape. TCI is calculated using an equation similar to the VCI but relates the current temperature to its maximum and minimum, since higher temperatures are likely to be unfavourable.

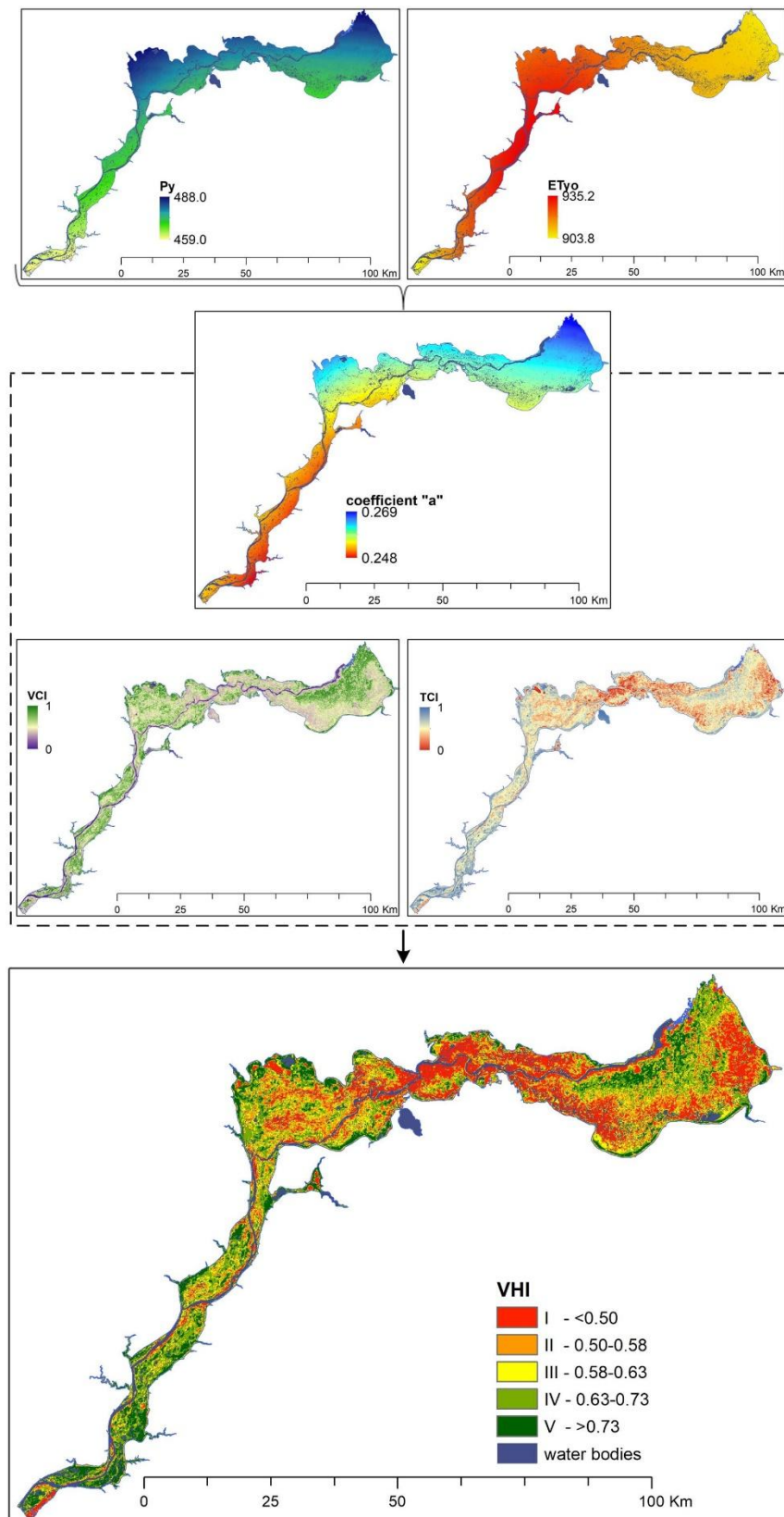


Figure 16. Natural-climatic transformation of the drained territory of the Kakhovka Reservoir in September 2023: P_y , mean annual precipitation 2020-2023; ET_{yo} , mean reference evapotranspiration in 2020-2023; a , coefficient of VCI and TCI; VCI, Vegetation Condition Index; TCI, Temperature Condition Index; VHI, Vegetation Health Index.

Figure 16 shows that, as of September 2023, the territory of the drained Kakhovka Reservoir is composed of: water bodies, 16%; I – bare ground, 21%; II – open landscapes with patches of stunted vegetation, 18%; III – sparsely vegetated land suffering water scarcity and significant temperature pressure, 13%; IV – landscapes with a satisfactory moisture level and plant biomass, 20%; V – a good level of moisture with healthy vegetation 12%. Vegetation is in good health where there is a hydrological connection with overflow from the Dnipro River, residual moisture in bottomland, and runoff from farmland. Two months after the destruction of the dam, isolated shallow water bodies covered 307km² but shrank to 196 km² by the end of September. Some will be filled by the spring flood and about 137 km² of water bodies and water courses in the drained territory of the water reservoir are functionally stable. The Great Meadow must have relied on seasonal flooding from the Dnipro River which is fed mainly from beyond the steppes and only 1.8% by local drainage from drained territory of the Kakhovka Reservoir. That flooding is now constrained by the remaining cascade of reservoirs.

Discussion

The Kakhovka Reservoir was hugely important to the southern steppe of Ukraine. It provided water for more than one million people in more than 140 settlements including Kryvyi Rih (population over 600,000), Nikopol (100,000) and several others, and for 800,000 ha of irrigated land via the Kakhovka Canal, the North Crimean Canal and the Dnipro-Kryvyi Rih Canal [7]. In total, 1.21-1.34BCM: 5% for domestic use, 8% for industry, and 74% for irrigation. Although the environmental condition of the reservoir was unsatisfactory, it was important for recreation and commercial fishing, providing 22% of the national catch. And it was an artery of navigation serving the port of Nikopol.

Destruction of the Kakhovka Dam was a catastrophe, terminating industries and livelihoods built up over 65 years, cutting off water for irrigation, setting in train desertification of the southern steppes. How great the damage might become may be envisioned in the example of the disappearance of the Aral Sea which gravely damaged ecosystems, public health, life security, and the quality of the national and regional development in neighbouring Uzbekistan [23,24]; its dry sea bed now the Aralkum Desert, salt flats subject to frequent dust storms; the number of days above 40oC has more than doubled; the biological productivity of the whole region has shrunk ten-fold and many Red Book species are lost.

The condition of the ecosystems and the territory impacted by the Kakhovka reservoir requires comprehensive, systematic monitoring, and exhaustive research to develop a better future than that for the south of Ukraine. Although the emergent vegetation is not capable of recreating a microclimate that will allow natural regeneration of the former Great Meadow, its beginnings have

been constrained by the available seed bank and raw, polluted soil parent materials. Things will improve as a new ecosystem develops with more fully developed soils and adapted, deep-rooted perennial vegetation. When we have peace, recovery of natural ecosystems and regenerative agriculture, not only on the former reservoir bed but across the southern steppes, may draw upon Europe-wide expertise, careful land preparation, introduction of adapted native species, and new farming systems including controlled flooding to combat heat and drought. We might call it The Great European Plan for working with Nature.

Conclusion

Construction of the Dnipro Reservoir cascade created a new hydrobiology: almost halving the flow of the Lower Dnipro but greatly increasing accessible water reserves. The Kakhovka Reservoir generated electricity, provided fresh water to more than one million people, heavy industry, irrigation of 800,000 ha, was an artery of navigation and an important centre of recreation and fishing. Stagnation of the retained water resulted in eutrophication and cyanobacterial blooms; water quality ranged from moderately polluted to very dirty in 81-87% of the reservoir area.

Destruction of the Kakhovka dam caused a 2°C increase in land surface temperatures and accelerated evapotranspiration across the drained area and the adjacent territories in 2023. Evapotranspiration in September (at maximum vegetation cover in the drained bed of the Kakhovka reservoir) increased two-fold and by 1.4-times across the 10km zone of adjacent land, *exacerbating water scarcity in almost 60% of the territory*.

At the maximum vegetation development in 2023, only 32% of the former reservoir bed supported lush vegetation. This cannot recreate the microclimatic conditions maintained by the reservoir, potentially increasing the frequency of droughts and water scarcity in the Steppe zone of Ukraine. We must do better.

Acknowledgments

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Geolocation

Polygon coordinates: 35.360596,47.71088; 33.333068,47.71088; 33.333068,46.72388; 35.360596,46.72388; 35.360596,47.71088

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Consequences of Sabotage of the Kakhovka Dam on the Waters of the Dniipro-Buh Estuary and the Black Sea

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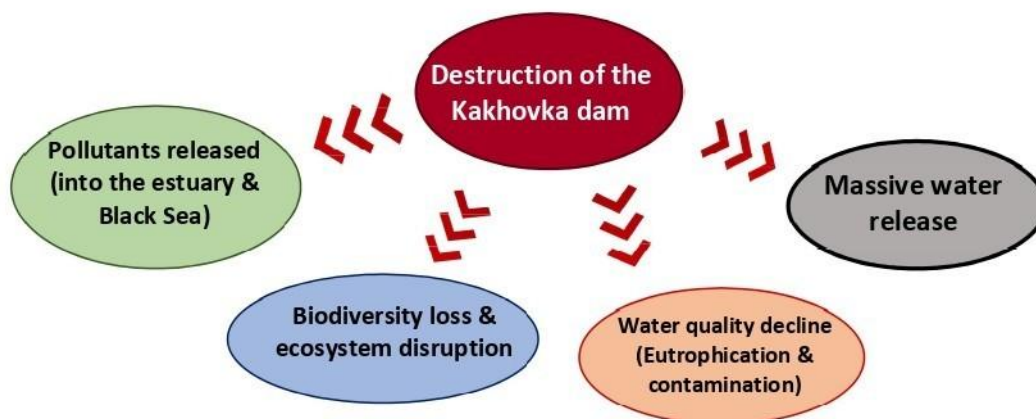
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Graphical Abstract

The destruction of the Kakhovka dam released pollutants up to 50 times above permissible levels across 6,800 km², severely impacting the Dniro-Buh estuary and the Black Sea. This disaster caused a deterioration in water quality, leading to eutrophication and excessive algal growth. The aquatic ecosystem is now disrupted, threatening biodiversity and hindering natural water regeneration.



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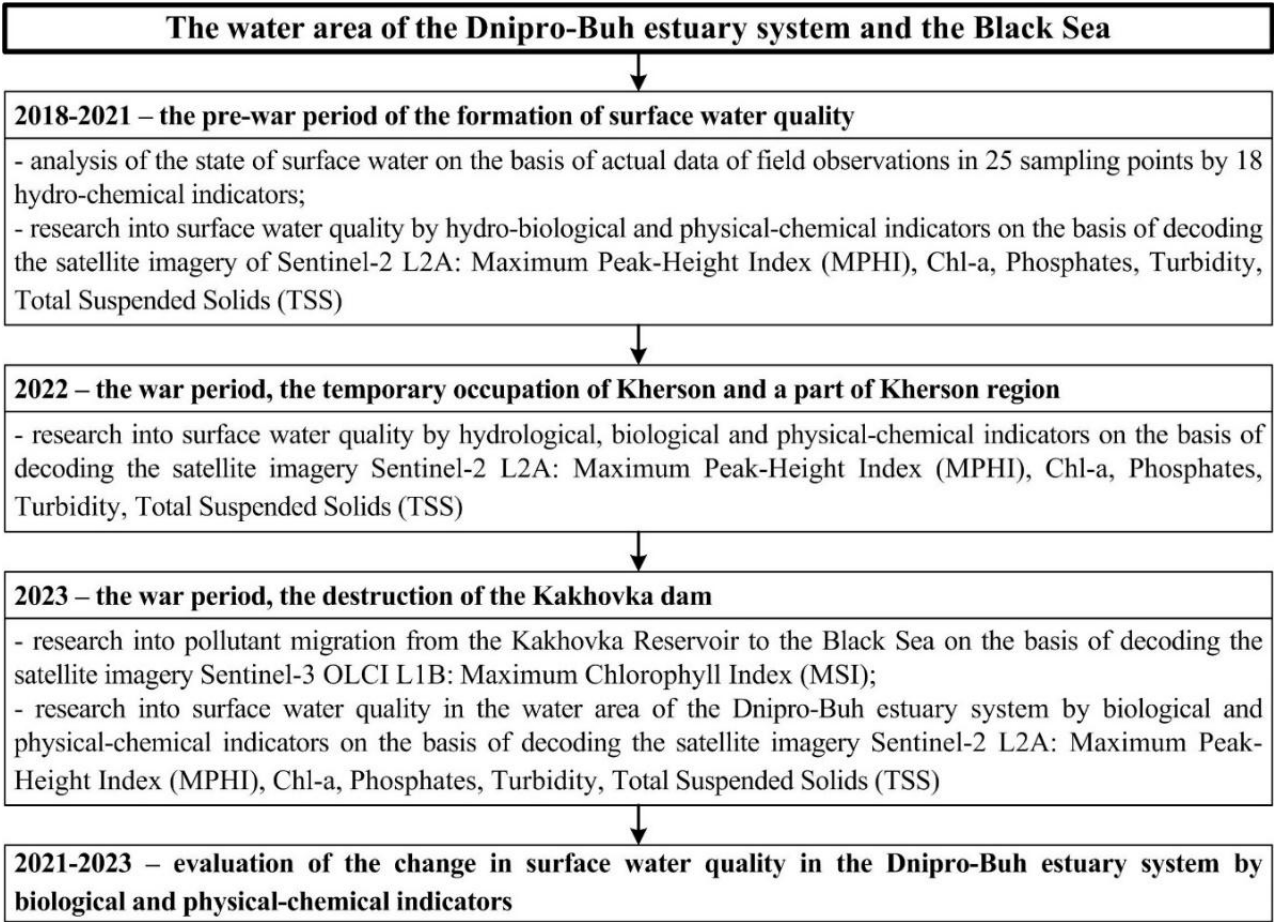
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Abstract: Sabotage of the Kakhovka dam released pollutants with concentrations up to 50 times their maximum permissible concentrations into 6,800 km² of the Dniro-Buh estuary and the Black Sea. The accumulation of biogenic elements doubled; there was a four-fold deterioration of physical and chemical properties, a three-fold increase in phytoplankton, and worsening of water quality to polytrophic status. The amplitude of freshwater flows to the estuary is now weakened and there is no effective mechanism to restore its status.

Keywords: *Armed aggression, Estuarine pollution, Spot samples, Remote sensing.*

Introduction

Russia’s threat is existential: Political, Economic, Cultural, Ecological. At a stroke, destruction of the Kakhovka Dam on 6th June 2023 damaged one third of Ukraine’s freshwater reserves and released the accumulated pollutants into the Dnipro-Buh estuary and the Black Sea [1-3]. However unsatisfactory its water quality, the Kakhovka reservoir supplied municipal and industrial needs, Europe’s biggest irrigation scheme [4, 5], and maintained the ecological flow of the lower Dnipro River and its estuary. In turn, the estuary is an important aquatic habitat [6,7]. When considering the necessity and feasibility of recreating the Kakhovka Reservoir, we should take account of changes in the hydrology and water quality of the Dnipro-Buh estuary which are the object of our research (Fig.1).



Materials and Methods

Table 1 lists our field observations and data of the State Environmental Inspectorate of the Southern Region (SEISR) of Ukraine that were used to trace surface water quality in 2018-23. Locations are plotted in Figure 2. Satellite imagery from the Sentinel 2 L2A and Sentinel-3 OLCI L1B, supplied by Copernicus Browser, was decoded to provide a dynamic spatial general view.

Table 1. Surface water samples from the Dnipro-Buh estuary.

№	Source of information	River	Name
Water samples 2018-21			
Dnipro river			
1	Authors	River bed	Ivanivka village shoreline
2	Authors	River bed	Ivanivka village 1, shallows
3	Authors	River bed	Ivanivka village 2, shallows
4	Authors	River bed	Ivanivka village, ship canal
5	Authors	River bed	ship canal
6	Authors	River bed	Kherson city 1, shallows
7	Authors	River bed	Kherson city 2, shallows
8	Authors	River bed	Rvach river 1, shallows
9	Authors	River delta	Rvach river 2, shallows
10	Authors	River delta	Rvach river, ship canal
11	Authors	River delta	Rvach river 3
Dnipro-Buh estuary			
12	Authors	Eastern	Shyroka Balka village
13	Authors	Eastern	Stanislav village, shallows
14	Authors	Eastern	Stanislav village, waterway
15	Authors	Eastern	Stanislav village, ship canal
16	Authors	Central	Oleksandrivka village
17	Authors	Central	Entrance to the Buh estuary 1
18	Authors	Central	entrance to the Buh estuary, 2
19	Authors	Central	entrance to the Buh estuary, ship canal
20	Authors	Central	Heroiske village, shallows
21	Authors	Western	Adzhyhiol village, shallows
22	Authors	Western	Adzhyhiol village, waterway
23	Authors	Western	Kutsurub village, shallows
24	Authors	Western	Pokrovski Khutory village, shallows
25	Authors	Western	Ochakiv city – exit from the estuary

Water samples 2023			
26	SEISR of Ukraine	Ingulets River bed	Darivka village, shoreline
27	SEISR of Ukraine	Dnieper River bed	Antonivka railway bridge
28	SEISR of Ukraine	Dnieper River bed	Kherson city, Mykola Sadovskyi Street
29	SEISR of Ukraine	Dnieper River delta	Koshova river, Kherson city, Prychalna street
30	Authors	Dnieper River delta	Pohorile lake
31	Authors	Dnieper River delta	Koshova River
32	Authors	Dnieper River delta	Chichkovate Lake
33	Authors	Dnieper River delta	Bezmen Lake
34	Authors	Dnieper River delta	Bile lake

The Dnipro-Buh estuary stretches from the former Kakhovka dam and the estuary of the Southern Buh River to the Kinburn spit, where it joins the Black Sea. The hyper-ecosystem encompasses 810 km² with a total length of 150.8 km (Fig. 1). The near-estuary between the Kakhovka dam and the city of Kherson and the delta includes numerous channels and floodplain lakes with a length of 94.4 km and an area of 492 km². Until 6/6/2023, its hydrology and ecology

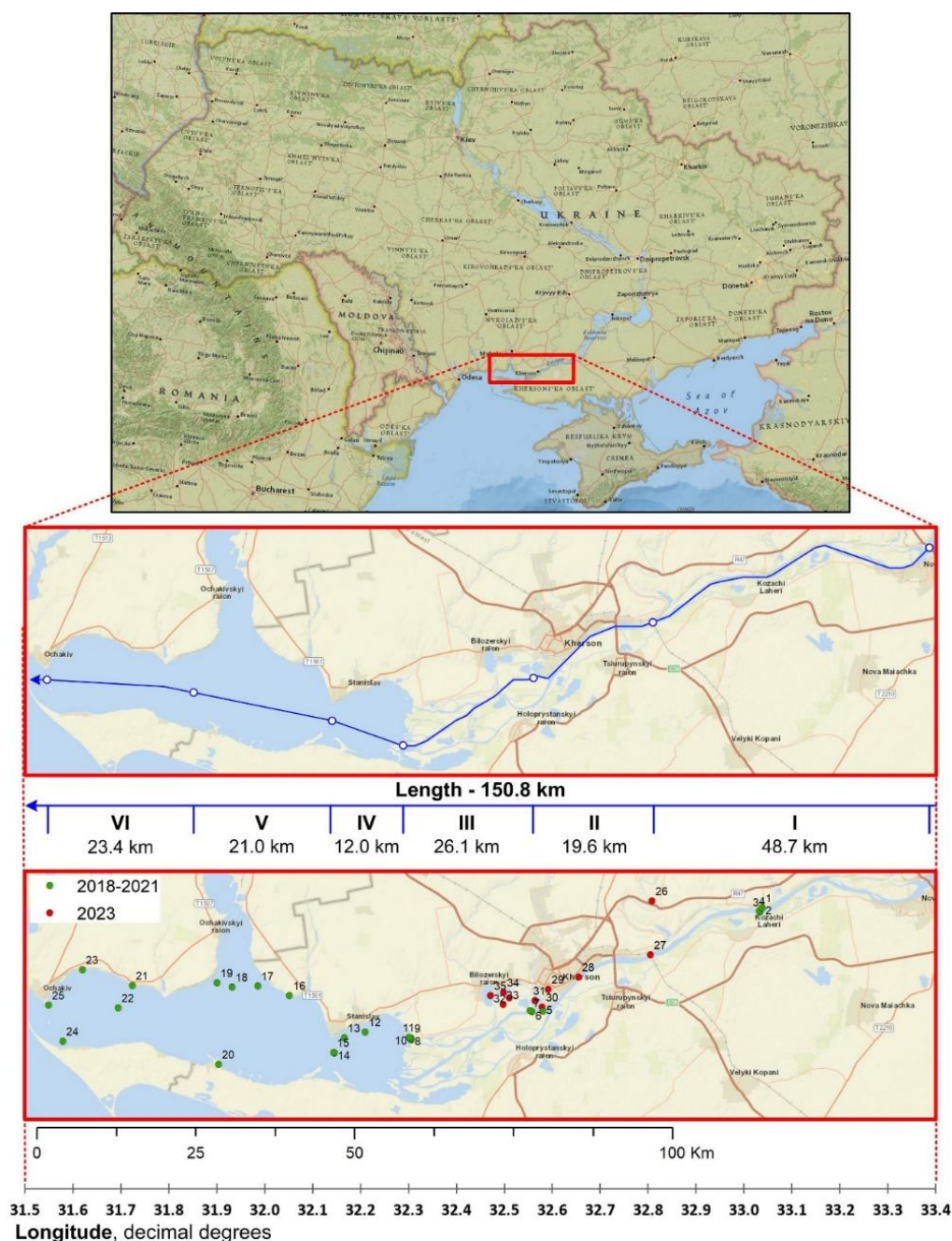


Figure 1. The water area of the Dnipro-Buh estuary system.

Lower Dnipro: length of the river course 94.4 km: I, from the Kakhovka hydroelectric power station dam to the Antonivka railway bridge within the mouth of the Inhulets river; II, from the Antonivka bridge to the beginning of the Dnipro delta within the city of Kherson; III, from the beginning of the delta to the Dnipro mouth. **Dnipro-Buh estuary:** length 56.4 km: IV, Eastern section; V, Central section; VI, Western section depended on the water flow through the Kakhovka hydroelectric plant and its dam. Now it is governed by the operation of the Dnipro hydroelectric plant, 230km upstream.

The 48.7 km reach from the Kakhovka dam to the Antonivka railway bridge receives surface waters similar to those of the Lower Dnipro but the 19.6 km reach from the Antonivka bridge to the beginning of the Dnipro delta receives the brackish Inhulets River and surface runoff from Kherson. Pollution of the Inhulets River stems from mine waters and wastewater from Kryvyi Rih and the Inhulets mine-processing plants that add dry residues of 2,150-3,900 mg/dm³, including chloride (425-1,365 mg/dm³) and sulphate (670-842 mg/dm³) [8]. Surface runoff from Kherson adds oil products entering the suburban river area with untreated municipal wastewater, so this section of the Dnipro River is classed as *dirty – very dirty* by the norms of water suitability for fisheries.

The third section of 26.1 km from the beginning of the delta to the Dnipro mouth is characterized by inflow of sewage from Kherson and stagnant lake waters with a low or medium flow capacity. The city's treatment plants, built in 1975, have a capacity of 45-50 thousand m³ of wastewater per day: filtration and primary settling tanks retain heavy pollution and effect 35-40% treatment; subsequent digestion by aerobic microorganisms achieves up to 90% treatment [9]. But, over the period 2016-21, sewage discharge into the Dnipro exceeded the *maximum permissible concentrations* (MPC) of suspended solids by 4.2 times, phosphate by 3.6 times, dry residue by 1.3 times, sulphate by 1.7 times, chloride by 1.2 times, sodium+potassium, by 2.6 times, ammonium nitrogen by 3.8 times, and oil products by 2.0 times. Moreover, releases of untreated sewage add 400 tonnes of surface-active agents, nitrogen oxides, sulphur, phosphorous and oil products [9].

After the Danube delta, the Dnipro delta is the second largest in Ukraine extending over about 350 km², including channels, floodplain lakes, backwaters, tributaries, and several small islands. The Dnipro-Buh estuary is an open, oligohaline water body with an average depth of 3.5-4 m and greatest depth 12 m. It connects with the Black Sea by a 3.6 km-wide strait between the Ochakiv Cape and the Kinburn Spit. The southern shore is low and sandy; the northern shore mostly high (up to 20-35 m) with steep, sandy clay banks and some sand and shell spits. The estuary proper comprises the Eastern section (IV) fed by the Dnipro which contributes 94 per cent of the outflow; the Central section (V) under the influence of river and sea waters that are vertically stratified, which brakes the mixing process and leads to anaerobic zones in hot weather; and the Western section (VI), also under the influence of river and sea waters. The average salinity is 3.6‰ but varies between 1.0 and 3.3‰ in the Eastern section, between 1.0 and 6.0‰ in the Central section, and between 1.0 and 11.0‰ in the Western section.

Research Methods and Techniques

We calculated the intensity of saturation of floating blue-green algae (cyanobacteria) with the Chl-a pigment ($\mu\text{g}/\text{dm}^3$) using the Maximum Peak-Height Index (MPHI) [10]:

$$\text{MPHI} = B5 - B4 - \frac{(B8 - B4) \times (705 - 665)}{842 - 665} \} \rightarrow \text{Chl-a} = 2223.18 \text{MPHI} + 24.03 \quad (1)$$

where B4, B5, and B8 are the reflectance and $\lambda B4 = 665\text{nm}$, $\lambda B5 = 705\text{nm}$, and $\lambda B8 = 842\text{nm}$ are the central wavelengths of the corresponding bands of Sentinel-2 L2A. MPHI ranges from -1.0 to 1.0; a value higher than 0.004 indicates presence of Chl-a in the blue-green algae of the surface water.

The content of phosphate (mgP/dm^3) was calculated on the basis of the established dependence (authors' research):

$$P = 0.0102 \text{Chl-a}^{0.6995}, \quad r^2 = 0.984 \quad (2)$$

The turbidity of the surface water (Turb, NTU) was determined by this formula [11]:

$$\text{Turb} = 194.79 \left(B5 \times \left(\frac{B5}{B2} \right) \right) + 0.9061 \quad (3)$$

where B4, B5, and B8 are the reflectance bands of Sentinel-2 L2A.

Total Suspended Solids (mg/dm^3) was calculated on the basis of the established dependence [12]:

$$\text{TSS} = 3.4216 \text{Turb}, \quad r^2 = 0.987 \quad (4)$$

Table 2 presents the ecological classification of surface water quality [13].

Table 2. Classes and categories of surface water quality.

Water Quality Classes	I	II		III		IV	V
Water Quality Categories	1	2	3	4	5	6	7
Chl-a, $\mu\text{g}/\text{dm}^3$	<2	2-4	5-10	11-30	31-50	51-150	>150
Phosphate, mgP/dm^3	<0.015	0.015 - 0.030	0.031 - 0.050	0.051 - 0.100	0.101 - 0.200	0.201 - 0.300	>0.300
Total Suspended Solids, mg/dm^3	<5	5 - 10	11 - 20	21 - 30	31 - 50	51- 100	>100
Water quality according to cleanliness (pollution)	Very Clean	Clean		Polluted		Dirty	Very Dirty
	Very Clean	Clean	Clean Enough	Slightly Polluted	Moderately Polluted	Dirty	Very Dirty
Trophic class	Oligotrophic	Mesotrophic		Eutrophic		Polytrophic	Hypertrophic
Turbidity, NTU							
Permissible turbidity	Low turbid	Fairly turbid		Rather turbid	Turbid		Very turbid
< 5	5-15	15-25		25-35	35-50		> 50

Class I waters are the least polluted with hydro-chemical and hydro-biological indicators close to natural values. Class II waters are changed in comparison with natural values but these changes do not disrupt the ecological balance. Class III waters significantly polluted, close to the limit of ecosystem sustainability. Classes IV-V waters are severely impaired [14]. Maximum permissible concentrations (MPCs) of substances in the waters of the river and estuary were determined by the most stringent standards of surface water quality and water bodies for potable, household, recreational and fishing purposes in Ukraine and the EU (Tab.3) [15].

Table 3. Surface water quality standards.

Indicator		Standards of water quality		
		for fishing	for drinking	for household and recreational purposes
Total Suspended Solids, mg/dm ³	TSS	≤25	≤25	≤30
Turbidity, NTU	Turb	≤3.5	≤3.5	≤5
Reaction	pH	6.5-8.5	6.5-8.5	6.5-8.5
Dissolved oxygen, mgO ₂ /dm ³	DO	> 6	4	> 4
Biochemical oxygen demand for 5 days, mgO ₂ /dm ³	BOD	≤2	≤3	≤3
Permanganate oxidation, mgO ₂ /dm ³	PO	≤5	≤5	≤5
Chemical oxygen demand, mgO ₂ /dm ³	COD	≤20	≤15	≤15
Dry residue, mg/dm ³	DR	≤1000	≤1000	≤1000
Iron, mg/dm ³	Fe	≤0.1	≤0.1	≤0.1
Chloride, mg/dm ³	Cl ⁻	≤300	≤200	≤200
Sulphate, mg/dm ³	SO ₄ ²⁻	≤100	≤150	≤150
Nitrite nitrogen, mg/dm ³	NO ₂ ⁻	≤0.02	≤0.1	≤0.1
Nitrate nitrogen, mg/dm ³	NO ₃ ⁻	≤9.1	≤25	≤25
Phosphate, mg/dm ³	PO ₄ ³⁻	≤0.2	≤0.2	≤1.14
Petroleum products, mg/dm ³	PP	≤0.05	≤0.05	≤0.05
Copper, mg/dm ³	Cu	≤0.001	≤0.02	≤0.02
Lead, mg/dm ³	Pb	≤0.10	≤0.01	≤0.01
Zinc, mg/dm ³	Zn	≤0.01	≤0.5	≤0.5

The transfer of pollutants to the Black Sea after the destruction of the Kakhovka dam was calculated using the Maximum Chlorophyll Index (MCI) [16]:

$$MCI = B11 - \left[B10 + (B12 - B10) \times \frac{709-681}{754-681} \right] \quad (5)$$

where B10, B11 and B12 are the reflectance and $\lambda_{B10} = 681$ nm, $\lambda_{B11} = 709$ nm, and $\lambda_{B12} = 754$ nm are the central wavelengths of the corresponding bands of Sentinel-3 OLCI L1B.

Correlation and regression were used to find relationships between concentrations of substances in the water. MS Excel 2016 was used to create graphs and perform cross-sectional analysis. Image processing, mapping and spatio-temporal analysis employed ArcGis 10.6.

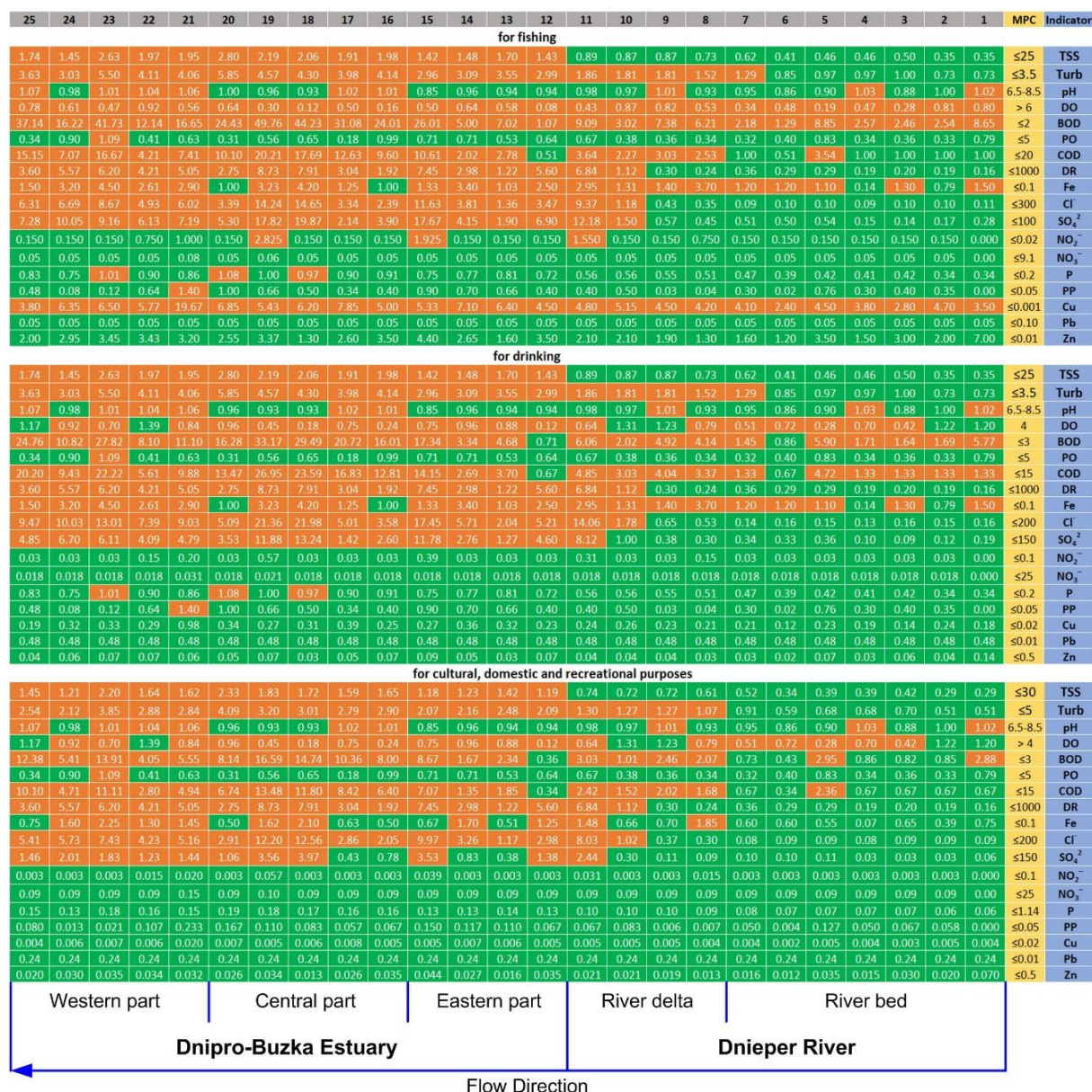
Results

Water quality in 2018-2021

In the pre-war period, surface water quality in the Dnipro-Buh estuary was driven by climate and human activities. Pollutants go with the flow, accumulating in excess of MPCs in the lower reaches of the river and the estuary, leading to eutrophication. In the spring floods, the Dnipro carries organic compounds, manganese, turbidity, chemical and biological oxygen demand from the upper reaches that drain upland peat bogs; but discharges from city sewage treatment plants have increased tenfold in the last decade and illegal construction in the water protection zone has caused bank abrasion. Above all, the cascade of huge reservoirs does not allow the river to function naturally. Increased anthropogenic load led to imbalance and a loss of self-regulation, self-purification and self-regeneration. Warm weather brought algal blooms, deoxygenation and mass fish deaths and microbiological and viral pollution of surface water required the closure of beaches.

During July-October 2018-21, MPCs of various contaminants were exceeded according to distance downstream from the Kakhovka Reservoir (Fig. 2). In particular, in the riverbed waters (samples 1-7), there was a deficiency in dissolved oxygen from 20 to 80% of the norm, and excess biological oxygen demand by 1.3-8.9 times from the multiplication of aerobic bacteria, causing fish kills. There was also an excess of chemical oxygen demand by 1.3-4.7 times, indicating input of untreated wastewater with organic and mineral substances, including humic substances, sulphides and iron. The concentration of iron exceeded the MPC for fisheries by 1.1-1.5 times. The excess of copper was 2.4-4.7 times MPC; enough to disrupt homeostasis and cause pathological changes of fish liver and other organs; human consumption of such fish is dangerous.

The waters of the delta (samples 8-11) were characterized by medium and low retention time but stagnation in floodplain lakes considerably worsened water quality. The turbidity of the surface waters was 1.3-1.9MPC, indicating the presence of fine impurities and plankton. Sentinel 2 L2A imagery (Fig. 3) also indicates algal blooms, turbidity and accumulation of suspended solids in the floodplain lakes. In July-October 2021, the average turbidity in the lakes varied from 13.5-26.9NTU (*i.e.* 3.8-7.4MPC), the maximum value being 52.5-74.1NTU (15-21MPC).



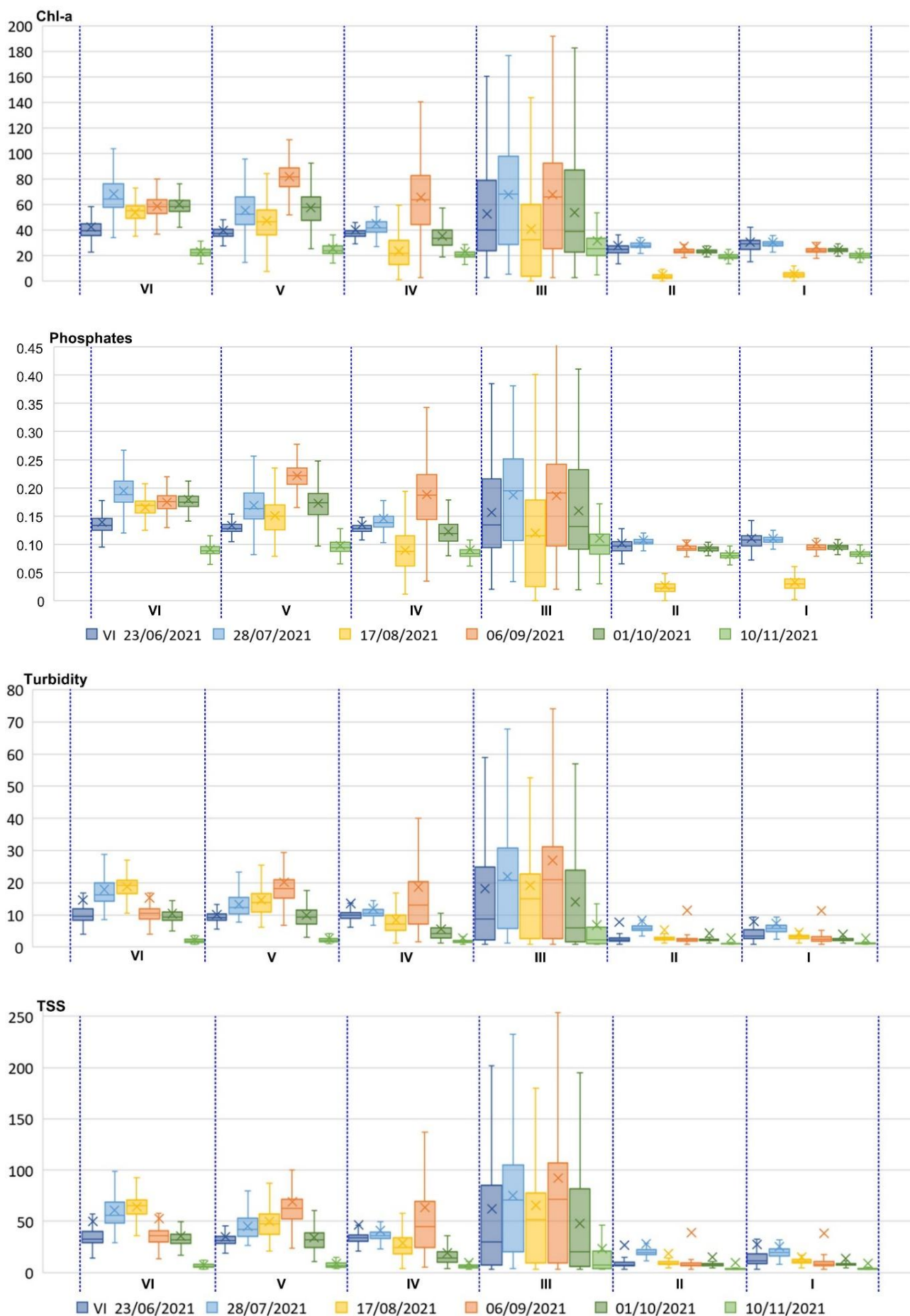


Figure 3. Surface water quality in the Dnipro-Buh estuary, 2021.

The dry residue (DR) measures the total amount of mineral salts and organic matter dissolved in water. In the period 2018-2021, DR was 1.1-6.8 MPC and, at the river mouth (samples 10 and 11), the content of chloride exceeded the MPC for drinking water by 1.8-9.4 times, the content of sulphate by 1.5-12.2 times. As well as tasting salty, salinity disrupts water-salt metabolism, suppresses gastric secretions, impedes food digestion and complicates hypertension; it also corrodes metal pipes and equipment used for transporting and storing water, and an increase in the sulphate content against the backdrop of the deficiency in dissolved oxygen, releases hydrogen sulphide. Sample 11 also exhibited excess nitrite, by 1.6 MPC, and a 4.2-5.2 MPC excess of copper.

In short, the surface waters of the Dnipro-Buh estuary in July-October were eutrophic or polytrophic. Throughout the estuary, there were mosaic processes of accumulation of pollutants and an increase in their quantity in the direction of water movement – from the western to the eastern section of the estuary and entry to the Black Sea shallows. The greatest pollution and a substantial phytoplankton biomass were observed at the beginning of September 2021 (satellite image dated 06/09/2021) when the concentration of Chl-a in the western section of the estuary ranged between 2.5 and 140 $\mu\text{g}/\text{dm}^3$ with a mean of 66 $\mu\text{g}/\text{dm}^3$; in the central section of the estuary, Chl-a ranged from 52-111 $\mu\text{g}/\text{dm}^3$ with a mean of 82 $\mu\text{g}/\text{dm}^3$; and in the eastern section ranged from 37-80 $\mu\text{g}/\text{dm}^3$ with a mean of 59 $\mu\text{g}/\text{dm}^3$. The minimum value of Chl-a was registered at the beginning of November when falling temperatures curtailed plankton growth; as of 10/11/2021, Chl-a ranged from 12.8 to 36.4 $\mu\text{g}/\text{dm}^3$.

Phytoplankton depend on the availability of phosphate. Their values change synchronously so that, on 06/09/2021, the mean phosphate concentration was 0.18mgP/dm³ (0.9 MPC) in the eastern section of the estuary, ranging from 0.03-0.35 mgP/dm³ (0.15-1.75MPC); in the central section the mean was 0.22 mgP/dm³ (1.1 MAC), ranging between 0.16-0.28 mgP/dm³ (0.8-1.4 MPC); and in the western section 0.17 mgP/dm³ (0.9MPC), in the range of 0.14-0.22 mgP/dm³ to 0.70-1.1 mgP/dm³. On 10/11/2021, the concentration of phosphate in the estuary water varied between 0.09 and 0.15 mgP/dm³ (0.5-0.8 MPC). In the same vein, turbidity and a high concentration of suspended solids (TSS) were observed in July-September 2021 when turbidity varied between 1.2 and 40.0NTU (0.3-11.4 MPC) and TSS varied between 4.1 and 136.9 mg/dm³ (0.2-5.5 MPC).

In summary, the July-October period throughout 2018-21 saw MPCs exceeded by most indicators *in all sections of the estuary*; in particular dissolved oxygen (0.08-0.8 MPC), biological oxygen demand (1.1-49.8 MPC), chemical oxygen demand (2.0-20.2 MPC), dry residue (1.2-8.7 MPC), iron (1.0-4.2 MPC), chloride (1.4-14.7 MPC), sulphate (1.9-19.9 MPC) and, sporadically, nitrite (1.6-2.8 MPC).

Water Quality in 2022

The Russian invasion of February 24, 2022, brought occupation of the Kherson region, seizure of water infrastructure, and transfer of enormous volumes of water from the Kakhovka reservoir to the occupied territories. According to the State Environmental Inspectorate of Ukraine, theft from the Kakhovka and the North Crimean main canals reached 50 m³/s; losses \$US 860,000 per day. Further water discharges to the Lower Dniro created significant fluctuations of the water level in the Kakhovka Reservoir (Fig. 4).

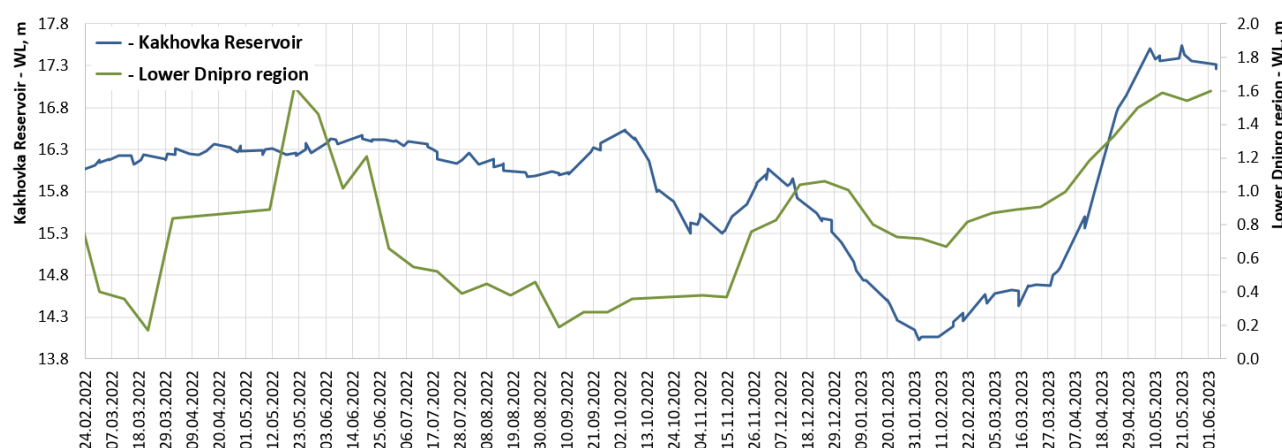


Figure 4. Dynamics of the water level in the Lower Dniro from the Russian invasion on 24.02.2022 to the destruction of the Kakhovka dam on 06.06.2023.

From the onset of hostilities until the liberation of Kherson (11/10/2022), unauthorised water discharges from the Kakhovka Reservoir produced pulses of flushing of the Lower Dniro and the estuary (Fig. 5). The water level in the Lower Dniro reached 1.46-1.62 m compared to the normative level of 0.57 m which reduced the background concentrations of pollutants in the river and the estuary: in particular, phosphate to (0.10±0.01 mgP/dm³), Chl-a (27.0±5.0 µg/dm³), turbidity (11.0±9.5 TU), and total suspended solids (37.5±32.6 mg/dm³).

From July to the second decade of September 2022 water levels in the reservoir and the Lower Dniro were lowered by diversion of water to the left bank of Kherson region and Crimea. Lesser river flow and stagnation of surface waters greatly increased the concentration of pollutants in the estuary in this warm season: there was a high concentration of phosphate in the river delta (0.18-0.22 mgP/dm³) and a record increase in the floodplain lakes (up to 0.44-0.45 mg/dm³ or 2.2-2.3MPC). The mean phosphate accumulation in different sections of the estuary ranged from 0.16-0.25 mgP/dm³ with spots of maximum concentration from 0.33-0.37 mgP/dm³ (1.7-1.9 MPC).

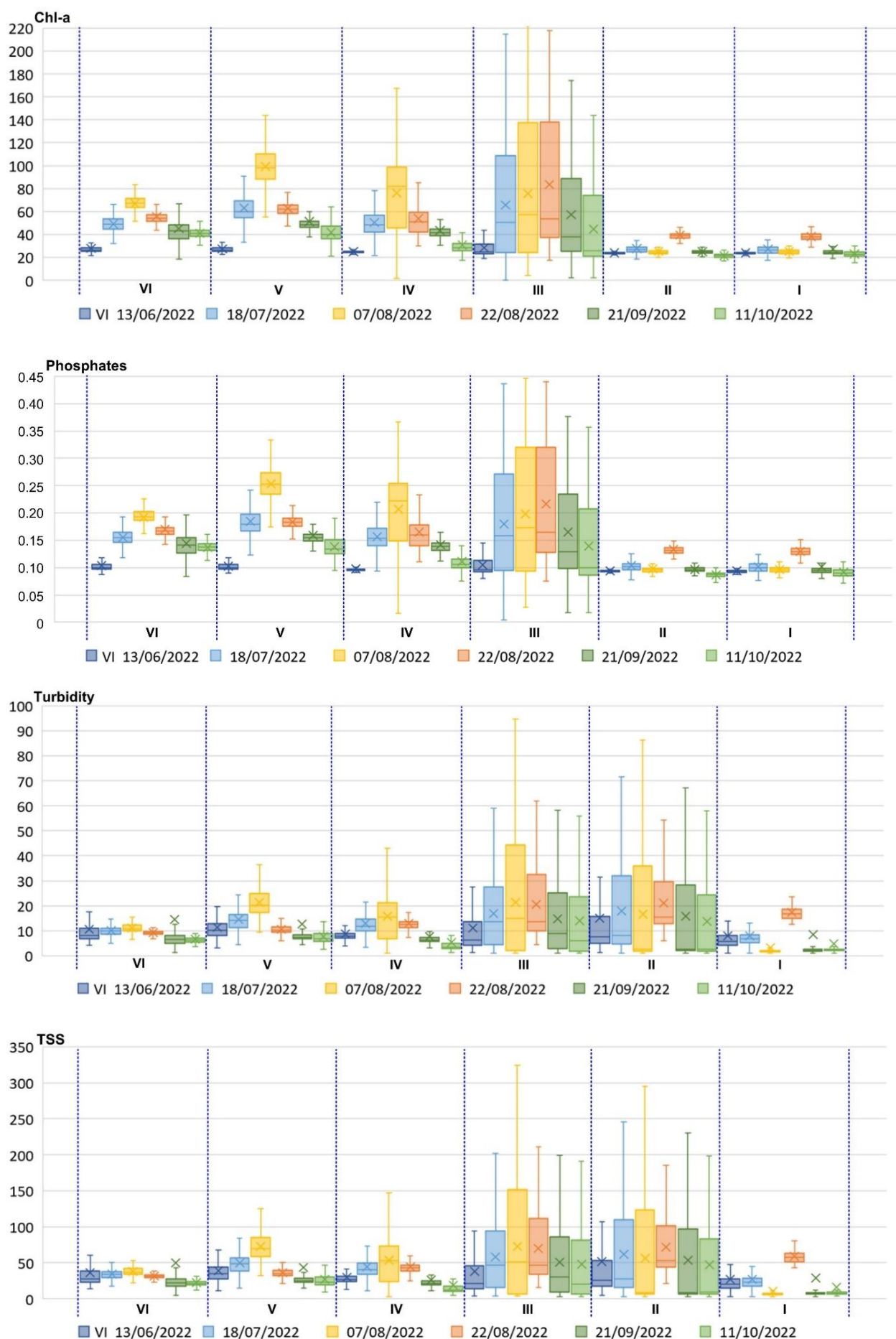


Figure 5. Surface water quality in the Dnipro-Buh estuary, 2022.

This accelerated the growth of algal blooms, shown by a rise in Chl-a content in the delta from 65.6 to 83.5 µg/dm³ with the spots of maximum concentration of 215-222 µg/dm³. The content of Chl-a in the estuary ranged from 49.1-99.2 µg/dm³ with a maximum of 144-168 µg/dm³. At the same time, occupying troops initiated shoreline destruction and diffuse pollution of the water sources in Section III of the Dnipro river area where turbidity varied between 4.3-24.7 MPC and TSS from 2.1-11.8 MPC.

During their retreat from the right bank to the left bank of the Dnipro from 04 to 30/10/2022, the occupying forces unloaded water to the main canals on the left bank and maintained spate flows in the lower reaches of the river; the reservoir level dropped sharply from 16.51 to 15.30 m, and the outflow was more than 2.6 BCM. After the Ukrainian army liberated the right bank of Kherson on 11.10.2022, Russian troops who remained in control of the dam kept the river in spate to prevent Ukrainian forces from crossing. A total outflow of 4.5 BCM brought a record decrease in the level of the reservoir from 16.07 to 14.03 m. The critical minimum water level was registered on 02/02/2023.

In the following period of winter water accumulation and spring floods, clearly with evil intent, Russian forces raised the water level in the reservoir to a record high of 17.5 m, 1.5 m above the norm, raising the water volume of the reservoir from 18.2 to 21.5 BCM. At 2.50 am on 06/06 2023, they detonated the dam.

Water Quality in 2023

At that time, the water level in the reservoir was 17.26 m. At 8:00 a.m on June 9 the water level in the reservoir had fallen to 11.74 m; at noon on June 11 the water level was 9.17 m; the losses being 17.5 BCM or 84 per cent of the storage. Some 600 km² of land along the Lower Dnipro was flooded, with a rise of water levels of 5.6-6.8 m in different places [1]. Hundreds of people drowned and innumerable plants and animals were destroyed along with the main water source for cities, towns and villages and 800 thousand ha of irrigated land; the emergency zone covered 180 settlements with over 900 thousand people. Washouts and bank abrasion created diffuse pollution of the Lower Dnipro and transport of pollutants to the Dnipro-Buh estuary and Black Sea. Sentinel satellite imagery from 08/06/2023 (Fig.6) shows concentrations of various pollutants in the Dnipro delta and estuary much in excess of their background values.

The maximum content of phosphate reached 0.21-0.23 mgP/dm³, exceeding its background value by 1.4-3.1 times, and Chl-a reached 77.5-86.9 µg/dm³, i.e. polytrophic levels. Turbidity was 33.9-50.7 NTU, exceeding the background level 3.5-8.7 times. Total suspended solids reached 116-173.5 mg/dm³ (4.6-6.9 MPC); chloride 3500-4200 mg/dm³ (17.5-21 MPC) sulphate 1,420-1,700 mg/dm³ (14-17 MPC);

and dry residue 8000-8700mg/dm³ (8.0-8.7 MPC). There was a sharp increase in biological oxygen demand to 90-103.5mgO₂/dm³ (45-52 MPC) and chemical oxygen demand to 370.0-425.6mgO₂/dm³ (18.5-21 MPC), and lack of oxygen caused mass fish kills.

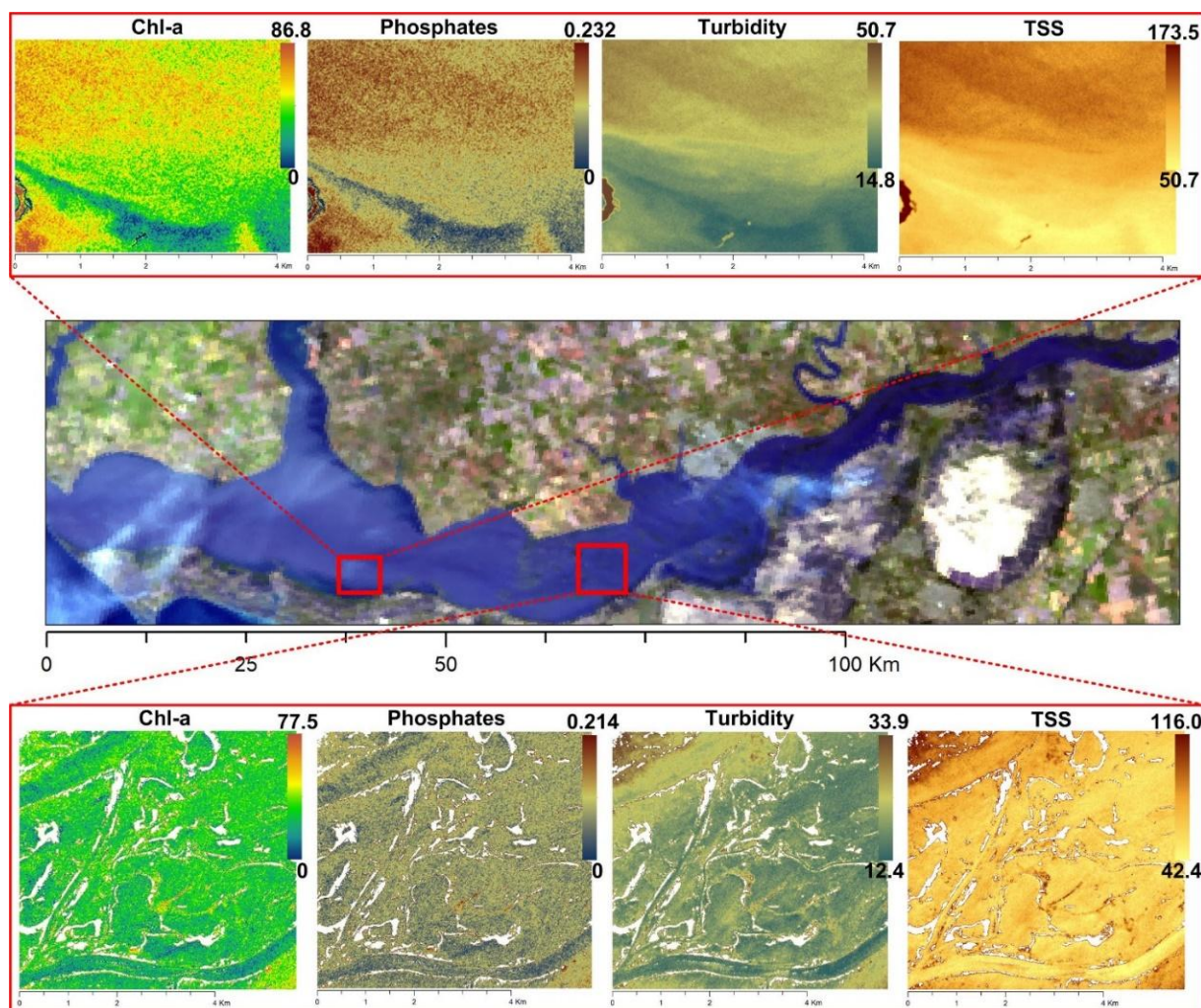


Figure 6. Transport of pollutants by surface flow in the Dniro-Buh estuary, as of 08/06/2023.

Calculation of Maximum Chlorophyll Index (MSI) according to the Sentinel-3 OLCI L1B imagery shows that polluted waters reached the Black Sea below the mouth of the Dniester River, the polluted area extending some 6800 km² by June 26, 2023 (Fig. 7).

At that time, in the coastal area of Odesa, the salinity of the sea decreased from 11.0 to 4.2g/dm³ and MPCs were exceeded for copper by 895 times, zinc 45 times, arsenic 3 times, and oil products by twice [1]. According to our calculations, the MCI in the Black Sea was 0.03, corresponding to a concentration of Chl-a of about 91.0 µg/dm³; phosphate 0.24 mgP/dm³ (1.2 MPC), turbidity 26.0NTU (7.4 MPC), total suspended solids 83.4 mg/dm³ (3.3 MPC), dry residue 9,220 mg/dm³ (9.2MPC), chloride 4260mg/dm³ (14.2MPC), sulphate 1,770 mg/dm³ (17.7 MPC), biological oxygen demand 110 mgO₂/dm³ (55.0MPC), chemical oxygen demand 451 mgO₂/dm³ (22.6 MPC). The MPC values were

calculated for fisheries; the maximum value of pollutant concentration was taken as 100%, and the sea areas with different levels of pollution were calculated on the basis of the ratio. The concentrations of pollutants were calculated in relation to the share of the total area of pollution in the Black Sea (6,800km²) where 29.3% of the water area was characterized by 0-10% of pollutant concentration, 31.8% of the water area accumulated 20-30% of pollutants, 22.9% of the water area had 30-40%; and 16.0% of the territory had >40%. One hundred and fifty dolphin deaths were officially recorded.

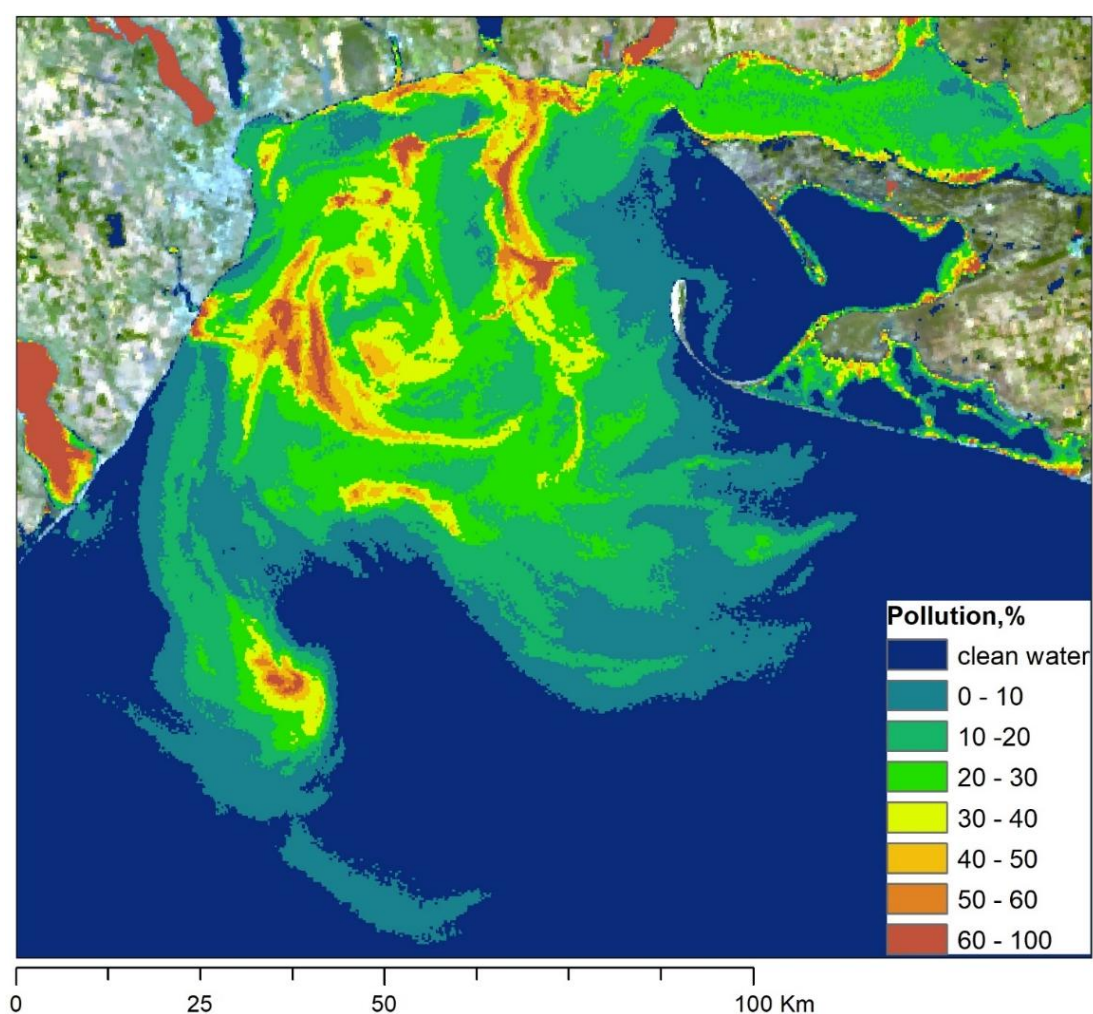


Figure 7. Spread of polluted waters in the Black Sea, 10-26/06/2023.

Destruction of the Kakhovka dam drained the reservoir that had extended over 2155 km², adding 230 km to the length of the Dnipro river bed that now has to be flushed by discharges from the smaller Dnipro reservoir upstream. Compared to the situation in 2018-21, the maximum seasonal fluctuations of the water level in the Lower Dnipro have decreased by 1.4-1.8 times, resulting in stagnant zones

in the Dnipro-Buh estuary and a sharp deterioration of water quality. Data of the State Environmental Inspectorate of the Southern Region of Ukraine were used to identify regularities of a change in dissolved oxygen (DO , mgO_2/dm^3).

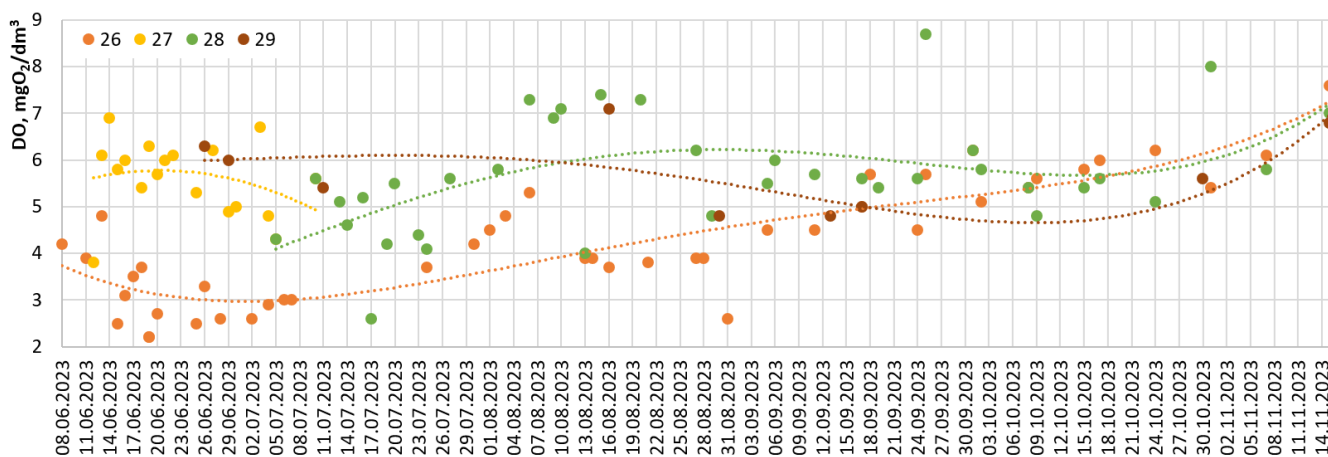


Figure 8. Dissolved oxygen in the waters of the Lower Dnipro and Inhulets River.

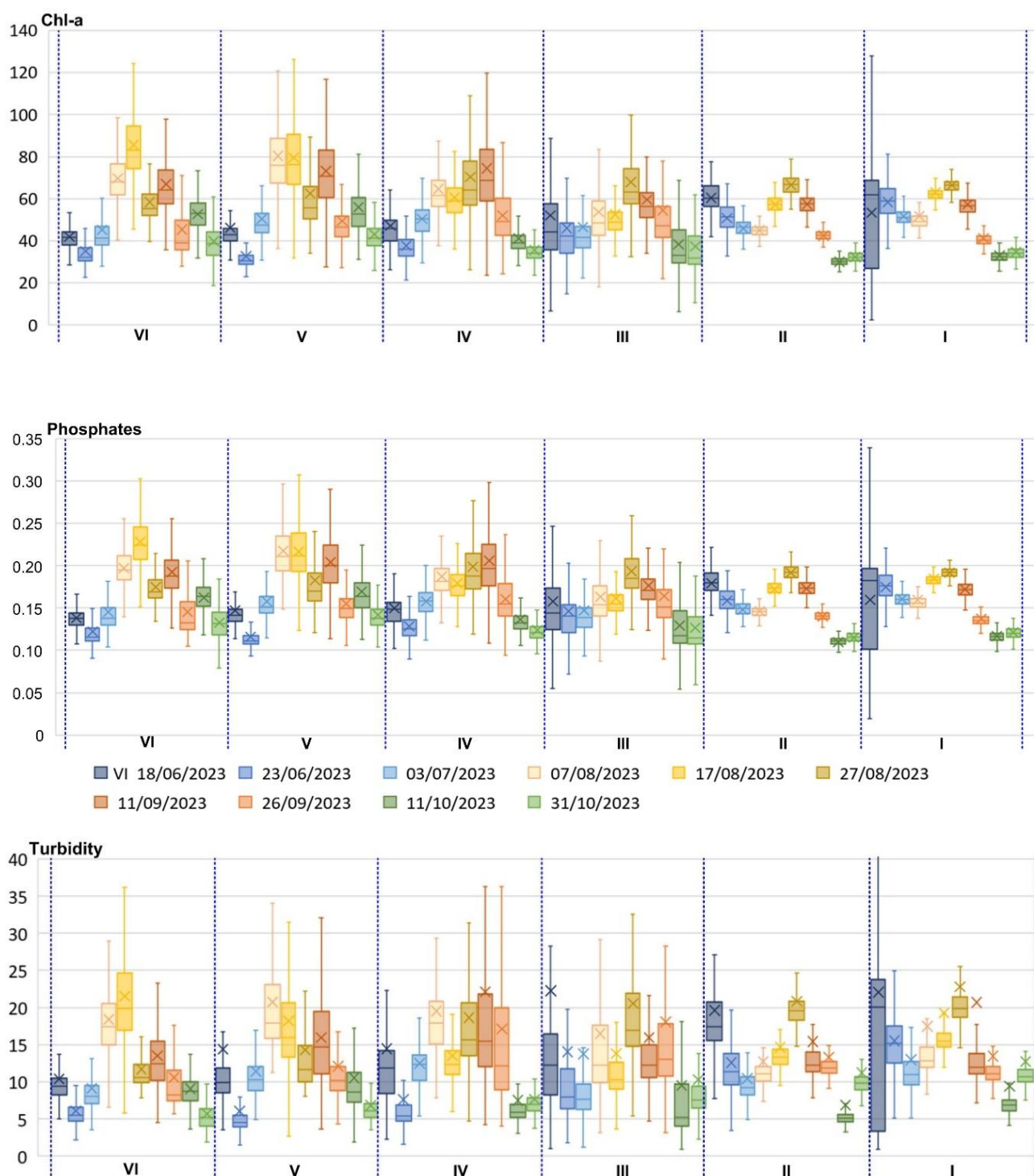
The deluge of polluted water sharply decreased dissolved oxygen in the period 08-15/06; DO reached a critical $4.8 \rightarrow 2.5 \text{ mgO}_2/\text{dm}^3$ ($0.80 \rightarrow 0.42 \text{ MPC}$) in the Inhulets River (sample 26) and in the Lower Dnipro near the Antonivka railway bridge (sample 27), DO fell from 6.9 to $5.8 \text{ mgO}_2/\text{dm}^3$ ($1.15 \rightarrow 0.97 \text{ MPC}$). In the period 15-25/06 when the water gradually receded, DO in the Inhulets River varied between 3.7 and $2.2 \text{ mgO}_2/\text{dm}^3$ ($0.62 \rightarrow 0.37 \text{ MPC}$); in the Dnipro River from 6.3 to $5.3 \text{ mgO}_2/\text{dm}^3$ ($1.05 \rightarrow 0.88 \text{ MPC}$).

As of June 18, 2023, there was a record high level of pollutants in the Lower Dnipro (Fig. 10).

In Sections I - III from the dam to the river mouth, Chl-a varied from $53.3\text{--}128.0 \text{ }\mu\text{g}/\text{dm}^3$ to $42.0\text{--}77.60 \text{ }\mu\text{g}/\text{dm}^3$; phosphate from $0.20\text{--}0.34 \text{ mgP}/\text{dm}^3$ ($1.0\text{--}1.7 \text{ MPC}$) to $0.1\text{--}0.25 \text{ mgP}/\text{dm}^3$ ($0.8\text{--}1.3 \text{ MPC}$); turbidity from $22.1\text{--}54.0 \text{ NTU}$ ($6.3\text{--}15.4 \text{ MPC}$) to $16.5\text{--}28.3 \text{ NTU}$ ($4.7\text{--}8.1 \text{ MPC}$); and total suspended solids from $76\text{--}185 \text{ mg}/\text{dm}^3$ ($3.0\text{--}7.4 \text{ MPC}$) to $57\text{--}97 \text{ mg}/\text{dm}^3$ ($2.3\text{--}3.9 \text{ MPC}$). From the end of June to the beginning of July 2023, pollutant concentrations in some riverbed areas were reduced as much as 3-fold by flushing into the estuary.

After the flood receded and until the beginning of August 2023, there was low but gradually increasing concentration of dissolved oxygen from 2.5 to $5.3 \text{ mgO}_2/\text{dm}^3$ ($0.42 \rightarrow 0.88 \text{ MPC}$) in the Inhulets River; from 4.3 to 5.8 ($0.72 \rightarrow 0.97 \text{ MPC}$) in the Dnipro River in Kherson (sample 28); and from 5.3 to 6.0 ($0.9 \rightarrow 1.0 \text{ MPC}$) within the river delta (sample 29). Chl-a varied between 46.6 and

67.2 $\mu\text{g}/\text{dm}^3$; phosphate 0.15-0.19 mgP/dm^3 (0.8-1.0 MPC); turbidity 10.5-17.3 NTU (3.0-4.9 MPC); and total suspended solids 35.9-59.2 mg/dm^3 (1.4-2.4 MPC). Low values of pollutants were observed in the estuary in relation to the background values of 2021, in particular: Chl-a, 27.8-69.8 $\mu\text{g}/\text{dm}^3$; phosphate 0.10-0.20 mgP/dm^3 (0.5-1.0 MPC); turbidity 3.5-18.6 NTU (1.0-5.3 MPC); and TSS 12.0-63.6 mg/dm^3 (0.5-2.5 MPC).



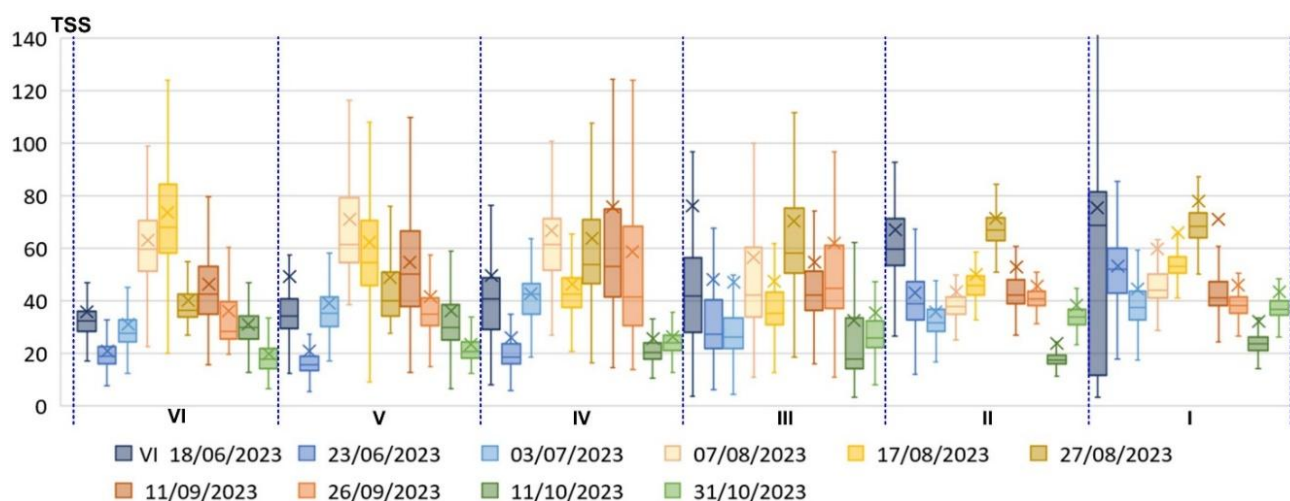


Figure 10. Water quality in the Dnipro-Buh estuary, 2023.

The seasonal maximum concentration of dissolved oxygen in samples 28 and 29 was registered at $6.9 \rightarrow 7.3 \text{ mgO}_2/\text{dm}^3$ (1.25 $\rightarrow$ 1.22 MPC) in the period of August 05-25 thanks to rainfall and slight flushing of the Lower Dnipro. But in the lower reach of the Inhulets riverbed there was a reversal, DO $5.3 \rightarrow 3.73 \text{ mgO}_2/\text{dm}^3$ (0.9 $\rightarrow$ 0.6 MPC) caused by accumulation of organic matter and an algal bloom. In this same period, there were concentrations of pollutants at the level of 1.1-5.5 MPC in the surface waters of the Dnipro riverbed and delta and they accumulated in the estuary at the level of 1.9-9.7 MPC.

Hot weather, systematic accumulation of pollutants and algal blooms caused eutrophication in the lower part of the river until the third decade of September: deficiency of dissolved oxygen (1.0 $\rightarrow$ 0.8 MPC) was observed in 80% of water samples and, in Dnipro riverbed Sections I and II, a polytrophic state prevailed with Chl-a, $54.9\text{--}78.7 \text{ }\mu\text{g}/\text{dm}^3$, phosphate $0.17\text{--}0.22 \text{ mgP}/\text{dm}^3$ (0.9 -1.1 MPC), turbidity 14.6-25.5 NTU (4.2-7.3 MPC), and TSS $50.0\text{--}87.3 \text{ mg}/\text{dm}^3$ (2.0-3.5MPC). The maximum content of pollutants in the waters of the estuary in the direction east $\rightarrow$ central $\rightarrow$ west varied at the level $4.3\text{--}9.0 \rightarrow 3.0\text{--}6.4 \rightarrow 2.2\text{--}4.6$ MPC.

As of September 25, dissolved oxygen peaked at $8.7 \text{ mgO}_2/\text{dm}^3$ (1.5MPC) thanks to water discharges from the Dnipro reservoir and flushing of the Lower Dnipro riverbed. In this period, pollutant concentration in the Dnipro riverbed was reduced by 40-47 %. The satellite image of September 26 shows a substantial release of pollutants in the Eastern estuary at the level of 5.0-10.4 MPC.

The seasonal decrease in surface and water temperatures from early October led to an increase in the concentration of dissolved oxygen in all the water samples by early November: $6.8 \rightarrow 7.6 \text{ mgO}_2/\text{dm}^3$ (1.1 $\rightarrow$ 1.3MPC). From 11-31 October 2023, the concentration of pollutants in the Lower Dnipro

decreased: in Sections I and II, Chl-a 25.2-34.8 $\mu\text{g}/\text{dm}^3$; phosphate, 0.10-0.12 mgP/dm^3 (0.5-0.6 MPC); turbidity, 3.3-14.1 NTU (0.9-4.0 MPC); TSS, 11.3-48.3 mg/dm^3 (0.5-1.9 MPC). In the delta (Section III), pollutant concentrations fell by 2.2-2.4 times, and in the estuary by 2.0-2.7 times in the eastern section (IV), by 1.9-3 times in the Central area (V), and by 2.2-3.8 times in the western area (VI).

Discussion

The wartime disruption of hydrology and biophysical processes in the Dnipro-Buh estuary can hardly be exaggerated: lesser flow velocity, lower water levels, worse water balance, stagnation and an increase in the water temperature and accumulation pollutants beyond maximum permissible concentrations – particularly in July-September with increased demands on water resources, diffuse pollution, surface runoff and sewage, and acceleration of biochemical processes.

The Lower Dnipro has been transformed from mesotrophic to polytrophic, water class from *clean* to *dirty*, saturation of surface waters with biogenic elements has increased twofold (Fig. 11a). In 2022-3, compared to 2021, algal chlorophyll density increased by 1.5→2.9 times (Fig. 11b) and turbidity (Fig. 12a) and total suspended solids (Fig. 12b) increased 4-fold.

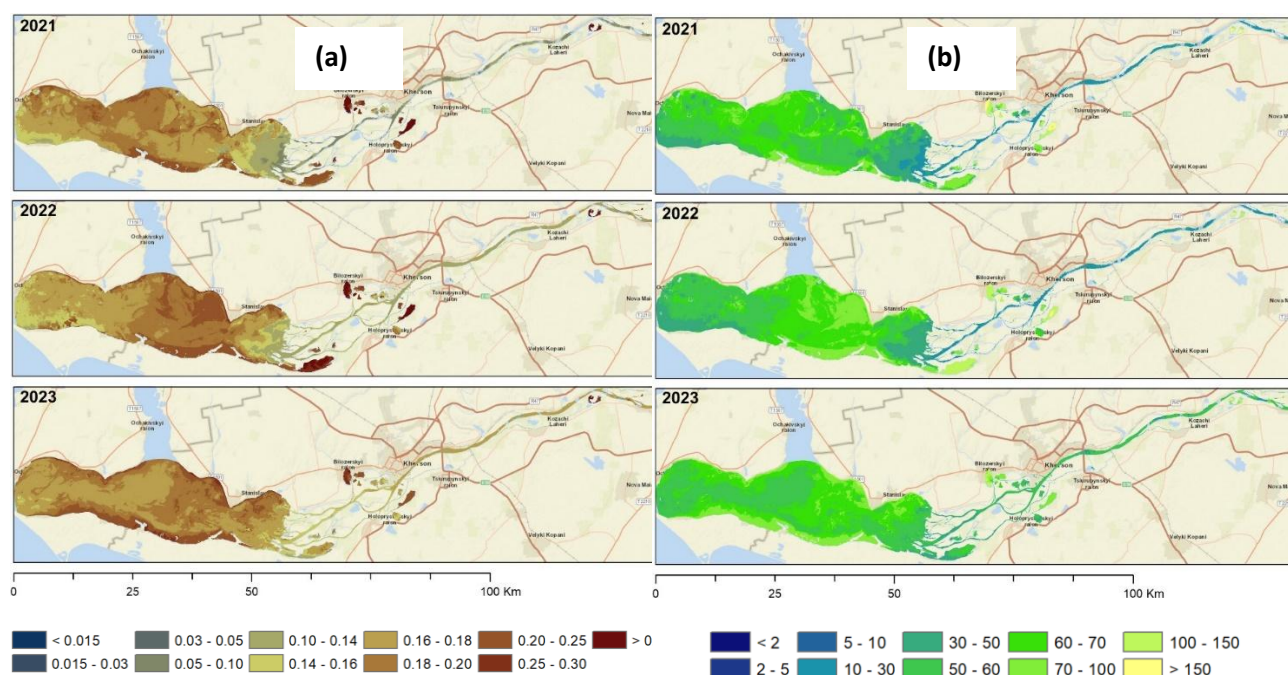


Figure 11. Concentration of phosphate (a, mgP/dm^3) and Chl-a (b, $\mu\text{g}/\text{dm}^3$) in the Dnipro-Buh estuary, July-September, 2021/23.

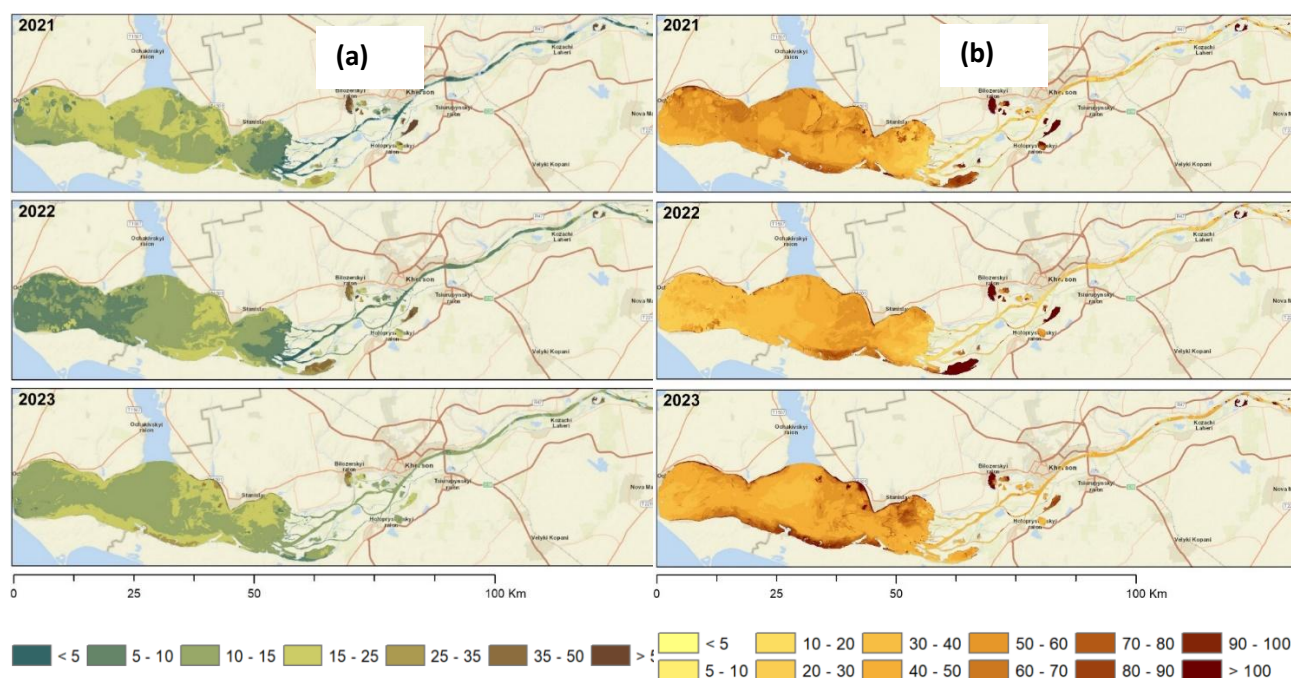


Figure 12. Water turbidity (a, NTU) and total content of suspended solids (b, mg/dm³) in the Dnipro-Buh estuary, July-September, 2021/3.

In the floodplain lakes of the delta (Section III), Chl-a increased by 1.3 times in 2022 compared to 2021. Their trophic status depends on a hydrological connection with the main river channel. Primary and secondary production contributes to intensive growth of bottom sediments; eutrophication and the volumes of runoff affect the balance of nutrients in the lakes - accelerating or restraining sedimentation. Floods flush out the bottom sediments, as we saw at the time of the destruction of the Kakhovka dam when the concentration of biological and physical-chemical substances in the floodplain lakes dropped by 35-55%.

Since the waters of the Dnipro River account for well over 90 per cent of the activity of the Dnipro-Buh estuary, the overwhelming effect of the destruction of the Kakhovka dam has been degradation. In the Eastern area of the estuary (Section IV) there was an increase in the average seasonal concentration of Chl-a by 42%, phosphate by 21%, turbidity by 98 %, TSS by 79%; in the Central area (Section V) there was a rise in the content of Chl-a 6.5%, phosphate by 6 %, turbidity and TSS by 7%; although additional accumulation of pollutants was insignificant in the Western area (Section VI). The estuary as a whole has become polytrophic. According to the level of biogenic pollution by phosphate, the water area lost patches with *Clean Enough* water, whereas the patches with *Moderately Polluted* and *Dirty* water expanded from 77.2→92.5% and this has slighted the integrity of the ecosystem.

Conclusions

- The pre-war management of aquatic ecosystems might be described as negligent but the Russian assault has brought catastrophic destruction of both water infrastructure and the natural ecosystems in the South of Ukraine, in particular the Dnipro-Buh estuary.
- Sabotage of the Kakhovka dam on 06/06/2023, released more than 20BCM of stored water carrying a mass of pollutants down the Lower Dnipro to the Dnipro-Buh estuary and the Black Sea. The excess concentration of pollutants amounted to 1.1-51.8 MPC, extending over about 6800km².
- As well as the loss of one third of the country's engineered fresh water reserves, the destruction of the Kakhovka dam and reservoir eliminated the main mechanism for flushing the lower Dnieper and its estuary.
- In turn, this has more than doubled the saturation of the river water with biogenic elements; increased growth of algal blooms threefold; and brought a 4-fold deterioration of transparency and total suspended solids. Compared to the pre-war period, the degraded area of the Dnipro-Buh estuary has increased by 15-28 %. In 2023, 95% of the water area was polytrophic; and more than 70% of the water areas have experienced disruption of hydro-ecosystem sustainability and ecological regression.

Acknowledgments

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Geolocation

Polygon coordinates: 30.380493,45.859787; 30.405213,46.734336; 32.609619,46.706099; 33.299011,46.826471; 33.381409,46.71363; 32.274536,46.415353; 32.26355,45.848311; 30.380493,45.859787

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The Biggest Explosions at Ammunition Depots During the Russia-Ukraine War

Viktor I. Vyshnevskyi

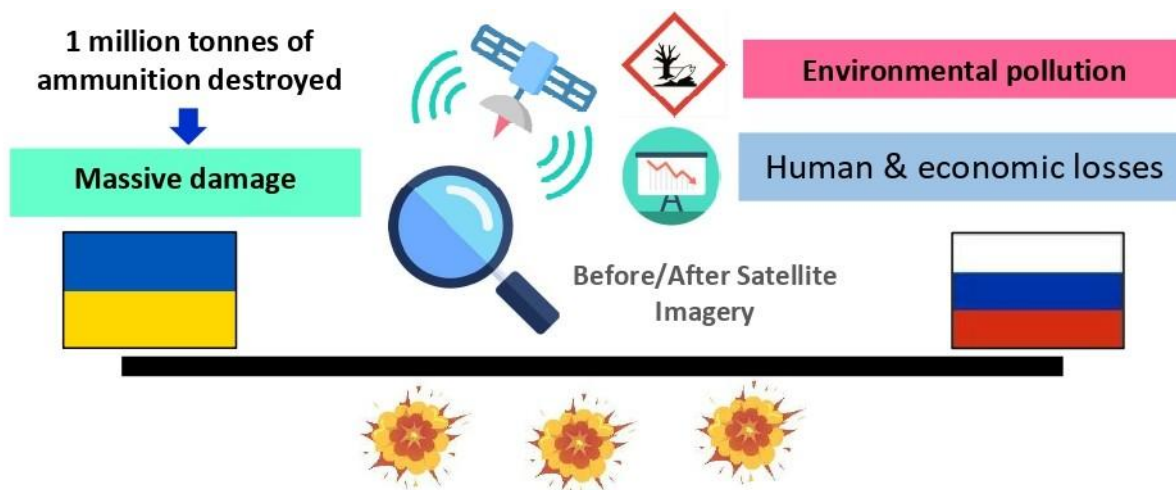
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Graphical Abstract

This study examines the largest ammunition depot explosions during the Russia-Ukraine war, highlighting their devastating consequences. Over one million tonnes of ammunition have been destroyed, leading to significant human casualties, economic losses, and severe environmental pollution. The use of remote sensing technologies has been crucial in assessing the scale of destruction and its long-term impacts



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Abstract: Since 2014, both Ukraine and Russia have witnessed big explosions at ammunition depots. The total mass of destroyed ammunition has now reached about one million tonnes. In the process, many people have been killed or maimed; economic losses are estimated in billions of dollars; and the air and soil have been significantly polluted.

Keywords: *Explosions, Ammunition, Russia-Ukraine, Remote sensing.*

Introduction

Every war is destructive – of the environment, economy and society – and scientists have contributed their particular insights to the sad chronicle [1-6]. The Russia-Ukraine war, which began in 2014 and intensified on February 24, 2022, is no exception [7-9]. By October 2023, almost 500 000 combatants and more than 30 000 civilians had been killed or wounded; more than 11 millions displaced; and damage to civilian infrastructure and the environment, including widespread chemical pollution of air, water and soil, was estimated at \$56.4 billion [7]. More than a year on, these losses have doubled, with the dead and injured now close to one million. The environmental impacts of the war are widely reported [8,10-19]; about 30% of Ukraine's territory has been sown with mines and unexploded ordnance, and landscape destruction, shelling, forest fires and pollution afflict 30% of Ukraine's protected areas.

Remote sensing provides invaluable evidence and oversight; thus Shevchuk *et al.* [15] identified the destruction of oil depots, forest fires, and pollution caused by the destruction of a water treatment plants. Hostilities are often accompanied by fires which can be studied through primary processing of remote sensing data. MODIS Terra and Aqua satellites have special channels designed for fire monitoring, and have become the primary sensors for active fire detection [20], as applied by Shevchuk *et al.* [15] and Tomchenko *et al.* [21].

Incidents at ammunition depots in Ukraine between 2014 and 2018 reported by Zabrodskiy *et al.* [9] destroyed over 210,000 tonnes of ammunition, a significant part of which was 152mm shells and rockets for multiple-launch systems. For comparison, in the first five years of the war in Donbas, the Armed Forces of Ukraine spent about 70,000 tonnes of ammunition. Chornohor [22] describes explosions at ammunition depots in Svatove and Balakliya, the latter the biggest in Eastern Europe extending over 3.7 km² and holding 125,000-175,000 tonnes of ammunition. A more precise quantity (138,000 tonnes) is given by Ovcharenko & Tabunenko [23], about half of which exploded, releasing three times the energy of the atomic bomb dropped on Hiroshima. Moreover, these munitions contain lead, zinc, nickel, barium, manganese, copper, antimony, depleted uranium, etc. – all toxic in varying degrees – that can enter our bodies not only with air or water but, also, through the skin and hair [12].

Beyond Ukraine, in 2011, Russian military intelligence (GRU) detonated an ammunition depot in the Bulgarian village of Lovnidol, near Gabrove, that contained 3120 152mm shells; secondary explosions lasted for several days [24,25]. There were two similar incidents on October 16 and December 3, 2014, in the Czech Republic, near Vrbetice, where about 7,000 tonnes of military material and about 1,000 tonnes of explosives were stored. The first, which claimed two lives, was

followed by 355 uncontrolled explosions; after the second incident on December 3, there were fewer uncontrolled explosions. Then five evacuations were ordered, 2 000 people were forced to leave their homes, work or schools [26,27]. The saboteurs included the two men who, in 2018, attempted to assassinate former Soviet spy Sergei Skripal, in Salisbury, England. Subsequently, the Czech Republic expelled 18 staff of the Russian embassy. The situation has deteriorated since Russia's invasion of Ukraine in 2022; there have been with several incidents at industrial enterprises in the UK, USA and elsewhere that supplied weapons to Ukraine [24].

In the face of the incessant hazard, this study is initiated as a tool for research and publicity for big explosions at ammunition depots.

Materials and Methods

Study Area

The Russia-Ukraine war has encompassed an extensive area not only of Ukraine but, also, of Russia. At the outset, incidents were confined to Ukraine but they soon spread to Russian territory; in some cases, as far as 500 km (Fig. 1).

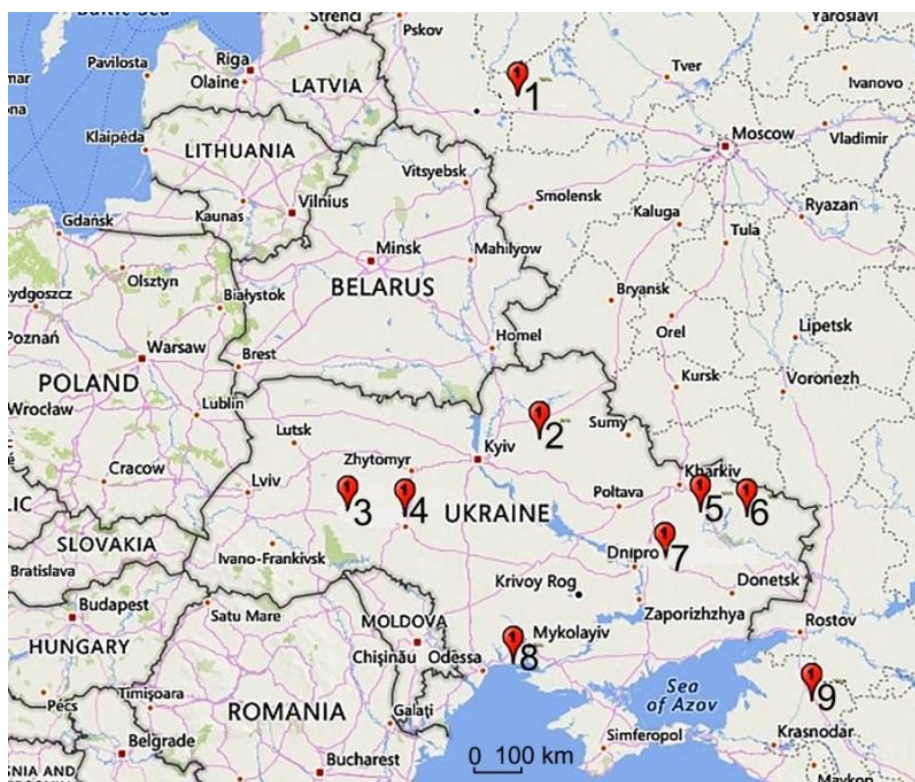


Figure 1. Location of ammunition depots experiencing significant explosions: 1, Toropets; 2, Druzhba; 3, Gruzevitsa; 4, Kalynivka; 5, Balakliya; 6, Svatove; 7, Pavlohrad; 8, Ochakiv; 9, Tikhoretsk.

Data and Methods

The main source of data is provided by remote sensing, especially the EU Copernicus website (<https://browser.dataspace.copernicus.eu>) that holds images taken by Sentinel 2 satellites, which have a revisit time of 3-4 days and a spatial resolution of 10m. Combination of spectral channels B12, B8 and B3 gives a good picture of fire; not quite so good by combination of B11, B8 and B4.

Information on thermal anomalies was also abstracted from <https://firms.modaps.eosdis.nasa.gov/map>, which holds data from the NOAA-2, Suomi NPP, Aqua and Terra satellites. Their spatial resolution is much poorer than Sentinel 2 but the site is good for quick searches for hotspots; <https://worldview.earthdata.nasa.gov> also presents the results of Earth surface imaging by the Aqua and Terra satellites, supplemented with information on air quality, cloudiness, etc. In some cases, we employed high spatial resolution data from Google Earth Pro. In many cases, the first alert was provided by the media, initiating the search for additional data on incidents.

Results and Discussion

Big explosions began early in the Russia-Ukraine war. Our research reveals about a dozen ammunition depots destroyed on the territory of Ukraine (Tab.1), which significantly depleted supplies to the Armed Forces of Ukraine.

Table 1. The biggest explosions at ammunition depots.

Date	Location	Coordinates
29-30.10.2015	Svatove, Luhansk oblast, Ukraine	49°24'03"N 38°11'36"E
23.03.2017	Balakliya, Kharkiv oblast, Ukraine	49°28'55"N 36°52'35"E
26-29.09.2017	Kalynivka, Vinnitsa oblast, Ukraine	49°24'37"N 28°29'56"E
03.05.2018	Balakliya, Kharkiv oblast, Ukraine	49°28'55"N 36°52'35"E
09.10.2018	Druzhba, Chernihiv oblast, Ukraine	50°48'17"N 32°19'46"E
09.01.2023	Ochakiv, Mykolaiv oblast, Ukraine	46°36'08"N 31°32'53"E
30.04.2023	Pavlohrad, Dnipropetrovsk oblast, Ukraine	48°34'42"N 35°50'50"E
13.05.2023	Gruzevitsa, Khmelnytskyi oblast, Ukraine	49°27'05"N 26°52'33"E
18.09.2024	Toropets, Tver oblast, Russia	56°30'29"N 31°42'52"E
20-21.09.2024	Tikhoretsk in Krasnodar oblast, Russia	45°53'08"N 40°02'27"E
21.09.2024	Toropets, Tver oblast, Russia	56°21'34"N 31°38'42"E

Explosions at Ammunition Depots on Ukrainian Territory

Svatove, Luhansk oblast on 29-30 October 2015

Svatove is located about 150km southeast of Kharkiv. About 3 000 tonnes of ammunition was stored at a depot in the southeastern outskirts of the town. Fires and explosions began on October 29, 2015, and continued the next day. Several members of the Armed Forces of Ukraine were killed and injured and the arsenal was practically destroyed. The consequences of this incident were studied using Google Earth Pro data (Fig. 2).



Figure 2. Satellite images from Google Earth Pro of ammunition depot in Svatove: on the left, taken in August 2013; on the right, in June 2016.

Balakliya, Kkarkiv oblast, 23 March 2017

Ammunition depot 65, belonging to military unit A1352, was located about 1 km north-east of Balakliya town (Fig. 3) and extended over 3.7 km².

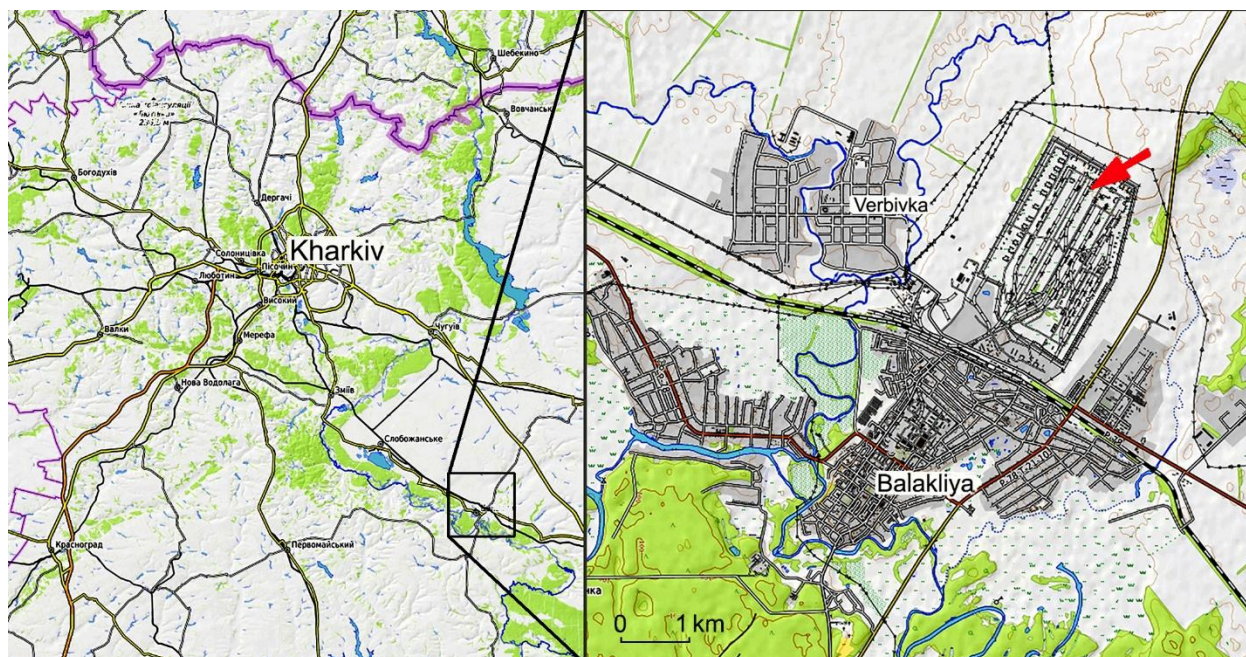


Figure 3. Location of Balakliya ammunition depot.

The depot held some 5million shells, more than all the munitions expended in the Gulf War in 1991 [3] and many left over from the days of the USSR. The ammunition was partly in underground storage, partly stacked haphazardly in the open; the ammunition boxes were rickety; the wooden underground storages were not treated with fire-retardant; and there was need to repair the lightning conductors and install a modern automatic fire alarm system [22].

Fire broke out at about 02:45 Kyiv time on 23 March 2017. Within 15 minutes, there were massive explosions; shells flew up to 1km, fragments spread from 5-10km; the column of fire reached 150-200m, and was seen at distances of up to 30km (Fig. 4); about 1kt of smoke, 35kt of carbon dioxide, 5kt of carbon monoxide, and about 500t of soot were released into the atmosphere [22].



Figure 4. Explosions at the ammunition depot in Balakliya (Chornohor 2018).

Fearing a Russian offensive, residents fled, even at night [22]. According to the Temporary Investigative Commission of the Parliament of Ukraine, more than 90% of the ammunition and military property stored there, valued at about \$US 400 million, was destroyed. The explosions damaged, also, several hundred residential buildings; and 20 thousand residents of Balakliya and nearby villages were evacuated. The loss of ammunition crippled resistance to the subsequent Russian invasion. For a more complete study, Sentinel 2 satellite images taken at the time were used. A combination of B12, B8 and B3 was used to identify fires (Fig. 5).

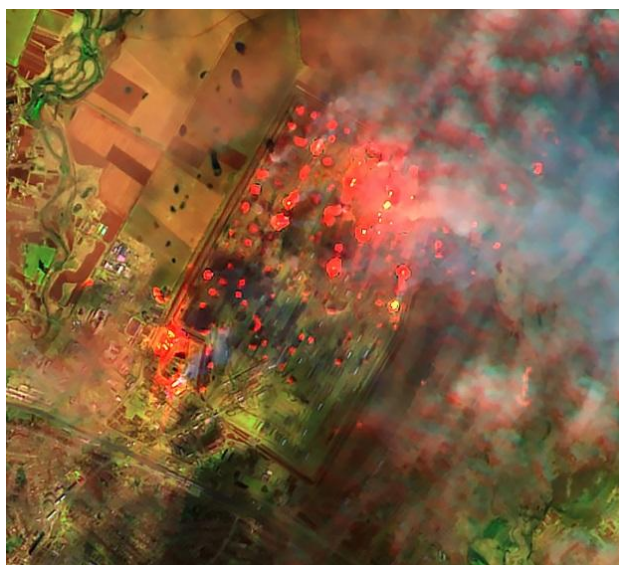


Figure 5. Sentinel 2A satellite image of explosions in Balakliya taken on 23 March 2017, obtained individually using bands B12, B8 and B3.

Google Earth Pro imagery reveals the destruction of dozens of buildings clearly visible in the image taken in 2014 but missing on the 2018 image; instead, numerous craters are visible on and around the ammunition depot (Fig. 6). Thermal anomalies after explosion in Balakliya were registered at FIRMS and WORLDVIEW websites on 23 March 2017, but disappeared next day.



Figure 6. Satellite images from Google Earth Pro at the Balakliya ammunition depot: on the left, taken in October 2014; on the right, in April 2018.

The remnants of the Balakliya arsenal were practically destroyed the following year, actually on May 3, 2018.

Kalynivka, Vinnytsa oblast, 26 September 2017

The arsenal in Kalynivka was one of the biggest in Ukraine; its area was about 5.9 km² and it held 188 000 tonnes of munitions [23]. The first explosions at the site occurred at around 9pm on 26th September 2017, and continued for several days. Several hundred people and more than 100 appliances were called on to contain the emergency and about 30 000 people were evacuated.

Satellite images show that some of the warehouses in Kalynivka survived (Fig. 7) but, on the day of the incident and later, cloud cover precluded high-quality pictures.



Figure 7. Sentinel 2 satellite images of the ammunition depot at Kalynivka: on the left, on 18 September 2017; on the right, on 18 October 2017.

Druzhba, Chernihiv oblast, 9 October 2018

The ammunition depot near Druzhba town was one of the three biggest arsenals in Ukraine, containing 69,500 t of ammunition, 43,000 t of which was usable, the remainder elements and components and ammunition subject to disposal. Explosions began at around 3 am on 9 October 2018. After the fire and explosions at the arsenal, only four protected concrete shelters survived, with ammunition losses amounting to about 95% of the 69,500 t of ammunition stored there. More than 12,000 people were evacuated and railway traffic through nearby Ichnya was suspended. Figure 8 shows Sentinel 2 satellite imagery of the incident.



Figure 8. Sentinel 2 satellite images of the Druzhba ammunition depot: on the left, on 7 October 2018; on the right, on 10 October 2018.

Ochakiv, Mykolaiv oblast on 9 January 2023

Ochakiv is a small town but an important centre of the land and naval Armed Forces of Ukraine with several ammunition depots. One of these, located on the seashore, stored very old anti-ship mines from the Second World War, buried in concrete. Obviously, the Russians had intelligence and, on 9 January 2023, deployed two missiles (presumably hypersonic Dagger missiles) to pierce the concrete and blow up these mines, smashing windows and roofs of more than 200 residential and public buildings, and leaving a crater 40 m in diameter (Fig. 9).



Figure 9. Satellite images of Ochakiv taken from the Google Earth Pro website: on the left, in July 2021; on the right, in March 2023.

Pavlohrad, Dnipropetrovsk oblast, 30 April 2023

The city of Pavlohrad is known for the chemical plant that produced rocket fuel – in particular for the Vilkha-M guided artillery missile and the Neptun anti-ship missile; and there is intelligence that fuel for SS-24 intercontinental ballistic missiles, decommissioned after the collapse of the Soviet Union, was stored here.



Figure 10. Sentinel 2 images of ammunition depot at Pavlohrad Chemical Plant: on the left, before the explosion on 30 April 2023; on the right, on 25 May 2023.

Sooner or later, there was bound to be a strike on this plant from the Russian side. It happened on 30 April 2023, injuring more than 30 people and damaging more than 100 residential, commercial, and municipal buildings (Fig. 10). More information, including high-quality satellite imagery distributed with permission, can be found at <https://www.twz.com/incredible-destruction-at-ukrainian-rocket-storage-site-that-detonated-from-russian-strike>.

Gruzevitsa village, Khmelnytskyi oblast, 12-13 May 2023

The 649th airborne ammunition and missile storage facility, located less than 10km from Khmelnytskyi city, was utterly destroyed on the night of 12-13 May 2023 (Fig. 11). According to available information (<https://www.youtube.com/watch?v=nAwgEcX7II8>), the cost of the destroyed ammunition was \$500million.



Figure 11. Natural colour imagery of ammunition depot near Gruzevitsa village taken by Sentinel 2A satellite: on the left, before the explosion on 30 April 2023; on the right, on 25 May 2023.

Explosions of Ammunition Depots on Russian Territory

Toropets, Tver oblast on 18 and 21 September 2024

The Russian arsenal near Toropets, 500 km from the nearest Ukrainian territory (Fig.1), became the first significant target on Russian soil struck by the Ukrainian Armed Forces. According to <https://en.wikipedia.org>, and the Institute of Study of War (Washington DC) website, Toropets hosted the 107th ammunition depot of the Main Missile and Artillery Directorate. About 30,000 t of munitions was stored there, including fuel tanks, missiles for Iskander and Tochka-U systems, guided aerial bombs, assorted artillery ammunition and, recently, munitions from North Korea.

Ukrainian drones struck the warehouse on the night of 17-18 September 2024, causing a series of explosions and fires, as well as breaking windows in neighbouring cities; the main explosion created an 82m-wide crater. Sentinel 2 imagery taken on 19 September 2024 (Fig. 12) shows fires even beyond the ammunition depot, and many more can be seen in a satellite image taken on 18 September 2024, presented on the FIRMS website (Fig. 13). Videos of many explosions in this area can be viewed on YouTube <https://www.youtube.com/watch?v=ukhqQRdhcMw>.



Figure 12. Sentinel 2A satellite images of the arsenal at Toropets: on the left, on 16 September 2024; on the right, on 19 September 2024.

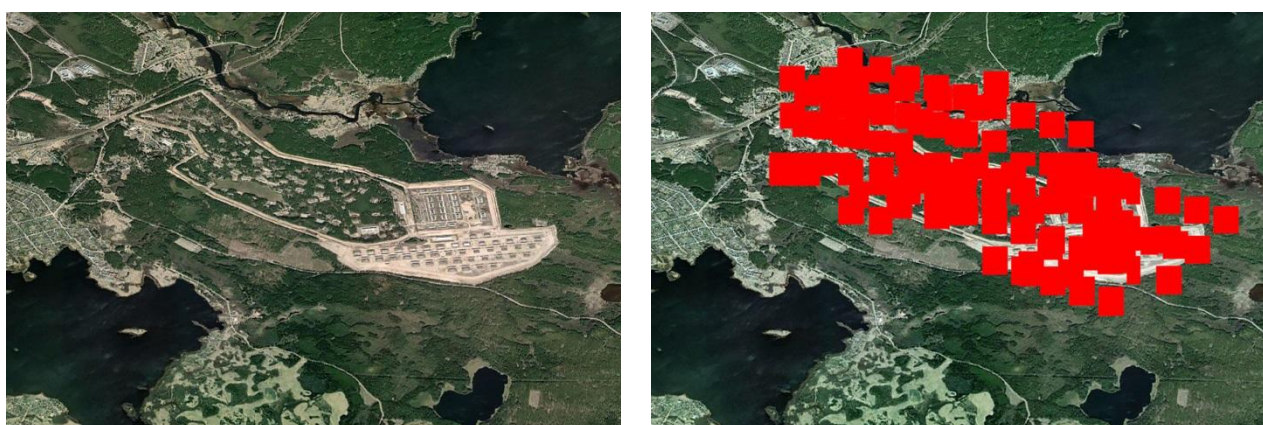


Figure 13. Satellite images of the arsenal at Toropets taken from the FIRMS website: on the left, on 16 September 2024; on the right, on 18 September 2024.

A few days later, on 21 September 2024, another ammunition depot located about 17 km south of Toropets was struck – with the same results (Fig. 14).

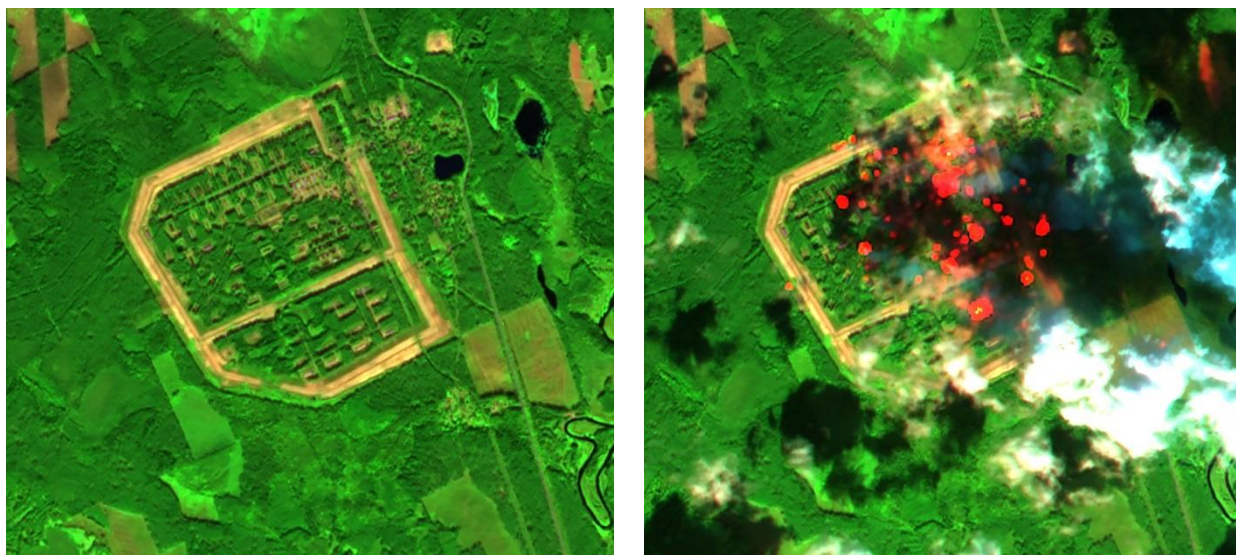


Figure 14. Satellite images of ammunition depot south of Toropets taken by Sentinel 2A: on the left, on 16 September 2024; on the right, on 21 September 2024.

Tikhoretsk, Krasnodar oblast, 20-21 September 2024

According to the media (<https://www.pravda.com.ua/eng/news/2024/09/21/7476177/>) the strike on the Tikhoretsk arsenal happened on the night of 20-21 September, destroying over 2 000t of ammunition, including supplies from North Korea, and also damaging the nearby military airfield (Fig. 15). Data of website <https://worldview.earthdata.nasa.gov> show that smoke from these fires was clearly visible from a distance of more than 30km.



Figure 15: Sentinel 2A satellite images of ammunition depot and **military airfield** near Tikhoretsk taken on 21 September 2024: above in natural colour; below, combination of B12, B8 and B3.

The war, and explosions at ammunition depots, continue: according to the media, two such cases occurred in November 2024. The first near Karachev town in Bryansk oblast of Russia (53°08'35"N 34°57'10"E) on 19 November; the second near Kotovo town in Novhorod oblast of Russia (58°28'56"N 33°30'10"E) on 20 November 20. Study of these cases is complicated by cloudy weather.

Conclusion

The unfinished war between Russia and Ukraine that began in 2014 has brought about many huge explosions at ammunition depots; the biggest in Balakliya, Kalynivka, and several other places. In 2024, a few arsenals have been struck in Russia, near the cities of Toropets, Tikhoretsk, *etc.*

About a million tonnes of explosives and several million shells were destroyed in these explosions; many people were killed or injured; the economic losses amount to billions of dollars; at the same time, the explosions have caused significant damage to the environment.

Cui bono?

CONFLICT OF INTEREST

The author declares no competing interests

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BOOK REVIEW

The Heart of the Wild: Essays on Nature, Conservation, and the Human Future, edited by B. A. Minter, and J. B. Losos; contributions by B. Adams, J. Berger, S. Clayton, E. Crist, M. L. Crump, T. L. Fleischner, H. W. Greene, H. Herzog, E. Marris, K. D. Moore, G. P. Nabhan, P. H. Raven, C. J. Schell, R. Shine, and K. Whyte, Princeton, NJ, Princeton University Press, 2024, 280 pp., \$26.95/£22.00 (hardback), ISBN: 978-0-691-22862-4.

The Heart of the Wild explores the raw beauty of a site that is both authentic and mysterious, an original place where nature and human emotions intersect. The various contributors allow one to feel deeply the power and splendour of the natural environment. The book cover touches upon an example that considers the conditions of our modern world. A straight road cuts through a coniferous forest, symbolising the coexistence between man and nature. This almost endless road leads towards imposing mountains, partially covered in snow, beneath a clear sky. The rays of the sun illuminate the mountain ridges, highlighting their magnificence. This contrast between the natural elements – trees, earth, snow, and sky – and the road that cuts through them invites reflection on the coexistence of man and nature, as well as the role of modernity in a conserved environment. The book teaches us how to keep these sanctuaries and their entire landscapes in their purest and most "wild" form, and to appreciate them for what they are.

Minter and Losos begin by presenting the central questions in this book (pp. 1-11). Their exceptional method is distinguished by the use of art, in its various purest and most famous forms, to touch hearts where words are insufficient or may fail to convey the message. Your reviewer feels deep admiration, for example, for the choice of paintings by 19th-century American artist Thomas Cole (pp. 1-11) as a starting point, to touch emotions and raise awareness about the relationship between civilisation and nature. Equally effective are the iconic illustrations of photographer Easton Adams (1902-1984) in environmental and cultural debates on the preservation of wild spaces, and to clarify the nuances of an idealistic view of "wilderness", often depicted as carrying no human trace, thus creating a "visual fiction" of pristine nature (pp. 100-107).

The Heart of the Wild also honours several other artists and thinkers who are all connected to how wild nature and its landscapes have been celebrated in culture, art, and ecology, such as Edward Abbey, writer and essayist, known for his work *Desert Solitaire* (pp. 11, 105, 115); Werner Herzog (p. 113), iconoclastic director and filmmaker, whose films on nature and the environment have often explored themes related to wild grandeur and human confrontation with nature; John Muir, Scottish-American naturalist and environmental activist, often regarded as the "father of the national parks system" in the United States (p. 75); and Rachel Carson (p. 105), ecologist and author, whose book *Silent Spring* played a fundamental role in raising public awareness about the dangers of pesticides and environmental protection. The book highlights their influence and the impact of their work on environmental awareness and the wild beauty of nature. The entire book is like unique poetry, through

the exceptional ability of the contributors to connect with nature to grasp every detail, and respond to the very complex questions of their work.

Their eyes perceive the slightest variation in colour, whether it be the nuances of a sunset or the reflections on a calm lake. Marris' curious gaze, for example, crossed paths with a badger near a retirement home, and described it in its natural habitat, seen by their consciousness as both proof and a symbol of the coexistence between human beings and wildlife (pp. 15-21). Marris thus proposed this event as demonstrating harmonious coexistence in "multispecies cities" or "wild urban cities" (p. 21).

The authors use diverse elements (animals, landscapes, ecosystems, human interactions) to convey central ideas. The dingo, for example, in the desert and semi-desert zones of Australia is seen by Losos as the master of survival and a guardian of balance in the arid ecosystem (pp. 36-45). It has been used to explore the complexity of human relationships with predators, highlighting their essential role in regulating ecosystems.

Symbols of the unintended consequences of human intervention include the infamous cane toad (p. 22) and several species of snakes, notably the garter snakes (*Thamnophis sirtalis*) in Manitoba, and as the pit vipers (*Gloydius shedaoensis*) on Shedao Island in China (pp. 34-39). Guided by awareness and wisdom, the book examines their cases with a perspective different from the usual one applied to what constitutes a nuisance in our environment. These cases remind me of a very interesting thesis defence on biodiversity in which I participated as a member of the jury. The candidate had presented as an issue the uncontrolled introduction since 2012 of the invasive alga *Sargassum muticum* (a brown alga) on the Moroccan Atlantic coast [1]. Although these invasive species are often seen as a threat to biodiversity, I instead see that nature has a unique ability to adapt.

A new balance, often influenced by anthropogenic factors, can emerge, transforming the ecosystem without necessarily causing its collapse. Generally, naturalists deeply connected to the elements of their favourite ecosystems feel the pain of the disappearance of many species and the degradation of nature. Shine, in *The Heart of the Wild*, encourages us to accept the current reality and find ways to conserve what remains, rather than lamenting what has been lost (p. 22). Thus, rather than attempting to eliminate or counter these species, it may be wiser to value this evolving dynamic. The prosperity of sardines along the Moroccan coasts may be a striking example of this successful adaptation and an opportunity to reinvent our relationship with changing ecosystems.

The book introduces an interesting reflection on the notion of "native" and "non-native" species. According to Shine, some argue that introduced species which eventually evolve and adapt to their new environment can be considered "native". The book also clarifies several other species categories to differentiate the effects and help understand the consequences of their introductions, including invasive species (e.g., ragweed in Europe, native to North America, which has invaded several European regions, disrupting ecosystems and increasing allergy problems); non-invasive species (e.g. bamboo - *Phyllostachys spp.* — in Europe); endangered species (e.g., the Iberian wolf, threatened with extinction in Spain because of the destruction of its natural habitat and competition with other species); and endomorphic species (e.g., cactus in arid regions of the Americas, sensitive to disturbances such as the introduction of non-native species). Shine maintains that truly "natural" ecosystems, i.e., those without human influence, are almost non-existent. "Shifting baselines" refer

to the phenomenon where each generation adjusts its expectations of nature based on what it knows, without always realising what has been lost (pp. 24-27).

Where art evokes an emotional response, science is thankfully present to strengthen and channel this emotion towards action. Wild nature is often an essential refuge for biodiversity. Its decline or collapse would lead to global crises (environmental, social, and economic) that directly threaten human communities. Scientists today propose ways to highlight any harm to biodiversity and, thus, indirectly to wild nature (Shannon-Wiener species diversity index, Simpson index, deforestation rate, abundance of key species, quality of natural habitats, etc.) [2].

Just as with the introduction of an invasive species, Emma Marris describes a walk along a concrete path in her neighbourhood. Moving away from the path, she discovers a small urban ecosystem that has formed around a drainage pipe, with plants and animals thriving despite the urban environment (p. 15). Marris thus suggests that nature is not simply defined by its purity or the absence of human intervention, but that human interaction — whether beneficial, harmful, or neutral — also influences *what nature becomes*. The interconnection between life and death in ecosystems is also perceived by Marris through the carcasses of dead animals in nature that she has observed. Their presence, according to the author, highlights how death contributes to life, reinforcing the interconnectedness of ecosystems.

This book subtly explores both the darkest and brightest corners of our relationship with domestic cats (p. 50). "What if cats had never been domesticated?" "Can we really 'own' a cat?" These are examples of questions that invite doubt about the idea of domesticating them, exploring the nuance between their independence and our desire for control. The question is indeed very difficult to resolve! It even took me back to some rather old family memories: the story of Mimi, a little cat that quickly won our hearts. But Mimi had a secret: an almost magical ability to multiply the members of our family. In no time, she gave birth to six adorable kittens that, in turn, followed her example with astonishing speed. What had started as a joyful experience quickly transformed into a complex management of this 'feline dynasty'. This personal experience taught me one thing: cats cannot easily be contained, neither by walls nor by our human plans. Currently, several practices are widely accepted and implemented to ensure harmonious cohabitation between animals and human beings, but many sensitive and complex questions remain open, ultimately demonstrating that these felines remain a little wild, even after millennia among us, and this is precisely what this book explores with great freedom and fascination.

This work left a significant impression on me with its evolving discourse. The position of "Man" in relation to "nature" lies at the heart of the authors' reflections. At the beginning of the book, Man is presented as a constituent part of wild nature, despite his disruptive effects. Gradually, we observe an increasing detachment of humanity from nature (p. 12). It is only at the end (p. 89) that the authors highlight that this "belonging" cannot justify destructive behaviour.

This change in perspective can also be interpreted as a tension between, on one hand, the acceptance of human impact on nature (seen as an integral part of our evolution) and, on the other hand, the recognition of humanity's responsibility towards this nature, which it continues to impact destructively. Kyle Whyte (pp. 71-83) presents the powerful and destabilising reality of "wild places" and "wildness," which were innocently "sought after." He skilfully deconstructs the myth of "pristine"

landscapes, national parks, and supposedly preserved environments, revealing how their creators, far from being innocent, often concealed aspects in the dominant narratives. Wildness has often been used to justify historical injustices, the dispossession of indigenous people, genocide, and violations of treaty rights. Furthermore, Eileen Crist advocates a state of wilderness (p. 76), with minimal human interference and less artificial modification, control, and use. For her, wild nature represents the original, dynamic, and unmodified manifestation of nature, uncontrolled by human hands. She argues for a world where wild spaces are left free to flourish, allowing non-human life forms to continue evolving and coexisting.

Eileen Crist perfectly complements Whyte's perspective (p. 84-89), defending the idea that wild nature is a space of freedom for ecosystems and non-human life forms. She sheds light on the tension between two opposing visions:

1. The utilitarian vision, where nature is seen as a resource to dominate and exploit.
2. The idealistic and ethical vision, where wild nature is valued for its autonomy, beauty, and ability to evolve independently of human intervention.

Crist strongly criticises the evolution of contemporary environmental discourse, which she calls "new environmentalism" (pp. 96-101). This trend, arising from an initial questioning of the ideal of wild nature (wilderness), focuses more on the sustainable use and management of nature to serve human needs. Crist dissects the fundamental difference between these approaches, mainly through their stance on human expansion on Earth. She thus introduces "Rewilding" as a philosophical vision that promotes a reduction in human activity and an active commitment to protecting and restoring wild areas.

Thus, through art, science, and well-established, conscious, and unique ideological reflection, the contributors to *The Heart of the Wild* remind us that wild nature, far from being an abstract notion, is at the heart of our survival and our future. This book is a vibrant call for the preservation of these vital spaces, for a more harmonious coexistence between humanity and the Earth, and for the awakening of consciences to protect the wild treasures that shape our world. With its rich and varied perspectives, the work encourages much-needed introspection on our responsibility towards the natural world and its inhabitants, at a time when ecological challenges and biodiversity crises demand urgent and informed responses.

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BOOK REVIEW

Garbocracy: Towards a Great Human Collapse, by Sayan Dey, Chennai, India: Peter Lang Group AG, Lausanne, 2025, XX + 128 pp., US\$72.95 (eBook), ISBN 978-1-80374-727-9 (PDF), DOI: 10.3726/b22308

At a time when right-wing populism is on the rise in India, when vast swathes of the Indian population experience hazardous air quality, and when dehumanizing and illegal waste-removal practices like manual scavenging continue despite legal prohibitions, this new book justifies big changes. Sayan Dey introduces the concept of "*garbocracy*" — a system in which the accumulation, disposal, distribution, and regulation of waste become central features of the social, political, and cultural fabric of contemporary India. Dey argues that with the advent of neoliberal policies, waste has become a pervasive material and immaterial presence, with implications that extend far beyond environmental issues.

In essence, *Garbocracy* interrogates how and why India's "*garbageous*" ways of living have contributed to—and become entangled with—larger socio-political and environmental crises that have, in turn, contributed to the rise of a "*garbocratic*" society characterized by the normalization of waste, the exploitation of certain communities, the erosion of critical thinking, and the systematic dismantling of democratic values and environmental protections.

As the third part of Dey's *Decoloniality, Eco-sustainability, and Cultural Studies* series, *Garbocracy* seeks to explore the "different patterns of garbageous existence that human civilization is entangled in" (p. xv), in contemporary India. Divided into eight chapters across four sections, the book begins with a rather uncharacteristic "(un)acknowledgment" statement that highlights the violent role played by the right-wing Bharatiya Janata Party (BJP) government in exacerbating caste, communal, and environmental tensions. These developments spurred the creation of this book; and to the extent that the BJP's divisive policies prompted Dey into research action, they are "warmly" (un)acknowledged from the start.

This incisive note sets the tone for the rest of the work. The first section, titled "*Accumulating*", contains two chapters. The first chapter, "*Introduction: The Politics and Optics of Garbaging*," introduces the monograph's theoretical and methodological intentions. It engages with the author's positionality and the contexts from which Dey's arguments emerge, discussing how material, spiritual, political, and governmental garbage evolves, accumulates, operates, and suffocates human beings and animals in India. The chapter also introduces the concept of "*garbo-beings*" as a way of explaining how Indians are collectively impacted by the physical and ideological presences of waste. The second chapter, "*Garbo-Imperialism: The Empire of Wastes*," develops the theories and methodologies introduced in the first chapter by discussing how garbage manifests imperialistic tendencies in India. This chapter examines how waste dictates people's existential patterns, both physically and cognitively. It delves into the ethics and politics of garbage disposal in Kolkata and Varanasi—locations that hold personal

and academic significance for the author, as he was born in Kolkata and has conducted extensive research in Varanasi. It examines how "*garbagescapes*"—spatially defined garbage kingdoms—are generated across residential and commuting areas through "*social, cultural, communal, and religious algorithms*" (p. 21).

The second section, "*Disposing*," shows how garbo-imperialism operates through paradigms of knowledge, power, and being; with the third chapter, "*Garbo-knowledge: Towards Garbo-intellectualism*," engaging with the idea of how the visible and invisible presence of garbage impacts knowledge production and relationships with diverse communities in India. This chapter examines how history, science, philosophy and other strands of intellectual thinking have today become negatively impacted by the "*decomposed intellectuality*," propagated by the policies of the right wing BJP government (pp. 31-33). The fourth chapter, "*Garbo-power and Garbo-being: The Onset of Garbo-ideologies*," explores how garbo-knowledge and garbo-power lead to the formation of "*garbo-beings*"—individuals whose functions are influenced by the physical and ideological garbage produced by political actors like the BJP. Dey highlights how the BJP's garbo-ideologies are manufactured and reinforced through a range of tools including radio, printings, posters, t-shirts, books, and flags.

The third section, "*Distributing*," comprises one chapter, —the fifth chapter, "*Garbo-pedagogies and Garbo-curricula: Schools as Knowledge-garbage Laboratories*," which examines how "*garbo-knowledge*," "*garbo-power*," and "*garbo-being*" are systematically produced in India through school curricula and pedagogies. Dey alleges that schools function as "*concentration camps, gas chambers, and mumbo-jumbo rooms*," (p. 59) systematically promoting unpractical, uncaring, and unsustainable modes of teaching and learning that lead to the production of physical and emotional wastes. It is difficult to see such savage language as justifiable.

The final section, "*Regulating*," consists of three chapters. Chapter six, "*Garbo-Citizenship: The Great Human Collapse*," explores the evolution of "*garbo-citizenship*" in India through three specific incidents: first, the destruction of the natural environment by the British; second, Dey's personal experience of teaching in a school with a discriminatory grading system; and third, the BJP's enactment of the constitutionally dubious Citizenship Amendment Act of 2019. Chapter seven, "*Digital Discardscapes, Digital Infrastructures, and Garbocratic Futures*," shifts the focus to the digital realm, exploring how the disposal of digital waste reflects social, cultural, and economic hierarchies in India, and how discarded electronic items, referred to as "*smart discards*" (p. 96), are used to display socioeconomic status. Chapter eight concludes with visions of alternative future.

What Dey does exceptionally well is weave together seemingly disparate events— across spatial and temporal boundaries— and link them to broader themes of marginality, disposability, and devaluation in the context of waste. By drawing readers' attention to the different kinds of garbage prevalent in India today—not just the physical, but also the ideological, political, and social—Dey reveals just how these seemingly unrelated forms of "garbage" are deeply interconnected and mutually reinforcing in creating a "*tentacular garbocratic empire, suffocating not only the present but also the future*." (p. 107).

Dey draws on several intellectual perspectives—from Zygmunt Bauman's concept of "*wasted humans*" (introduced in Chapter three) to Foucault's concept of the panopticon and

Deleuze's concept of the rhizome (p. 69), from Latour's work on actor-network theory (introduced in Chapter seven) to Siegfried Kracauer's concept of "sollen" knowledge systems (p. 40)—and in tying these ideas together, Dey enriches his explanation of what it means to produce, consume, and dispose waste in contemporary India.

That said, Dey seems to place disproportionate blame for the rise of garbocracy on the BJP, without adequately addressing the political processes, systems, and ideologies that both preceded its ascendancy and continue. Perhaps it is necessary to examine a broader range of actors intertwined with the BJP's agenda of producing "garbo-citizens," such as, for example, India's deteriorating legal system, the poor state of environmental governance policies in India, and the role of non-BJP political actors in making these problems *alongside* the BJP. It is notable that many of the BJP government's beneficiaries are Muslims and Dalits.

Although *Garbocracy* is very full of neologisms and sociology, it also offers a view of waste problems mediated by environmental justice.

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BOOK REVIEW

Before They Vanish: Saving Nature's Populations—and Ourselves, edited by *Paul R. Ehrlich, Gerardo Ceballos, and Rodolfo Dirzo*, Johns Hopkins University Press, 2024, 320 pp., \$29.95 (hardback), ISBN: 978-1-4214-4969-2.

It has always been easier to condemn tragedies once they belong to the past—always after the fact, when the damage has already been done. In this work, Ehrlich, Ceballos and Dirzo boldly sound the alarm in time. The question is whether people will act to preserve endangered species before it is too late and before the consequences weigh heavily on ecosystems. The authors emphasise that the issue extends beyond mere environmental concerns; it is a fundamental challenge for humanity itself. Saving biodiversity also means protecting the conditions of our existence, often without realising it. The urgency is real, and the book urges action before disaster becomes irreversible, while populations can still be saved (p. 128).

From the very first pages, we are plunged into a gripping narrative that blends exploration, discovery, and tragedy. Vitus Bering's expedition in the 18th century (pp. 1-6), with all its details, invites us to grasp its lesson and engage in deep reflection on the multiple causes of past and future extinctions of species. After all, it is by understanding these causes that we can find solutions.

Gradually, awareness intensifies—everyone is, in fact, concerned: yet the sixth extinction is advancing. The authors demonstrate an unprecedented rate of species disappearance, far faster than that observed during past biological crises. Rising temperatures, ocean acidification, and man-made pollution are affecting the living conditions of countless species. Are we doomed to suffer the same fate as the species once discovered, then wiped out by trophic cascades, as in the five previous mass extinctions caused by natural disasters? Today, what we are experiencing is the result of our own actions—the product of our own hands.

The book recounts, through a poignant narrative, that once upon a time, there was a continent—the cradle of humanity—where *Homo sapiens* emerged about 300,000 years ago. Within these conditions, our ancestors learned to integrate into their environment without disrupting it—a land rich in biodiversity, teeming with diverse flora and fauna. Yet, other territories existed, just beyond. Driven by an insatiable curiosity, *Homo sapiens* decided to leave

Africa, its first continent, to explore these new lands—territories unprepared for its arrival (pp. 1-14). This highly intelligent predator chose to force its adaptation, exert a dominant influence, and impose a way of life born of its own imagination—unleashing a massive wave of extinctions.

Several magnificent birds have completely vanished. Species such as the moas (*Dinornithiformes*) in New Zealand (p. 14), the dodo (*Raphus cucullatus*) from the islands of the Indian Ocean (pp. 14-18), and the passenger pigeon (*Ectopistes migratorius*) (pp. 18, 69) have all fallen victim to extinction. So have certain mammals. Indeed, the dark record of extinction highlights the disappearance of the thylacine (*Thylacinus cynocephalus*) and the Bramble Cay melomys (*Melomys rubicola*), the latter being recognised as the first documented case of a mammal's extinction directly caused by contemporary climate change (p. 17).

And so, the authors compile an extensive list of species that have vanished, including amphibians, reptiles, and mammals (p. 80), as well as vertebrates as a whole (pp. 80-98) and invertebrates (p. 125), not to mention plant species (pp. 131-144), eliminated by deforestation, climate change, and the introduction of invasive species (p. 140). The authors reconstruct, like detectives, the sequence of events that led to these extinctions (p. 79), scrutinising fossilised clues and every trace left behind. Each lost species has its own meticulously documented case file, and with every page, a new culprit is unveiled.

The number one culprit—the most powerful of all—is Man, the great predator (p. 36), the serial killer with varied methods (p. 39), the poacher (p. 176), the faceless industrialist who, though he does not wield a weapon (p. 187), is nonetheless responsible for a massive decline through his factories (p. 188). He clears hectares of jungle (p. 227), poisons the air and water, drains groundwater reserves, depletes fertile soils, and devours the Earth's resources (pp. 131-145). His thirst for accumulating wealth is like a desert swallowing a river.

He is the master of global warming—through his carbon emissions, he burns the planet's lungs, heats the oceans, and disrupts biodiversity. He introduces invasive species, rewrites the rules of evolution, decides which animals will survive in his farms and which will perish, enclosing them until they disappear (p. 178).

Yet, healthy nature shelters us, nourishes us, protects us, heals us, purifies our air and water, and regulates the climate (pp. 166-189). Every individual, every species, every plant serves mankind—this has been the deeply rooted belief since the dawn of civilization, and so it remains. But without them, our harvests collapse, our rivers dry up, and our delicate balances are shattered (p. 146). And still, we mistreat them, exploit them, as if they could never fail us. How far will we go before we understand—before we ensure the right change?

The authors take us into another dimension, allowing us to observe with great precision our total dependence on our smallest yet most powerful allies (pp. 6, 7, 145-156): microbes—now threatened by intensive agriculture, pesticides, and pollution. They brilliantly succeed in exposing the major risks associated with their collapse. A dark future looms ahead: the COVID-19 pandemic was merely a warning (pp. 151, 152). Is there an emergency exit, a way to make amends before it is too late? Yes, apparently, according to this book. But, are we truly ready to implement these best solutions? (p. 205).

Like a breath stirring the dust of the past, *Before They Vanish* confronts us with our responsibility. It takes inventory of the species wiped out, urging us to listen to their silence, hear their stories, understand their absence—and, above all, to act in order to prevent an even greater catastrophe. This book is The Codex of Lost Life. It provides essential insights and irrefutable evidence for those who refuse to remain mere spectators, passively awaiting extinction. Read this book!

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BOOK REVIEW

Managing Biodiversity in Agricultural Landscapes: Conservation, Restoration and Rewilding, edited by Nick Reid and Rhiannon Smith. Burleigh Dodds Series in Agricultural Science, Cambridge, 2024. 543 p., ISBN: 978-1-80146-454-3 (hardback) £150; ISBN: 978-1-80146-456-7 (PDF); ISBN: 978-1-80146-455-0 (EPUB).

There are two literatures: of what we know (and find useful) and what is new (which we find interesting). Therefore, it seems to me better ... that we should translate certain books which are most needful for all men to know, into a language that we can all understand.

Alfred the Great to Bishop Waerferth, c890 [1]

This book comes late on the scene of what is most needful for all men to know because we have been slow to realize that our daily bread depends as much on unseen, hard-to-see and largely unknown biota as on our artifice and a handful of domesticated species. Biodiversity has been consigned to David Attenborough; alternatively, to Aldo Leopold: *‘If the biota, in the course of aeons has built something we like but do not understand, then who but a fool would discard seemingly useless parts? To keep every cog and wheel is the first rule of intelligent tinkering* [2]. The Burleigh Dodds Series on Agricultural Science offers a reliable map. This is a text book; it weighs two and a half pounds; 16 essays cover principles, farmland and conservation practices, the role of government and the private sector, and rewilding; and this review is only a sketch.

Agriculture is driving mass extinction. If there is a will, it might be managed to retain essential biodiversity but crops and pasture already occupy one third of the land – another third is desert – so future biodiversity will depend on how well species and communities can be sustained within and alongside farmland. Andrew Bennett and colleagues from La Trobe University lead off with *Key concepts* in such a way that novices will not flounder and experts can nod sagely.

Species move between natural elements of the landscape like forest patches and wetlands; modified elements like hedgerows; and manmade ponds, ditches and cropland. So, enough – and big enough – habitats are needed for viable populations; and key issues include the frequency and scale of changes, interactions between them, time lags, and irreversible effects. Land is limited so there must be trade-offs between farmland and land for nature: *land sparing* aims for intensive production on the best farmland so that other land can be spared for conservation, *land sharing* requires wildlife-friendly practices on farmland. For most species, land sparing is the least-worst option but most agricultural landscapes occupy a middle ground. The people who live there must be part of the solution but engagement has had mixed success and it is too early to say whether market-based solutions will be any more successful.

Geoff Squire and Cathy Hawes of the James Hutton Institute follow up with *Integrated farm management*. Every productive process depends on wild plants, the soil microbiome, detritus feeders, pollinators, pests and their natural predators, pathogens of crops and livestock, and higher organisms living within managed crops and grassland: defined as Biodiversity for Agriculture (B for A), to distinguish it from Agriculture for Biodiversity (A for B) where management supports iconic plants or animals. Integration implies that the various activities on a farm, and ecological processes they support, are complementary – crop rotation is a long-standing example of integration – but recent intensification has abandoned many traditional, integrated systems in favour of extractive production of very few crop species and varieties, exacerbating soil erosion, pollution and loss of biodiversity.

Early farming systems cropped a small area supported by a hinterland from which nutrients were scavenged by cutting and carrying vegetation or by grazing livestock; or, in slash-and-burn, moving on when fertility was depleted. Both need a lot of land per person. The *green revolution* raised production per unit area by new crop varieties, manufactured or mined fertilizer, irrigation, mechanization, and chemical pest control. Societies that could afford these inputs could dispense with scavenging and recycling but, now, depend on a narrow genetic base susceptible to pests and disease, severe and unpredictable weather, and inherent imbalances. For instance, when livestock are raised on feed grown elsewhere, cycling can be achieved by spreading their manure on the homefields; but the mass of manure from intensive livestock cannot be contained, and the outflow pollutes land and water downstream.

Regeneration may be achieved through increasing species, functional and habitat diversity; safe ranges for soil organic matter, nutrients and seedbanks; chains-of-effect of crop choice, application of fertilizer and pesticide, or tillage; complementary crops, wild plants, invertebrates and soil microbes, and ecological processes such as primary production and creation of soil architecture – *i.e.*, A for B. But all of these require rare skill and judgement. Moreover, action on the field and farm cannot, by itself, arrest the decline of land area per person and depletion of soil resources: the main impediments to sustainability remain extractivism, conflict over land and water resources, and lack of political will.

We are in a world of wicked problems [3] where different people see and know the world differently – not just concerning what should be done but what is the problem, or whether there is one! Theodore Alter and colleagues from Penn State University and Lauren Hull and colleagues from Energy, Environment and Climate Action, Victoria, survey the philosophies, processes and politics of *Engaging local voices*. It may be that individual selfishness impedes social change. And, yet, Elinor Ostrom illustrated case-upon-case of resilient, adaptable natural resources management by local communities drawing on trust, reciprocity and cooperation [4]. Our authors present two current case studies. In Victoria, 150 years of campaigns against rabbits, gorse, blackberry and serrated tussock grass established a range of effective controls but no coordinated action. On the premise that common ownership of the problem and collective action required parties with differing interests to come together to find possible solutions, in 2016, Community Pest Management Groups were established wherein the people most affected by the pest define the problem, create strategies to deal with it, and share investment decisions: a big shift from government-led decision making that marginalized rural and regional stakeholders. There have been no ‘quick fixes’ but empowering people to take personal and collective action has raised community capacity deal with these and future issues.

The Chesapeake Bay catchment embraces six states with a population of 17million whose water is contaminated by sediment, nitrates and phosphates from agricultural runoff. Individual landholders have rights up to heaven and down to hell but not the means to tackle the problem. Yet, over the last decade, community-led initiatives have doubled the no-till acreage, and increased cover crops and edge-of-field practices that have cut phosphate loading by one third and nitrates one sixth. Community engagement requires time, effort and humility. Millions of conversations, public information sessions and negotiations have generated precious public information; and local leadership committees including farmers, agriculture organisations, local government and state agencies, technical service and educational providers focus on understanding the impacts of proposed programs, prioritizing goals, bridging information gaps, and ensuring that all the stakeholders know that their voices are heard and valued. Our authors summarize an immense, inchoate literature on public engagement with check lists of engagement principles but, in one phrase, *we need each other*.

Alice Day and David Johnson of Manchester University and Ezekiel Njeru of Kenyatta University boil down the fuzzy concepts of *Soil health and ecological restoration* to plant-soil feedbacks and biodiversity-ecosystem functional relationships – in particular, plant-microorganism interactions like symbioses with nitrogen fixing bacteria and mycorrhizal fungi, which have co-evolved with green plants over 450 million years. Webs of mycorrhizal hyphae vastly extend and combine the root systems of partner plants, offering: inter-plant signalling to respond to stress, pests and diseases; decomposition of waste and toxins; better access to water and nutrients; stabilization of soil architecture, increased aeration, water infiltration and storage; enhanced soil formation and less erosion; all combining to enhance primary production and, potentially, crop yields. Only potentially, because these biodiversity-soil function relationships evolved in nutrient-limited environments, not fertilized fields and we know little about the interrelationships of 250 000 plant species that form mycorrhizal relationships with a few thousands of fungal species and all their intra-species variations. Transferring theory to practice might require diversifying the plant community; introducing microbial inocula, and changing cultivation practices, in particular by minimum or no-till to avoid disruption of hyphal networks.

The impact and design of field margins by Jane Morrison of Bishop's University, Quebec, is a gem. Agricultural landscapes are increasingly uniform, natural habitats more fragmented, but every field has a margin that may provide a cost-effective and minimally invasive biosphere reserve. It may be a fence, hedge, wall, terrace, track, waterway, grass strip or some combination of these. The biodiversity supported by field margins is only a fraction of what would have been present in the natural landscape but it makes a difference and pays its keep: agronomically – by keeping livestock or trespassers in or out, cutting runoff and erosion, sheltering crops and livestock, enhancing pollination and pest control, providing organic matter, fruit and wood; environmentally – by promoting farm wildlife conservation including soil microorganisms and as a corridor or refuge for beneficial plants and animals, buffering pesticide drift and reducing the need for pesticides, improving soil carbon capture; and socially – by providing access, supporting game, and enhancing the landscape. Our author gives detail on design and management for pollinators, seeding mixes, optimum width and configuration; and the need for policies to support farmers and develop effective context-based management plans.

Ian Montgomery and Neil Reid of Queen's University Belfast, present a paean on *The impact and management of hedgerows*: their history, ecology, biodiversity and benefits of increased diversity,

management, and barriers to their conservation. Hedgerows are unrivalled habitats for farmland biodiversity while providing pest, pollination and other environmental services that can be multiplied by not flailing them to within an inch of their lives every year. But farmers remain sceptical, daunted by the scientific complexity and contradictions; demonstration of improved hedgerow management drawing on the experience of local farmers is more effective than convoluted ecological advice from other sources. But our authors offer no clear policy direction other than diversity is *a good thing* [5].

This inability to trigger the existential-threat reaction in society confronts the following section on the role of government and the private sector in promoting on-farm conservation. Jennifer Dodsworth and Rachel Lasko from Oxford University and Ruth Little from Sheffield University lead off with *England's new Environmental Land Management policy* following the UK's departure from the EU, devolved particularity in Wales and Scotland, and dithering as the Dept for Environment, Food and Rural Affairs struggled to co-design the policy of public money for public goods with the people expected to deliver the goods. The sentiment acknowledges that farming simultaneously delivers private goods paid for by the market, and public benefits not paid for by the market but it also, wrongly, assumes that the market adequately pays the production costs of private goods (*i.e.*, food) [6]. Farmers are frustrated by a lack of engagement; rushed and overlapping timelines, with their positions undermined by public pronouncements about policy that their insights were supposed to shape; and eventually abandonment of hope for innovative policy that might meet anticipated needs.

Gordon Rausser and David Zilberman of UC Berkely consider *Developments in agri-environment schemes: North America*. Economic theory suggests that government intervention is needed to counter *externalities* inflicted by private and corporate activities – amongst them pollution and loss of biodiversity. Taxes raise objections from affected parties; subsidies are costly; tradeable permits may introduce markets for externalities that may decrease costs to polluters; regulations are blunt and inefficient; voluntary agreements have limited capacity unless there is the threat of regulation; education that internalizes environmental values among the population is effective but limited in scope. For all these reasons, payments for environmental services became the preferred government intervention, *e.g.*, in agri-environment schemes, until the Trump administration abdicated any responsibility and scrapped them all.

Dean Ansell and colleagues at the Australian National University consider *Developments in agri-environment schemes in Australia* in the context of abject failure of conservation policies implemented over the last two or three decades, and a scramble to shift the cost of any action to the private sector. Following the financial if not environmental success of carbon-offset schemes, biodiversity is being linked in the statutory Australian Carbon Credit Unit (ACCU) scheme that registers carbon-offset projects for tradeable credits that are supposed to represent real and additional abatement of greenhouse gas emissions. This offers an opportunity for income diversification and the long shot of agricultural exports being able to claim both carbon and biodiversity neutrality. In the Australian Government's Carbon+biodiversity pilot, landowners can contract environmental planting of 5-200ha either to restore the pre-clearing vegetation or with simple mixed-native planting. Participants receive initial payments to cover their costs for the first 2-3 years and receive credits for the carbon sequestered over 25 years which they can sell in the carbon market. Regional biodiversity benefit scores also contribute to payment offers that should achieve a prescribed internal rate of return. But, the complexity of arrangements and their contract length are unattractive and the risk is greater

than in traditional agri-environment schemes, operation of the market being left mainly to the private sector.

The fourth section of the book covers *rewilding* of ecosystems from human dependency to some degree of self-sustaining autonomy. In *Restoring peatlands in European landscapes*, Rudy van Diggelen and colleagues from the Low Countries note that vast, undisturbed peatlands are no more in densely populated parts of Europe; the isolated remnants have lost many of their characteristic species, most of the survivors are endangered, and recolonisation by typical species is unlikely because of short-lived seed banks and the distance from source populations. Restoration to wilderness would need a lot of space and regional re-engineering of the hydrology; but semi-natural wetland or productive functional landscapes are achieved through closure of drainage ditches, cessation of fertilization, and suppression of competitive plants by mowing or grazing. There is also a need for wet-agriculture buffer zones for sites of higher biodiversity value; several crops are being tested, economic models developed. The search is on for sustainable systems in which multiple ecosystem services can be restored or conserved on degraded peatlands still under cultivation.

Worldwide, grasslands and rangelands have been the main target for takeover by farmers. *Rewilding grasslands and rangelands* by Thomas Jones of USDA Forest and Range Research discusses the ecological principles that drive grassland and rangeland function, the diversity of best practices, and emerging technologies. Grasslands are disturbance-driven ecosystems: walking away rarely restores anything like the original vegetation. Abandoned land commonly reverts to scrub: active restoration by reseeding, controlled grazing, mowing and/or burning may be required; even so, sustainability has been elusive. Long-term field experiments are invaluable for informing seed-mix design and management; and native-seed industries have developed to meet the demand.

A case study of sagebrush steppe rangelands in Utah reports encroachment of woodland from less than 3million ha pre-European settlement to 50million ha today. The Utah Watershed Initiative in 2006 covered some million ha with state-assisted community projects combining herbicide and drill seeding, chaining or harrowing and drill seeding, or aerial seeding followed up by shredding with a toothed drum, and controlled burning. Recovery of herbaceous perennials has been greater on seeded sites but, in the case of sagebrush, establishment may be limited by the infrequency of regeneration episodes, dry spring weather, grazing by big game, and competition from seeded herbaceous perennials. Rewilding is challenged by climate change but diversion of marginal agricultural land is opportune for many urban populations. Three things are necessary: ecological monitoring, plant materials that are more effective in modified environments, and reliable demand to support seed production. The big challenge is to apply science in the face of economic and political realities.

David Lindenmeyer and colleagues from the Australian National University draw *Lessons from reforestation of agricultural landscapes in SE Australia*. Twenty-four years of empirical data on restoration projects on some 800 sites on more than 300 farms reveal that biodiversity depends on a hierarchy of factors from individual features like paddock trees, fallen logs, mistletoe, rocky outcrops, and farm dams; to the site level – topography, shape and size, connectivity with other plantings or remnants; to the farm level – the variety of assets across the farm and the amount of native woody vegetation; to the landscape level – the amount of native woodland and the climate. Each and every feature is important and everything is connected to everything else. Larger plantings are better than smaller; blocks better than strips; plantings connected to others or near to others or remnants are better than isolation; those around streams, paddock trees, rocks and logs are better than those without them

– *e.g.*, scattered paddock trees can be stepping stones for bird movements in agricultural landscapes and foraging sites for other animals. Greater diversity on farms and in landscapes with higher levels of native trees is likely to be related to greater connectivity. Restoration is most popular where there are multiple benefits; *e.g.*, restoration of a farm dam might entail controlling livestock by fencing, creating vegetation cover across the catchment to filter animal waste and runoff, establishing trees to shade the water body, and encouraging vegetation cover at the margins of the dam and in the water. The cost can soon be recouped through improved livestock weight gain as a result of better drinking water; at the same time, methane emissions are halved and there is a biodiversity dividend from water quality.

Animal rewilding is generally believed to be incompatible with farming but *The future of animal rewilding in agricultural landscapes* by Kiarrah Smith and colleagues, also from ANU, places it between wilderness and conventional intensive agriculture, within the land-sharing part of the spectrum. There are trade-offs between the ecosystem services and disservices of wild animals but, within heterogenous landscapes, there are opportunities from hunting, shooting and fishing to flood control; wildlife habitat within croplands is increasingly appreciated for pollination, pest and weed control, soil and water conservation; and money can be made by running traditional native livestock breeds or species best suited to local conditions, including the threat of natural predators. Ecosystem engineering by beavers and re-introduction of top predators takes an order of magnitude more adjustment but *co-existence conservation*, originally proposed for managing predation of threatened species by invasive predators may be transferred to animal rewilding in agricultural landscapes. In the conservation context, the vulnerable organism is a threatened rare plant or animal: in the agricultural context it is crops or livestock that are at risk and predation (*i.e.*, consumption) rather than the predator (consumer, herbivore or carnivore) becomes the focus of management strategies.

The mobility of animals makes rewilding a landscape-scale venture. Heterogenous landscapes that might include intensive agriculture for land sparing provide a diversity of habitats, support greater animal diversity, and may benefit from complementary species that may reduce the disservices of otherwise beneficial herbivores. Our authors conclude with an optimistic case study of kangaroos and dingoes worthy of David Attenborough.

Both climate science and biodiversity science have drawn lines in the sand. History has already washed away the climatic +1.5°C sandcastle and, in *Looking ahead*, Nick Reid surveys the sand lines of the UN Convention on Biodiversity [7]: cease transforming natural areas, restore at least 30% of degraded ecosystems by 2030, conserve and manage for biodiversity conservation at least 30% of the world's lands, coastal areas and oceans by 2030 – noting that only 17% of land and 8% of marine areas were so managed in 2022. In 2025, the issue becomes 'How best to restore habitats in agricultural regions so that at least 30% of the land is managed for biodiversity conservation?' Bottom-up, we have farmers' own initiatives. Top-down, we have statutory and policy interventions that prohibit, encourage or require farmers to undertake specified management actions, extension and training programs, market incentives, reversal of pest-animal policies to permit the return of wild animals, government purchase of land with high potential for restoration of high conservation-value species and ecosystems, and outlawing conversion of natural habitats.

Nick Reid highlights regional-scales issues in two case studies. Despite extensive clearance, Mount Lofty in South Australia is one of the continent's biodiversity hotspots, reflecting its isolation and the impending collapse of biodiversity. Since there is a time lag between initial clearance of

vegetation and extinctions; the critical requirement is to put back substantial plots of appropriate habitat, especially on better soils. The competing claims on the waters of the Murray-Darling Basin have already featured on these pages [8]. The 30,000 wetlands, 16 of them Ramsar listed, are priceless flood-related habitats: but the basin also sources almost 40% of national farm output and water supply for more than 3 million people. Prior to abstraction for irrigation, an average of 12,500 GL per annum flowed to the sea, although with dramatic variations. By the turn of the millennium, more than 13,000 GL per annum was abstracted and, during the Millennium drought, the Murray mouth was kept open only by continuous dredging. The best-available science suggested that between 3,000 and 7,600 GL should be returned to the environment to secure the health of the various ecosystems, the lower figure offering little chance of success. The Water Act was promulgated in 2007 to drive water reform, including buying back over-allocated water from irrigators, and the 2012 Murray-Darling Basin Plan undertook to return a modest 2,750 GL annually by 2024. Politics, rival claims of riverine states, has prevented this, which suggests that the much more ambitious UN targets are unreachable.

If indeed the big challenge is to apply science in the face of economic and political realities, how much more factual or persuasive must researchers be before society listens?

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BOOK REVIEW

Biomass-based Clean Technologies for Sustainable Development: Sustainable Resources of Energy Production and Utilization, edited by *Dan Bahadur Pal and Ashish Kapoor*, Singapore, Springer, 2024, 268 pp., EUR 159.99 (hardcover), ISBN 978-981-97-0846-8.

This book serves as an essential reference particularly for chemists, to understand the role of biomass-based clean technologies in supporting the current trend of sustainable development. It highlights various innovations in using agricultural waste and biomass to produce bioenergy, biochar, and high-value chemical materials, significantly contributing to carbon footprint reduction. The book sees biomass-based clean technologies as a key element in sustainable development because of their potential to reduce reliance on fossil fuels and mitigate environmental impacts. Each chapter addresses specific topics, including nanocellulose for energy storage, agricultural waste valorization for biorefinery applications, and biochar innovations for water treatment. By focusing on process development, sustainability assessment, and practical applications, the book provides a holistic view of green technologies and the role of chemists in creating environmentally friendly solutions.

The editors aim to provide a comprehensive view of biomass-based clean technologies, emphasizing process development and sustainability evaluation. The book seeks to integrate concepts from various disciplines to develop innovative strategies that support sustainable development. These technologies, if applied worldwide, can benefit the energy and environmental sectors. This book not only offers technical insights but also encourages collaboration in addressing current energy challenges through sustainable practices.

The first chapter focuses on the potential of nanocellulose derived from agroindustrial waste as an innovative material for sustainable energy storage devices. It discusses the production processes of nanocellulose through chemical and mechanical methods, as well as surface modifications to enhance performance. The applications of nanocellulose in batteries, supercapacitors, and fuel cells are great improvements in energy capacity, efficiency, and mechanical stability. Nanocellulose is an environmentally friendly solution that can help reduce dependence on fossil fuels.

Chapter 2 discusses the use of agricultural biomass waste through effective pre-treatment for biorefinery applications. This process aims to enhance the economic value of waste by converting it into high-value products such as bioenergy, biochemicals, and biomaterials while optimizing the

process to ensure high efficiency and sustainability. The pre-treatment methods include chemical, physical, and biological approaches, to remove lignin and improve cellulose availability.

Chapter 3 discusses the conversion of biological waste into biofuels to support a bio-based economy. Organic waste, such as agricultural residues and food waste, can be feedstock for biofuel production, including biodiesel and bioethanol. Various technological approaches, such as fermentation and catalysis, can maximize energy conversion efficiency. They can also offer environmental benefits like reduced carbon emissions and improved waste management.

Chapter 4 discusses the synthesis of agricultural resources as biomass for biodiesel, via the use of agricultural waste such as vegetable oils and animal fats. The transesterification process uses efficient catalysts to convert raw materials into high-quality biodiesel. It delves into the challenges faced during production, including feedstock processing and by product management. Biomass use is a renewable energy solution that helps reduce greenhouse gas emissions.

Chapter 5 concerns the role of microorganisms in biofuel production from various types of biomass through fermentation, via the use of bacteria, yeast, and fungi to produce bioethanol, biobutanol, and biogas. Optimizing fermentation conditions and genetic manipulation can enhance productivity and also bring environmental benefits like biomass waste management and greenhouse gas emission reduction. In contrast, Chapter 6 focuses on clean energy technologies, particularly hydrogen production using solar-based methods. This chapter details solar energy conversion techniques, including photocatalysis and water electrolysis, which use innovative materials to improve efficiency. High costs and material stability are challenges. Mitigation strategies exist.

Chapter 7 covers the optimization of processes in biofuel production using various types of biomass. Efficiency and yield depend on variables. Different methods and techniques for achieving this optimization include an analysis of factors affecting the final output. Chapter 8 focuses on the optimization of process variables as a crucial step to improve biofuel efficiency and productivity. The goal is to reduce production costs and enhance the quality of biofuels produced.

Chapter 9 discusses biochar, which has a high capacity for absorbing pollutants, making it an effective solution for improving water quality. This chapter outlines various methods and technologies for the adsorption process, as well as the potential applications of biochar in sustainable water treatment systems: all very fully detailed. Better water management is an element of the discussion.

Chapter 10 explains chitosan and cellulose-based materials for sustainable wastewater remediation. The emphasis is on adsorption mechanisms. Additionally, the chapter covers synthesis and characterization methods for these materials in water treatment applications.

Chapter 11 discusses biochar-based mixed matrix membranes, which represent an innovative approach in water treatment. These membranes can enhance the efficiency of water filtration processes by using biochar as a key component. Biochar, produced from biomass, possesses high adsorption properties. The use of mixed matrices in the fabrication of these membranes aims to improve their durability and performance in water treatment applications.

Chapter 12 concerns pollution control through bioelectrochemical systems, microbial fuel cells, and microalgae-based wastewater treatment strategies. Bioelectrochemical systems work by converting the chemical energy from organic materials into electrical energy, and microbial fuel cells use microbial activity to generate energy while treating waste.

This book's main strength lies in the data provided, but it lacks discussion on the global challenges of biomass implementation, such as policy and funding issues. Nevertheless, it remains an important reference for researchers and practitioners seeking to understand the potential of biomass as a renewable energy source and to raise awareness about the transition to more environmentally friendly energy solutions.

The book also advocates policy development to support research and innovation in biomass technology, aligning with the Sustainable Development Goals (SDGs). The use of biomass can be an effective strategy to achieve sustainability and confront climate change challenges.

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