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CO-OCCURRENCE OF TWO INVASIVE PLANTS IN A TROPICAL SAVANNA ECOSYSTEM: A TOP PRIORITY FOR MANAGEMENT

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ABSTRACT

In this study, we assessed the co-occurrence influence of *Hyptis suaveolens* and *Urena lobata* on native plant species and soil properties in a guinea savanna vegetation in Nigeria. We sampled 120 plots of 10×10 m^2 with 30 plots each in sites invaded by *H. suaveolens*, *U. lobata*, mixed site and in sites with none of the species (control). A sparse partial least square discriminant analysis was used to assess the effect of invasive plant treatments on the plant diversity and soil properties, whereas the relationships between the soil properties, plant diversity and invasive species treatments were assessed using the canonical correspondence analysis. The indices of diversity of the control were significantly higher than all the other treatments ($p < 0.001$) with the mixed site having the lowest. There were significant differences in phosphorus, calcium, aluminium, soil alkalinity and diversity indices among the treatments. The results also indicated that the diversity indices and some soil properties were negatively associated with the mixed site. The negative impacts on the native diversity and change in the soil properties caused by the co-invasion of these two plants are more additive than non-additive. Therefore, priority should be placed on the management of co-invaded sites.

Keywords: *Hyptis suaveolens*; invasion; Nasarawa State; species diversity; *Urena lobata*

Introduction

There has been a proliferation of research on biological invasion in the last 20 years (Gurevitch et al. 2011). This is partly due to the realization of the negative impacts of the invasives on ecosystems and economy of nations of the world (Mack et al. 2000; Pyšek and Richardson 2010). History recorded that most of the studies on invasion have emphasized more on single species and factors enhancing their impacts on their invaded ecosystems (Davis 2006; Simberloff 2011a). Advances in plant invasion studies have produced trait-based approaches and mechanistic and probabilistic models in predicting the areas susceptible to such invasive plants (Kuebbing et al. 2013; Ordonez et al. 2010), produced potential environmental factors that drive the invasion (Fridley et al. 2007; Simberloff 2009; Drenovsky et al. 2012), and robust understanding of the mechanisms which produce much invasion impacts (Levine et al. 2003).

Among several effects, invasive plants have been reported to produce the following impacts on the native communities they invaded: they disrupt the plant-pollinator relationship by reducing visitation rates or making the habitats unconducive for pollinators (Brown et al. 2002; McKinney and Goodell 2010); releasing of allelopathic chemicals thereby reducing the growth rates of native plants (Stinson et al. 2006); altering the ecosystem services, such as nutrient cycling by changing the litter quality (Liao et al. 2008; Ehrenfeld 2010) or changing the intensity and timing of natural fire regimes, and modifying the structure of habitats (Simberloff 2011b).

There are relatively fewer studies on the impacts of biological invasions in tropical ecosystems around the world (Hulme et al. 2013; Zenni et al. 2017). Many other types of research in savanna ecosystems have focused on the community-level impact of invasive species rather than the impacts at ecosystem levels (Almeida-Neto et al. 2010; Rossi et al. 2014). Some ecosystems are invaded by multiple alien species. However, researchers don't focus on understanding the ecological combined impacts of co-occurring invasive species (Kuebbing et al. 2013; Lenda et al. 2019). Ecosystems invaded by co-occurring alien species do exhibit different ecological impacts depending on the nature of the species and their patterns of co-habiting (Zenni et al. 2020). This is because individual invasive species has a peculiar impact niche, which is described as the number and magnitude of ecological impacts it produces in the invaded ecosystem (Tekiela and Barney 2017).

Therefore, the interactions and overlap in the impact niches of each invasive species will determine how the impacts of their co-occurrence will be. This may be additive, non-additive, independent, antagonistic or synergistic. Literature has described these terms in clearer forms (Kuebbing et al. 2013). But independent means the impact niche of individual invasive species does not overlap one another.

In the present study, we filled the gap in the invasion ecology of plants by assessing the impacts of two co-occurring invasive plants *Hyptis suaveolens* and *Urena lobata* on soil chemical properties and resident plant communities in a guinea savanna ecosystem of North Central

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Nigeria. We hypothesize that the co-occurring invasive plants have higher impacts on the soil's chemical properties and resident plant communities. Consequently, the following research questions were asked: (1) Are there differences in resident plant communities and soil chemical properties among the treatments (control, *H. suaveolens* invaded, *U. lobata* invaded and two co-occurring species invaded communities)? (2) Does the co-occurrence of these two plants have a higher invasion impact? (3) Do relationships exist among resident plant diversity and soil chemical properties and the invasive species treatments?

Materials and Method

Study area

This study was conducted in Nasarawa State which is predominated by typical guinea savanna vegetation in Nigeria. The guinea savanna vegetation is characterized by abundant grasses and woody shrubs with few trees (Akomolafe et al. 2024). This State has a land area of 27117 km^2, a population of about 1,826,883 people and 13 local government areas also known as districts (Fig. 1). Nasarawa State occupies the central part of the middle belt region (north-central) of Nigeria. It has a topography ranging from lowland, undulating plain land and hills. This State is typified by a tropical rainy climate with a distinct dry season. It usually experiences up to seven months of rainy period and five months of the dry season. The annual rainfall ranges from 1200–2000 mm while the temperature ranges from 22.5 to 27.5 °C which may be higher during the peak of the dry season (Binbol and Marcus 2010). The indigenous people of the State are mainly farmers who are into the production of food crops such as groundnut, soybeans, melon, sesame, millet, yam and maize (Kwon-Ndung et al. 2016).

Study species

Hyptis suaveolens (L.) Poit. (family Lamiaceae) originated from the Neotropical regions and has now spread across several tropical and sub-tropical regions after its introduction. Its high adaptability to climatic conditions made it easier for this plant to naturalize itself in its introduced ranges (Padalia et al. 2015). The severity of its invasion has been reported in Nigeria, particularly in the savanna ecosystems Nigeria (Akomolafe et al. 2024; David et al. 2021). In the same vein, *Urena lobata* L. (family Malvaceae) has also been found to be a noxious weed in many parts of northern Nigeria (Akomolafe and Nkemdy 2020). Its aggressive invasiveness enabled it to outcompete native species in the invaded ecosystems.

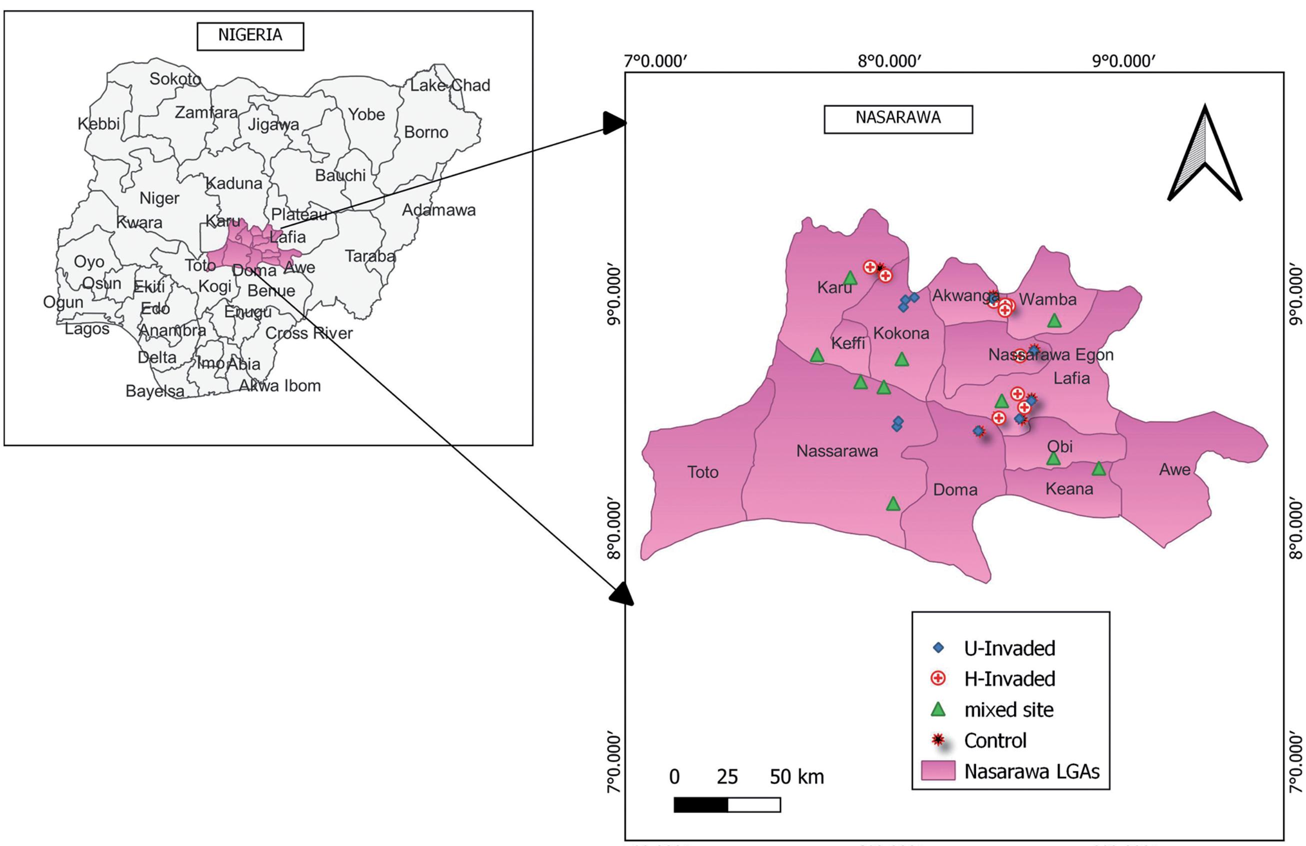

Fig. 1 Study area map showing the sampling sites.

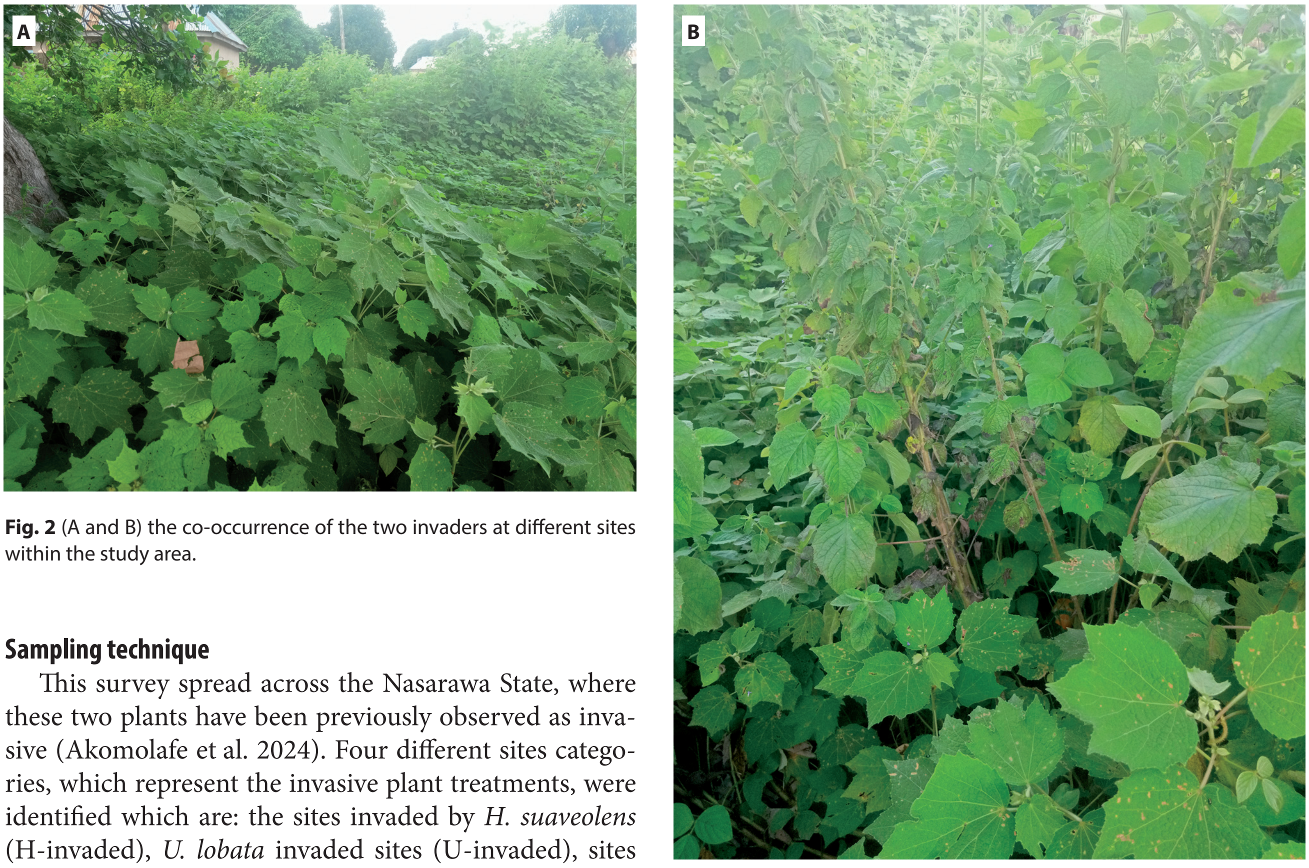

Fig. 2 (A and B) the co-occurrence of the two invaders at different sites within the study area.

Sampling technique

This survey spread across the Nasarawa State, where these two plants have been previously observed as invasive (Akomolafe et al. 2024). Four different sites categories, which represent the invasive plant treatments, were identified which are: the sites invaded by *H. suaveolens* (H-invaded), *U. lobata* invaded sites (U-invaded), sites where both plants co-invaded (mixed, Fig. 2), and uninvaded sites (control). We sampled 120 plots of 10×10 m^2 with 30 plots each in the invasive plant treatments mentioned earlier (i.e., H-invaded, U-invaded, mixed and control). In each plot, the abundance and frequency of all the plants found there were documented. Further precaution was taken to ensure that the selected plots have the same land use history, elevation range and soil type, so that the observed differences can be attributed only to species invasion (Coppi et al. 2022).

Soil sampling and chemical analysis

Ten soil samples were taken at ten different plots in each invasive plant treatment site using a soil core of 2.5 cm in diameter to a depth of 0–10 cm. This gave rise to a total of 40 soil samples. The samples were thereafter conveyed to the laboratory for drying at room temperature and sieving using a 2 mm sieve. The chemical contents of the soil such as the pH, phosphorus, nitrogen, calcium, potassium, magnesium, aluminium, soil organic matter and soil alkalinity were analysed using standard methods. The soil pH was determined using the glass electrode method in water suspension. Available phosphorus and potassium in the soil were determined using ammonium lactate extraction (Egnér et al. 1960). Other elements such as Al, Ca, and Mag were analysed after the digestion of the soil samples using concentrated acids (HNO_3 and H_2O_2) by following the methods described by Vujanovic et al. (2022).

Statistical analyses

We addressed the first question by determining the differences in resident plant communities and soil chemical properties among the treatments (i.e.: control, *H. suaveolens* invaded, *U. lobata* invaded and two co-occurring species invaded communities). The plant community structure was measured as alpha diversity (i.e.: species evenness, species richness, Simpson, and Shannon indices). The differences in the diversity indices among the treatments were determined using the Monte-Carlo permutation test. After this, generalized linear models with normal distribution for soil parameters and Poisson distribution for plant diversity indices were employed. In each of the univariate models, we chose soil chemical properties and plant diversity indices as the dependent variables, while the treatments were selected as the independent (categorical) variables. The significant difference among the treatments in each model was determined using the 95% confidence interval (CI). We also ran separate models using the invasive species treatments as numerical linear variables, and the results obtained were the same qualitatively as those of the previous models (Supplementary Table 1). All these analyses were performed in palaeontological statistics (PAST 3.0) software.

To determine the effect of invasive plant treatments on the resident plant diversity and soil chemical parameters, we performed a sparse partial least square discriminant analysis (sPLS-DA) using the XLSTAT package. In this analysis, we used the already identified significant plant diversity indices (Simpson, Shannon, and evenness indices) and soil chemical parameters (i.e.: phosphorus, calcium, aluminium, and soil alkalinity). Out of these variables, the analysis still removed some variables of lesser influence. Thereafter, we extracted the loadings and did a linear regression model using the treatments as the predictor and loadings as the dependent variable (Legendre and Legendre 2012).

The relationships between the soil chemical properties, plant diversity indices and invasive species treatments were assessed using the canonical correspondence analysis (CCA). In this CCA, the treatments were chosen as the response variables (the number of each plant species was the entry for each treatment), while the significant soil chemical properties (i.e.: phosphorus, calcium, aluminium, and soil alkalinity) and plant diversity indices (Simpson, Shannon, and evenness indices) were selected as constraining variables. The significance of the model was assessed using a permutation test (ANOVA-like) with 1000 bootstrap replicates. The CCA model loading was used to estimate the relationship between the plant species, soil chemical properties and treatments.

Results

Differences in plant diversity and soil chemical properties among the treatments

The different diversity indices of the invasive plant treatments are presented in Table 1. The indices of species richness and diversity of the control treatment are significantly higher than in all the other treatments ($p < 0.001$, Table 2). In comparing the mixed site with the U-invaded and H-invaded treatments, the mixed site had the significantly (at different p-values) lowest species richness and diversity, as revealed by several indices (Taxa_S, Shannon_H, Margalef and Fisher_ alpha). However, between U-invaded and H-invaded treatments, there were no significant differences in their diversity indices.

Our results showed general significant differences in phosphorus, calcium, aluminium, soil alkalinity, Simpson index, Shannon index, and evenness index among the invasive species treatments (Table 3), whereas the other soil chemical properties and species richness exhibited little variation across the treatments (Table 4). Phosphorus significantly increased in mixed site and control as compared to U-invaded and H-invaded, which were not significantly different from each other (Fig. 3A). Soil calcium and alkalinity in U-invaded, H-invaded, and mixed sites were significantly higher than in the control treatment (Fig. 3B and 3D). Also, aluminium content was significantly higher in the control than in the other treatments (Fig. 3C). The mixed site was observed to have the highest nitrogen and soil organic matter, though not significantly different from the single-species treatments. Simpson and Shannon indices in the control were significantly higher than in the other treatments (Fig. 3E and 3F). The evenness index in the control and mixed site were significantly higher than in the U-invaded and H-invaded treatments (Fig. 3G).

Effect of invasive plant treatments on plant diversity and soil chemical parameters

The results of the PLS–DA revealed that the first and second components explained 92.17% and 7.26% of the variance among the variables. Aluminium, phosphorus, Shannon index, Simpson index, and evenness index were positively correlated (each having correlation values of 0.43, 0.11, 0.43, 0.38, 0.29 respectively), while calcium and soil alkalinity were negatively correlated (each having correlation values of −0.44 and −0.45 respectively) with component 1. The regression analysis revealed that mixed treatment correlated most negatively and

Table 1 Diversity indices of the invasive plants invaded site treatments.

Diversity indices	U-invaded	H-invaded	Mixed site	Control
Taxa_S	16	15	4	68
Dominance_D	0.6286	0.5279	0.4496	0.02049
Simpson_1-D	0.3714	0.4721	0.5504	0.9795
Shannon_H	1.037	1.175	0.885	4.042
Evenness_e^H/S	0.1762	0.2158	0.6058	0.837
Brillouin	0.9635	1.07	0.8553	3.858
Menhinick	0.8889	1.115	0.2843	2.527
Margalef	2.595	2.693	0.5673	10.17
Equitability_J	0.3739	0.4337	0.6384	0.9578
Fisher_alpha	3.532	3.883	0.7099	18.39
Berger-Parker	0.7901	0.7127	0.4949	0.04696
Chao-1	16	17.5	4	68

Table 2 Significant differences in the diversity indices between the invasive plant treatments.

Diversity indices	U-invaded & H-invaded	U-invaded & Mixed site	U-invaded & Control	H-invaded & Mixed site	H-invaded & Control	Mixed site & Control
Taxa_S	0.9486	0.0001	0.0001	0.0003	0.0001	0.0001
Dominance_D	0.0067	0.0001	0.0001	0.0689	0.0001	0.0001
Simpson_1-D	0.3402	0.2824	0.0001	0.0305	0.0001	0.0001
Shannon_H	0.5324	0.0001	0.0001	0.0001	0.0001	0.0001
Evenness_e^H/S	0.0067	0.0001	0.0001	0.0689	0.0001	0.0001
Brillouin	0.3232	0.0002	0.0001	0.0001	0.9314	0.0001
Menhinick	0.8987	0.0001	0.0001	0.0001	0.0001	0.0001
Margalef	0.179	0.0001	0.0001	0.0001	0.0001	0.0001
Equitability_J	0.7481	0.0001	0.0001	0.0002	0.055	0.0001
Fisher_alpha	0.0928	0.0001	0.0001	0.0001	0.0001	0.0001
Berger-Parker	0.9486	0.0001	0.0001	0.0003	0.0001	0.0001
Chao-1	0	0	0	0	0	0

NB: The values represent p-values of the ANOVA-live permutation test.

Table 3 The soil chemical properties across the invasive plant treatments.

Soil properties	U-invaded	H-invaded	Mixed site	Control
pH (water)	5.7 ± 0.3	6.9 ± 0.2	6.2 ± 0.2	4.9 ± 0.3
pH (CaCl2)	5.3 ± 0.1	5.8 ± 0.2	5.4 ± 0.1	4.2 ± 0.2
Phosphorus (dag/kg)	0.5 ± 0.1	0.3 ± 0.1	1.5 ± 0.5	1.2 ± 0.1
Nitrogen (dag/kg)	0.32 ± 0.02	0.51 ± 0.02	1.62 ± 0.3	0.82 ± 0.1
Potassium (dag/kg)	34.1 ± 6.7	54.3 ± 12.3	96.71 ± 15.4	19.74 ± 6.5
Magnessium (cmol/dm^3)	1.2 ± 0.4	1.7 ± 0.2	1.6 ± 0.1	0.8 ± 0.1
Calcium (cmol/dm^3)	10.3 ± 1.2	10.8 ± 1.2	11.1 ± 1.4	4.5 ± 1.2
Aluminium (cmol/dm^3)	nd	nd	0.02 ± 0	0.84 ± 0.1
Soil organic matter (dag/kg)	12.1 ± 2.2	12.6 ± 2.2	16.6 ± 2.4	5.6 ± 1.5
soil alkalinity (sum of bases)	11.6 ± 2.0	12.7 ± 3.1	12.7 ± 3.2	5.4 ± 1.3

Nd means not detected.

Table 4 Summary of the regression model with invasive species treatments as predictor.

Response variable	Chi-square	P-value	R2
pH (water)	1.94	0.45	0.23
pH (CaCl2)	1.28	0.17	0.49
Phosphorus	1.09	0.03	0.86
Nitrogen	1.64	0.3	0.35
Potassium	2.5	0.99	0.02
Magnesium	2.08	0.53	0.17
Calcium	1.26	0.04	0.79
Aluminium	0.95	0.05	0.72
Soil organic matter	2.02	0.49	0.19
soil alkalinity	1.33	0.01	0.87
Species richness	1	0.41	0.43
Shannon index	1	0.02	0.56
Simpson index	1	0.05	0.85
Species evenness	1	0.05	0.93

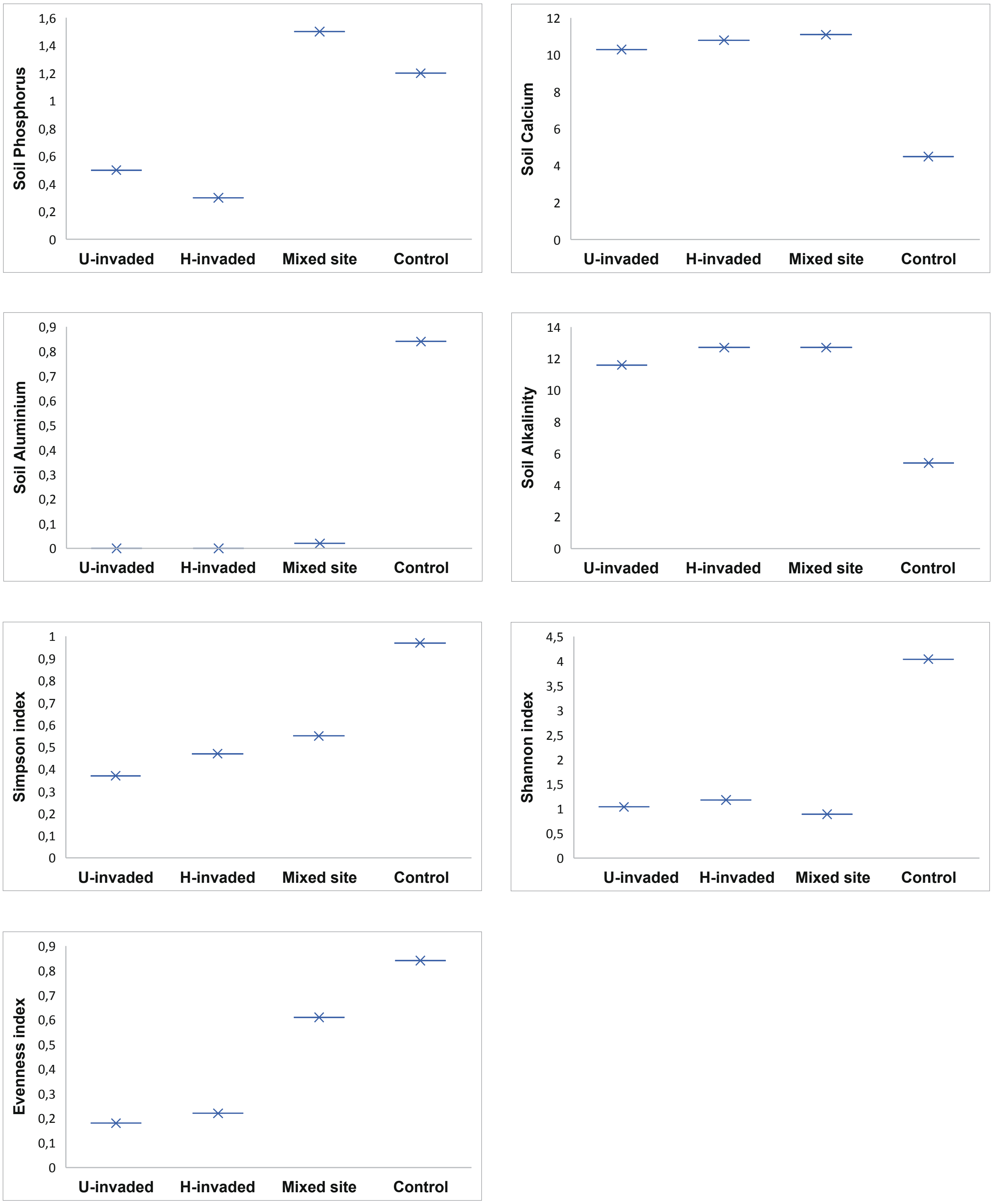

Fig. 3 Effects of invasive species treatments (U-invaded, H-invaded, Mixed site, and control) on different soil chemical properties phosphorus; calcium; aluminium; soil alkalinity and Plant community diversity indices Simpson index; Shannon index; evenness index.

significantly with the first axis (β = −2.47 ± 0.56, P = 0.02), while control was most positively correlated with the first axis (β = 0.12 ± 0.05, P > 0.05). The H-invaded (β = −0.84 ± 0.16, P > 0.05) and U-invaded (β = −0.04 ± 0.03, P > 0.05) which are single-species treatments were also negatively correlated with axis 1, though not significantly so.

Relationship among plant diversity, soil properties and the invasive species treatments

The multivariate canonical correspondence analysis revealed that the first two axes represent 100% of the total variance in the plant species distribution across the invasive species treatments (Fig. 4). The first axis represents 79.74% of the total variance. The mixed site was on the first axis (x_{mixed} = 5.38), while the other single-species treatments and control were distributed along the second axis ($x_{U\text{-}invaded}$ = 1.41, $x_{H\text{-}invaded}$ = 1.57 and $x_{control}$ = −0.69). Calcium and soil alkalinity were positively correlated with the first axis (0.93 and 0.91 respectively), while phosphorus (−0.11), aluminium (−0.93), Simpson index (−0.89), Shannon index (−0.96), and evenness index (−0.61) were negatively correlated with it.

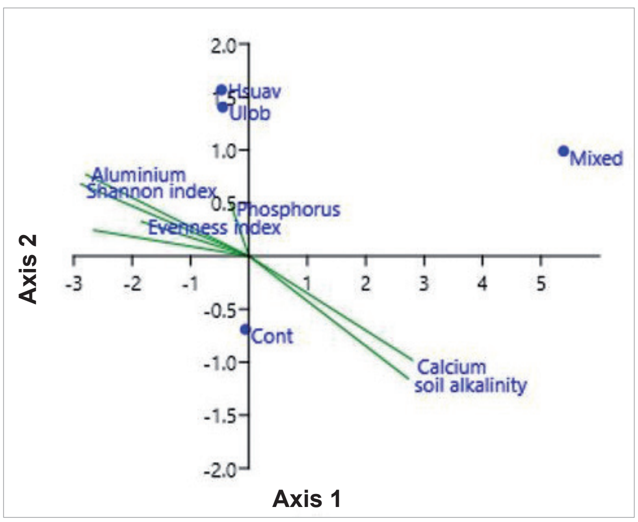

Fig. 4 CCA biplot showing the relationship among plant diversity, soil properties and the invasive species treatments.

Discussion

It is a well-known fact that invasive species are co-occurring in several ecosystems worldwide. However, studies exploring the impacts of the co-occurrence of these invaders (on both soil and native plant diversity) in West African ecosystems are very rare. Our study has revealed the differences in the impacts of the single-species invasion and co-occurrence in the study area. The greater impact of these co-occurring invasive species was observed in the lower plant species richness and diversity of the mixed site in comparison with the control and all the other single-species invaded treatments. The rate of dominance and colonization of invasive plants is normally expressed in the impacts they made on the native plant communities (Chmura et al. 2015; Czarniecka-Wiera et al. 2019). Our observation at the mixed sites where the two species co-occurred shows that both *H. suaveolens* and *U. lobata* contributed almost equally to the total plant cover. This could be a strong reason to deduce that the lower species richness and diversity observed at the mixed site were due to the harmonious impacts of these two invasive plants.

The cumulative allelopathic effects of the invaders could also be partly responsible for the reduction in the native species diversity of the mixed site since most invasive plants are known for exhibiting allelopathy (Coppi et al. 2022). It has not been clearly reported that *U. lobata* exhibits strong allelopathy in literature. However, several studies have reported the strong allelopathic effects of *H. suaveolens* on other plant species (Islam et al. 2013; Maiti et al. 2015; Poornima et al. 2015; Suntia and Singh 2015). At the mixed site, the reduction of the native species diversity affected both annual and perennial herbaceous and woody plants. This further proves the strong effects of the synergistic influence of the two invaders. Specifically, it was only two species *Hyptis lanceolata* and *Heteropogon contortus* that were found together with *H. suaveolens* and *U. lobata* at the mixed sites.

Our observations showed that there were variations in the soil's chemical properties among the invasive plant treatments. As revealed by the PLS-DA and regression analyses, the co-occurring species at the mixed site exerted a more significant negative influence on the soil properties and diversity indices, such as aluminium, phosphorus, Shannon index, Simpson index, and evenness index than when occurring singly at U-invaded and H-invaded sites. This further indicates the joint effects of these two invaders on the soil properties, when co-occurring. Soils invaded by co-occurring invasive plants have been described as normally having a high amount of nitrogen, carbon, and organic matter (Coppi et al. 2022). This was not totally true in our study, where the mixed site had a higher amount of nitrogen and organic matter, but not significantly different from the other treatments. A large amount of nitrogen and organic matter was said to have been due to the higher amount of litter produced by the co-occurring high-impact invasive plants, which decomposed at a more rapid rate than in other treatments (Krevš et al. 2013; Jo et al. 2016; Incerti et al. 2018).

Although our study did not involve the determination of the soil microbial communities of these invasive plant treatments, past studies have related the increase in soil organic carbon and nitrogen directly to the increased activities of soil microbes (Jo et al. 2017; Coppi et al. 2022). The results of the CCA also indicate that the mixed site had a strong negative relationship with the diversity indices. This further suggests that the influence of the joint invasion of the two plants reduces the plant diversity and richness of the areas affected.

Conclusion

From our observations in this study, we conclude that the joint invasion by *U. lobata* and *H. suaveolens* in the study area could promote their persistence in the ecosystem through the strong negative impacts on the native species diversity and change in the soil properties. The impacts of these two co-occurring invaders in the study area tend to be more additive than non-additive. By implication, restoration efforts on these co-invaded sites might be more difficult in the future if neglected or not prioritized now. Therefore, it is recommended that more priority be placed on the application of integrative control and management methods on these two invasive plants in the study area and any other part of the world, where co-occurring invasive species have threatened the natural ecosystems. This will help in reducing the negative effects of these plants on the native plant species.

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INTERNATIONAL HUMANITARIAN LAW AND ECOCIDE: THE WAR IN UKRAINE AS A CASE STUDY

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ABSTRACT

Ecocide, an illegal act that causes serious damage to the environment, has become one of the greatest threats to the planet. Ukraine, which has recently been exposed to ecocide, strives to bring the perpetrators to justice by applying to international judicial bodies and creating mechanisms for collecting evidence. Ecocide should be included in the list of crimes against humanity and properly regulated at the international level, which will contribute to the preservation of the environment and human security. The purpose of this article is to define the crime of ecocide under international humanitarian law, apply it to the conflict in Ukraine, and discuss possible international mechanisms for bringing to justice those who have committed ecocide. This paper showcases Ukraine's legal and factual basis for holding Russia accountable for ecocide, which includes the destruction of natural resources, water and air pollution and actions that could result in environmental catastrophes. This paper emphasizes the importance of filing lawsuits with international bodies, international cooperation, public engagement, and resource mobilization for effective prosecution. Ukraine's commitment to environmental protection through the pursuit of ecocide accountability is of global significance for the preservation of the planet and well-being of future generations.

Keywords: crime of ecocide; ecocide; environmental safety; international legal responsibility; international mechanism

Introduction

One of the main problems facing humanity today is the need to address the issues of world-wide environmental security and preservation of biodiversity that are under threat due to intense pollution and environmental degradation caused by various economic, military, and political factors and, above all, deliberate actions. Ecocide is one of the most serious offences in this regard, which gives grounds for considering it as a crime against the security of humanity and the environment.

Ecocide is the commission of any deliberate unlawful act that causes serious, irreversible, and long-term damage to the environment. This term comes from Greek, meaning killing or destroying one's home. Ecocide, a negative human effect on the environment, being committed deliberately or due to lack of responsibility, causes massive destruction of entire ecosystems that sustain all life on Earth in the long term. One example is the Chornobyl disaster in 1986 when an explosion released toxic radioactive substances into the atmosphere (even more than the atomic bombing of Hiroshima). Currently, Russia's actions have caused fires to break out in the Chornobyl forest exclusion zone in Ukraine. In addition, the Kakhovka hydroelectric power plant was blown up resulting in severe and irreversible damage to the environment in the south of the country.

From the first days of the war, Ukraine established an Operational Headquarters at the State Environmental Inspectorate of Ukraine to compile a list of all environmental violations and bring Russia to justice. To collect data on environmental crimes, an app was created that allows anyone to record information about the environmental damage caused by the aggressor state. Thus, the ongoing Russian-Ukrainian war led to changes in climate in Ukraine and other countries, including Russia and Belarus, which are now committing these crimes, will also suffer from pollution. Currently, hundreds of thousands of square kilometers of soil have been degraded and land severely disturbed, forests burnt, large quantities of toxic substances emitted into the atmosphere, animals killed and due to the hostilities changes in bird migration routes have been recorded in Ukraine. The Ministry of Environmental Protection and Natural Resources collects information on such crimes against Ukraine committed by the Russian Federation to file lawsuits in international courts.

An urgent problem for civilization is the creation of a mechanism to ensure environmental safety. Environmental lawyers actively discuss and attempt to include ecocide in the list of crimes against humanity, such as war, genocide, etc. This, in turn, would criminalize ecocide at the international level and trigger the mechanism of international legal liability. Thus, the purpose of this article

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is to define the crime of ecocide under international humanitarian law, apply it to the conflict in Ukraine and discuss possible international mechanisms for bringing to justice those who have committed ecocide.

To achieve this, numerous methods and approaches were used. For example, a literature review provided a theoretical basis for understanding and analyzing the key ecocide concepts, principles, and international documents, including the Rome Statute of the International Criminal Court. This made it possible to determine the rules of international humanitarian law applicable to environmental crimes committed in armed conflicts. National legislation and policy documents related to the war in Ukraine were studied to assess its effect on the environment and identify legal frameworks for accountability. International mechanisms for prosecuting perpetrators of ecocide, including the International Criminal Court, were analyzed.

The method of synthesis and analysis ensured a comprehensive understanding of the interconnection between ecocide, humanitarian law, and the war in Ukraine. Induction was used to draw general conclusions and helped establish cause-effect relationships between ecocide and liability. Analogical reasoning made it possible to compare cases of ecocide in Ukraine with those in other countries or conflicts and by abstraction key concepts and rules of humanitarian law were highlighted.

Thus, based on analytical research and the literature review, this article formulates a definition of the crime of ecocide under international humanitarian law, considers its application to the conflict in Ukraine and discusses possible international mechanisms for bringing to justice those who commit ecocide.

Results

The concept of ecocide in international legal literature: the problem of definition

Attention to environmental pollution is fully justified since human activity, industrial processes, and wars destroy the ecology of the planet, thereby causing irreversible damage and bringing humanity closer to an environmental catastrophe. Such actions are defined as ecocide, which, in turn, is a partially criminalized act under both international and national criminal law in many countries.

The term ecocide was first used in 1972 after the United Nations Conference on the Human Environment considered environmental destruction caused by bombing, herbicide use and large-scale construction in natural areas. The problem of ecocide as an independent type of international crime became more relevant during the Vietnam War when the United States used chemical weapons, which destroyed a significant part of the forests. A special place in the theoretical development of the concept of ecocide as an international crime and the methods of its commission in the context of an aggressive war was initiated by Vietnamese lawyers.

In international legal literature, ecocide is defined as the use of geophysical, meteorological, and other means to alter the dynamics, composition, or structure of the Earth (its biosphere, lithosphere, hydrosphere, and atmosphere) and outer space, which may or has led to a massive loss of flora or fauna, poisoning of the atmosphere, water resources or other serious consequences (Baranenko and Rusin 2023). For example, Article 1 of the Convention on Environmental Impact Assessment in a Transboundary Context prohibits resorting to any means of affecting the environment "that cause its widespread, long-term or severe damage when attempting to injure or destroy health or territory of any other State Party" (United Nations Economic Commission for Europe 2004).

According to the position of the International Criminal Court (ICC), ecocide can be considered one of the crimes against humanity and may fall under its jurisdiction in the future. The act of ecocide was first officially enshrined at the international legal level in 1991. Thus, Article 26 of the Code of Crimes against the Peace and Security of Mankind (International Law Commission 1991) stipulates that "a person who intentionally causes or orders damage to the environment shall, upon conviction, be punished." However, after many legal discussions, Article 26 was excluded in 1995, and it became impossible to bring individual actors to international legal responsibility under the article on ecocide (Hasler 2022).

The next attempt to criminalize ecocide was an indirect mention in the Rome Statute of the International Criminal Court (United Nations General Assembly 1998a) in 2002. However, no separate article enshrined this crime, but it was mentioned in the context of war crimes, armed conflicts, or crimes against humanity, which include the destruction of the natural environment resulting from attacks on the population of a region. There is no direct definition of ecocide at the level of international legal documents, although global lawyers actively discuss this issue. Thus, a group of experts on international law, with the support of the Stop the Ecocide Foundation, tried to develop a clear definition of ecocide in 2021. This, with the support of Swedish parliamentarians, was launched in November 2020 on the 75th anniversary of the opening of the Nuremberg War Crimes Trials of Nazi leaders in 1945. The coordinators were Philip Sands of University College, London and Florence Mumba, a former judge of the International Criminal Court. The Stop Ecocide Foundation hoped governments and the International Criminal Court would consider their work when investigating crimes involving the environment (Mynkovich-Slobodianyk 2023).

Even though ecocide is not defined and codified in international law, there are many definitions of this term in legal literature. Thus, it is advisable to formulate the authors' definition of ecocide. Ecocide encompasses deliberate and unlawful actions resulting in systematic and large-scale damage to the environment or its constituent components, as a subject of international law. This includes various actions, such as deforestation, excessive discharge of toxic substances, pollution of water bodies, as well as other types of crimes that lead to a serious disruption of the ecology in an area. Ecocide has severe consequences for biodiversity, climate, and human health. The term is defined not only by the execution of actions but also by their systematic and intentional nature. As a result of ecocide, there may be a need for legal mechanisms for determining liability, compensation for damage and measures for restoring and protecting nature. This term emphasizes the need to take measures to prevent and punish serious environmental violations.

Legal frameworks for prosecuting ecocide: international agreements and mechanisms

International agreements on environmental protection are crucial elements and effective mechanisms of cooperation between states and international organizations to prevent damage to the environment and human health. Therefore, the existence of a variety of international environmental treaties is quite reasonable. First of all, it is necessary to consider the following statutory acts: the World Charter for Nature (United Nations General Assembly 1982), the Rio Declaration on Environment and Development (United Nations General Assembly 1992), the Convention on Access to Information, Public Participation in Decision-making and Access to Justice in Environmental Matters (United Nations General Assembly 1998b), the Johannesburg Declaration on Sustainable Development (United Nations Department of Public Information 2002), etc. Equally important is the work of various international intergovernmental and non-governmental organizations dealing with environmental issues, including the UN, IAEA, UNESCO, IMO, FAO, etc. All of them pay great attention to the issue of ecocide.

While there is no single international law covering ecocide, some tools and procedures can be used to bring perpetrators to justice. Here are some examples of international mechanisms used for this purpose (Heidary and Vaezi 2020). Articles 35 and 55 of the Additional Protocol (AP) I to the Geneva Conventions of 12 August 1949 contain provisions directly aimed at protecting the environment during international armed conflicts (High Contracting Parties 1977). Paragraph 3 of Article 35 prohibits the use of methods or means of warfare intended or expected to cause widespread, prolonged, and severe damage to the natural environment. Article 55 stipulates that care should be taken to protect the natural environment from widespread, prolonged, and severe damage during hostilities. Such protection involves prohibiting the use of methods or means of warfare that are intended or expected to cause such damage to the natural environment and thereby endanger the health or survival of the population. Damage to the natural environment as a form of reprisal is prohibited.

These articles differ in that, firstly, Art. 35 prohibits the intentional use of methods and means of warfare that may cause damage to the environment, while Art. 55 imposes on states the duty of care to protect the environment during armed conflicts. Secondly, Art. 35 is aimed at specifically protecting the environment, because of its importance and intrinsic value, and Article 55 is aimed at protecting the environment to the extent necessary for the protection and defense of victims of war, the civilian population (Mynkovich-Slobodyanyk 2023). To be held liable for violations of the environmental protection requirements of AP I, the damage must be extensive, long-term, and severe (all three conditions should be met simultaneously). However, the concepts of "large, long-term and severe damage" are not defined in the AP I. The preparatory documents of the AP I used for its interpretation and doctrinal opinions make it clear that damage is considered extensive if it covers an area of about 20 thousand square kilometers. Damage is specified as long-term, if it lasts for several decades and is classified as severe if it also causes damage to the health or survival of the population.

It is controversial whether damage caused by war involving conventional weapons falls within the scope of Article 35(3) or Article 55 of the AP I: the impossibility of their further use is due to the danger associated with the explosives. The narrow interpretation of Article 35(3) and Article 55 can be considered outdated since "large, long-term and severe damage" is not legally defined in the Protocol; thus, the nature of the latter is relative and subject to change depending on the assessment of the qualifying authority (Panigaj and Bernikova 2023).

Even though the consequences of an attack fall outside the definition of "large, long-term and severe damage," it does not mean they are lawful. These consequences may violate other principles of International Humanitarian Law (proportionality, distinction, humanity, military necessity). The International Criminal Court has jurisdiction over war crimes, among which Article 8.2.b.iv of the 1998 Rome Statute distinguishes the intentional commission of an attack that causes damage to civilian objects or extensive, long-term, and severe damage to the natural environment if it is manifestly disproportionate to the specific and directly anticipated overall military advantage. In addition to the need to meet the cumulative standard, the offense requires proof of intent to commit an attack. Furthermore, the awareness of the significant destructive consequences of such an attack should be proved. Finally, the damage should be recognized as disproportionate in terms of the anticipated overall military advantage.

The cumulative standard is complemented by a requirement to apply a proportionality and military necessity test. Article 8 qualifies "crimes against the environment" as war crimes in section (b) instead of section (a), which, in turn, defines grave breaches of the Geneva Convention. In addition, the provision of this article applies exclusively to international armed conflicts. However, crimes against the environment may constitute a material element of other crimes, such as genocide, crimes against humanity and other war crimes. In the 2009 Prosecutor v. Omar Al-Bashir decision, the International Criminal Court did not deny a link between environmental degradation and genocide (Borshchevska 2023).

The provisions of the main treaties on liability for environmental damage caused by armed conflict (AP I, the Environmental Modification Convention, and the Rome Statute) were not developed according to the logic of international environmental law but international humanitarian law. This is evident, firstly, in the subject matter of their regulation. Thus, one can find "hostile use of means of influence" in the Environmental Modification Convention, "methods and means of warfare", in AP I, and "attack" in the Rome Statute. Secondly, the prevailing doctrinal view is that IHL rules are *lex specialis* about international environmental law. However, the recommendations of international environmental law also contain provisions prohibiting damage to the environment. The World Charter for Nature stipulates, "Nature shall be protected from pillage because of war or other hostilities. Military actions which cause damage to nature should be refrained from."

Prosecution for environmental damage caused in times of war is more problematic than in peacetime. International liability under international criminal law may be incurred both for the state and state officials. At the same time, no international tribunal or criminal court has yet ruled on criminal liability for purely "environmental" crimes committed during an armed conflict (Hotz 2021). The International Tribunal for the Law of the Sea (ITLOS) is one of the international mechanisms for resolving disputes related to the United Nations Law of the Sea (UNCLOS) (United Nations 1982). ITLOS deals with disputes arising out of the application or interpretation of the UNCLOS, including those relating to the marine environment and environmental issues. ITLOS resolves disputes over the establishment of maritime boundaries and exclusive economic zones as per the requirements of the UNCLOS. ITLOS has jurisdiction over cases for protecting and preserving the marine environment. This involves prosecuting activities that cause damage to the marine environment, such as marine pollution, violation of the law of the sea, loss of biodiversity, etc. The ITLOS can resolve disputes related to compensation for environmental damage, in particular the determination of the liability of states or entities for violations of the law of the sea and determine the amount of compensation (Killean 2021).

Another example is the International Convention for the Conservation and Use of the Marine Environment of the Atlantic Ocean (OSPAR) (1998), which also provides mechanisms for the regulation and protection of the marine environment. The Convention is aimed at conserving and restoring marine biodiversity in the region, in particular, ensuring the sustainability of ecosystems and maintaining species in their natural environment. The Convention defines measures to prevent, reduce and control marine pollution by limiting emissions and discharges of hazardous substances. OSPAR provides a forum for cooperation between countries so that they can jointly develop and implement measures to protect the marine environment. On the legal side, the OSPAR Convention contains legal obligations for participants to implement measures to ensure the protection and sustainable use of the marine environment. In cases of violation of the Convention's provisions, it provides for the possibility of bringing a complaint by people or other participating states to people or other participating states before arbitration or the International Court of Justice. OSPAR is an important tool for regulating and controlling human activities in the marine environment and ensuring its sustainable use. Both mechanisms, ITLOS under UNCLOS and conventions such as OSPAR, establish a legal framework for handling cases related to environmental damage and provide opportunities for judicial resolution of issues related to marine environmental conservation (Artamonova et al. 2022).

If the ICC finds a person guilty of a war crime, in particular, environmental destruction under Article 8(2)(b)(iv) of the Rome Statute, possible consequences include imprisonment for the convicted person. ITLOS is responsible for resolving disputes between states regarding the establishment of maritime boundaries, decisions on environmental issues and the obligation of states to comply with the tribunal's decisions. The OSPAR Convention aims to protect and preserve the marine environment and determine mechanisms for compensating for the damage caused. States may be obliged to stop or reduce certain activities that cause damage and to compensate for the damage caused. Both mechanisms, the ICC and ITLOS/OSPAR, play a crucial role in establishing liability and ensuring the protection of the marine environment. However, their consequences are considered at different levels, namely, individual criminal liability or the collective responsibility of states (Moribe et al. 2023).

Environmental devastation caused by Russian military aggression in Ukraine

It is worth noting that the ICC already criminalizes environmental destruction during wartime or armed conflict under Article 8(2)(b)(iv) as a war crime. This provision places a significant emphasis on the protection of the natural environment in times of war, recognizing the importance of avoiding the unintended environmental consequences of hostilities. Article 8(2)(b)(iv) demonstrates that the ICC recognizes the importance of protecting the environment as a component of the general welfare in times of war. This provision is designed to prevent unforeseen environmental consequences of hostilities.

Article 8(2)(b)(iv) establishes legal responsibility for war crimes involving the environment and provides a mechanism for investigation and prosecution. When a case of environmental degradation is brought to the ICC, it can pave the way for a trial where an objective investigation and conviction for these crimes during war can be carried out. The general purpose of Article 8(2)(b)(iv) is to establish responsibility for protecting the environment in wartime and to create a legal mechanism for the protection of ecosystems in times of military conflict (Branch and Minkova 2023).

Even though environmental reparations are an exception in the ICC's history, they mean that the aggressor country must pay for such damage. The issue related to the fact of reparations is the proven amount of damage caused by the actions of the Russian army. According to the Ministry of Ecology of Ukraine, Russia is responsible for numerous natural disasters, such as large-scale explosions, ammonia storage explosions and attacks on nature reserves. There are also indirect consequences, such as dangerous amounts of greenhouse gases, carbon dioxide, or methane emissions into the atmosphere, which harm human health in the long term. Russian aggression against Ukraine has led to numerous local disasters; therefore, Ukraine's legal position on damage to the environment in claims against Russia should focus on documenting direct, hidden, and long-term damage from Russia's post-invasion actions (Rybachek 2022b). Although the environmental dimension will be presented as part of the broader genocide proceedings, it is possible to maximize the punishment for damage to the environment committed by the Russian Federation.

Currently, Russian troops harm the environment in Ukraine both intentionally, for military and political reasons, and indirectly. The short list of threats posed by Russian military aggression involving the environment in Ukraine includes forest fires caused by shelling, military equipment, missiles, ammunition, oil products poisoning soil and water resources, chemical emissions from shelled industrial enterprises, and risk of radiation accidents. According to the State Environmental Inspectorate, Russian aggression has caused more than UAH 1 trillion 743 billion worth of damage to the environment over eleven months of the war. Damage due to soil and land pollution amounted to over UAH 688 billion, air pollution to UAH 998 billion, and water pollution and contamination over UAH 56 million. Furthermore, over 6,000,000 farm animals and about 50,000 dolphins have been killed so far during this war. Despite the existence of a criminal article, it was only in 2022 that the Prosecutor Geiseral's Office reported suspicions of ecocide for the first time in Ukraine's history. The main reason for this was the results of the State Environmental Inspectorate's control measures conducted from 2015 to 2022. It took experts seven years to create an evidence base for pollution of rivers, which led to irreversible changes in the ecosystem (UKRAINFORM 2023).

The enemy troops are trying to do as much damage to the environment, infrastructure, and the economy of Ukraine as possible. The invaders deliberately degrade or even destroy Ukrainian nature. The first cases of deliberate arson by Russian troops began in early March 2022 in the Chornobyl Exclusion Zone. The fire destroyed 15,000 hectares of forests (including those contaminated with radiation) in the area from Irpin to Chornobyl. Equally large-scale fires raged in the Kherson region (in the Black Sea Biosphere Reserve, Sviatoslav's Biloberezhzhia National Nature Park) and the Donetsk region in the Holy Mountains National Nature Park. This includes the so-called Sarmatian forests, the sodden pine forests on the left bank of the Siverskyi Donets River in Kharkiv, Donetsk and Luhansk oblasts (UKRAINFORM 2023).

According to experts, it is impossible to restore Ukrainian forests under current conditions. In Kherson, the occupiers set fire to the Dnipro floodplains that are part of the Emerald Network on the Lower Dnipro. Such actions lead to a decrease in biodiversity, environmental pollution and damage or destruction of valuable habitats. More than 1,000 square kilometers of the Black Sea surface are polluted by oil from Russian military aircraft and ships, killing about 50,000 Black Sea dolphins.

The list of Russia's crimes against the environment also includes the following: the bombing of fuel and lubricant warehouses, oil product storage facilities and enterprises that may use hazardous and (or) chemical substances in production; damage, destruction, or suspension of the operation of wastewater treatment facilities; destruction of filtration field dams and leakage of waste onto the terrain; destruction of treatment or hydraulic structures; ignition (burning, smoldering) of landfills; degradation of soil and deforestation (Shamsutdinov 2023). The movement of heavy military equipment, explosions and fires contaminate natural and agroecosystems and degrade soil. The latter is due to mixing and over-compaction of soil horizons, disturbance of the upper fertile layer, chemical degradation, burnt soil, contamination with explosives, remnants of munitions, or various military equipment. Significant damage is caused to the air, soil, and water because of missile strikes on infrastructure facilities.

Russia has exposed Ukraine and the world to nuclear danger by resorting to international terrorism at nuclear facilities. This involves seizure, damage and affecting their functioning, which could lead to global accidents. The cost of the damage caused by Russian troops at the Chornobyl Nuclear Power Plant is at least UAH 2.5 billion. The level of nuclear threat remains high at Zaporizhzhia Nuclear Power Plant. Russian troops regularly shell the plant, deploy heavy weapons close by and do not allow Ukrainian experts and IAEA representatives to carry out repairs. The exclusion zone around the Zaporizhzhia Nuclear Power Plant in the event of a potential nuclear explosion could be up to 30,000 square kilometers (Rybacek 2023). The blowing-up of the Kakhovka hydroelectric power plant by Russian troops has already had many catastrophic consequences and will have even more in the long term. The consequences of such actions are the flooding of dozens of settlements and civilian casualties, the erosion of minefields and waste dumps, the unavailability of water for irrigation, the killing of Red Book species and the threat to the operation of the Zaporizhzhia Nuclear Power Plant.

Russia must be held accountable for its crimes against the environment. To this end, the Operational Headquarters of the State Environmental Inspectorate of Ukraine was established to compile a list of all violations involving the environment, record ecocide and bring Russia to justice. On the initiative of the Verkhovna Rada Committee on Environmental Policy and Nature Management, the Ministry of Environmental Protection and Natural Resources, the State Environmental Inspectorate of Ukraine, NGOs and eco-warriors, information on Russia's crimes involving the environment is being collected. To collect data on these crimes, it was agreed to allow anyone to record information about damage caused to the environment by an aggressor state. In addition, the Ministry of Environmental Protection and Natural Resources of Ukraine, with the support of the Ministry of Digital Transformation of Ukraine and other partners, created EcoThreat, a separate official resource on the national online platform EcoSystem, for collecting and recording information on threats to the environment in real-time.

Currently, Ukrainian government agencies (the Verkhovna Rada, the Committee on Environmental Policy and Nature Management, the Ministry of Environmental Protection and Natural Resources and the State Environmental Inspectorate of Ukraine), volunteers, eco-warriors and citizens are actively working to record and document ecocide caused by Russia's military activity in Ukraine. This information will form the basis for determining the extent and cost of damage caused by military aggression, which the International Criminal Court needs, if it is to investigate war crimes against humanity and damage to the environment committed in Ukraine (Syvodyed 2022).

Legal framework and challenges in prosecuting ecocide

Responsibility for ecocide is stipulated in Article 441 of the Criminal Code of Ukraine (CCU) (Verkhovna Rada of Ukraine 1998). The legislator defines this crime as "mass destruction of flora or fauna, poisoning of the atmosphere or water resources, as well as other actions that may cause an environmental disaster." According to Article 12(6) of the CCU, the punishment for acts of ecocide is imprisonment for a term of 8 to 15 years, which indicates ecocide is a very serious crime. Similar provisions are provided for in the criminal laws of some other countries, including Australia, England, Estonia, Canada, Germany, Finland, Croatia, Slovenia, Serbia, and Montenegro. The generic object of this offense is public relations to ensure peace, human security, and international law and order.

The crime of ecocide is included in Chapter 20 of the CCU, "Criminal Offences against Peace, Human Security and International Law and Order." However, the CCU has Chapter 8, "Criminal Offences against the Environment." Therefore, ecocide is a more global and widespread crime. The disposition of Art. 441 of the CCU contains many evaluative concepts. What do "mass destruction," "poisoning," and "environmental catastrophe" mean? What is the difference between "illegal logging" (punishable under Article 246 of the CCU) and "mass destruction of flora" (Article 441 of the CCU)?

The subject of ecocide is a person of sound mind who is 16 years of age or older. The subjective side of ecocide is criminal intent. It should be noted that direct intent is more typical for this crime. Such a criminal offence is committed when any of the acts specified in Article 441 of the CCU could have caused an environmental disaster. An act is classified as a criminal offence, when its consequences are mass destruction of flora or fauna or poisoning of the atmosphere or water resources. For example, marine pollution that causes mass deaths of flora and fauna or other grave consequences is classified under Part 2 of Article 243 of the CCU (Prodan et al. 2023).

However, Russian troops are not the only perpetrators of ecocide. Furthermore, this crime can be committed not only in wartime. Currently, the Specialized Environmental Prosecutor's Office of the Prosecutor General's Office is working on a case against employees of a cardboard and paper mill in Khmelnytskyi Oblast. For several years, this company discharged contaminated water into the small Khomora River. As a result, several kilometers of the river were damaged to the extent that it is almost impossible to restore nature. This case qualifies as ecocide (McIntyre-Mills 2021).

Under Section 11.4 of the draft CCU, one of the manifestations of war crimes is the knowledge that a directed attack will, among other things, cause large, long-term, and severe damage to the environment, which is disproportionate to the specific and directly anticipated overall military advantage. Thus, liability is established for an environmental effect, which is when a prohibited means of warfare is used. However, as provided for in the current CCU, ecocide (on a par with genocide) should be recognized as an independent international crime. The draft CCU does not provide for a separate article on liability for such actions as an element of crimes against international law and order, which needs to be amended. It is important not only to punish Russia for all crimes of ecocide committed in Ukraine since 2014, but also to force the terrorist state to compensate for all the environmental damage caused (Mynkovich-Slobodyanyk 2023).

Currently, the priority task for Ukraine is to estimate the ecosystem services lost by the Ukrainian people and include this in the total reparation to be paid by the Russian Federation. However, the current methods of calculating environmental damage discussed above do not fully consider the damage caused by the loss of ecosystem services. Ecosystem services are all the useful resources and benefits obtained from nature. Ecosystem services are crucial for satisfying fundamental human needs for habitat and food and directly affect our standard of living. The loss of ecosystem services has quite devastating consequences. Thus, the destruction and damage of forests will lead to large-scale wind erosion and desertification of entire regions in the future.

All ecosystem services are free, but some of them should be monetized, i.e., valued in monetary terms. Such monetization is necessary to include the value of the ecosystem services lost in the reparation to be paid by Russia to Ukraine. When creating a compensation mechanism, it is crucial to consider the international practice of compensation for environmental damage caused by military operations, particularly the practice of the International Court of Justice. For example, the concept of ecosystem services was used in the case of Costa Rica v. Nicaragua. Costa Rica used the "ecosystem services approach" to determine the amount of environmental damage, according to which the value of the environment was assessed through the services and goods it could provide and which might or might not be sold on the market (Borshchevska 2023). It is necessary to consider the direct and indirect use value of ecosystem services and goods as it allows the assessing of the full and potentially long-term damage caused to the environment.

In developing doctrinal approaches to these issues, the historical experience of establishing special compensation bodies should be analyzed. For example, the UN established a separate body, the United Nations Compensation Commission, to compensate for environmental damage and other losses caused by Iraq during its armed aggression against Kuwait (1990–1991). The United Nations Compensation Commission was established as a temporary subsidiary body of the UN Security Council in 1991 to consider claims and payment of compensation for losses and damage caused by the illegal invasion by Iraq and occupation of Kuwait. The Compensation Commission operated for 31 years and was terminated in 2022. Iraq paid about 52.4 billion in damages as of 2022 for all claims of all categories (5.26 billion was paid to compensate for environmental damage).

Currently, it is crucial for Ukraine to actively and systematically collect evidence, record, and store environmental monitoring data, document environmental effects and assess environmental damage using standard methods and be prepared to substantiate claims against the aggressor state at the international level (Rybachek 2022a). Nevertheless, the formalization of the public danger and punishment for ecocide in no way indicates the effectiveness and proper environmental protection. Therefore, in addition to legislation, it is necessary to study the practical dimension, i.e., the Unified register of pre-trial investigations. The number of criminal proceedings, the number of verdicts and their ratio are a measure of the effectiveness of a particular law on criminal liability so that pre-trial investigation bodies, procedural managers and courts know how to "work" with this type of criminal offense.

In this context, it should be noted that problems with the enforcement of Article 441 of the Criminal Code of Ukraine existed even before the full-scale invasion. For example, against the backdrop of large-scale floods in the Carpathian region in June 2020, a coordination meeting on environmental issues was held for the first time in Ukraine since its independence. The former Prosecutor General Iryna Venediktova noted that more than four dozen laws regulated environmental protection. However, the system of liability for environmental offences and guaranteeing respect for the environment in terms of human rights remains relatively weak. For this reason, 2021 should be defined as a period of active environmental protection and effective cooperation between law enforcement agencies in this area (Pavlova 2023).

At the same coordination meeting on environmental issues, Ivan Bakanov, the former Head of the Security Service of Ukraine, reported 184 criminal proceedings for crimes involving the environment. Ivan Bakanov identified environmental issues as one of the elements of national security. This raises the question whether 2021 was the start of a period of active environmental protection and effective cooperation between law enforcement agencies in this area. The answer to this question can be found in the Unified State Register of Court Decisions (USRCD). This document reveals that there were no verdicts in the USRCD for cases of ecocide or Article 441 of the Criminal Code of Ukraine. Thus, pre-trial investigations of criminal proceedings for environmental crimes, including ecocide, conducted by the Security Service of Ukraine showed their inefficiency, as no act was convicted by a local general court (Security Service of Ukraine 2021).

Today, the issue of liability for environmental crimes has taken on new significance. Pollution of air, water and soil, forests and agricultural land and attacks on nuclear facilities are considered war crimes. People in Ukraine and beyond will feel the consequences of these actions for decades to come, as the effects will extend beyond the borders of Ukraine. The vulnerability of nature to such effects endangers ecosystems and leads to large-scale environmental problems that are not limited to the area of conflict. It is worth noting that pre-trial investigations are currently underway on more than two hundred proceedings on Russian war crimes involving the environment, including 14 acts classified as ecocide. It will take Ukraine 20 to 30 years to fully assess the damage caused by Russian aggression and calculate direct and indirect losses.

Given that ecocide is not subject to the rule set out in Part 5 of Article 49 of the CCU, it can be concluded that the perpetrator will be released from criminal liability upon the expiry of the fifteen years from the date of the criminal offence and the proceedings closed. Therefore, it is reasonable to assume that all 14 proceedings for acts classified as ecocide are unlikely to result in a verdict.

However, exemption from criminal liability due to the expiry of the statute of limitations is not exoneration. Having collected all the necessary evidence, the state still has the right to file a claim for damages in civil proceedings, but it is not possible to bring the perpetrators to criminal liability in such circumstances. Thus, there is no case law under Article 441 of the CCU. Given the consistent lack of initiative in the exercise of law enforcement functions, it can be concluded that until such a case is considered by the Criminal Court of Cassation of the Supreme Court, neither pre-trial investigation bodies nor local courts will know how to work with this article. Therefore, Article 441 of the CCU is currently only declarative and formal.

Discussion

Currently, Article 5 of the Rome Statute (United Nations General Assembly 1998a) contains only four crimes, namely genocide, crimes against humanity, war crimes and crimes of aggression. Damage to the environment is a war crime, but the wording of Article 8 does not provide a clear definition and can hardly be proved in court. In addition, the Rome Statute does not address severe damage to the environment that occurs in peacetime. The Stop Ecocide Foundation and the Swedish Parliament are currently working on amending the Rome Statute. The initiative group suggests amending Article 5 with the crime of ecocide and adding an article to the international legal act defining its elements.

According to the proposed Article 8, ecocide means unlawful or unjustified acts committed with the knowledge that there is a substantial likelihood that such acts will cause severe and widespread or long-term damage to the environment. Experts have also developed a clear definition of the terms used in this proposed article. In particular, unreasonable means reckless disregard for harm that would be manifestly excessive bearing in mind the expected social and economic benefits; serious means harm that involves adverse changes, disruption or damage to any element of the environment, including effects on human life or natural, cultural or economic resources; widespread means damage that spreads unrestrictedly, crosses national borders, affects entire ecosystems or individual species, or large numbers of people; long-term means damage that is irreversible or cannot be compensated for by natural recovery within a reasonable time; environment means the Earth, its biosphere, cryosphere, lithosphere, hydrosphere and atmosphere, and outer space (Negri 2022).

A very important step towards a clear standardization of the crime of ecocide was the adoption of a resolution on the negative effect of war on the environment by the Parliamentary Assembly of the Council of Europe. This happened on 25 January 2023. In it, PACE deputies stressed the importance of defining the concept of ecocide in both national legislation and international law. The resolution recognizes the need to amend the Rome Statute of the International Criminal Court and add ecocide as a new crime. If these changes are introduced, Ukraine will be able to sue Russia in the ICC for crimes involving the environment, such as, destroyed ecosystems, contaminated soil, burnt forests, etc.

The world already has a precedent for punishing an aggressor for environmental damage. In the spring of 1991, the UN Compensation Commission was established to consider all claims for compensation for losses and damages caused to Kuwait because of the Iraqi military invasion and occupation. A separate area of the Commission's work was the consideration of claims for compensation for environmental damage worth USD 80 billion. As a result of its work, the Commission partially satisfied these claims, with a payment of just over five billion dollars (Prakasa 2021).

In general, ecocide has become a recognizable crime against humanity and others. Both in Ukraine and in the world, there are active discussions about compensation for environmental damage caused by Russia. There is a high probability that the Rome Statute will be amended, and Ukraine will be able to receive full compensation through the International Criminal Court. All that remains for Ukraine to do is to record Russian war crimes carefully and meticulously.

Conclusions

Currently, there is no universally recognized definition of the crime of ecocide in international humanitarian law. Ecocide is a concept that has emerged in the context of debates on environmental damage and human rights, and its legal definition has not yet been finalized or accepted by international treaties or courts. However, recently, there has been an active discussion about the possibility of recognizing ecocide as an independent crime in international humanitarian law. The idea is to provide international legal protection for the environment and prevent serious environmental disasters by criminalizing actions aimed at systematically damaging the environment. Different organizations and groups propose different definitions of ecocide, but a specific legal definition still requires further discussion, diplomatic efforts, and possibly new international treaties.

Ukraine has entered the fight for accountability for ecocide based on international norms and instruments. The filing of lawsuits against Russia in international judicial bodies, particularly the International Court of Justice, demonstrates Ukraine's seriousness in holding the aggressor state accountable for damaging the environment during the invasion. Although the concept of ecocide does not yet have a universal definition in international law, there are initiatives and proposals to establish the international legal status of ecocide as a separate crime. Researchers and experts are already working on defining ecocide and establishing its status as an international crime. This raises the possibility of holding the aggressor state accountable for damaging the environment, the use of prohibited methods of warfare and environmental pollution.

Filing lawsuits with the International Criminal Court and the possibility of considering environmental crimes within the framework of genocide create chances for bringing to justice those responsible for ecocide. In addition, the possible filing of a separate lawsuit with the International Tribunal for the Law of the Sea will allow us to focus on issues related to environmental crimes committed during the blockade of Ukrainian ports and toxic substances leaking into the water. Prosecution for ecocide requires not only legal measures but also international cooperation, public pressure, and mobilization of resources to collect evidence. It is important to maintain a dialogue with international partners and engage civil society organizations and activists to ensure proper investigation and prosecution of perpetrators.

Ukraine has a strong legal and factual argument to hold Russia accountable for ecocide, which includes the destruction of natural resources, water and air pollution, and other actions that could result in an environmental catastrophe. Bringing the perpetrators to justice and obtaining compensation for the damage will be important steps in protecting the environment and ensuring the vital interests of people.

By continuing to fight for accountability for ecocide, Ukraine shows its determination to ensure environmental protection and prevent future environmental crimes. International efforts and cooperation will be important factors in this process, as prosecution for ecocide is of global importance for the preservation of our planet and well-being of future generations.

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CRIMINAL RESPONSIBILITY FOR ECOCIDE RESULTING FROM THE MILITARY AGGRESSION OF RUSSIA

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ABSTRACT

Russia's military aggression against Ukraine has led to the destruction of natural resources, ecosystems, and infrastructure. These actions have violated international principles of environmental safety. The hostilities have caused serious damage to nature reserves, wetlands, and soil. Air and water pollution have a transboundary effect. Russia's actions threaten future generations and the climate. That is why the creation of a mechanism to ensure environmental safety is an urgent problem for world civilization. Therefore, the aim of this study is to analyze the criminal responsibility for ecocide in the context of Russia's military aggression and identify the existing legislative problems in this area and ways to overcome them. The methodology of the study of ecocide and its connection with Russia's military aggression includes analysis, synthesis, induction, deduction, dialectic, analytics, analogy, abstraction, and generalization. These methods help to reveal the essence of the problem, establish legal norms, and develop recommendations and priorities for regulating ecocide.

Keywords: criminal responsibility; ecocide; environmental damage; environmental losses; environmental safety; legal protection of the environment

Introduction

Russia's full-scale military aggression against Ukraine that started on February 24, 2022, has created a new reality. Crimes against the Ukrainian environment and people are being committed every day. Since the beginning of the invasion, the deliberate destruction of Ukrainian natural resources, ecosystems, and industrial and infrastructure facilities, resulting in many environmental problems have occurred. Russia's attack is a strong reason for revising international guidelines and frameworks that link environmental damage to crimes against humanity. This is why ecocide is of particular relevance in the context of Russian aggression, as any war is "the greatest threat to humanity and the environment".

Due to the ongoing hostilities, the functioning of ecosystems is being disrupted, which negatively affects the state of natural resources and their survival. The Ukrainian environment is a silent victim of war. According to the official website of the Ministry of Environmental Protection and Natural Resources of Ukraine, EcoThreat, as of January 5, 2023, there were 2,278 reports of crimes against the environment recorded in Ukraine and the estimated cost of the minimum damage is 441 billion UAH. In addition, 42,371 tons of aerial emissions, 372,877 tons of waste and almost 20 thousand pieces of Russian equipment are recorded. The hostilities have affected one-third of Ukraine's nature reserves and large areas of wetlands designated for protection under the Ramsar Convention on Wetlands of International Importance. In addition, the movement of heavy equipment, construction of fortifications and military operations has damaged the soil, resulting in the degradation of vegetation and an increase in erosion by wind and water. Approximately 2.9 million hectares in the Emerald Network (an ecological network consisting of areas of special conservation interest), in other words, approximately 200 sites of scientific importance are likely to be destroyed.

War affects every natural object, and the resultant pollution has long-lasting negative transboundary effects. Experts, politicians, scientists, and civil society in Europe should bear this in mind. Currently, the intervention in the performance of the Zaporizhzhia NPP, the fires and occupation of the Chornobyl exclusion zone and the missiles flying over the South Ukrainian NPP are of great concern. On August 25, 2022 the Zaporizhzhia NPP was completely disconnected from the Ukrainian power grid for the first time in its history. Fires in ash dumps at the Zaporizhzhya TPP, located next to the Zaporizhzhya NPP, resulted in the last (fourth) power line being disconnected twice. However, Russia has only been excluded from one Environmental Convention for such violations of international law. In addition, in June 2023, the explosion caused by the Russian Federation at the Kakhovka hydroelectric power plant resulted in ecocide and had extremely harmful consequences for the environment not only in Ukraine but also around the world.

An analysis of these violations indicates that crimes against the environment, humanity, and war crimes disrupt the international balance, thereby leading to dissonance in global international environmental security that jeopardise the right to a safe environment for future generations. Thus, Russia's invasion has harmed the international community and violates the norms of

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international law. Against this background, the relevance of research into the environmental and legal aspects of criminal responsibility for ecocide is very important. An urgent problem for world civilization is the creation of a mechanism for ensuring environmental safety.

In this regard, there is a need to determine criminal responsibility for ecocide at the national and international levels. Environmental lawyers actively discuss and attempt to include ecocide in the list of crimes against humanity, which would create a mechanism for determining international legal responsibility for damaging the environment. Many scholars, such as Nigreeva (2021), Martynenko (2021), Sivodyed (2022), Borshchevska (2023), Mynkovich-Slobodyanyk (2023) have studied this issue. Therefore, the aim of this study is to analyze the criminal responsibility for ecocide in the context of Russia's military aggression and identify the existing legislative problems in this area and ways to overcome them.

Materials and Methods

In this research several approaches and methods are used to analyse the concept of ecocide and its connection with Russia's military aggression against Ukraine, to determine legal norms for the environment and provide recommendations aimed at preventing and punishing crimes against the environment.

The analysis of international legal acts related to environmental protection and countering military aggressions helped in the identification of existing legal norms and obligations that define the legal framework for regulating ecocide. A comparative analysis allows the national and international legislations on ecocide and its relation to military aggression to be compared. Moreover, the legal analysis presupposes the analysis of international humanitarian law, international environmental law, and human rights in the context of regulating ecocide caused by military aggression. Moreover, the principles applicable to ecocide in the context of military aggression are determined.

Generalization and systematization are used to formulate conclusions and recommendations on criminal responsibility for ecocide. Apart from that, synthesis allows different aspects and approaches to be combined in the regulation of ecocide. As a result, recommendations for amending the legislation on ecocide are formulated. Moreover, they help in determining general patterns in ecocide based on the analysis of specific cases. By analysing examples of ecocide due to Russia's military aggression, general trends, and characteristics of crimes against the environment are established. Deduction indicates how international law should be applied in each case.

The dialectical method facilitates considering ecocide as a complex social and legal problem. It is used to understand the causes, consequences and relationships between ecocide and Russia's military aggression. Furthermore, the analytical method is used to reveal the essence and features of the problem of ecocide in the context of military aggression. The effect of military aggression on the environment is studied by dividing the problem into separate components and analysing the causes and consequences. Moreover, the method of analogy helps compare situations like cases of ecocide in the context of Russia's military aggression in order to determine the specific features of ecocide and identify those in common with other legal issues. The use of abstraction highlights the main aspects of the problem of ecocide and Russia's military aggression and thus the key factors and characteristics, the main provisions and formulation of the principles of regulation.

The scope of these methods helps in the systematic analysis of the problem of ecocide and its connection with Russia's military aggression, formulate recommendations for criminal responsibility and ensure proper environmental protection. The combination of these methods provides for a comprehensive and in-depth understanding of the problem and identifying effective ways to regulate ecocide in the context of Russia's military aggression.

Results

The international community and governments of most countries have realized the need and urgency of ensuring environmental safety, not only at the national level, but also on a global scale. The deterioration of the environment can be caused by various intentional economic, military, and political factors. Thus, the attacks of Russia on Ukrainian nuclear power plants, infrastructure and industrial facilities, nature reserves and ecosystems and water resources are the most dangerous for the environment. These actions can be classified as ecocide, which in turn provides grounds for considering it a crime against human security and the environment (Negri 2022; Yaroshenko et al. 2023).

International agreements on environmental protection are one of the most effective mechanisms for cooperation between states and international organizations for preventing damage to nature and human health, such as the World Charter for Nature (1982), the Rio Declaration on Environment and Development (1992), the Convention on Access to Information, Public Participation in Decision-making and Access to Justice in Environmental Matters (Aarhus Convention 1998), the Johannesburg Declaration on Sustainable Development (2002). Apart from these agreements, various international intergovernmental and non-governmental organizations dealing with environmental issues (UN, IAEA, UNESCO, IMO, FAO, etc.) are interested in ecocide.

Attention to environmental pollution is quite justified because of human activity, production processes and wars, men destroy the ecological safety of the planet, pollute and degrade the quality of the environment, thereby causing irreversible damage and bringing us closer to an environmental catastrophe. Such actions are defined in international law as ecocide, which is a partially criminalized act both under international criminal law and in the national law of many countries (Prakasa 2021).

The term "ecocide" comes from Greek and Latin, with "oikos" meaning house and "credo" meaning to destroy or kill. Currently, its meaning is the destruction of large areas of the natural environment because of human activity in peacetime or wartime. In addition, in international legal space, there is an approach whereby ecocide also means the use of meteorological, geophysical and other means to change the composition, dynamics, or structure of the Earth, including all layers of its atmosphere and outer space, which in turn will entail mass destruction of the living world, poisoning of the air, water and other serious consequences (Mynkovich-Slobodyanyk 2023). The term "ecocide" was first used at the 1972 UN Conference on the Human Environment, when the destruction of the environment because of bombing, herbicide use and large-scale constructions in natural areas was discussed. In addition, the fact that the United States used chemical weapons during the war in North Vietnam is important as it resulted in the destruction of a significant part of its forests (Rawal 2022; Yaroshenko and Lutsenko 2022).

Considering the legality of ecocide, it should be noted that it was first officially enshrined in 1991 in Article 26 of the Code of Crimes against the Peace and Security of Mankind. This article states that "a person who intentionally causes or orders damage to the environment shall, if found guilty, be punished". This wording, although indirect, includes ecocide. However, already in 1995, after many legal discussions, this article was deleted, making it impossible to bring individuals to international legal responsibility under the article on ecocide. The next attempt was the international criminalization of this crime in the Rome Statute of the International Criminal Court in 2002, but it is not defined as a separate offense in the context of war crimes and armed conflicts, or crimes against humanity, which involves the destruction of the natural environment through mass attacks against the population of a region (Borshchevska 2023).

Therefore, at present, there is no direct enshrining of ecocide in international legal documents. However, discussions on this issue started on June 22, 2021, when a group of international law experts, with the support of the Stop Ecocide Foundation, presented a definition of the concept. This was launched with support of Swedish parliamentarians in November 2020 on the 75th anniversary of the opening of the Nuremberg War Crimes Trials of Nazi leaders in 1945. The work was coordinated by Philip Sands of University College London and Florence Mumba, a former judge of the International Criminal Court. As a result of their work, governments and the International Criminal Court should investigate crimes committed against the environment (Panigaj and Bernikova 2023).

Thus, Article 5 of the Rome Statute of the International Criminal Court (International Criminal Court 1998) states that it can consider "questions relating to four categories of crimes: genocide, war crimes, crimes against humanity and crimes of aggression", which is widely criticized. In response, Philip Sands stated that "the time has come to use the power of international criminal law to protect our global environment – 75 years ago crimes against humanity and genocide were first spoken of in the hall of Nuremberg, and I hope that this group can build on the experience of that day to formulate a definition that is practical, effective and sustainable, and that can attract support to allow for amendments to the ICC Statute".

It was proposed that the preamble to the Rome Statute of the International Criminal Court should be amended, noting that the environment is being seriously destroyed and degraded every day, which, as a result, poses a threat to humanity and the world. In addition, it is suggested to supplement Article 5 "Crimes within the Jurisdiction of the Court" with clause "e" "The crime of ecocide" and to add Article 8 to the international legal act, which sets out its composition. According to the proposed Article 8, ecocide is defined as "unlawful or unjustified acts committed with the knowledge that there is a substantial likelihood that such acts will cause serious and widespread or long-term damage to the environment" (Allouzi 2019). Moreover, in its advisory opinion on the Legality of the Threat or Use of Nuclear Weapons of July 8, 1996, the International Criminal Court, recognized that "the environment is under daily threat" and confirmed that "it is not an abstraction but represents the living space, quality of life and very health of people, including unborn generations" and confirmed that "the general obligation of States" is to protect it within international law.

Although the term "ecocide" emerged only in the 20th century, cases of ecocide have occurred throughout history. For example, in Ancient Rome, during the wars, it was common for Germanic soldiers to poison water to kill the enemy army (Artamonova and Kutnyakova 2022). Moreover, during the First World War, German troops first used chemical weapons of mass destruction near the city of Ypres in Belgium, which contained mustard gas and chlorine, both of which are hazardous to the environment. There were also cases of ecocide during World War II such as the export of soil from Ukraine to Germany, subsequently affecting soil fertility in Ukraine (Hasler 2022).

Furthermore, the international legal responsibility was first used during the Vietnam War. The reason for this was the scale of the environmental crimes committed by the United States in this war. The U.S. Army conducted large-scale chemical attacks, which damaged the environment of Vietnam, which were later called "scorched earth tactics". According to unofficial data, 96 thousand tons of herbicides were used during these attacks on the jungles of Cambodia and Vietnam, including 57 thousand tons of dioxin; official data is currently classified as "secret" (Robinson 2022). The use many such weapons led to the conclusion that the United States was testing a new type of weapon of mass destruction, namely, ecocide. The consequence of such an effect on nature was the transformation of parts of the tropics into savannah and the loss of fertility of the land. In addition, the consequences for the local population are no less catastrophic. Most of the residents developed hormonal disorders affecting thyroid and pancreatic hormones, sexual and faetal development, and the percentage of stillbirths and children with congenital diseases increased (Sivodyed 2022).

Apart from that, during the Gulf War, 1,200 oil fields, bases, and tankers were deliberately blown up, resulting in many contaminations of soil and sea. The use of phosphorus bombs in military conflicts is also very common. They were used by the US army during the bombing of Iraqi cities, in the military conflict between Armenia and Azerbaijan in Nagorno-Karabakh and by the Russian army in Ukraine.

In addition, the damage to industrial enterprises is also significant because they can result in an environmental disaster. This is exemplified by the military conflict in Yugoslavia, during which many chemical, oil, and pharmaceutical facilities were damaged. In our opinion, it would be appropriate to legalize the international responsibility for developing various weapons that could cause ecocide in the future, such as phosphorus bombs, depleted uranium missiles or nuclear weapons.

Furthermore, there are cases of ecocide related to the economic goals of a state. For example, the Amazon forests were deliberately burnt by the local population to create agricultural land and industrial complexes. Forests in Siberia were set on fire for the same purpose and in the same way (Anisimova and Donets 2022). At the end of the 19th century, the extermination of bison began in America, and in 2002, there was a fish farm in Kamchatka where young salmon were being poisoned.

These examples demonstrate that it is essential to understand the consequences of ecocide and prevent them in the future. To achieve this, it is necessary to improve legislation and monitor its implementation. Thus, having studied the criminal responsibility at the international legal level, it can be argued that the term "ecocide" has not yet been used in criminal proceedings. However, there are several countries, including Ukraine, that have criminalized ecocide and established legal liability for its commission.

However, today's crime of ecocide committed by Russia is not the first in Ukrainian history. In 1941, Soviet troops blew up the upper part of the Dnipro hydroelectric dam to halt the German offensive. Several thousand people drowned in the subsequent flood. On March 13, 1961, one of the largest man-made disasters in the Soviet Union occurred in Kyiv. On that day, the slurry waste from brick factories that was stored at Babyn Yar behind an earthen dam that collapsed resulted in a huge stream of slurry travelling at 3–5 meters per second that inundated Kurenivka and washed away vehicles, residential and administrative buildings, the tramway park, and

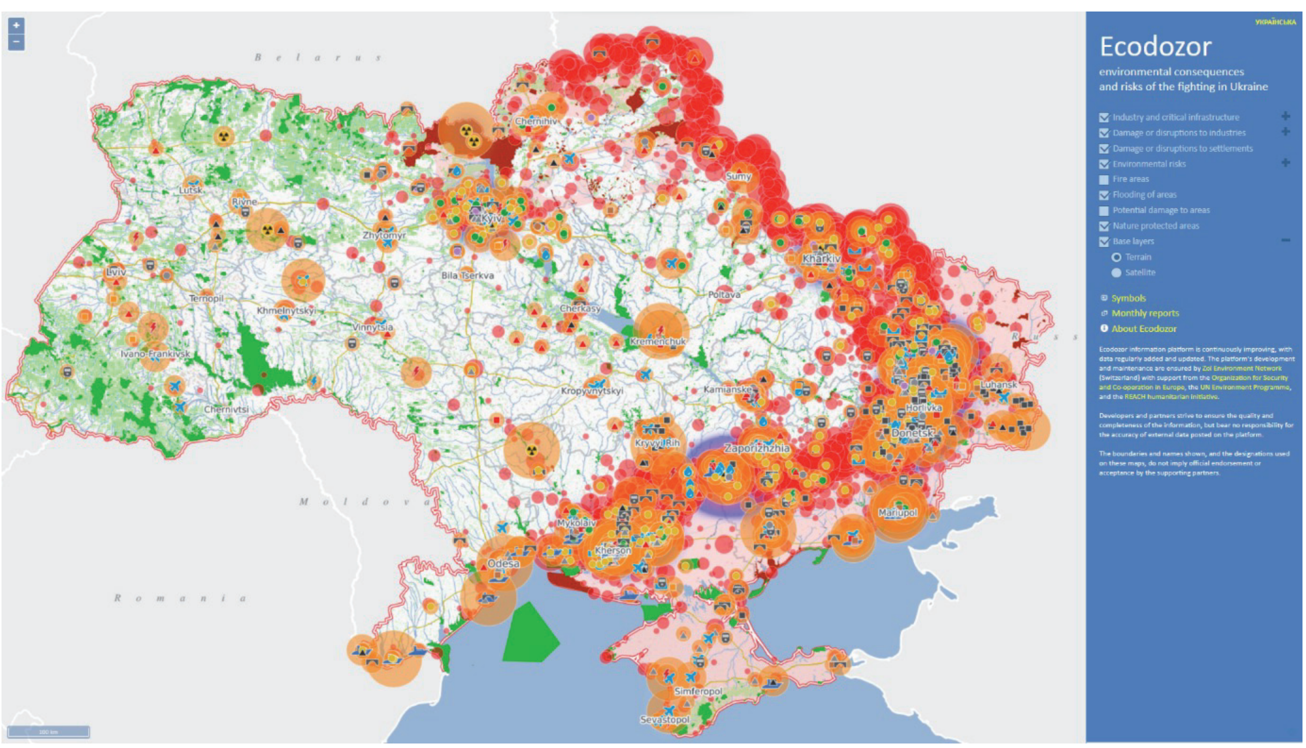

Fig. 1 Map of Ukraine showing the environmental consequences and risks of the war in Ukraine. Source: Ecodozor (2024).

Spartak stadium. The human toll, according to official figures, was about 200 people. The area covered by the slurry was 30 hectares (Martynenko 2021).

The numerous violations of international law by the Russian Federation are unprecedented (Fig. 1). The Ecoaction NGO team documented more than 337 cases of potential environmental damage caused by the war, including the shelling of industrial areas and oil depots that may cause environmental pollution, violation of nuclear safety at Chornobyl and Zaporizhzhia NPPs, oil pollution of the sea, etc. Data gathered from public sources is currently updated on an online map. The highest number of such cases are documented for the Luhansk, Kyiv and Kharkiv regions (Ratushna 2022).

The Ukrainian authorities calculate that ecological damage recorded in the last year amount to no less than $441 billion, with a proposed reparation demand of $51 billion from Russia (Rubryka 2023). This war has resulted in an unparalleled instance of environmental devastation in Ukraine, making it the most severe armed conflict Europe has witnessed since World War II. A summary of the most serious environmental effects of the war in Ukraine is presented in Table 1.

As the UNEP (United Nations Environment Programme 2023) reports, this war has caused extensive harm in numerous areas of Ukraine, resulting in incidents at nuclear power plants and installations, energy infrastructure such as oil storage vessels, refineries, drilling platforms and gas facilities, as well as the destruction of rare flora and fauna, natural parks pollution of water and air.

International institutions and international law have not been able to influence the aggressor and limit its criminal actions. Thus, there are many legal gaps in terms of investigating, opportunities for compensation for environmental damage and restoration of violated environmental rights. Crimes against the environment during this war should be recognized as a component of

Table 1 The most serious environmental effects of the war in Ukraine.

Event	Location	Ecological damage
Temporary occupation of the Chornobyl NPP	Chornobyl, Kyiv Oblast, Ukraine	Russian soldiers damaged a power line, resulting in the plant losing power. Consequently, the cooling systems responsible for preventing the overheating of spent nuclear fuel in storage pools ceased functioning. This situation posed the risk of nuclear waste and pool water overheating, potentially leading to evaporation and subsequent release of radioactive materials into the environment.
Temporary occupation of the Zaporizhzhia NPP	Enerhodar, Zaporizhzhia Oblast, Ukraine	Zaporizhzhia NPP remains under Russian occupation, posing continuous threats to global nuclear safety. Since the start of the occupation, numerous incidents have occurred at this facility, including fires, explosions of Russian ammunition and damage to power lines caused by the Russians.
Attack on the Kakhovka Dam	Nova Kakhovka, Kherson Oblast, Ukraine	77 settlements were inundated with 19.9 billion cubic meters of water, affecting over 100,000 hectares of agricultural land and nature parks. This resulted in the loss of over 50 lives and the destruction in the area of the 2023 harvest. Rare flora and fauna, including approximately 20,000 species, were killed due to the flooding of nature parks (for example, Oleshky Sands). The Dnipro River was contaminated by over 150 tons of machine oil and significant volumes of organic waste. Unique ecosystems were destroyed.
Extensive pollution by explosives	All the areas affected by the hostilities	30% of the territory of Ukraine is mined. Unexploded bombs and rockets can lead to the release of soot, carbon, and lead into the air. The remnants of ammunition can also result in immediate and long-term health effects due to the presence of iron, carbon, sulphur, and copper. When these substances seep into the soil, they contaminate water sources and poison people and animals.
Askania-Nova	Kherson Oblast, Ukraine	Under Russian occupation, a biosphere preserve has experienced a shortage of resources to maintain the dozens of species from all around the world.
Bombing of oil refining factory	Lysychansk, Luhansk Oblast, Ukraine	Blazes occurring at oil storage facilities result in the discharge of detrimental substances such as soot, sulphur dioxide, nitrogen oxides, heavy metals, carbon dioxide and various other pollutants into the atmosphere. The combustion byproducts pose risks to human health and contaminate soil, thereby jeopardizing surface and groundwater. Furthermore, these fires have the potential to trigger acid rain due to the interaction of sulphur dioxide and nitrogen oxide with water vapour, resulting in the formation of sulphuric and nitric acids.
Bombing of the Sumy-KhimProm chemical plant	Sumy, Ukraine	As a result of the Russian bombing of the city of Sumy, an ammonia leak occurred at the SumyKhimProm chemical plant, leading to contamination of the neighbouring village of Novoselytsia.
Destruction of the Azov Sea and Black Sea marine ecosystems	Azov Sea and Black Sea, Ukraine	The presence of naval mines, sunken wrecks, damage to coastal infrastructure have resulted in chemical contamination of seawater by oil-based pollutants, which is adversely affecting biodiversity in the Azov and Black Seas.
Mass death of dolphins in the Black Sea	Black Sea, Ukraine	The sonar used by the Russian fleet emit very strong and low-frequency signals that affect the inner ear of dolphins, their organ of navigation and hearing. Because of this, they cannot orientate, find food and die from hunger.

Source: Ratushna (2022), Rubryka (2023), Hryhorczuk et al. (2024).

genocide or enshrined in international law as a separate type of international crime. Today, the Rome Statute of the International Criminal Court and other international legal acts do not consider ecocide a separate type of crime. Consequently, there is no legal basis for combating ecocide. This situation in the current technocracy and economic development of mankind is unacceptable, as there is no mechanism to bring international environmental criminals to justice, which are the officials who make decisions and give orders that result in environmental damage.

Discussion

Environmental protection is part of national security, as any local damage to the environment can eventually cause irreversible consequences and large-scale destruction of the whole country, even beyond its borders. However, this analysis shows that international and national environmental law do not meet the challenges posed by Russia's war against Ukraine. Current legal provisions do not ensure the inevitability of punishment for crimes against the environment because of legal uncertainty. Therefore, compensation for environmental damage caused by military operations is a complex legal issue. Unfortunately, it is at the cost of Ukrainians' lives and well-being that a new international and national system for the protection and restoration of environmental rights and the creation of a mechanism for compensation for environmental damage must be developed.

The national legislation of Ukraine, namely Article 441 of Section XX of the Criminal Code, which provides for criminal offenses against peace, security, humanity and international law and order, has implemented the crime of ecocide and defined its characteristics. The concept of ecocide should be understood as "mass destruction of flora or fauna, poisoning of the atmosphere or water resources, as well as other actions that may cause an environmental disaster".

Accordingly, it is appropriate to consider and characterize each element of the crime of ecocide. Thus, the object of ecocide should be understood as "the safety of nature as a human habitat". In this regard, environmental safety can be defined as "a state of the environment when prevention of deterioration of the ecological situation and human health is guaranteed". The subject is flora, fauna, atmosphere, water resources, land, subsoil, other components of the ecosystem and outer space (Hnedina and Nagorny 2022).

The disposition of Article 441 covers the objective side in such forms as mass destruction of flora or fauna; poisoning of the atmosphere or water resources; committing other actions that may cause an environmental disaster. Mass destruction of flora or fauna means their complete or partial extermination and poisoning of the atmosphere or water resources involves the dispersal of a high number of toxic substances of biological, radioactive, or chemical origin in the air, rivers, lakes, seas, oceans and other water bodies, which can cause severe forms of illness and even the death of people. An environmental catastrophe is fairly rapid damage to the natural environment and humanity within a certain region on Earth or the entire planet. To define it in each case, it is appropriate to refer to such criteria as "a large area of the territory where adverse environmental changes have occurred; significant restriction or exclusion of human life or plant or animal life in a certain area; duration of adverse environmental changes or their inevitability; significant negative changes in the ecological system".

However, it should be noted that the current legislation does not provide an exhaustive list of acts that can be classified as ecocide. This position of the legislator is correct because we live in a rapidly developing environment, which both opens up new opportunities for solving environmental problems of mankind and can create new challenges in the form of unexplored or poorly studied environmental pollution problems, such as the use of genetically modified organisms, new pesticides and fertilizers, the effect of various kinds of radiation on living organisms and the environment, the development of nanotechnology in biotechnology, energy, medicine, etc. (Kirin 2022). Nevertheless, it turns out that enshrining this criminal offence in law is not enough because there are currently no decisions in Ukraine under Article 441 of the Criminal Code "Ecocide" (Verkhovna Rada of Ukraine 2001). In support of this, the following statistics can be cited. As of September 15, 2021, law enforcement agencies have opened 19 criminal proceedings under the relevant article, of which 4 have already been closed and others are under pre-trial investigation. In addition, the Office of the Prosecutor General of Ukraine opened criminal proceedings under Article 441 "Ecocide" due to Russia's actions at the Rivne oil depot, Chornobyl and Zaporizhzhia NPP since such actions pose a threat to nuclear safety.

One of the main problems in implementing the concept of ecocide are the shortcomings in its regulation. It is extremely difficult to prove guilt because having fixed the forms of the objective side, the legislator did not provide for their interpretation. In particular, the Criminal Code of Ukraine or any other regulatory legal act of environmental legislation does not define the concepts of mass destruction, poisoning or environmental disaster, which raises questions about the qualification of a particular act under the above article. In addition, it is difficult to prove the perpetrator's intent because ecocide is distinguished by the fact that it is subjectively a crime in which the mental attitude to the act and its consequences is characterized by guilt in the form of direct intent, while in practice, there are quite primitive intentions, such as, saving on equipping enterprises up to environmental standards or, conversely, enrichment by illegal logging, etc.

Furthermore, Article 35, paragraph 3, of the Protocol Additional to the Geneva Convention Relative to the Protection of Victims of International Armed Conflicts (Protocol I) (Verkhovna Rada of the Ukrainian SSR 1949) prohibits the use of methods or means of warfare that are intended to cause or will cause widespread, long-term, and severe damage to the natural environment. Moreover, Article 55 of the same Protocol requires that in the conduct of hostilities, care shall be taken to protect the natural environment from widespread, prolonged, and severe damage. It also prohibits environmental damage as a reprisal (coercive measures used by one state in response to the illegal actions of another state to force it to stop these actions and accept the demands it made) (Barabash et al. 2020; Zaveryuha 2022).

These elements of a war crime against the environment in international law have legally undefined criteria. In most cases, this will make it impossible to compensate for environmental damage. After all, the criteria of extent, duration and seriousness are not legally enshrined. Therefore, there is a high risk of not receiving compensation for environmental damage caused by the military actions of the Russian Federation. Natural resources and the environment are an invaluable heritage of Ukrainians that we must preserve for our descendants. Therefore, a legal definition of the criteria for a war crime against the environment it is necessary to ensure compensation (Chizh et. al. 2022).

Article 441 of the Criminal Code of Ukraine provides for criminal responsibility for ecocide. This article is used to start criminal proceedings for environmental damage caused by Russia. At the same time, national legislation does not define the term "environmental disaster" and does not establish criteria for the massive destruction of flora and fauna and the degree of poisoning of the atmosphere and water resources that trigger criminal responsibility.

Thus, this provision of the Criminal Code of Ukraine, as well as the provisions of international legal acts protecting the environment, lack legal certainty. Accordingly, the absence of clear criteria that should form the corpus delicti under Article 441 of the Criminal Code creates the basis for corruption during the investigation and sentencing. The legal uncertainty of the concepts of environmental catastrophe and mass destruction makes Article 441 of the Criminal Code declarative both in peacetime and during military aggression against Ukraine. The opening of criminal proceedings under this article in wartime will not ensure that the offenders are brought to justice and, accordingly, that the damage to the environment is remediable. However, the investigation within the framework of criminal proceedings under Article 441 of the Criminal Code of Ukraine will ensure the collection of evidence (interrogation of witnesses, attachment of photographic and video evidence, conducting examinations, obtaining expert opinions) of crimes against the environment, which in turn can become the evidence for applying to the International Criminal Court (Khaletska and Sydorenko 2019).

At the same time, according to Article II of the Convention on the Prevention and Punishment of the Crime of Genocide, which is the deliberate creation of living conditions for a group that is intended to lead to the physical destruction of the group in whole or in part. The Russian attacks on hydraulic structures and thermal power plants, chemical plants, oil refineries, seizure of nuclear facilities and their shelling, use of chemical weapons and other war crimes aimed at the physical destruction of Ukrainians are considered to be acts of genocide (Nigreeva 2021).

To solve these problems, it is necessary to amend the legislation and provide a common interpretation of the terms specified in the disposition of the article that establishes the criminal offence of ecocide. In 2000, Ukraine signed the Rome Statute of the International Criminal Court which has yet to be ratified. The ratification of the Rome Statute is also required by the Association Amendment with the European Union. Meanwhile, Ukraine recognized the jurisdiction of the International Criminal Court under Articles 6, 7 and 8. Thus, on May 4, 2015, the Verkhovna Rada of Ukraine adopted a resolution approving the Statement of the Verkhovna Rada of Ukraine "On Ukraine's recognition of the jurisdiction of the International Criminal Court over crimes against humanity and war crimes committed by senior officials of the Russian Federation and leaders of the DPR and LPR terrorist organizations, which led to particularly grave consequences and mass murder of Ukrainian citizens" (Verkhovna Rada of Ukraine 2015).

In addition, the Order of the Prosecutor General's Office of August 2, 2021, established the Specialized Environmental Prosecutor's Office. This is an independent structural unit that organizes and procedurally manages pre-trial investigations, resolves other issues in criminal proceedings by the law and supports public prosecution in criminal proceedings for criminal offences in the field of environmental protection.

Furthermore, on June 2, 2022, an interview with the Minister of Justice of Ukraine Denys Maliuska was published on the Radio Liberty website, in which he explained the impossibility of Ukraine ratifying the Rome Statute while at war. In his opinion, the reason for this is the lack of proper communication with the Ukrainian military, which has led to a misconception that ratification would be harmful to Ukrainian defenders. Therefore, in the opinion of the Minister of Justice of Ukraine, it is not time to amend national legislation and ratify the Rome Statute of the International Criminal Court (Gerasimenko 2020).

Apart from that, the issue of international legal protection of the environment during a military conflict is regulated by international law, which falls under the jurisdiction of the International Criminal Court. Therefore, Ukraine recognizes the jurisdiction of the International Criminal Court under Articles 6, 7 and 8 of the Rome Statute or have started the procedure for its ratification. This must be done to ensure compensation for crimes against the environment to be paid as environmental damage.

Investigating the war crimes of the Russian Federation under the Rome Statute will allow for the involvement of the international law on criminals, raise the standards of investigation, and ensure that the evidence collected is adequate for the consideration of criminal cases in the International Criminal Court. Another step to ensure compensation for environmental damage should be to legislate the concept of environmental damage. According to national legislation, in particular Article 22 of the Civil Code, damage is divided into real and lost profits (Haltsova et al. 2021; Vedkal 2021). Therefore, environmental damages should include the costs incurred by the state in connection with the damage to the environment and the costs that the state must incur to restore the environment; the income that the state could receive under normal circumstances if there had been no damage to the environment and compensation when it is not possible to restore the environment.

Moreover, the responsibility for violation of the Protocol Additional to the Geneva Convention of August 12, 1949, and Relating to the Protection of Victims of International Armed Conflicts (Protocol I) of June 8, 1997, is provided for in Article 91 of this Protocol (Verkhovna Rada of the Ukrainian SSR 1949). This provision stipulates that a party to a conflict that violates these documents must compensate for all damage if there are grounds for doing so. This party is also responsible for all actions committed by persons who are members of its armed forces. Such a formulation of responsibility for war crimes does not always correspond to modern methods and means of warfare, which are developed and used by the Russian aggressor. For example, the Russian occupier widely uses methods of hybrid warfare during its aggression against Ukraine (irregular armed units "Kadyrovites", "Wagnerites", etc.). Consequently, there are high risks of manipulation by Russia to avoid responsibility for war crimes, including those against the environment. Therefore, Ukraine could initiate amendments to this provision of the Additional Protocol to the Geneva Convention and extend its application to irregular armed units (Yelaev 2021).

Conclusions

Environmental security and biodiversity are among the most important issues facing humanity. Russia's full-scale invasion of Ukraine has resulted in serious destruction and pollution of natural resources. As a crime against humanity and the environment, ecocide has become an alarming phenomenon in the context of Russian aggression. International treaties and environmental organizations play an important role in ensuring environmental safety. However, the implementation of these agreements and bringing perpetrators to justice for ecocide are insufficient. In this regard, more attention needs to be paid to the regulation of ecocide in international law and to ensure its effective prevention and detection to protect the environment for future generations.

The explosion at the Kakhovka hydroelectric power plant carried out by Russian forces has serious consequences that are constantly escalating and will have long-term effects. This includes the flooding of many settlements, civilian casualties, destruction of minefields and waste dumps, loss of access to water for irrigation of agricultural land, destruction of Red Book species and cultural monuments, and a threat to the safety of the Zaporizhzhia NPP.

The international and national legal systems do not meet the challenges posed by Russia's war against Ukraine. The legal provisions protecting the environment do not ensure the inevitability of punishment for environmental crimes and cause legal uncertainty. Accordingly, compensation for environmental damage caused by military operations has become a complex legal issue. To ensure justice, crimes against the environment during the war should be recognized as a component of genocide or a separate international crime. The concept of ecocide should be enshrined in international law, with clear legal criteria for crimes against the environment being established. Thus, national legislation should define the term "ecological catastrophe" and the criteria for the massive destruction of biodiversity and the degree of environmental poisoning. Obtaining compensation and bringing war criminals to justice is important to preserve natural resources and the environment for future generations.

In addition, Ukraine should recognize the jurisdiction of the International Criminal Court under Articles 6, 7, and 8 of the Rome Statute or start the procedure for its ratification. The recognition of the jurisdiction of the International Criminal Court will allow the involvement international criminalists, improve the standards of investigation, and ensure the adequacy of the evidence collected. In addition, Ukraine should initiate the recognition of the concept of ecocide as one of the most serious crimes under the Rome Statute. This will allow for compensation for crimes against the environment and strengthen the international protection mechanisms.

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EFFECTS OF URBANIZATION AND KANIV RESERVOIR ON THE THERMAL CHARACTERISTICS IN THE REGION

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ABSTRACT

This study contributes to understanding the effects of urbanization and a large body of water, Kaniv Reservoir, on thermal characteristics in the region. This was done by recording trends in air and surface temperatures in the growing seasons in the period 1985–2022. Specifically, the study's objectives were to 1) detect and quantify changes in built-up areas using Landsat satellite data, and 2) analyse trends in land surface temperature (LST) in several shoreland zones of the reservoir with different land covers. To identify built-up areas, Landsat data was processed. LST was calculated using Google Earth Engine for the period studied for three shoreland zones and six types of land cover, including sites with substantial, partial and no change in land use. The built-up area increased unevenly over time and differently in each of the shoreland zones. The growth in built-up area was greatest, increasing by 3.7 times, in a zone close to the city of Kyiv. The highest mean LST values were recorded in the zone that was mainly agricultural. Positive LST trends were recorded throughout the entire period of the study, albeit with different trend slopes in individual months. Statistically significant trends were recorded only in August and September.

Keywords: built-up area; land surface temperature; land cover change; satellite remote sensing; regional

Introduction

Kaniv Reservoir, also known as Kaniv Hydroelectric Power Plant Reservoir, is a large body of water in central Ukraine and the second reservoir in the Dnipro Cascade. It was established in the 1970s by the construction of the Kaniv Dam on the Dnipro River. Construction of the Kaniv Dam and the reservoir's creation had significant effects on the surrounding area.

Recent studies (Starodubtsev et al. 2021; Vyshnevskyi and Shecvhuk 2021) report the spatiotemporal dynamics of this hydromorphic landscape, shoreland erosion, overgrowing of the reservoir with vegetation, and other ecological aspects related to development in and around Kaniv Reservoir. In wet years flood water is released from the reservoir through culverts into low-lying areas adjacent to the reservoir. The capacity of the low-lying areas was sufficient to hold the flood water even in very wet years. Urbanization and development in the low-lying areas greatly reduced this capacity. For this reason, Kaniv Reservoir in the future may not be able to prevent the flooding in vast areas of the southern part of the city of Kyiv. Knowledge of the changes in the urban land cover in the low-lying areas around the shores of Kaniv Reservoir might be helpful in estimating actual capacity of the whole area to hold flood water in very wet years. Moreover, it potentially could account for some of the ecological and anthropogenic changes that have occurred in recent years, namely the newly built-up areas close to the reservoir. This study reports the changes in land cover and reflected changes in land surface temperature (LST) in shoreland zones and neighbouring regions of the Kaniv Reservoir. This was done using satellite data that are available for almost the entire period of the reservoir's operation, recent field observations and standardized maps.

There are two maps of land cover known to the authors that include the shoreland zones around the Kaniv Reservoir. The first is that for Ukraine for 1990, 2000 and 2010 with a 30-meter spatial resolution (Kussul et al. 2016). This map includes a neural network classification of satellite Landsat images that identifies six classes of land cover within the European Land Use and Cover Area Frame Survey (LUCAS) nomenclature: artificial surfaces, cropland, grassland, forest, bare land, and water. The second is GlobeLand30, which consists of global land cover maps for 2000, 2010 and 2020 with a 30-meter spatial resolution (Chen et al. 2014). The maps include images from the Landsat satellite and HJ-1 (China Environment and Disaster Reduction Satellite) that identify 10 land cover classes: namely cultivated land, forest, grassland, shrubland, wetland, bodies of water, tundra, artificial surfaces, bare land and perennial snow and ice. Artificial surfaces in these two maps differ in their accuracy because of the different methods used. The layers have slightly different definitions and include several types of artificial objects, such as settlements, mining areas, transportation facilities and so forth, whereas the current study is focused on the increase in built-up areas at the local level. Also, these two maps do not cover the period at the start of the operation of Kaniv Reservoir. The changes in urban area throughout the entire period of the operation of this reservoir have not so far been studied.

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Land surface temperature (LST) is an important component of the Earth's energy budget and is increasingly used in various applications related to the assessment of land surface conditions, including mapping the urban extent (Trlica et al. 2017; Bala et al. 2021; He et al. 2021) and the nature of urban microclimates (Li et al. 2020a). The Landsat satellite time series can be used to provide LST estimates at high spatial resolutions, which are particularly appropriate for local and small-scale studies. Google Earth Engine (GEE) (Gorelick et al. 2017) is an online platform created for users of remote sensing to carry out big data analyses in a user-friendly manner and without the need for computation.

Changes in urban area, among other factors, can potentially influence the thermal regime of the shoreland zones of a reservoir (Dawson et al. 2018). Human pressure resulting from urbanization, is one of the main factors leading to increasing risk to shorelands, bearing in mind future climate change scenarios. The recent study of Arulbalaji et al. (2020) reports a marked increase in built-up area and in mean LST in a coastal town in India during the period of their study. The temperature in built-up areas is higher than in the surroundings even in a shoreland zone with abundant vegetation (Tarawally et al. 2018).

In recent decades, the water temperature in the Dnipro Cascade increased in summer by 0.74 °C per decade and during May–October by 0.65 °C per decade (Vyshnevskyi and Shecvhuk 2021). Apart from the effects of climatic factors, the temperature of the water in the Kaniv Reservoir is influenced by the fact that the reservoir discharges water from its lower layers, where the processes of heating and cooling are very slow. During spring and summer, water temperature recorded in the upper zone of the Kaniv Reservoir is lower than that recorded in the surroundings and in autumn it is higher. The temperature of the water in Kaniv Reservoir also depends on the intensity of algal blooms, with higher temperatures recorded in areas where there are large blooms.

This study aims to improve the level of understanding of the effect of ongoing land use/cover changes and a large water reservoir on the thermal characteristics of different land use/cover types in the region and to identify the trends in surface temperature recorded during the growth seasons in the period 1985–2022. In particular, the objectives were to 1) detect and quantify changes in built-up areas using Landsat satellite data and 2) analyse trends in surface temperatures in several shoreland zones around the reservoir and at sites with different types of land cover.

Materials and Methods

Area studied

Kaniv Reservoir is one of six water reservoirs on the Dnipro River. Construction of the Kaniv Dam began in 1963 and the reservoir began to be filled in 1972. Filling to the normal water level (NWL) was completed in 1976. Kaniv is classified as a large dam (Obodovskyi 2002). The area of the water surface at NWL 91.5 m is 581 km^2 and the volume is 2.62 km^3. At the level dead storage (LDS) 91.0 m, the volume is 2.20 km^3. The so-called "working" volume between NWL and LDS is 0.30 km^3. Length of the reservoir is 123 km, maximum width is 8 km, mean width is 5.5 km, mean depth is 3.9 m and maximum depth is 21 m (Vyshnevskyi et al. 2011).

Kaniv Reservoir's maximum operating level (MOL), also termed full supply level, is 92.7 m (Decree of Ministry of Environmental Protection and Natural Resources of Ukraine, 2022). This water level and ASTER digital elevation model (NASA/METI/AIST/Japan Spacesystems and US/Japan ASTER Science Team 2019) were used to define the area of interest, delineated as the area that would be flooded when water in the dam reaches MOL (Fig. 1). Based on local expert knowledge about the processes prevailing in certain parts of the water body and nearby shore, the area of interest was divided into 3 zones:

Zone_1 is located adjacent to the city of Kyiv. It is undergoing intensive construction and expansion of infrastructure, mainly residential and personal recreation in nature. Water covers approximately 25% of the area and there are islands of alluvial vegetation. In contrast, *Zone_2* is predominantly covered by open water, accounting for about 50% of the area. It also includes an army-expropriated territory with long-term disturbed soil and vegetation cover, making up around 15% of the total area. *Zone_3* is a flat area with no direct contact with the water reservoir. It is mainly an agricultural area where

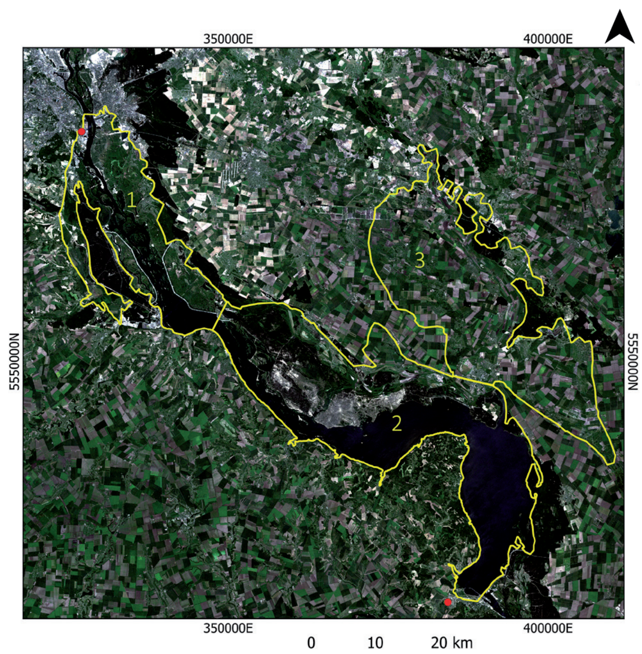


Fig. 1 Photograph of the general area with the three areas studied (1, 2 and 3) demarcated by yellow lines. Red points are the locations of the Kyiv (north) and Kaniv (south) meteorological stations.

crops of winter wheat, corn, sunflower, soybean, winter rapeseed, barley and sugar beet are grown between scattered villages and small towns.

The region has a moderately cold climate, classified as Dfb (**warm-summer humid continental climate**) (Köppen 1936). Precipitation is substantial, even in the driest month and amounts to 677 mm per year. The mean annual temperature is 9.0 °C (Martazinova 2019). The growth period starts in May and ends in September.

Data

Satellite data

Multispectral satellite Landsat (NASA) data for the years 1985, 2000 and 2022 were used to analyse changes in built-up area (Table 1).

Table 1 Satellite data used to analyse changes in built-up area.

Data	Date	Processing level	Source
Landsat-5	6 June 1985	Surface reflectance, L2A	https://earthexplorer.usgs.gov/
Landsat-7	7 June 2000		
Landsat-9	20 June 2022		

Land surface temperature

LST values were estimated using the Statistical Mono-Window algorithm developed by the Climate Monitoring Satellite Application Facility and the GEE online platform (Ermida et al. 2020), which enables large amounts of satellite thermal time-series data to be analysed. The program code returns mean LST value for an object (shape file) and date specified by the user. The number of available mean LST values derived from Landsat data for each zone or site varied between months and hence also between years because of different zone or site locations, layout of acquired satellite data and cloudiness. In the analyses, only those LST values for the same date for all zones and sites were used. The LSTs for the three zones and six small sites with different types of land cover in Zone 1 were recorded. The six small sites are places where there have been dramatic changes in land use, with one land cover type being replaced by another (e.g., vegetation to built-up area), places where there have been partial changes within a land cover, and places without any land use change (Table 2), (Fig. 2). LST values were obtained for available dates from May to September for 1985 to 2022. The code of Ermida et al. (2020) was complemented with *ee.Reducer.count* procedure to count the

Table 2 Land cover types at six small sites (S1–S6) in Zone 1 for 1985, 2000 and 2022.

	S1	S2	S3	S4	S5	S6
1985	fields	vegetation	forest	built-up area	vegetation with built-up areas	water
2000	fields	vegetation with built-up areas	forest	built-up areas	built-up areas	water
2022	fields	built-up areas	forest	built-up areas	built-up areas	water

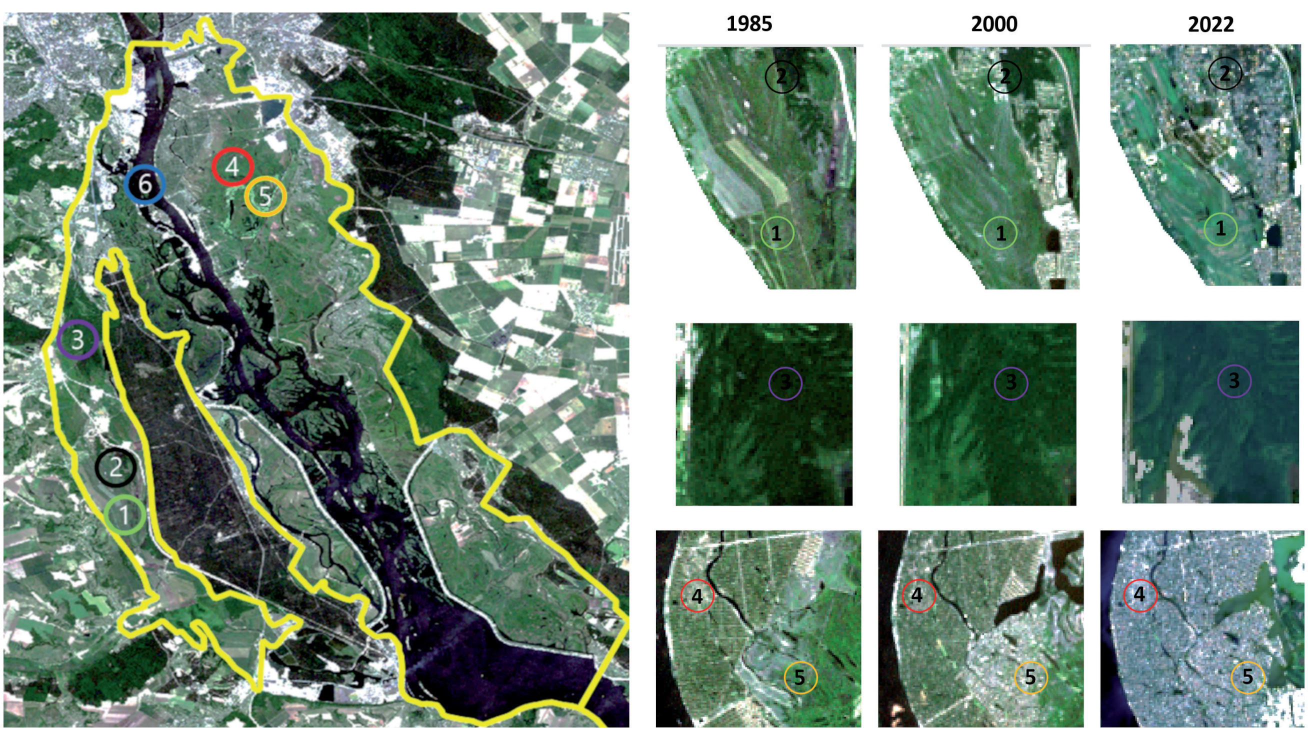


Fig. 2 Small sites used in the analysis of changes in surface temperature for the period 1985–2022: (1) site where there was no change in land use (agricultural), (2) site where there was great change (vegetation to built-up area), (3) and (4) sites where there was no change (forest and built-up area, respectively), (5) site with small changes (sparsely built-up area to densely built-up area) and (6) site where there was no change (water).

number of valid pixels (i.e., without clouds and shadows) for each location and each date involved in calculating mean LSTs. The LST data (Landsat 4, 5, 7, 8, 9) was sorted for each location and each date, and the values used were those with more than 75% valid pixels.

Climate data

Air temperature data was provided by the Central Geophysical Observatory of Ukraine (http://cgo-sreznevskyi.kyiv.ua/?lang=en) from the Kyiv and Kaniv meteorological stations. The daily mean air temperatures were used to analyse the difference between the Kyiv and Kaniv measurements, and 9:30 a.m. air temperature for Kyiv based on the analysis of Landsat LST. The dates of the air temperature records are the same as the dates of the LSTs obtained from satellite Landsat imagery using the GEE algorithm.

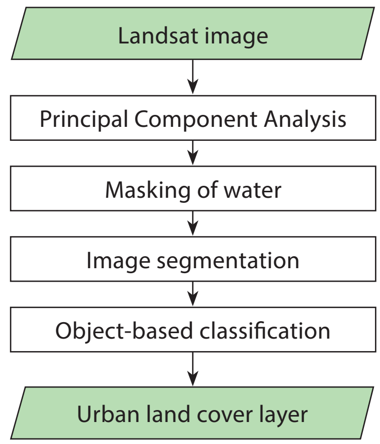


Fig. 3 Main steps in the processing of Landsat data.

Processing of Landsat satellite data

To identify built-up areas, the same steps were used for the classification of Landsat-5, Landsat-7 and Landsat-9 data so that the classification results are comparable (Fig. 3).

The principal component analysis (PCA, also referred to as "PC transform") of the Landsat-5 and Landsat-9 scenes was used to remove redundant information from the dataset while retaining as much of the original spectral information as possible. This resulted in a set of uncorrelated image bands, termed *PC_bands* or components (Chuvieco 2016). The explained variance indicated how much of the total variation in the original data is included in each component. Generally, the first few components of the PCA correspond to dominant features, whereas the later components may represent noise or subtle variations. The first two *PC_bands* from Landsat data PCA analysis accounted for 93.4% of the total variance and were used in subsequent analyses.

Significant parts of Zone 1 and Zone 2 and was covered with water and were not included in the urban land cover classification. PCA_band_1 was used to remove water from each Landsat scene with thresholds, 9450 ≤ PCA_band_1 ≤ 10090.

Fig. 4 Photographs of the Kaniv Reservoir shoreland zone landscapes (2021).

Segmentation was used to divide an image into segments of similar spectral and/or textural characteristics. The spectral and textural attributes were computed for each band of the input image (two *PC_bands* from Landsat image PCA). The value of the spectral attribute for a particular segment was computed from the input data band where the segmentation label image had the same value (ENVI Reference Guide). The spectral attributes include mean, maximum, minimum, and standard deviation values for the pixels in the image of the region in each band. Computation of the textural attribute was a two-step process wherein the first applied a square kernel of predefined size to the input image. The textural attributes were calculated in terms of pixels in the kernel window (7 × 7 pixels) and the result was referenced to the central kernel. Next, mean values of the attribute results were calculated for each pixel in the segment to create the attribute value for that band's segmentation label. The textural attributes consisted of the range, mean, variance and entropy of the pixels in the kernel. The watershed transform algorithm (Roerdink and Meijster 2001) with scale level of 50 and merge level of 80 was used for the image segmentation.

Training regions for built-up areas for supervised object-based classification were selected using archival and current high-resolution satellite imagery (visualization in Google Earth) and photographs taken in the field in 2021 and 2022 (Fig. 4). Overall, 52 training regions were selected from Landsat scene 1985, 106 from Landsat scene 2000 and 156 from Landsat scene 2022.

The object-based classification was done using the K Nearest neighbour algorithm (Cover and Hart 1967) with a threshold value of 5%, meaning that segments having a less than 5% confidence in each class were set to "unclassified." The overall accuracy and kappa coefficient were used to estimate the classification results.

Statistical analysis of air temperature data and land surface temperature

Analysis of air temperature data and land surface temperature was done using the Statistica (StatSoft) software TIBCO Data Science (https://www.statistica.com/en/). One-way ANOVA was used to test for significant differences among the LST of zones where there was no water cover in months or years during the growth season. A simple linear regression model was used to estimate the LST for each month during the growth season for the six cover classes.

Results

Changes recorded in built-up areas from 1985 to 2022

The accuracy of the estimate of the built-up area based on kappa coefficients for 1985, 2000 and 2022, respectively, were 80% and 0.82, 78% and 0.82 and 82% and 0.84.

The built-up area increased from 1985 to 2022 (Fig. 5). This increase was uneven over time, however, and different in each of the three zones (Table 3, Fig. 6). Built-up

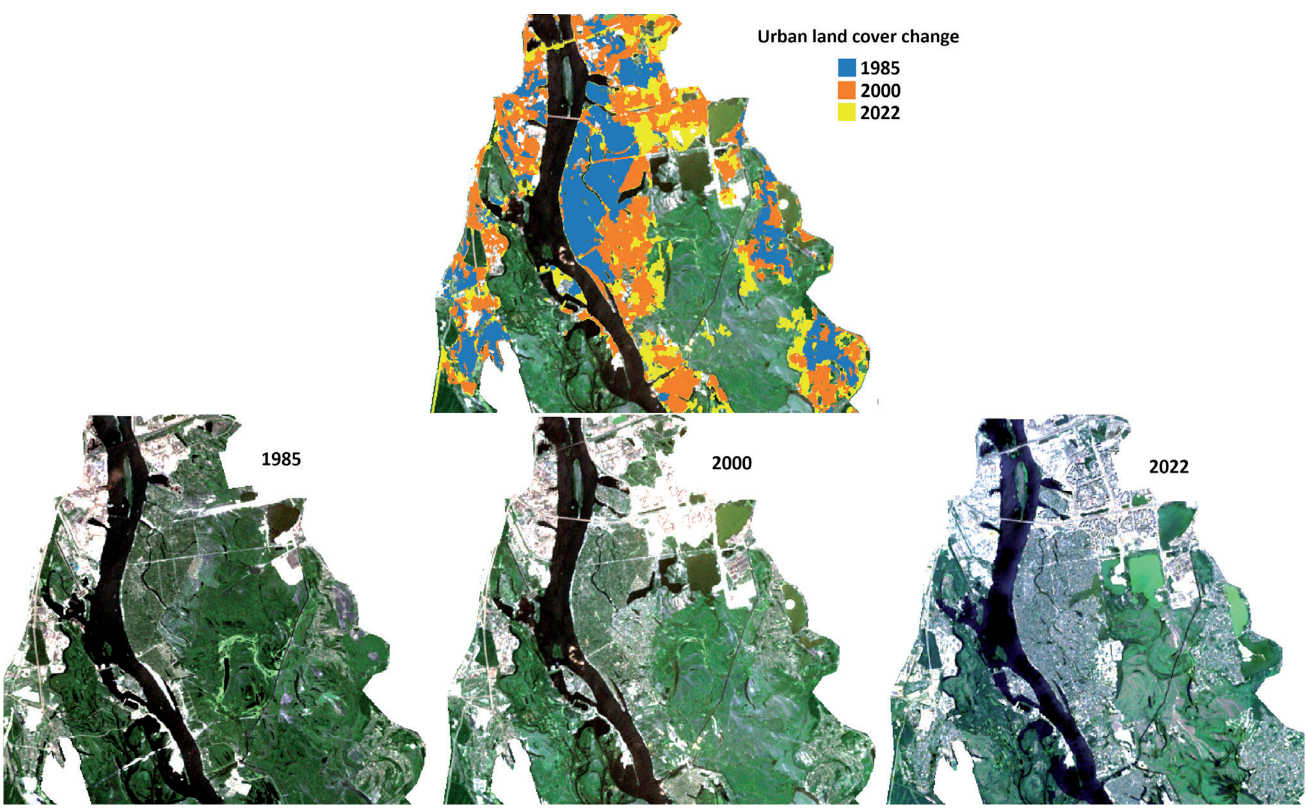


Fig. 5 Part of the map showing the change in the area built-up (at the top) and Landsat scenes (at the bottom).

area increased by 3.7 times in Zone 1, and approximately doubled in zones 2 and 3 from 1985 to 2022.

Table 3 Built-up area recorded in zones 1, 2 and 3 of the area studied in 1985, 2000 and 2022.

Zone	1985	2000	2022
1	24.81 km^2	56.18 km^2	93.40 km^2
2	13.66 km^2	20.38 km^2	29.75 km^2
3	43.75 km^2	57.93 km^2	84.41 km^2
Total	82.22 km^2	134.49 km^2	207.56 km^2

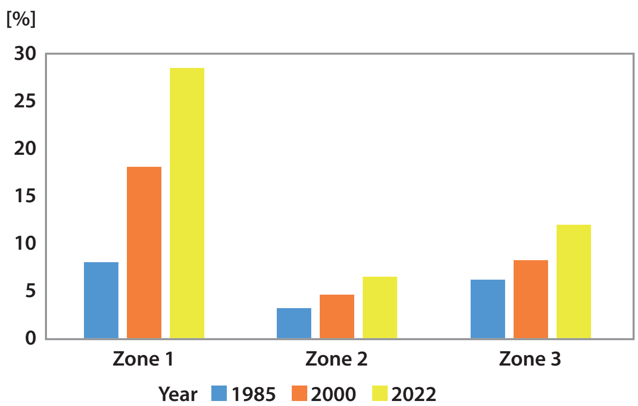


Fig. 6 Changes in the built-up area recorded in zones 1, 2 and 3 of the area studied in 1985, 2000 and 2022. The built-up area is a percentage of the total land area in each zone (areas of water were excluded from the analysis).

Trends in the temperature of the air and surface of the land

Based on the trend in the daily mean air temperature recorded by the Kyiv and Kaniv meteorological stations, the temperature increased by approximately 4 degrees over a period of 37 years (Fig. 7).

LST patterns for zones 1, 2 and 3 without water followed that of the air temperature (Fig. 8a). Zone 3 nearly had the highest LST values for each month from May to September compared to other zones, followed by Zone 2 and Zone 1, both of which lack open water. There was a significant difference ($p < 0.01$) between Zone 3 and the other two zones in mean LST for the months of May and September. Moreover, Zone 3 reached its LST peak throughout summer while both Zone 2 and Zone 3 had a prominent peak in July, which coincided with the peak in air temperature. A similar trend in LST occurred in the yearly means for the growth season. This development followed an evenly increasing trend until 2002, after which the trends for all zones, including air temperature, were very variable (Fig. 8b).

The objective was to determine if the trend in LST is common to all the small sites and therefor attributable to climate change or if the trend in LST differed and is attributable to changes in land cover and/or use (Fig. 9). There are two months, namely August and September, for which the trends (regression coefficients) are similar and statistically significant ($p < 0.01$) and differences in LST values for different classes can be attributed to type of surface cover. There were not statistically significant ($p > 0.05$) trends in LST for other months and classes or for air temperature. For additional information (regression coefficients) see Appendix.

Discussion

The largest increase in built-up area over the period 1985 to 2022 occurred in Zone 1 (3.7 times increase) from 1985 to 2022 (Fig. 6). This may be due to its closeness to the city of Kyiv, the growing interest of the population in life outside the city, and, therefore, intensive construction and expansion of infrastructure. Built-up area in zones 2 and 3 approximately doubled. This can be accounted for by the growth in the rural population within the Boryspil Raion during the period of this study (Timonina 2020).

The accuracy of estimate of the area built-up in this study is less than in other recent publications based on satellite data. The accuracy of the dataset for this study was limited by that of the Landsat satellite data, as the interest in this study was in changes in built-up area since

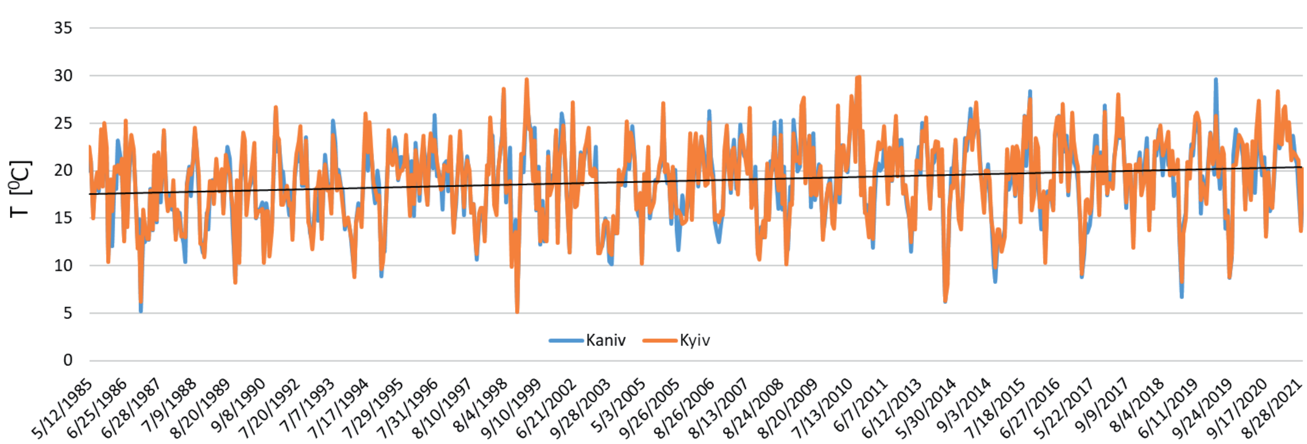
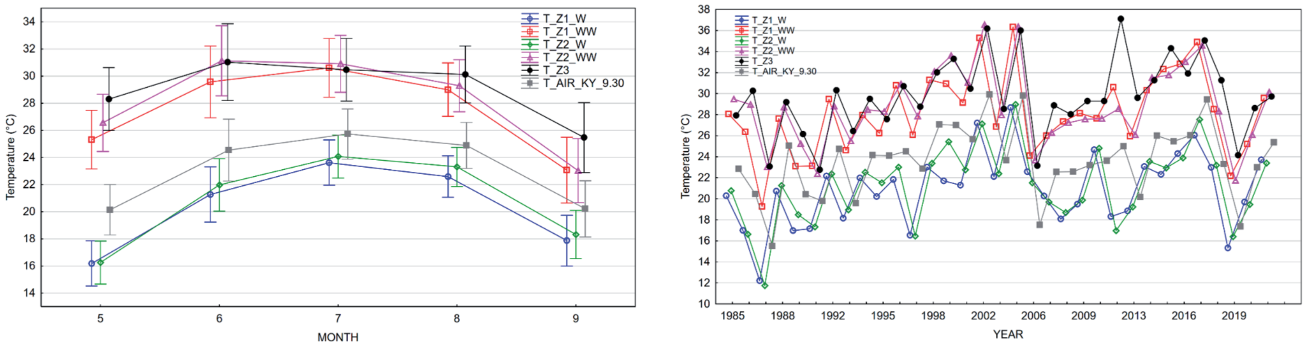
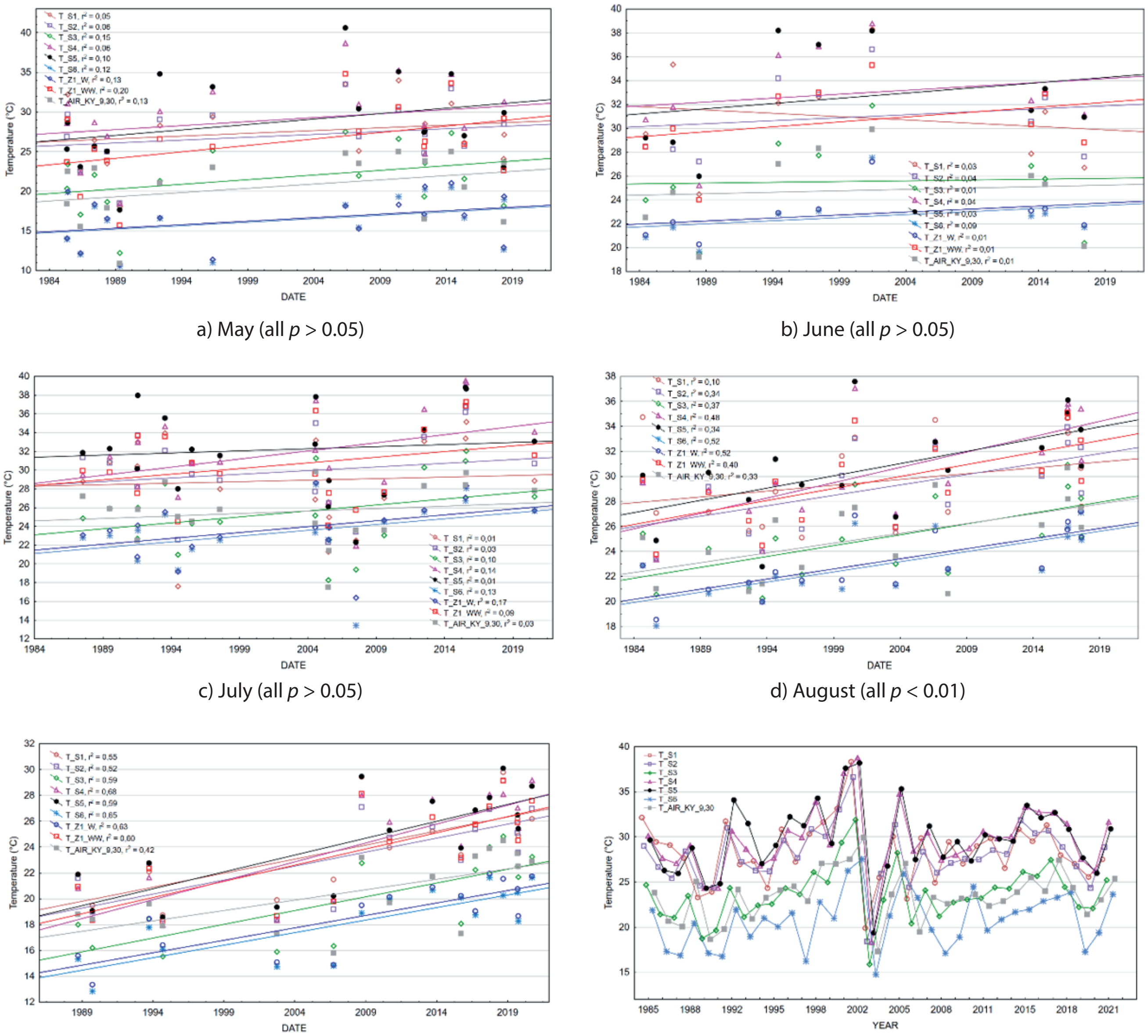


Fig. 7 Daily mean air temperature recorded by the Kaniv and Kyiv meteorological stations over the 37year period from 1985 to 2022.



a) b)

Fig. 8 Mean land surface temperature (LST) for the months May through to September (a) and (b) for the period of 37 years for zones with open water (T_Z1_W, T_Z2_W) and without open water (T_Z1_WW, T_Z2_WW, T_Z3) together with air temperature (T_Air_KY_9.30).



a) May (all $p > 0.05$)

b) June (all $p > 0.05$)

c) July (all $p > 0.05$)

d) August (all $p < 0.01$)

e) September (all $p < 0.01$)

f) May–September

Fig. 9 Trend in surface temperature for the period 1985–2022. (a)–(e) individual months from May to September and (f) all months. The graphs are for six small sites (T_S1–T_S6) in Zone 1 (T_Z1), with an area of reservoir water (T_Z1_W), without an area of reservoir water (T_Z1_WW) and air temperatures for Kyiv (T_AIR_KY_9.30).

the beginning of the operation of the water reservoir in the 1970s to 2022 calculated using the same algorithm and source of satellite data. Whereas the recent publications mentioned below use a single land use classification and/or combined Landsat satellite data with other imagery, which accounts for the slightly higher classification accuracy compared to that of this study. Kussul et al. (2016) report an overall accuracy of classification for the whole of Ukraine in 2010 of about 89% using an unsupervised neural network algorithm for Landsat-8 and Sentinel-1 satellite data. Prasomsup et al. (2020) using a Modified Built-Up Index based Landsat-8 data reports an 82–83% accuracy for the estimates of the built-up areas in Bangkok.

The higher LST values for Zone 3 compared with the other zones for each month from May to September (Fig. 8a) can be accounted for in the remoteness of Zone 3 from the water reservoir, lack of inland water and agriculture, which dominates almost the entire zone. Agricultural fields can create microclimates in their surroundings. Fields with bare soil or sparse vegetation have a higher LST because of their low evapotranspiration compared to fields with dense vegetation. Morsy and Aboelkhair (2021) report that the relative effect of agriculture on LST in Egypt (calculated as the ratio between the change in each area and the sum of all changes in all areas multiplied by the change in LST or normalized difference vegetation index) was 45%, followed only by deserts with an effect of 50%. According to Vendrov et al. (1968) and Kurbanov and Kurbanov (2014), the direct influence of water reservoirs on the microclimate of nearby areas extends for distances of several hundred meters and in the direction of the wind it can be up to 10 km. Zone 3 was about 14 km from the Kaniv Reservoir.

Zone 1 had the greatest growth in built-up area throughout the entire study and it was expected that it would have the highest LST. Being on the bank of the reservoir, the LST of Zone 1 was affected this body of water. The LST of built-up areas combined with the water's moderating influence resulted in a low LST in Zone 1.

Water surfaces have the lowest daily surface temperature compared to other land covers like forests, grasslands, or urban areas during the growth season each year. This phenomenon can be attributed to differences in the heat-absorbing and heat-retaining properties of water and land (Li et al. 2020b). In this study, small site "water" (T_S6) and Zone 1, excluding the area covered by the reservoir (T_Z1_WW), had lower surface temperatures than other small sites (Fig. 9). Small sites where there were built-up areas (T_S2, T_S4, T_S5), in contrast, had higher surface temperatures than other small sites, because buildings absorb and store energy from the sun's radiation more effectively than natural surfaces (Nugroho et al. 2022). The difference in surface temperature of small site "water" (T_S6) and those where there are built-up areas (T_S2, T_S4, T_S5) was significantly higher both in August and September since 1985. In these months, the greatest increase was recorded for the permanently built-up site (T_S4; see regression coefficients in Appendix). This is mainly due to the thermal lag of water bodies (Pickens et al. 2022), which is supported by the thermal regime of the water in the Kaniv Reservoir (Vandiuk 2013).

A positive trend in surface temperature in the three zones without water or open water bodies (Z1_W, Z2_W) and six small locations throughout the entire period studied (1985–2022) was recorded (Fig. 8b, 9). Vyshnevskyi and Shecvhuk (2021) report an increase in mean water temperature in the Dnipro Cascade during summer of 0.74 °C per decade since the late 1970s. A statistically significant trend in the LST for all sites in August and September can be attributed to a natural cycle in the climate and presence of the reservoir, as air temperature is lower in spring and the first half of summer (cooling effect), then increases in the second half of summer and autumn (warming effect).

Conclusion

This study detected and quantified an increase in built-up areas in the shoreland zone of Kaniv Reservoir throughout this 37-year study (1985–2022). Surface temperature in the built-up area increased unevenly over time and differently in each of three zones studied. The zone with the biggest increase in built-up areas is located close to the city of Kyiv along the shore of the reservoir. The LST in this zone was mitigated by the presence of the reservoir. The highest LST values were recorded in the zone where agriculture dominated. Positive LST trends were recorded throughout the entire study, albeit with differences in the slopes in individual months and these were statistically significant only in August and September.

This study provides valuable insights into the thermal dynamics associated with reservoirs adjacent to urban developments. The findings increase our understanding of the multifaceted effects of large reservoirs and urbanization on regional climates, especially the variations in temperature and land cover dynamics.

Acknowledgements

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Appendix

Table 4 Regressions and regression coefficients for surface temperature trend during the period of 37 years for individual months from May to September. Information is presented for six small sites (T_S1–T_S6), conditional Zone 1 (T_Z1), conditional Zone 1 with reservoir water area (T_Z1_W), conditional Zone 1 without reservoir water area (T_Z1_WW), and air temperatures for Kyiv (T_AIR_KY_9.30). This information complements the graphs in Fig. 9 of the main text.

Month	Regression coefficients	Month	Regression coefficients
May	T_S1 = 20.33 + 0.0002 × x; T_S2 = 19.63 + 0.0002 × x; T_S3 = 9.72 + 0.0003 × x; T_S4 = 18.77 + 0.0003 × x; T_S5 = 14.73 + 0.0004 × x; T_S6 = 7.30 + 0.0002 × x; T_Z1_W = 7.35 + 0.0002 × x; T_Z1_WW = 9.47 + 0.0004 × x. T_AIR_KY_9.30 = 9.75 + 0.0003 × x	June	T_S1 = 36.50-0.0002 × x; T_S2 = 25.88 + 0.0001 × x; T_S3 = 24.21 + 3.6173E-5 × x; T_S4 = 26.28 + 0.0002 × x; T_S5 = 23.76 + 0.0002 × x; T_S6 = 17.34 + 0.0001 × x; T_Z1_W = 17.70 + 0.0001 × x; T_Z1_WW = 22.30 + 0.0002 × x; T_AIR_KY_9.30 = 22.36 + 0.00006 × x
July	T_S1 = 25.52 + 0.00008 × x; T_S2 = 21.70 + 0.0002 × x; T_S3 = 12.27 + 0.0003 × x; T_S4 = 13.84 + 0.0005 × x; T_S5 = 27.53 + 0.0001 × x; T_S6 = 10.57 + 0.0003 × x; T_Z1_W = 10.85 + 0.0003 × x; T_Z1_WW = 18.17 + 0.0003 × x; T_AIR_KY_9.30 = 20.17 + 0.0001 × x	August	T_S1 = 19.98 + 0.0003 × x; T_S2 = 11.84 + 0.0005 × x; T_S3 = 7.10 + 0.0005 × x; T_S4 = 4.97 + 0.0007 × x; T_S5 = 10.47 + 0.0005 × x; T_S6 = 6.11 + 0.0004 × x; T_Z1_W = 6.40 + 0.0004 × x; T_Z1_WW = 9.85 + 0.0005 × x; T_AIR_KY_9.30 = 8.98 + 0.0004 × x
September	T_S1 = 0.20 + 0.0006 × x; T_S2 = -0.23 + 0.0006 × x; T_S3 = -3.30 + 0.0006 × x; T_S4 = -7.88 + 0.0008 × x; T_S5 = -4.04 + 0.0007 × x; T_S6 = -3.01 + 0.0005 × x; T_Z1_W = -2.50 + 0.0005 × x; T_Z1_WW = -3.73 + 0.0007 × x; T_AIR_KY_9.30 = 2.97 + 0.0004 × x		

EFFECT OF GLYPHOSATE ON GERMINATION AND SEEDLING DEVELOPMENT OF FOUR NATIVE PLANTS OF DUNES IN SPAIN

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ABSTRACT

Using glyphosate is easier than manually controlling the invasive plant *Carpobrotus* in dune ecosystems. However, before it is used its effect on native species in this ecosystem needs to be determined. This study reports the effects in the laboratory of different concentrations of glyphosate (1.0, 0.5, 0.3, 0.1, 0.05, 0.01, and 0.005 g/m^2) on germination and seedling emergence of four species of Mediterranean dune plants, *Lotus creticus*, *Medicago marina*, *Ononis ramosissima* and *Ammophila arenaria,* used in the restoration of this natural ecosystem. Germination was completely inhibited when glyphosate was applied at 0.5, and 1.0 g/m^2. The recommended dose (0.3 g/m^2) also inhibited the germination of *L. creticus* and *M. marina*. The percentage germination of *O. ramosissima* and *A. arenaria* was around 40% and 30% respectively when a dose of glyphosate of 0.1 g/m^2 was used, whereas for seeds of *L. creticus* and *M. marina* it was lower than 5%. The T_{50} at germination of seed incubated with glyphosate increased in all species independently of the dose of glyphosate. Seedling emergence from seeds previously germinated in the presence of glyphosate was only recorded for *O. ramosissima* and *A. arenaria.* The results indicate that seeds of *O. ramosissima* are more tolerant of glyphosate, followed by those of *A. arenaria* and those of *L. creticus* and *M. marina* are the most sensitive.

Keywords: germination; glyphosate; herbicide tolerance; chemical control; dune restoration; native plant reintroduction

Introduction

The invasive non-native succulent plant genus *Carpobrotus* (L.) N.E. Br. (*Aizoaceae*) is one of the most important weeds in Mediterranean coastal ecosystems and numerous studies have shown that it has strong adverse effects on native plants and animals (Campoy et al. 2018). Attempts to eradicate this invasive weed have been implemented in many places around the world (Buisson et al. 2020; Lazzaro et al. 2020, 2023; Fos et al. 2021, 2022) including in Mediterranean habitats (Campoy et al. 2018). Although the most common method of eradicating *Carpobrotus* is removal by hand, the use of herbicides has been an effective means of control. Glyphosate, N-(phosphonomethyl) glycine, was the herbicide most often used (Lazzaro et al. 2020; Fos et al. 2021, 2022).

Glyphosate-based herbicides are among the most widely used broad spectrum herbicides in the world (Henderson et al. 2010; Myers et al. 2016). Glyphosate is a non-selective herbicide that inhibits plant growth by interfering with the production of essential aromatic amino acids, which do not share the same biosynthetic pathways with members of the animal kingdom (Henderson et al. 2010; Velmourougane et al. 2021). Glyphosate is assimilated by leaves and other green plant tissues and then rapidly translocated via the phloem through the entire plant, including the roots (Henderson et al. 2010; Badani et al. 2023). The large-scale use of glyphosate is currently restricted or banned by legislation in many European countries (Lazzaro et al. 2020), but the context and scale must be considered when applying such bans on a small scale, such as for the purpose of controlling an invasive plant (Pergl et al. 2020).

However, before deciding to use glyphosate to eradicate invasive plants, and in particular, the eradication of *Carpobrotus* in coastal areas, the potential negative effects of this herbicide must first be identified and understood. For this purpose, the germination and emergence of seedlings of four native plants growing in dunes around the Mediterranean were recorded after their seeds were incubated in solutions of different concentrations of glyphosate and the effect of glyphosate exposure on germination and seedling development evaluated.

Material and Methods

Plant material

The effect of glyphosate on germination and seedling emergence was evaluated for four native plants of dunes in the Mediterranean area: *Lotus creticus* L, *Medicago marina* L, *Ononis ramosissima* Desf. (*Fabaceae*) and *Ammophila arenaria* (L.) Link. (*Poaceae*). These species are very common and abundant in the natural vegetation growing along the Valencian coast and belong to the association *Medicagini mariane-Ammophiletum australis,* found in embryonic dunes and those that are moving. Seed of these species was supplied by the seed bank at the Centre for Forest Research and Experimentation (CIEF, Generalitat Valenciana).

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Tests used to evaluate the effect of glyphosate on dune plants

Germination

The seeds of *L. creticus*, *M. marina*, *O. ramosissima* and *A. arenaria*, which were previously scarified (15 minutes in H_2SO_4), sterilized by placing them in 0.5% sodium hypochloride (NaClO) solution for two minutes and thoroughly rinsed with deionized water before sowing.

These seeds were placed in 9 cm diameter Petri dishes on two layers of filter paper (Whatman nº 1) moistened with 20 ml of distilled water or glyphosate solutions of a particular concentration. Four replicates of 50 seeds were used in each treatment. Seeds were considered to have germinated when approximately 2 mm of the primary root had emerged. Seeds were germinated in a growth chamber (16 h light/8 h darkness, 21 °C, 60% relative humidity).

Glyphosate (36% p/v, as an ammonium salt; Touchdown, Syngenta Iberica, Spain) was used to obtain solutions of the desired concentration for moistening seeds (20 ml/germination box). For each species, seeds were germinated in glyphosate solutions equivalent to doses of 1.0, 0.5, 0.3, 0.1, 0.05, 0.01 and 0.005 g/m². The minimum effective glyphosate concentration for controlling *Carpobrotus* is 0.4 g/m² (Fos et al. 2021) and the recommended dose of glyphosate is 0.3 g/m² (EFSA 2017).

Percentage germination was recorded over a period of 40 days. At the end of the experiment, non-germinated seeds were dissected in order to determine their viability and only seeds with a complete embryo were considered as viable. Percentage seed germination (G) for each replicate was calculated at the end of the experiment as: $\%G = 100 \times (GS/FS)$, where *GS* is the number of seeds that germinated and *FS* the number of viable seeds. Rate of germination was estimated using T_{50}, which is the time in days to when 50% of the seeds had germinated.

Seedling emergence

Germinated seeds incubated in a solution of glyphosate (0.005 to 0.1 g/m²) were sown in trays containing 0.5 l of sterilized beach sand to evaluate the effect of glyphosate on seedling emergence and development. For each species and glyphosate treatment twenty germinated seeds were used. The emergence tests were carried out in a chamber under controlled conditions (16 h light/8 h darkness, 20 °C, 60% relative humidity). The emergence and development of the aerial parts of seedlings was monitored for at least 60 days.

Statistical analysis

The analysis of percentage germination of the seed of four native species in solutions of glyphosate equivalent to different doses was done using analysis of variance (ANOVA) and Statgraphics plus software (5.1 for Windows, 1994, Statistical, Corporation, Warrenton, VA). The statistical analysis of the data was done separately for each species using the glyphosate doses as the main factor. Tukey multiple comparison test was used for each ANOVA to determine the significance of any differences in germination between the different glyphosate doses with respect to that on the control substrate ($p < 0.05$).

Results

Effect of exposure to glyphosate on time to and percentage germination of dune plants

Percentage germination of *L. creticus* seeds after 40 days of incubation in the control was higher than 90% (Fig. 1a). It was significantly reduced by up to 50% and 45% at glyphosate doses of 0.005 and 0.01 g/m² (Fig. 1a) and less than 10 and 5% for the doses of 0.05 and 0.1 g/m² (Fig. 1a). No germination was recorded when incubated with doses of glyphosate equal to or greater than 0.3 g/m² (Fig. 1a). The T_{50} for the control was 4 days and increased to over 20 days when incubated in the presence of glyphosate (Fig. 2a). No differences in T_{50} were recorded between the different doses of glyphosate.

Percentage germination of *M. marina* seeds after 40 days of incubation in the control was approximately 70% (Fig. 1b). It was significantly reduced by up to 75% at glyphosate doses of 0.005 and 0.01 g/m² (Fig. 1b) and was less than 3% for doses of 0.05 and 0.1 g/m² (Fig. 1b). No germination was recorded when incubated with doses equal to or greater than 0.3 g/m² (Fig. 1b). The T_{50} for the control was 8 days and increased to 15–18 days when incubated in the presence of glyphosate (Fig. 2b).

Percentage germination of *O. ramossisima* seeds after 40 days of incubation in the control was approximately 30% (Fig. 1c). It was significantly reduced by 50% at a glyphosate dose of 0.005 g/m² and 60% at glyphosate doses between 0.01 to 0.1 g/m² (Fig. 1c) and 3% at a dose of 0.3 g/m². The T_{50} for the control was 4 days and increased to 16–22 days when seeds were incubated in the presence of glyphosate (Fig. 2c). No differences in T_{50} were recorded for the different doses of glyphosate (Fig. 2c).

Percentage germination of *A. arenaria* seeds after 40 days of incubation in the control was approximately 42% (Fig. 1d). It was significantly reduced by about 40% compared to control at glyphosate doses of 0.005 and 0.01 g/m², about 70% at doses between 0.05 to 0.1 (Fig. 1d) and 1–2% at a dose of 0.3 g/m². The T_{50} for the control was 13 days (Fig. 2d) and increased to 20–25 when incubated in the presence of glyphosate (Fig. 2d).

Effect of exposure to glyphosate on seedling emergence and development of dune plants

Seedling emergence in the control ranged from 70% for *A. arenaria* and *M. marina,* 75% for *O. ramosissima* and a maximum of 85% for *L. creticus* (Table 1). Seedling emergence of *O. ramosissima* was 40% for doses of 0.005 and 0.01 g/m² and 10% for 0.1 g/m² (Table 1 and Fig. 3). In the case of *A. arenaria,* it was 10% for a dose

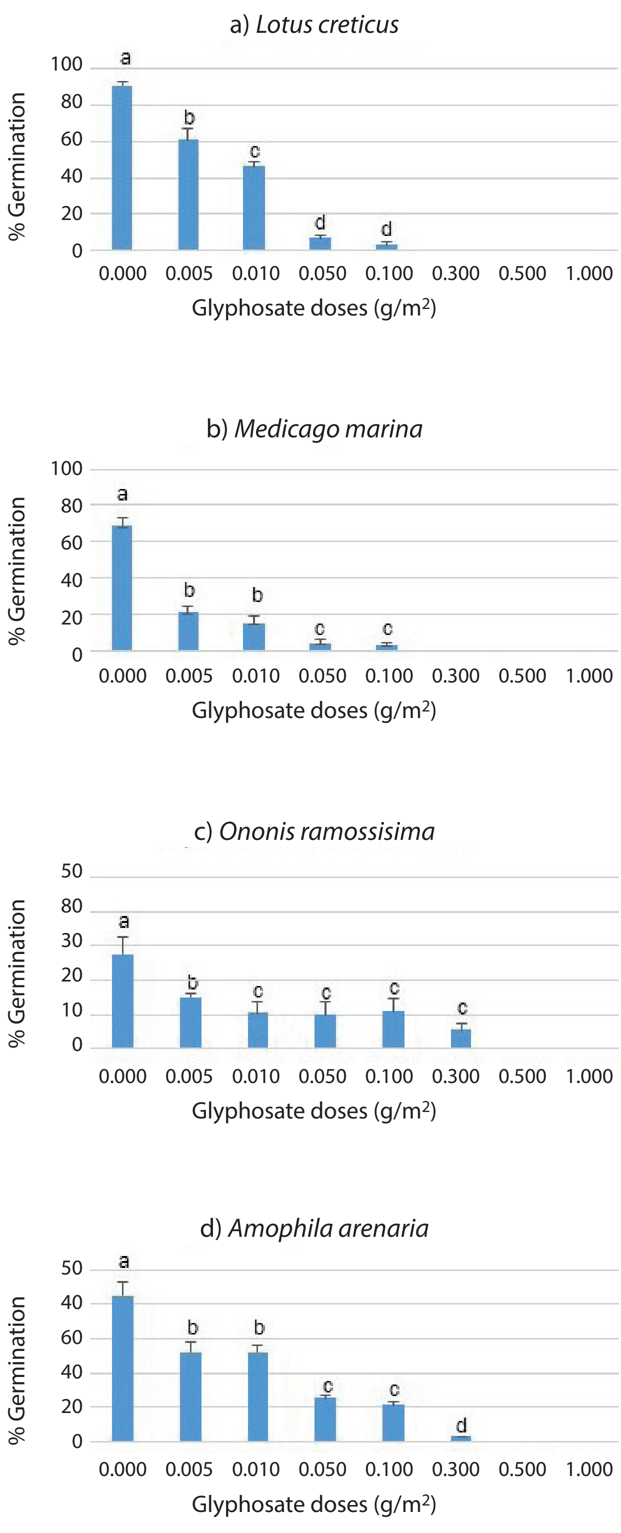

Fig. 1 Percentage germination of the seed of *Lotus creticus*, *Medicago marina, Ononis ramosissima* and *Ammophila arenaria* previously exposed to different doses of glyphosate. Percentage germination was recorded after 40 days (mean values ± standard error). Different letters denote statistically significant differences in the percentage germination at different doses of glyphosate based on Tukey's multiple comparison test ($p < 0.05$).

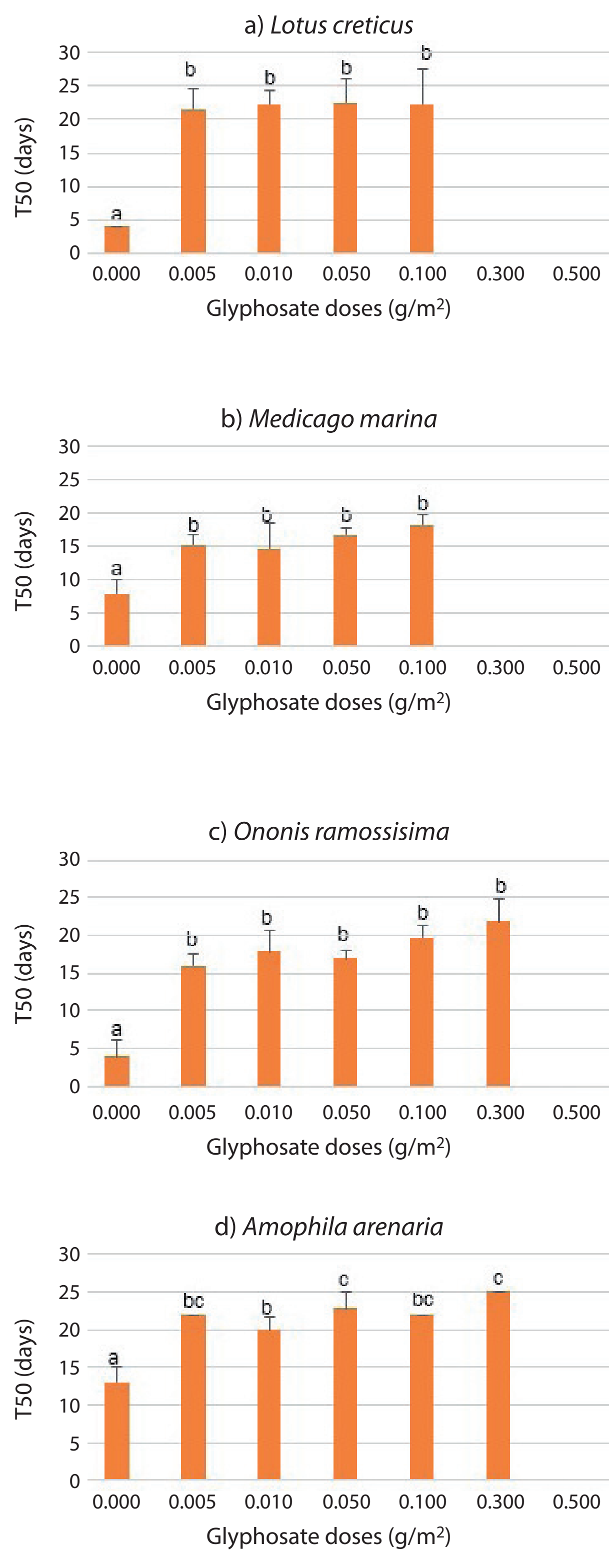

Fig. 2 Time to germination of seed of *Lotus creticus*, *Medicago marina, Ononis ramosissima* and *Ammophila arenaria* treated with different doses of glyphosate. T_{50} (mean values ± standard error). Different letters denote statistically significant differences based on Tukey's multiple comparison test ($p < 0.05$).

of 0.005 g/m^2 and 30% for 0.01 g/m^2 (Table 1 and Fig. 3). No seedling emergence was recorded for *L. creticus* and *M. marina* previously incubated in presence of glyphosate (Table 1).

The height of the seedlings of *O. ramosissima* that germinated from seeds incubated with glyphosate decreased relative to the control from 30% for the dose of 0.005 g/m^2 to 50% for 0.1 g/m^2 (Table 1 and Fig. 3). The decrease in development relative to the control was greater for the *A. arenaria* seedlings that germinated from seed incubated with glyphosate, with the height 40% lower for the dose of 0.005 g/m^2 and 60% lower for the dose of 0.01 g/m^2 (Table 1 and Fig. 3).

Discussion

In summer in the Mediterranean area, the seeds of many species of *Fabaceae* are dormant, which prevents germination during the dry hot summers and postpones germination to the following spring. Germination of *M. marina* seeds is less than 10% (Scippa et al. 2011) and in the case of *O. ramosissima* it is between 6 and 16% (Devesa et al. 2000). Seed dormancy in these species is associated with the condition of the testa, which after mechanical or chemical scarification, results in almost 100% germination in *L. creticus* (Hajri et al. 2018; Rejili et al. 2009), *M. marina* (Scippa et al. 2011) and *O. ramosissima* (Devesa et al. 2000). The scarification treatment used has been highly effective for *L. creticus* (92%) and *M. marina* (70%) (Fig. 1). The percentage germination of scarified seed of *O. ramosissima* (30%, Fig. 1) was higher than that of non-scarified seed (Devesa et al. 2000). The time taken to complete dormancy may be longer than the time to germination after scarification (Devesa et al. 2000). The percentage germination of the *A. arenaria* seeds that were not exposed to glyphosate was higher than 42% (Fig. 1). This is lower than previously reported for this species (Chergui et al. 2013).

Glyphosate is known to affect germination or seedling quality when applied directly to seed of species of different families, such as: *Poaceae* (Yenish and Young

Table 1 Seedling emergence and development of *Lotus creticus*, *Medicago sativa*, *Ononis ramosissima* and *Ammophila arenaria* from seed incubated in different doses of glyphosate (n = 20). Final seedling emergence and percentage emergence compared to the control (%) after 60 days. Height of seedlings (mean) and percentage height compared to the control (%) after 60 days.

Lotus creticus				
Glyphosate doses (g/m2)	**Final emergence (n)**	**%**	**Seedling height (cm)**	**%**
0.000	17	85	13.1	100
0.005	0	0	0	0
0.010	0	0	0	0
0.050	0	0	0	0
0.100	0	0	0	0
Medicago marina				
Glyphosate doses (g/m2)	**Final emergence (n)**	**%**	**Seedling height (cm)**	**%**
0.000	14	70	9.3	100
0.005	0	0	0	0
0.010	0	0	0	0
0.050	0	0	0	0
0.100	0	0	0	0
Ononis ramosissima				
Glyphosate doses (g/m2)	**Final emergence (n)**	**%**	**Seedling height (cm)**	**%**
0.000	15	75	4.4	100
0.005	8	40	3.1	70
0.010	8	40	2.6	59
0.050	0	0	0	0
0.100	2	10	2.3	52
Ammophila arenaria				
Glyphosate doses (g/m2)	**Final emergence (n)**	**%**	**Seedling height (cm)**	**%**
0.000	14	70	13.9	100
0.005	2	10	8.0	57
0.010	6	30	5.6	40
0.050	0	0	0	0
0.100	0	0	0	0

Fig. 3 Seedling emergence and development of *Ononis ramosissima* and *Ammophila arenaria* seeds previously incubated in solutions of different doses of glyphosate.

2000; Piotrowicz-Cieślak et al. 2010) *Fabaceae* (Piotrowicz-Cieślak et al. 2010; Gomes et al. 2017; Mondal et al. 2017) and *Brassicaceae* (Piotrowicz-Cieślak et al. 2010; Kashyap et al. 2023). As expected, based on the widespread use of glyphosate for pre- and post-emergence control, the presence of this herbicide in the incubation medium inhibited seed germination of all the dune plants used in this study (Fig. 1). In addition, the percentage inhibition of germination by glyphosate was clearly associated with the dose (Fig. 1). The germination was completely inhibited at doses of 0.5 and 1.0 g/m^2 (Fig. 1), which are both above the recommended dose of 0.3 g/m^2 (EFSA 2017). The inhibitory effect of exposure to glyphosate decreased progressively for doses equal to or lower than the recommended dose (Fig. 1).

The results indicate that the percentage inhibition of germination depends on the species. Thus, the presence of glyphosate at the recommended dose (0.3 g/m^2, EFSA, 2017) did not completely inhibit the germination of the seed of *O. ramosissima* and *A. arenaria* (Fig. 1), whereas it did for that of *L. creticus* and *M. marina* (Fig. 1). Differences were also recorded for the percentage germination of the seed of the 4 species of dune plants at doses below the recommended dose (Fig. 1). An equivalent dose of 0.1 g/m^2 was used in a glyphosate tolerance screening of wild relatives of crop plants belonging to the *Brassicaceae* (Kashyap et al. 2023). At this dose, the percentage germination relative to the control was reduced by more than 95% for *L. creticus* and *M. marina*, 75% for *A. arenaria* only by 40% for *O. ramosissima* (Fig. 1). The results indicate that *L. creticus* and *M. marina* are more sensitive to glyphosate than the other two species (Fig. 1).

Glyphosate not only reduced percentage germination, but also significantly increased the time to germination (Fig. 2). Relative to the control the T_{50} of glyphosate treated seed of *M. marina* and *A. arenaria* doubled and that of *L. creticus* and *O. ramosissima* increased 4 to 5fold (Fig. 2). The general trend in the 4 species is that the increase in T_{50} is very similar regardless of the dose of glyphosate used (Fig. 2). The results also indicate that germination of seed incubated with glyphosate starts after a similar length of time for all species (Fig. 2). These results might indicate that the time to germination is associated with the loss of glyphosate toxicity. Glyphosate has a half-life of 2 to 14 days in water (Badani et al. 2023), thus the inhibitory effect of glyphosate applied to beach sand on the emergence of dune species would begin to decline after 15 days (Fos et al. 2022).

The results also indicate that glyphosate affected the emergence and development of seeds germinated previously in the presence of this herbicide (Table 1 and Fig. 3). Seedling emergence from germinated seeds previously incubated in the presence of all the doses of glyphosate was not recorded for 2 of the 4 species, *L. creticus* and *M. marina.* These results reinforce the different sensitivity of the species to the inhibiting effect of this herbicide (Table 1) as recorded for the wild relatives of different crop plants belonging to the *Brassicaceae* (Kashyap et al. 2023). In addition, the seeds of *A. arenaria* and *O. ramosissima* were more resistant to glyphosate.

The results, however, are based on laboratory tests under very homogeneous and controlled conditions. The seeds were soaked in a solution containing the herbicide. These conditions do not correspond to those in the dunes where glyphosate is used to control the non-native invasive plant *Carpobrotus.* In a real situation, in which this herbicide is applied by professionals, the seed in the soil seed bank would only be slightly wetted by the herbicide in areas adjacent to monospecific carpets of *Carpobrotus*. In addition, the seeds used in the laboratory tests were not subjected to changing environmental conditions as they would be in the field. Previous results of a study on seedling emergence of Mediterranean dune plants on substrates collected after spraying with glyphosate under real conditions reports higher emergence values (Fos et al. 2022) than those obtained under laboratory conditions (Table 1).

Conclusions

In conclusion, the presence of the herbicide in the incubation medium inhibited seed germination of four species of Mediterranean dune plants and the inhibition was dependent on the dose of glyphosate used. The increase in T_{50} of seeds incubated in the presence of glyphosate was independent of the dose used. Glyphosate also affected the later emergence and development of seedlings from seed germinated previously in the presence of this herbicide. The four dune plants differed in their sensitivity to glyphosate, with *A. arenaria* and *O. ramosissima* the least sensitive to the herbicide. Under field conditions, the negative effect of glyphosate is significantly less than that recorded in the laboratory.

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NATURAL ATTENUATION OF PHARMACEUTICALS AND PERSONAL CARE PRODUCTS PERCOLATING THROUGH ROCKY SUBSTRATES – AN EXAMPLE BASED ON THE KÁRANÝ WATERWORKS

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ABSTRACT

This survey focused on a detailed analysis of the ability of fluvial Quaternary sediments to remove pharmaceuticals and personal care products (PPCPs) from drinking water. Thirty-eight PPCPs were detected in the Jizera River, which is used after infiltration to produce drinking water by the Káraný waterworks. Several PPCPs occurred in the water at concentrations exceeding 100 ng/l, some of which are not possible to remove (oxypurinol, acesulfame). The presence of PPCPs was monitored after infiltration and during passage through sandy gravels to the receiving well (total distance of 180 m) at monthly intervals in 2022–2023. PPCPs can be divided into different groups based on the results. Iohexol, iopromide, metoprolol, cetirizine, valsartan and clarithromycin were already below the established threshold after passing through 60 m of gravel. Other substances were gradually attenuated and a diverse group of PPCPs remained in the groundwater even after passing through 180 m of subsoil. Surprisingly, the PPCP with a high degree of attenuation, such as metformin, whose concentration drops from the original value of 677 ng/l to 16 ng/l, was in this group. The member of this group with lowest degree of attenuation was sulfamethoxazole with a value of 9%. Five substances (benzotriazole, propylparaben, bisphenol S, hydrochlorothiazide, ibuprofen-2-hydroxy) were identified as the most problematic since they passed through the quaternary fluvial aquifer practically unchanged and the process of qualitative treatment using artificial infiltration appears to be ineffective.

Keywords: artificial infiltration; natural attenuation; pharmaceuticals; personal care products

Introduction

Artificial recharge is a process by which various types of surface water (e.g. that from rivers, lakes, captured precipitation, but in extreme cases also treated wastewater) are transformed into groundwater. Primary aim is to slow down water runoff and retain water in the landscape, which can greatly benefit nature. This technology is becoming more and more popular globally. Artificial recharge is often used to revitalize and save wetlands or other water-bound ecosystems (Ruiz et al. 2023; Gómez-Escalonilla et al. 2024). Another common goal of artificial recharge is to create new water source for irrigation, especially in regions with a high level of groundwater overexploitation, such as India, Pakistan, USA, but also in Mediterranean countries in the EU (Maskey et al. 2022; Perdikaki et al. 2022). Artificial recharge is a very popular technology for producing high-quality sources of drinking water, e.g. via building artificial infiltration systems that use the natural attenuation properties of rocky substrates. Percolation through an unsaturated zone and aquifer, can result in a great improvement in the physical-chemical properties, removal of nitrogen and phosphorus compounds, as well as organic compounds, heavy metals and other undesirable components (Eisfeld et al. 2021; Sarfaraz et al. 2021; Hassan et al. 2023).

However, with advances in analytical techniques, such as, liquid or gas chromatography coupled with highly sensitive (tandem) mass spectrometry, a new qualitative problem has emerged in recent years. A wide range of previously unknown substances have been identified and quantified in the aquatic environment in ng/l to μg/l concentrations (Fawell and Ong 2012; Rivera-Utrilla et al. 2013; Yao et al. 2022) and in other parts of the ecosystem (Badani et al. 2023), pharmaceuticals and personal care products (PPCPs). Over 400 of them are recorded in surface and groundwater and constitute a large and diverse group of chemicals of mainly synthetic origin (e.g. drugs used to treat human and animal diseases, household chemicals and disinfectants, cleaning products, lotions, suncream agents, fragrances). Some of them are extremely persistent in the environment and have toxic and bio accumulative properties. Untreated and treated sewage, landfills, hospital waste, veterinary drugs and agricultural wastewaters are the main sources of PPCPs (Grasserová et al. 2020; Yao et al. 2022).

The most problematic drugs are the ones that are also the most representative in terms of numbers. Already more than a decade ago, global annual per capita use of pharmaceuticals was 15 g, which is three to ten times higher (50–150 g) in developed countries (Zhang et al. 2008). Moreover, the use of pharmaceuticals has

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continued to rise in recent years, driven by a growing need for drugs to treat age-related and chronic diseases (González Peña et al. 2021). Oncology is the other top therapeutic area driving drug sales globally and likely to show the largest growth in the see able future, followed by drugs for treating autoimmune diseases and diabetes. The COVID-19 pandemic also needs to be included, as vaccine Comirnaty was the world's top revenue generating pharmaceutical product during the height of the COVID-19 pandemic (Mikulic 2024). The total global pharmaceutical market was estimated at around 1.6 trillion U.S. dollars in 2023, which is an increase of over 100 billion dollars compared to 2022 (Mikulic 2024). The study of PPCPs in drinking water is even more complicated as the pharmaceutical industry currently produces more than 7,100 approved drugs for human or veterinary use. The total number of experimental or investigational drugs is also high, increasing by 38% from 3,394 to 6,231, between 2018 and 2023 (Knox et al. 2024). Not only do primary substances fall into this category, but also their by-products, which sometimes have much more negative properties. It is likely that these substances are present everywhere to varying extents and concentrations, and the awareness of their presence depends on which chemical substance laboratories focus on identifying in the water/environment.

The toxicity of drugs, especially endocrine disruptors, has recently become the subject of scientific debate and a global public health problem, primarily because of their individual or synergistic effects on humans and the ecosystem (Trapido et al. 2014; Barton-Maclaren et al. 2022; Wu et al. 2023; Dalamaga et al. 2024; He et al. 2024). These substances cause dysfunction of the endocrine system, manifesting in a variety of diseases, including developmental and metabolic disorders (Schneider et al. 2019; Robles-Matos et al. 2021; He et al. 2024). Another fundamental problem is that these pollutants occur in the environment almost exclusively as mixtures (Kolpin et al. 2002; Loos et al. 2009). The negative effects of PPCPs on animal species, especially those in the aquatic environment, are well documented (Hoeger et al. 2005; Lyssimachou and Arukwe 2007; Adeleye et al. 2022; Iturburu et al. 2024; Li et al. 2024). In contrast, there are very few studies on the negative effect on human health, especially clinical studies (Huang et al. 2020). Intensive and long-term studies of this issue are needed, especially of the drugs in drinking water, as this is one of the main sources of these substances for humans. The Káraný waterworks, where the occurrence and fate of PPCPs after percolating through a rocky environment is monitored, is the first to do this in the Czech Republic.

The Káraný waterworks, which is located in Central Bohemia (see Fig. 1), is a unique facility in the Czech Republic in using two different technologies. Between 1906 and 1913, a bank infiltration system was established and is still operating. It consists of 685 wells with a depth of 8–12 m, which are 20–40 m apart. The receiving wells are located mainly in gravel-sand fluvial sediments at about 250 m from the Jizera River. The total capacity of this system is 86 400 m^3/day.

Fig. 1 The location of the Káraný waterworks in the Czech Republic.

Since 1968, the waterworks has used an additional method to produce drinking water based on the principle of artificial recharge. The first step in this process is a simple mechanical treatment of the surface water from the river. The water is filtered through sand and pumped into infiltration basins, from where it seeps into sandy fluvial sediments with a thickness of 20 m. A system of large-diameter wells. with a total capacity of 77,760 m^3/day, is located about 200 m from the infiltration basins. The water from these wells is a mixture of infiltrated surface water and groundwater from the sand-gravel terrace in the east between the waterworks and the Jizera River.

The Káraný waterworks supplies approximately one third of the drinking water for the capital city of Prague, with more than 1.3 million inhabitants. In the last decade, monitoring the concentrations of PPCPs in drinking water has come to the fore. The source of this pollution is water from wastewater treatment plants (WWTP) the out flow from which goes into the Jizera River. The most important source of PPCPs is the city of Mladá Boleslav (45,000 inhabitants), in which is the Kosmonosy psychiatric hospital with a capacity of up to 600 patients.

Systematic monitoring of the water quality in the Jizera River between 2017 and 2021 in the section between Mladá Boleslav and Káraný (Hrkal et al. 2018; Hrkal 2022) revealed a wide range of PPCPs leaving the WWTP in Mladá Boleslav. Oxypurinol and telmisartan occurred in the treated wastewater at concentrations in the order of tens of μg/l; the other four drugs, namely diclofenac, tramadol, lamotrigine and hydrochlorothiazide, were present at concentrations in the order of units of mg/l. For the other 44 drugs analysed, concentrations systematically ranged from units to hundreds of ng/l. Even though treated wastewater is greatly diluted (the average flow of the Jizera River in Mladá Boleslav is 24 m^3/s), 38 PPCPs were recorded in the river water. Table 1 provides an overview of the most important substances whose average concentrations in the water used to produce drinking water, at the Káraný waterworks, exceed 100 ng/l.

Table 1 Concentrations of PPCPs (ng/l) in the water of the Jizera river (monthly average for 2017–2018) (Hrkal et al. 2018).

PPCP substance	Concentration (ng/l)
Oxypurinol	379
Acesulfame	291
Telmisartan	210
Paraxanthine	209
Caffeine	172
Saccharin	156
Gabapentin	121
Iomeprol	110

Detailed monitoring revealed a significantly higher removal of PPCPs in the case of bank infiltration. The clogging zone of the riverbed was able to capture the vast majority of PPCPs thanks to the sorption properties of the clay minerals it contains. None of the PPCPs were regularly detected in the river during the monitoring, except very rarely in concentrations at the limit of detection.

A worse situation was revealed in the case of artificial recharge. In this case, the river water passes through the gravel in the infiltration basins and then through fluvial sediments of similar lithological composition. A thin clogging layer on the surface of the basins, which forms during the infiltration process, reduces the efficiency of infiltration. For this reason, it is mechanically removed approximately every two years. The sorption capacity of artificial infiltration is therefore less effective than bank infiltration. This was manifested in the presence of six substances, of which acesulfame and oxypurinol exceeded a concentration of 100 ng/l (Fig. 2).

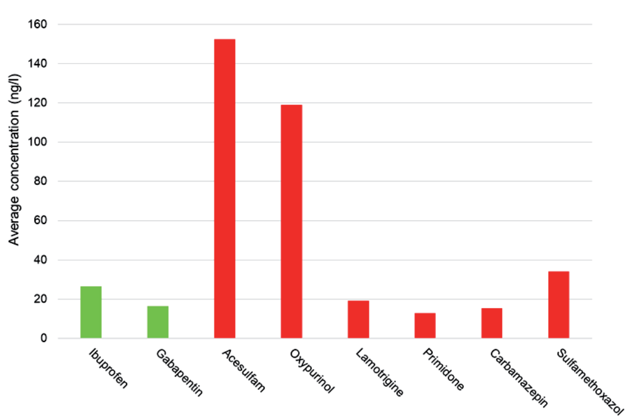

Fig. 2 Average concentrations (ng/l) of PPCPs in drinking water produced using artificial recharge (monthly averages from 2017–2018) (Hrkal et al. 2018) – unsystematic occurrences are in green, regularly occurring substances are in red.

Monitoring carried out between 2017 and 2018 revealed a problem. It assumed the process of artificial recharge was a "black box" and the chemistry was monitored in the input (raw river water) and output (drinking water produced). However, this monitoring did not reveal what happens to individual PPCPs when they pass through the infiltration basin and subsequently the rocky substrate. The new method of monitoring, the results of which are presented in this article, set out to answer this question. The main objective of this paper was to follow up and analytically extend the outputs of the previous monitoring system of PPCPs, which occur in the water used in the Káraný waterworks for producing potable water using an artificial filtration system. The objective was to describe what happens to PPCPs as they pass through this system, monitor changes in the concentrations of individual PPCPs, identify groups of PPCPs that are affected similarly and evaluate the effectiveness of natural attenuation for removing PPCPs from water. A secondary objective was to propose possible other solutions and identify technologies by which PPCPs can be destroyed or their concentrations can be further reduced .

Methodology

This two-year monitoring project focused on a detailed analysis of the behaviour of 43 PPCPs, the selection of which was based on the substances detected during previous monitoring between 2017 and 2018.

Monitoring and sampling

From May 2022 to April 2023, four samples of water were collected at the following locations at monthly intervals: river water entering the infiltration basin (i), monitoring well HV1 (ii), monitoring well HV2 (iii) and collection well R14 (iv). Locations monitored and schematic cross section of the site are presented in Fig. 3.

The wells HV1 and HV2 were located at a distance of 60 m and 120 m from the infiltration basin, respectively. They are 20 m deep with the following lithological profile: 0–2 m clayey sand (Quaternary), 2–19 m sandy gravel (Quaternary) and 19–20 m marl (Cretaceous). The diameter of both wells was between 2–19 m. Well, R14, is 145 m from the outer edge of the infiltration basin and has a diameter of 5 m. It is a Raney-type well with a depth of 20 m and the bottom of which is impermeable chalk clinker. Two drainage pipes 300 mm in diameter, located parallel to the axis of the infiltration basin, extended horizontally from both sides of the bottom of this well. The southern drain is 57 m long, the northern 45 m long. Thus, they can collect groundwater flowing from the infiltration basin, which includes that flowing from opposite sides of the Quaternary aquifer. The water in the R14 well is therefore a mixture of water from artificial recharge and groundwater, the quality of which is that of natural background water. Understanding the

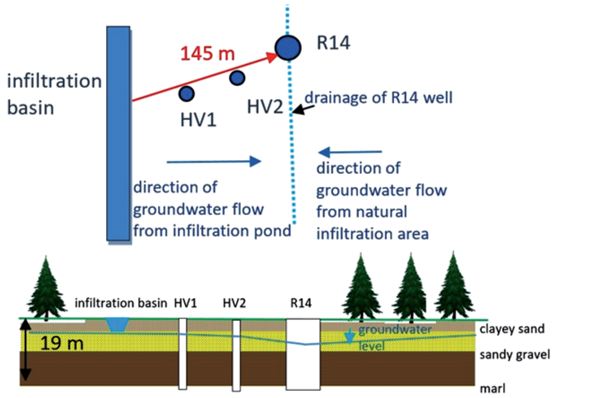

Fig. 3 Schematic presentation of the location and spatial distribution of sites monitored.

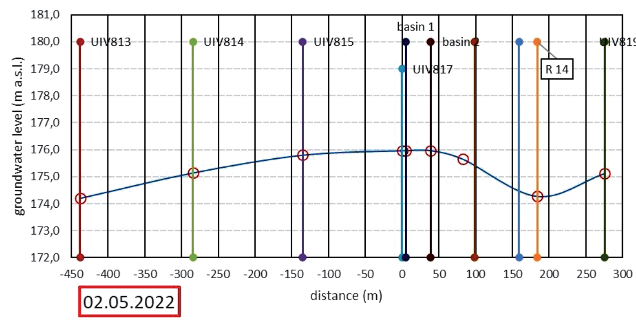

Fig. 5 Level of groundwater, basin infiltration = 0 l/s, R14 pumping = 19.88 l/s.

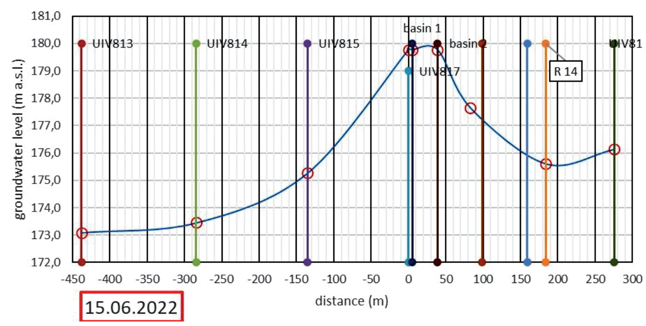

Fig. 6 Level of groundwater, basin infiltration = 22.12 l/s, R14 pumping = 20.47 l/s.

attenuation processes depends on a knowledge of the hydraulic properties of the Quaternary aquifer. Daily measurements of the levels in the observation wells 813, 814, 815, 817, 818 and 819, and in the infiltration basin and well R14 are available based on previous measurements (see Figs 4–6). The volumes of water flowing into the infiltration basin and withdrawn from well R14 were included in the data collected. Based on this data, the mean value of the saturated hydraulic conductivity of the aquifer k_f (4.2×10^{-4} m/s), the mean value of the effective porosity (27.5%) and range of the size of the hydraulic gradients I between the infiltration basin and R14 well in a situation corresponding to the sampling of groundwater (1×10^{-4} – 3×10^{-4}), were calculated. The actual rate of movement of groundwater between the infiltration basin and R14 well varied from 1×10^{-4} to 3×10^{-4} m/s (16.8 to 5.6 m/day). The retention time thus varied during the monitoring period, depending on the specific hydraulic situation, from 8.8 to 25.9 days.

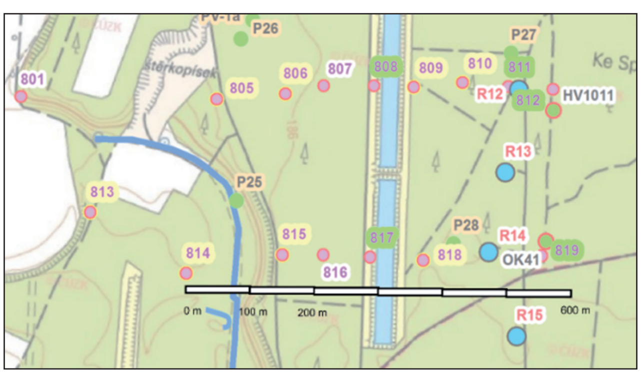

Fig. 4 Location of wells and data on levels of groundwater.

Analytical methods

Sample preparation and analysis were done using the method of Dume et al. (2023). A reducing agent was added to the aqueous samples, the pH was adjusted to 3.5, the samples were placed in an ultrasonic bath (10 min, RT) and then centrifuged (10 min, 6,000 rpm). A 1.8 mL aliquot of supernatant from each sample was collected in a glass HPLC vial and analysed in triplicate.

The treated aqueous samples were analysed using an Agilent 6470 Triple Quadrupole LC-MS System liquid chromatograph with mass detection. The system was equipped with an Agilent Poroshell 120 EC-C18 column (100 × 3 mm; 2.7 µm) with an Agilent Poroshell 120 EC-C18 pre-column (5 × 3 mm; 2.7 µm) and an Agilent 6470 triple quadrupole. 0.5 mM ammonium fluoride in MiliQ water with addition of 0.01% formic acid (the mobile phase A) and 100% methanol (the mobile phase B) were used in the analyses. The gradient elution was as follows: (time [min], % phase B): 0, 5; 0.5, 5; 12.5, 100; 14.5, 100. The duration of an analysis was 16.5 minutes, the flow rate of the mobile phase was 0.6 ml/min. The chromatographic column was heated to a temperature of 40 °C, the temperature of the MS source was 210 °C, the gas flow in the source was 6 l/min, the capillary voltage was 2500 V. Substances were analysed in the dynamic MRM mode, when usually 2 MRMs were selected for each analyte transition. The standard addition method was used for quantification, when the concentration of the standards added to the sample was always 5, 25 or 125 ng/ml. Samples with a high concentration of analytes of interest outside the calibration range were diluted with water (MilliQ) and reanalysed. Data evaluation was done using the MassHunter Workstation Quantitative Analysis program (Version 10.0, Agilent).

Table 2 Changes recorded in the concentrations of different PPCPs in the infiltration basin and at the locations where samples were collected (after 60 m in well HV1, after 120 m in well HV2 and after 180 m in well R14), where effectiveness of natural attenuation of PPCPs passing through 180 m of fluvial deposit is expressed in %, (–) means concentration below the limit of quantification and N/A means "not applicable".

Median concentration (ng/l)

Monitoring location	GROUP 1					
	Iohexol	Iopromide	Metoprolol	Cetirizine	Valsartan	Clarithromycin
Infiltration pond	39	29	17	18	16	18
HV1	–	–	–	–	–	–
HV2	–	–	–	–	–	–
R14	–	–	–	–	–	–
Attenuation efficiency (%)	N/A	N/A	N/A	N/A	N/A	N/A

Monitoring location	GROUP 2		
	Iomeprol	Tramadol	Sulfapyridine
Infiltration pond	180	54	7
HV1	38	9	3
HV2	–	–	–
R14	–	–	–
Attenuation efficiency (%)	N/A	N/A	N/A

Monitoring location	GROUP 3A							
	Metformin	Oxypurinol	Paraxanthine	Gabapentin	Caffeine	Sucralose	Carbamazepine	DEET
Infiltration pond	677	623	99	174	130	1,422	24	47
HV1	33	340	45	76	74	1,318	22	29
HV2	24	148	46	45	79	910	15	31
R14	16	83	23	45	43	619	11	23
Attenuation efficiency (%)	98	87	77	74	67	56	55	51

Monitoring location	GROUP 3B					
	Celiprolol	Diclofenac	Acesulfame	Primidone	Lamotrigine	Sulfamethoxazole
Infiltration pond	24	37	224	57	47	35
HV1	20	22	162	50	44	55
HV2	18	23	134	48	29	40
R14	15	23	139	44	37	32
Attenuation efficiency (%)	41	38	38	22	20	9

Monitoring location	GROUP 4				
	Benzotriazole	Propylparaben	Bisphenol S	Hydrochlorothiazide	Ibuprofen-2-hydroxy
Infiltration pond	230	4	35	13	56
HV1	277	5	34	24	51
HV2	217	4	34	14	57
R14	265	5	36	14	52
Attenuation efficiency (%)	N/A	N/A	N/A	N/A	N/A

Results

The results of the two-year monitoring revealed that the PPCPs could be placed into different groups based on their presence in the rocky environment. The presence of a specific substance in at least 3 of the 14 samples analysed was chosen as an indication of systematic occurrence. The initial information indicates that a total of 38 substances meet this criterion in the inlet to the infiltration basin. Subsequently, 24 PPCPs were regularly detected in the outlet from well R14.

Changes in concentrations of PPCPs

Changes in concentrations of the important PPCPs detected during water treatment in the artificial recharge system are summarized in Table 2. For easier description and explanation of the presence of these substances they are divided into several groups in this table, the characteristics of which are described in the text below.

Group 1 includes pharmaceutical substances that were completely removed by the artificial recharge process, namely iohexol, iopromide, metoprolol, cetirizine, valsartan and clarithromycin. Concentrations of these substances fell below the limit of quantification as early as in the section between the infiltration basin and monitoring well HV1. They were either adsorbed directly by the clogging layer of infiltration basin, or natural attenuation occurred when they passed through the first 60 m of the sandy-gravel aquifer.

Group 2 includes three pharmaceuticals, namely iomeprol, tramadol and sulfapyridine, the concentration if which fell below the limit of quantification when passing through the long section of the 120 m fluvial aquifer. Their concentrations in well HV1 revealed a great decrease, but they were still systematically present (see Table 2). Saccharin, which was irregularly present in the infiltrated water and in very different concentrations from tens of ng/l to 250 ng/l. It was only very rarely detected at concentrations of around 20 ng/l in wells HV1 and HV2. Saccharin was not recorded in well R14 and is thus not included in Table 2.

PPCPs of Group 3 are those substances that passed unchanged in terms of concentration along the entire 180 m between the infiltration basin and well R14, but their concentrations decreased as they passed through the rocky substrate. These substances are divided in Table 2 according to their attenuation efficiency (percentage ratio between the initial and the final concentration) into two sub-groups (Group 3A, Group 3B), which emphasizes the differences in the effectiveness of natural attenuation in the decrease in the concentration of these substances in the samples. Group 3A consists of substances that are degraded very well or excellent with an efficiency above 50%. This group mainly includes metformin, a pharmaceutical for the treatment of diabetes, whose initial concentration decreased by 98%, and oxypurinol, an active metabolite of allopurinol, which is used to treat uric arthrosis, which was degraded by a similar amount. In contrast, the antibiotic sulfamethoxazole, the concentrations of which also decreased, but the attenuation efficiency was only 9%. The group of PPCPs for which the attenuation efficiency was evaluated at less than 50% also includes the sweetener acesulfame and five pharmaceuticals (see Group 3B in Table 2).

Finally, Group 4 includes five pharmaceutical substances that are the most problematic as they passed through the quaternary fluvial aquifer almost unchanged, which indicates that artificial recharge appears to be ineffective for eradicating them. This group includes the organic pollutant benzotriazole, preservative propylparaben, endocrine disruptor bisphenol S and pharmaceuticals hydrochlorothiazide and ibuprofen-2-hydroxy.

The relevant limits of quantification (LOQ) of the analytical methods used are summarized in Table 3.

Role of clogging layer

Until recently, the operators at the Káraný waterworks regarded the clogging layer on the surface of the infiltration basins as a nuisance. It gradually reduces the efficiency of infiltration. Therefore, approximately 10 cm of the uppermost layer was removed at more or less two-year intervals. However, the monitoring results indicated that the clogging layer might have a positive role in the elimination of some types of PPCPs. Since the clogging layer was present for two years, a sample of sediment from it was analysed for the presence of 43 PPCPs. The median content of these substance in the infiltrated water was used to calculate the amount of these substance trapped in the clogging layer during the two years. The results are summarized in Table 4.

The amount of PPCPs present in dry clogging layer, which accumulated over two years compared to the total amount of PPCPs in the infiltrated water over the same period is significantly lower than the sorption capacity of this layer. This indicates that it is highly likely that biodegradation of these PPCPs occurs in the clogging layer. This is also supported by the data reported in the EPI Suite database (US EPA 2012), which is based on using BioWin models.

Discussion

Monitoring PPCPs detected in surface water used to produce drinking water by means of artificial recharge at the Káraný waterworks showed very good attenuation of the substances detected. Concentration of most of them decreased after percolation through a 180 m gravel-sand aquifer and some PPCPs were even below the limit of quantification of the analytical method used. The artificial recharge process had no effect on only six pharmaceuticals. Artificial recharge and natural attenuation are not simple processes as they consist of a combination of many variables, which include the physicochemical parameters

Table 3 The relevant lower limits of quantification (LOQ) of the analytical methods used.

Substance	LOQ (ng/l)	Substance	LOQ (ng/l)	Substance	LOQ (ng/l)
Acesulfame	6.3	**Hydrochlorothiazide**	10.5	**Primidone**	2.5
Benzotriazole	12.5	**Ibuprofen-2-hydroxy**	12.5	**Propylparaben**	2.5
Bisphenol S	2.5	**Iohexol**	13.4	**Saccharine**	25.0
Caffeine	12.5	**Iomeprol**	23.9	**Sucralose**	123.0
Carbamazepine	8.5	**Iopromide**	35.1	**Sulfamethoxazole**	6.3
Celiprolol	10	**Lamotrigine**	2.5	**Telmisartan**	13.5
Cetirizine	32.7	**Metformin**	28.2	**Tramadol**	2.5
Clarithromycin	33.7	**Methylparaben**	32.2	**Valsartan**	6.3
DEET	10.0	**Metoprolol**	2.5	**Sulfapyridine**	2.5
Diclofenac	1.5	**Oxypurinol**	25.2		
Gabapentin	6.3	**Paraxanthine**	2.5		

Table 4 Estimated contents of PPCPs in the clogging layer on the surface of the infiltration basin.

Substance	Substance content (ng/g)	Substance content (kg/kg)	Estimated content in the entire volume of the clogging layer of the infiltration basin (kg)	Sorbed in the clogging layer over the 2 years (%)	Total amount of substance recorded over the 2 years in infiltrated water (kg)
Acesulfame	94.64	9.46E-08	0.188	11.6%	1.61
Caffeine	10.76	1.08E-08	0.021	2.3%	0.93
DEET	168.84	1.69E-07	0.335	99.2%	0.33
Gabapentin	1.87	1.87E-09	0.004	0.2%	2.46
Metformin	65.18	6.52E-08	0.129	8.0%	1.61
Methylparaben	19.42	1.94E-08	0.038	2.4%	1.61
Oxypurinol	187.63	1.88E-07	0.372	8.3%	4.50
Paraxanthine	6.89	6.89E-09	0.014	1.9%	0.72
Telmisartan	5.84	5.84E-09	0.012	v0.9%	1.33
Tramadol	3.83	3.83E-09	0.008	v2.1%	0.36

of the treated water and the geological-chemical composition of the given subsoil. The physical-chemical properties of PPCPs also affect their movement and transformation in aquifers (Wu et al. 2020). Thus, variability in the concentration of $PPCP_S$ is to be expected. Different frequencies of detection and rate of PPCPs removal are reported in other studies (Zou et al. 2011; Wu et al. 2020; Labad et al. 2023). For example, Wu et al. (2020) identified sulfamethoxazole and ibuprofen as the most frequently detected substances in groundwater passing through an artificial recharge system. This also corresponds with the current results, as sulfamethoxazole passed through the entire artificial recharge system at the Káraný waterworks and the efficiency of natural attenuation for it was only 9% and there was no change in the concentration in ibuprofen, which is a priority PPCPs as it belongs to a group of substances with middle or high ecological risk (Wu et al. 2020).

One of the simple measures to achieve an even greater reduction in the concentration of PPCPs, is to extend the clogging zone removal period. Although this will reduce the efficiency of infiltration, this is balanced by an increase in sorption capacity and longer duration of the action of biochemical processes that destroy PPCPs. The importance of biotransformation and sorption as the most important mechanisms for the natural attenuation of PPCPs is confirmed by several studies (Schaffer et al. 2012; Regnery et al. 2017). Muñoz-Vega et al. (2023) describe the influence of soil biofilms on hydraulic conductivity, reduction and fate of three pharmaceuticals with different physicochemical properties: carbamazepine, diclofenac, and metoprolol; PPCPs that were also recorded in the current study. They observed enhanced adsorption and biodegradation of all pharmaceuticals in the system, with high biological activity both in batch and column experiments. However, the measurable effectiveness of this process at Káraný needs to be tested in the future. The current results are not sufficient for a relevant evaluation and final conclusion.

Other options for greater or even complete elimination of PPCPs from drinking water include using activated carbon (AC) filters as an additional part of the treatment (Rao et al. 2021; Adegoke et al. 2022; Zhu et al. 2022) or an advanced oxidation processes (AOPs)

(Krishnan et al. 2021; Chen et al. 2023). Although these methods are very effective, they are relatively financially and technologically demanding, as well as time-consuming. Moreover, according to the EPI Suite database (US EPA 2012) some of PPCPs present in water are not subject to oxidative decomposition.

The question is whether this additional cleaning is really necessary. Natural attenuation, with few exceptions reduce the concentrations of PPCPs either below the limit of detection or to extremely low values in the tens of ng/l. Assessment of the risk posed by these substances at these concentrations to human health or the ecosystem, is practically non-existent, or is based on theoretical models and predictions. When using reference values for PPCPs in drinking water, several factors need to be considered because the level of exposure and sensitivity in humans is very variable. When there is more than one PPCP present in drinking water, which is almost always the case, it is difficult to predict the health risk of individual contaminants, as they may act independently or synergistically (Tijani et al. 2015).

Of the few studies on the effect of PPCPs on human health, there is one that reports that endocrine disruptors are responsible for low sperm count or reduced fertility (Andersson et al. 2007; Bolong et al. 2009; Benkhalifa et al. 2023; Mascarenhas et al. 2024). Probably the most serious problem is bacterial resistance to various antibiotics prescribed for humans and used in veterinary medicine, which may result in bacteria producing endocrine disruptors. PPCPs are known to be drivers of antibiotic resistance (Lu et al. 2018; Thiroux et al. 2023). A typical example is tetracycline, which often becomes a completely ineffective (Daghrir and Drogui 2013; Zhao et al. 2023).

Even if the concentrations of PPCPs are low in aquatic environments and even more so in drinking water, some older publications report that it is not a threat to human health (Houtman 2010; Stanford et al. 2010), but the concentration of PPCPs in drinking water has to be considered in a wider context. These substances are commonly ingested by people in other products, such as meat (veterinary medicines) or fruit and vegetables irrigated with water that contains PPCPs (Fromme et al. 2009; Zeng et al. 2022; Johnson and Bell 2023; Li et al. 2023), it is therefore necessary to consider the possibility of an additive negative effect when ingesting PPCPs from many sources. Moreover, since PPCPs are mainly man-made substances artificially introduced into an ecosystem that is vulnerable to them, it is incumbent on humanity to eliminate or reduce their concentration in the environment.

Conclusions

1) Artificial recharge appears to be an effective method for removing PPCPs, especially pharmaceuticals.
2) Of the 38 substances detected in infiltrated water, the concentration of six substances was reduced below the limit of quantification either by the clogging layer in the infiltration basin, or by percolating through the first 60 m of the gravel-sand aquifer.
3) Other substances were removed either by percolating through another 60 m of infiltration and were at extremely low concentrations in the water supply well.
4) Only five substances (benzotriazole, propylparaben, bisphenol S, hydrochlorothiazide and ibuprofen-2-hydroxy) passed through the Quaternary fluvial aquifer practically unchanged.
5) Residual concentrations in the order of tens of ng/1 of PPCPs remained in the drinking water, with the exception of benzotriazole and sucralose. Although these could be removed using an AC filter or the AOPs method, the question is whether these economically demanding cleaning methods are necessary. There is no clinical evidence that these substances negatively affect human health at such extremely low concentrations.
6) There is a need to consider utilizing the natural attenuation properties of the clogging layer in infiltration basin, which for certain pharmaceuticals is a very effective at destroying them or reducing their concentrations.

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