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Research Article

Winter Activity of Reptiles in the Anatolian Peninsula

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ABSTRACT

The effects of global climate change and the related temperature increases on the behavior and life adaptation of organisms are a significant concern for ectothermic species. In this study, the winter activities of reptiles in the Anatolian Peninsula were examined by considering the potential effects of climate change on their behavior. In this context, the importance of thermal fluctuations was emphasized by focusing on the responses of different reptile species to sudden changes in winter conditions. For this purpose, 23 reptile species showing winter activity in Anatolia were evaluated by considering the data obtained from social media platforms and online sources. Among these species, 9 are snakes, 13 are lizards, and 1 is a tortoise. Cluster analysis was performed using the UPGMA method to reveal the models in the geographical distribution of the evaluated species. Winter activity for many reptile species was reported for the first time. As a result of the obtained findings, important information was provided on how reptile species are affected by winter warming in temperate regions and the unusual winter behaviors of different species. As a result, it is shown that understanding the effects of climate change on ectothermic species is essential for correctly predicting the biological responses of species and creating conservation strategies.

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Anadolu Yarımadasındaki Sürüngenlerin Kış Aktiviteleri

ÖZET

Küresel iklim değişikliğinin ve buna bağlı sıcaklık artışlarının, organizmaların davranışları ve yaşam uyumları üzerindeki etkisi, ektotermik türler için büyük bir endişe kaynağıdır. Bu çalışmada, Anadolu Yarımadası'ndaki sürüngenlerin kış aktivitelerini, iklim değişikliğinin davranışları üzerindeki potansiyel etkileri dikkate alınarak incelenmiştir. Bu doğrultuda, kış koşullarındaki ani değişimlere karşı farklı sürüngen türlerinin tepkilerine odaklanılarak termal dalgalanmaların önemi vurgulanmıştır. Bunun için, sosyal medya platformları ve çevrimiçi kaynaklardan elde edilen veriler dikkate alınarak, Anadolu'da kış aktivitesi gösteren 23 sürüngen türü değerlendirilmiştir. Bu türlerin 9'u yılan, 13'ü kertenkele, 1'i ise kaplumbağadır. Değerlendirilen türlerin coğrafi dağılımındaki modeller ortaya konularak kümeleme analizi UPGMA yöntemi ile gerçekleştirilmiştir. Birçok sürüngen türünde kış aktivitesi ilk kez rapor edilmiştir. Elde edilen bulgular neticesinde, sürüngen türlerinin ılıman bölgelerdeki kış ısınmalarından nasıl etkilendiklerine ve farklı türlerin alışılmadık kış davranışlarına dair önemli bilgiler sunulmuştur. Sonuç olarak, iklim değişikliğinin ektotermik türler üzerindeki etkilerinin anlaşılmasının türlerin biyolojik tepkilerinin doğru tahmin edilmesi ve koruma stratejileri oluşturması için önemli olduğunu göstermektedir.

Anahtar Kelimeler:

Kış ısınması, Kertenkele, Yılan, Kaplumbağa, Türkiye

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1. INTRODUCTION

Temperature increases associated with global warming have significant implications for the fitness of organisms. In contrast to endothermic species, which maintain a stable body temperature, ectothermic species depend physiologically on the surrounding environment to regulate their behavior, development, and reproduction [1,2]. Consequently, these species are particularly susceptible to anticipated thermal fluctuations. Most studies investigating the effects of temperature changes have primarily focused on temperature fluctuations during active annual periods—specifically when mean temperatures surpass the minimum thresholds necessary for growth and reproduction—. However, thermal conditions can also profoundly affect ectotherms during periods of inactivity or dormancy, such as winter [1,2]. According to the Intergovernmental Panel on Climate Change (2014), winter temperatures have increased more than summer temperatures. Failing to account for potential biases introduced by asymmetric warming patterns could undermine the validity of climate change research findings [3]. The phenomenon known as "winter warming," which has conventionally been investigated in the context of cold-adapted mammals and high Arctic species, is expected to have significant but previously overlooked consequences for species inhabiting temperate zones as well [4]. Moreover, it could be a critical vulnerability factor for ectotherms [5]. In temperate regions, winter marks the onset of colder temperatures, diminished food sources, and limited thermoregulatory opportunities for reptiles. The majority of reptiles are capital breeders, fueling reproduction in the spring with conserved energy [6]. An optimal overwintering strategy should optimize opportunities to replenish and conserve energetic reserves while minimizing the risk of cold exposure [7]. The general patterns observed in the winter season for reptiles are hibernation, brumation, seeking warmth, and burrowing. However, the specific winter behaviors of reptiles can vary significantly among species and are influenced by factors such as geographic location, climate, and the reptile's adaptations. In this study, we aim to collect data on the unusual winter activities of many reptile species observed in the Anatolian Peninsula.

2. MATERIALS and METHODS

2.1. Study area

The Anatolian Peninsula, also known as Anatolia, is a vast landmass located in Western Asia, forming the majority of the territory of Türkiye. It is bordered by the Aegean Sea to the west, the Mediterranean Sea to the south, and the Black Sea to the north. The climate of Anatolia varies significantly across the regions. Coastal areas generally experience a Mediterranean climate, characterized by hot, dry summers and mild, wet winters. In contrast, the inland areas, particularly in the central plateau, have a more continental climate with hot summers and cold winters [8]. For this study, we comprehensively searched various social media platforms, such as Facebook, Instagram, iNaturalist, and Twitter. This search examines our friend lists as well as the public group "Türkiye Wildlife Association" on Facebook. Additionally, amateur naturalists and students (as mentioned in the Acknowledgements section) frequently shared relevant information from their Facebook profiles with the first author between November 2020 and March 2021.

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2.2. Data collection

To assess the winter activity of reptiles, individuals who uploaded photos on social media were requested to provide the location data. If GPS coordinates were inaccessible, contributors were interviewed to obtain information that allowed for determining the position with an accuracy of at least ±0.5 km. Data on air temperature, soil temperature, cloudiness and moisture estimate rates were retrieved from a real-time online platform [9].

2.2. Data analysis

A grouping analysis of the observed species was conducted based on the Euclidian Distance to ascertain whether the species were geographically grouped. The UPGMA (Unweighted Pair Group Method with Arithmetic Mean) technique was used to verify if the species clustered by region.

3. RESULTS

A total of 29 observations from 23 reptile species were recorded, revealing a winter activity pattern (Figure 1). Among these species, 9 were snakes, 13 were lizards, and 1 was a tortoise. The records, along with their localities, are listed in Table 1. Eleven records were obtained from the western part of the peninsula, seven from the southeastern, six from the southern, and five from the northern regions. *Ophisops elegans* was the only species recorded from two different areas (western and southeastern). Additionally, winter activity was observed in six species in at least two distinct locations. The mean temperatures in western localities were 16.2 ˚C, 15.35˚C in the south, 16.8˚C in the north and 12.3 ˚C in the southeastern localities. Similarly, the mean soil temperatures in the west, south, north, and southeast were 18.06 ˚C, 17.6 ˚C, 18.3 ˚C and 14.4 ˚C, respectively. The cluster analysis revealed two major groups: southeastern and the rest (Figure 2). However, two inner Aegean observation records were also nested within the southeastern clade. Finally, no discernible classification pattern was observed based on the species' taxonomic hierarchy.

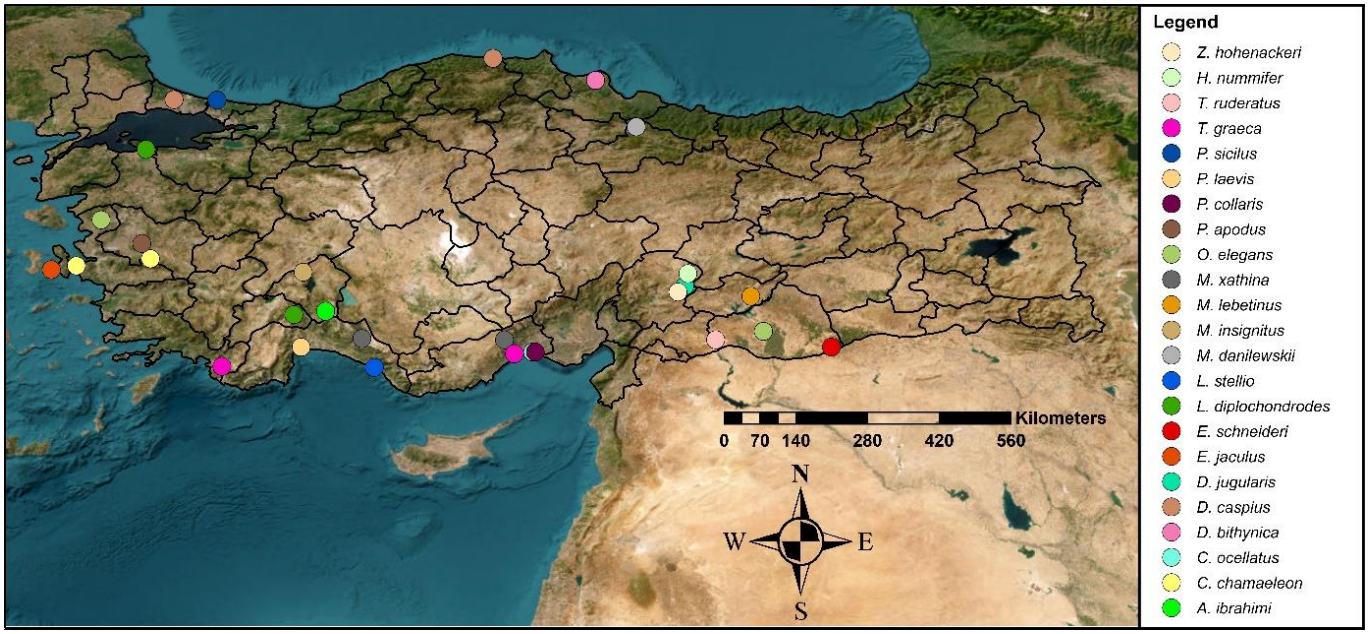

Figure 1. Distribution of reptile species exhibiting winter activity across in the Anatolian Peninsula

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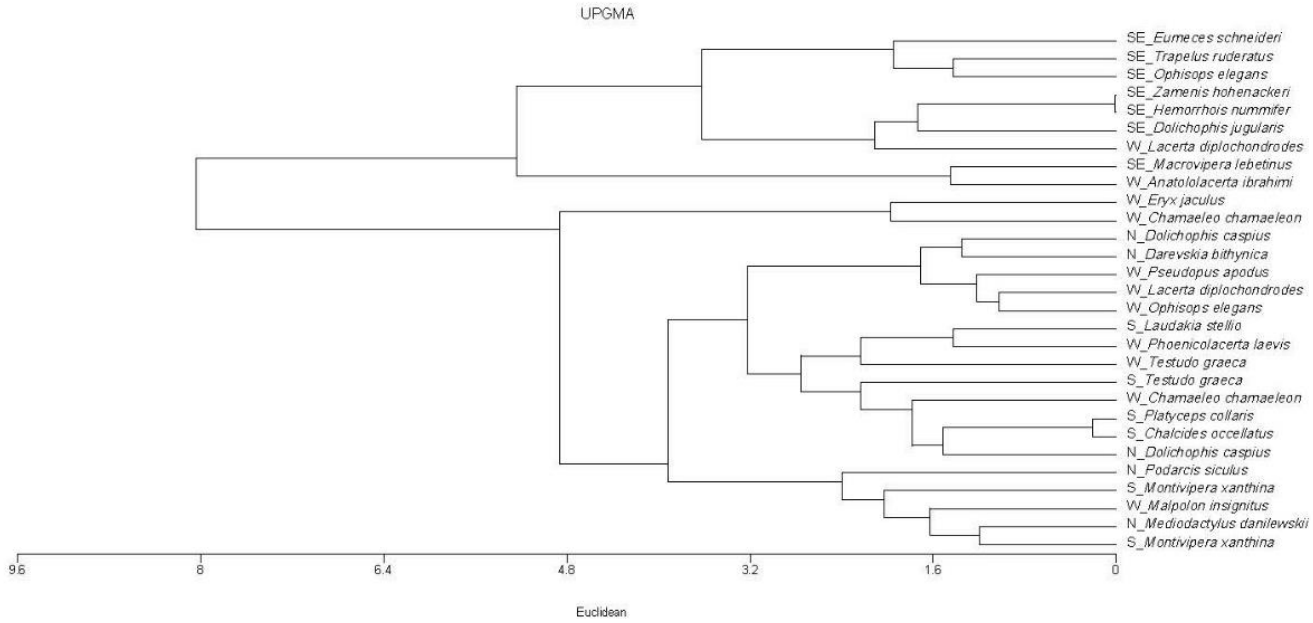

Figure 2. UPGMA cluster analysis for winter activity patterns based on geographic distribution

Table 1. Winter activity records of reptile species in the Anatolian Peninsula (T_{air}: air temperature, T_{soil}: soil temperature).

Family	Species	English name	Locality	Date	T_{air}	T_{soil}
Testudinidae	*Testudo graeca*	Common Tortoise	Muğla/Fethiye/Çaltıözü	08.12.2020	17.1	20.1
			Mersin/Çevlik	27.02.2021	15.2	19.2
Agamidae	*Laudakia stellio*	Starred Agama	Antalya/Alanya	30.01.2021	16.1	18.8
	Trapelus ruderatus	Horny-scaled Agama	Şanlıurfa/Birecik	14.02.2021	13.2	16.1
Gekkonidae	*Mediodactylus danilewskii*	Danilewski's Bent-Toed Gecko	Tokat/Erbaa/Karayaka	12.11.2020	15.9	17.1
Chamaeleonidae	*Chamaeleo chamaeleon*	Mediterranean Chameleon	İzmir/Urla	30.11.2020	19.4	21.4
			Manisa/Salihli/Gökköy	13.02.2021	17.3	18.7
Anguidae	*Pseudopus apodus*	European Glass Lizard	Manisa/Akpınar/Gölmarmara	12.01.2021	18.1	19.3
Scincidae	*Chalcides ocellatus*	Occellated Skink	Mersin/Tarsus	04.02.2021	15.8	17.3
	Eumeces schneideri	Orange-tailed Skink	Şanlıurfa/Ceylanpınar	07.03.2021	14.1	17.2
Lacertidae	*Ophisops elegans*	Snake-eyed Lizard	İzmir/Bergama	14.11.2020	17.8	18.3
			Şanlıurfa/Eyyübiye	07.01.2021	13.3	15.4
Lacertidae	*Lacerta diplochondrodes*	Rhodos Green lizard	Burdur/Bucak	13.12.2020	13.5	14.2
			Balıkesir/Bandırma	05.01.2021	18.1	19.5
	Podarcis siculus	Italian Wall Lizard	İstanbul/Riva	07.02.2021	15.2	16.7
	Phoenicolacerta laevis	Lebanon Lizard	Antalya/Konyaaltı	02.01.2021	17.2	20.7
	Anatololacerta ibrahimi	Anamur Lizard	Isparta/Sütçüler	13.11.2020	9.9	11.3
	Darevskia bithynica	Uludağ Lizard	Samsun/Bafra	15.01.2021	18.1	19.4
Erycidae	*Eryx jaculus*	Sand Boa	İzmir/Çeşme	30.01.2021	17.6	20.8
Colubridae	*Dolichophis caspius*	Caspian Whip Snake	İstanbul/Çatalca/Oklalı	13.11.2020	16.4	18.7
			Kastamonu/Abana	12.02.2021	18.4	19.6
	Dolichophis jugularis	Large Whip Snake	Kahramanmaraş/Nurhak	28.02.2021	11.2	12.8
	Platyceps collaris	Collared Dwarf Racer	Mersin/Tarsus	27.02.2021	16.6	17.7
	Hemorrhois nummifer	Coin-marked Snake	Kahramanmaraş/Nurhak	03.03.2021	12.5	13.7
	Zamenis hohenackeri	Transcaucasian Rat Snake	Kahramanmaraş/Nurhak	04.03.2021	12.4	13.6
Psammophiidae	*Malpolon insignitus*	Eastern Montpellier Snake	Isparta/Senirkent/Gençali	06.11.2020	12.1	14.4
Viperidae	*Montivipera xanthina*	Ottoman Viper	Between Antalya - Konya, Toroslar	03.11.2020	15.1	17.3
			Mersin/Toroslar/Arslanköy	03.11.2020	13.3	15.4
	Macrovipera lebetinus	Levantine Viper	Adıyaman/Kahta	02.12.2020	9.4	11.8

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4. DISCUSSION

Although many organisms depend on photoperiodic cues to indicate seasonal transitions, underground-dwelling reptiles might not be significantly exposed to such stimuli. For most temperate reptiles, ambient temperature is the primary factor determining the onset and cessation of dormancy [10]. In other words, environmental temperature regulates their reproductive cycles and energy expenditure. However, sudden temperature fluctuations may also trigger the temporary awakening of the reptiles [11].

The investigation of biological reactions to climate change has significantly emphasised reptiles. A range of fitness responses has been linked to shifts in thermal maxima and minima [12,13]. To forecast the short- and long-term impacts of warming winters on temperate reptiles, it is crucial to establish direct correlations between winter temperatures and survival or reproductive success and identify temperature-sensitive biological processes. These processes must be integrated into a broader understanding of fitness [14].

For effective conservation efforts, information on the habitat and activity of tortoises during the winter months is crucial, as they spend over half of their lives in hibernation [15,16]. According to Özgül et al. (2022), *Testudo graeca* was observed in Bozcaada in February at an air temperature of 12 ˚C. Other observations of this species in Fethiye, Muğla (December) and Çevlik, Mersin (February) revealed higher air temperatures of 17.1˚C in Fethiye and 15.2 ˚C, respectively. In these locations, soil temperatures were consistently higher than the air temperatures, at 20.1 ˚C and 19.2, ˚C respectively [17]. This suggests that ambient temperature dynamics might trigger the winter awakening of the tortoises.

Cold climates significantly impact the hibernation patterns of lizards. According to Adolph and Porter (1993), low air temperatures negatively affect key functions, such as movement, food availability, and escape behavior. Temperature fluctuations in air temperature can prompt lizards to end their hibernation prematurely [11]. In this context, winter activity of the following lizard species has been documented: *Apathya cappadocica* [18], *Darevskia rudis* [19], *Lacerta media* [20], *Lacerta viridis* [21], *Hemidactylus turcicus* [22], *Mediodactylus kotschyi* [23], *Ophisops elegans* [24,25], *Podarcis erhardi* [26,27], *Podarcis muralis* [28 – 31], *Sceloporus jarrovi* [32] and *Zootoca vivipara* [33]. Our study contributes to the case of lizards' winter activity via the observations for *Anatololacerta ibrahimi*, *Chamaeleo chamaeleon*, *Lacerta diplochondrodes*, *Phonenicolacerta laevis*, *Pseudopus apodus*, *Darevskia bithnyica*, *Eumeces schneideri*, *Chalcides ocellatus*, and *Trapelus ruderatus* for the first time in the literature.

Winter activity in *H. turcicus* was previously reported in January – February at air temperatures ranging from 8.89 to 18.89˚C [22]. In the Anatolian Peninsula, similar temperature ranges were observed for geckos and agamids. For instance, *Mediodactylus danilewskii* was recorded in Tokat, North region (T_{air}: 15.9 ˚C, T_{soil}: 17.1 ˚C in October 2020) and, *Laudakia stellio* in Antalya, South region (T_{air}: 16.1 ˚C, T_{soil}: 18.8 ˚C in January 2021). Moreover, *Trapelus ruderatus* was observed in Şanlıurfa, Southeast region, at 13.2 ˚C air and 16.1 ˚C soil temperatures (February 2021).

Rock lizards, such as *D. rudis*, have shown winter activity in Trabzon, North region, at air temperatures of 10-16.5 ˚C [19]. Our findings for *D. bithnyica* in Bafra, Samsun, North region showed similar activity

patterns at 18.1 ˚C air and 19.4 ˚C soil temperatures (January 2021). [25] revealed that *O. elegans* individuals were active between December and February with minimum T_{air}: 15.4 ˚C and minimum T_{soil}: 16.2 ˚C. Our observations for *O. elegans* were T_{air}: 17.8 ˚C, T_{soil}: 18.3 ˚C in Bergama, İzmir, West region (October 2020) and T_{air}: 13.3 ˚C, T_{soil}: 15.4 ˚C in Eyyübiye, Şanlıurfa, Southeast region (January 2021). Therefore, these findings demonstrated that this species was active at ~2 ˚C lower than the literature knowledge.

Chameleons and legless lizards exhibited activity at temperatures significantly above seasonal averages [34]. For example, *C. chameleon* was observed in Urla, İzmir, West region: (T_{air}: 19.4 ˚C, T_{soil}: 21.4 ˚C in November 2020, T_{season}: 11.04 ˚C) and in Salihli, Manisa, West region: (T_{air}: 17.3 ˚C, T_{soil}: 18.7 ˚C in February 2021, T_{season}: 9.4 ˚C); *P. apodus* was recorded in Gölmarmara, Manisa, West region (T_{air}: 18.1 ˚C, T_{soil}: 19.3 ˚C in January 2021, T_{season}: 8.9 ˚C). These observations indicate that poikilothermic animals awaken as microhabitat temperatures rise, enabling their vital activities [35]. Moreover, our results suggested that lacertids might still be active even in relatively low temperatures (i.e. *A. ibrahimi*: T_{air}: 9.9 ˚C, T_{soil}: 11.3 ˚C in Sütçüler, Isparta, West region, 13 October 2020).

To date, there has been no observed winter activity for the following snake species in the Anatolian Peninsula: *Montivipera xanthina*, *Macrovipera lebetinus*, *Eryx jaculus*, *Platyceps collaris*, *Dolichophis caspius*, *Dolichophis jugularis*, *Hemorrhois nummifer*, and *Zamenis hohenhackeri* up to date. Snake winter activity has primarily been associated with basking and movement behaviors, which incur costs such as increased energy expenditures during non-feeding periods and vulnerability to predators [36 – 39]. Various hypotheses propose that winter basking activity in snakes "enables the continuation of gonadal activity" [40] and could potentially serve as an indicator of the impending mating season [6]. The present study's findings showed that the winter activity could be seen in extremely low temperatures in snakes for this reason: i.e. *M. lebetinus* in Kahta, Adıyaman, Southeast region: (T_{air}: 9.4 ˚C, T_{soil}: 11.8 ˚C in 2 December 2020). In addition to this viperid species, similar low-temperature activity was recorded in other snake species in southeast region: *D. jugularis* (T_{air}: 11.2 ˚C, T_{soil}: 12.8 ˚C), *H. nummifer* (T_{air}: 12.5 ˚C, T_{soil}: 13.7 ˚C, *Z. hohenhackeri* (T_{air}: 12.4 ˚C, T_{soil}: 13.6 ˚C) in Nurhak, Kahramanmaraş in March 2021. Moreover, in the West region, *M. insignitus* from Senirkent, Isparta, was recorded in November 2020 (T_{air}: 12.1 ˚C and T_{soil}: 14.4 ˚C). However, snake activity in other parts of Anatolia was generally at higher temperatures. Therefore, it can be concluded that the soil temperatures were slightly higher than the air temperature in all cases.

Variation in winter activity arises from species-specific, thermoregulation behavior and geographic conditions [41]. Our cluster analysis demonstrated that air and soil temperatures and other meteorological and geographical factors may influence early awakening in reptiles. However, further data is required to confirm these patterns.

In conclusion, recent observations suggest that reptiles might exhibit increased winter activity due to global warming [20, 42 – 46]. Recognizing these atypical behaviors enhances our understanding of reptile biology and provides crucial initial data for evaluating climate change models.

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The Declaration of Conflict of Interest/ Common Interest

No conflict of interest or common interest has been declared by the author.

Author's Contribution

MKŞ and MK wrote the main manuscript. TB collected the meteorological data. MK prepared the map. MKŞ did the cluster analysis. All authors contributed substantially to the final version of the manuscript. All authors have read and approved the manuscript.

The Declaration of Ethics Committee Approval

This study does not require ethics committee permission or any special permission.

Declaration of research and publication ethics

The authors of the paper declare that we followed the scientific, ethical and citation rules of Environmental Toxicology and Ecology in all processes of the paper and that we did not make any falsification of the data collected. Furthermore, we declare that ETOXEC and its Editorial Board are not responsible for any ethical violations that may have occurred and that this study has not been evaluated in any other academic publication environment than ETOXEC.

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Review Article

Environmental Pollutants and Their Impact on Prenatal Brain Development and Later Neurodegenerative Diseases

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ABSTRACT

Background: Neurodegeneration is the progressive loss of neurons' structural and functional components, a common feature of many neurodegenerative disorders. Its neurological side effects may significantly impact patients' mental and physical health. Due to their widespread prevalence, some neurodegenerative pathologies have gained international attention in recent years. Although its etiology is not fully known, it is suggested that environmental factors and genetic predisposition are responsible. Understanding the mechanisms that drive the development of neurodegenerative diseases will allow the development of new therapeutic strategies for their prevention and treatment. In particular, the postnatal effects of prenatal exposure have been investigated for many years. **Summary:** It has been long known the role of pregnancy-related factors on brain development and the impact of intrauterine changes on the development of many neurodegenerative diseases. The main mechanisms crucial in neurodegeneration are loss of neuronal function and cell death. Currently, the drugs used in the treatment of neurodegenerative diseases are used for symptomatic treatment and cannot stop the process of neuron loss. Identifying molecular commonalities of neurodegenerative diseases will help discover effective drugs for treating neurodegenerative diseases in the future. **Key Messages:** This review provides a comprehensive overview of the role of environmental neurotoxic exposures on prenatal neurodegeneration, the specific sensitivity of the nervous system to toxicant exposure, and the mechanisms of neurodegeneration. In a pathophysiological context, deciphering these mechanisms of prenatal neurodegeneration contributes to the discovery of therapeutic targets.

ÖZET

Arka Plan: Nörodejenerasyon, birçok nörodejeneratif hastalığın yaygın bir özelliği olan nöronların yapısal ve fonksiyonel bileşenlerinin progresif kaybıdır. Bu durumun nörolojik yan etkileri, hastaların mental ve fiziksel sağlıklarını önemli derecede etkileyebilir. Yaygın prevalansları nedeniyle bazı nörodejeneratif patolojiler son yıllarda uluslararası ilgi görmüştür. Etiyolojisi tam olarak bilinmemekle birlikte, çevresel faktörlerin ve genetik yatkınlığın bu duruma neden olduğu öne sürülmektedir. Nörodejeneratif hastalıkların gelişimini tetikleyen mekanizmaların anlaşılması, bunların önlenmesi ve

tedavisi için yeni tedavi stratejilerinin geliştirilmesine olanak tanıyacaktır. Özellikle, prenatal maruziyetin postnatal etkileri uzun yıllardır araştırılmaktadır.

Özet: Gebelikle ilgili faktörlerin beyin gelişimindeki rolü ve intrauterin değişikliklerin birçok nörodejeneratif hastalığın gelişimi üzerindeki etkisi uzun zamandır bilinmektedir. Nörodejenerasyonda kritik olan ana mekanizmalar, nöronal fonksiyon kaybı ve hücre ölümüdür. Şu anda nörodejeneratif hastalıkların tedavisinde kullanılan ilaçlar semptomatik tedavi için kullanılmakta olup, nöron kaybı sürecini durdurmamaktadır. Nörodejeneratif hastalıkların moleküler ortak yönlerinin belirlenmesi, gelecekte bu hastalıkların tedavisi için etkili ilaçların keşfedilmesine yardımcı olacaktır.

Ana Mesajlar: Bu derleme, çevresel nörotoksik maruziyetlerin prenatal nörodejenerasyon üzerindeki rolü, sinir sisteminin toksinlere karşı özel duyarlılığı ve nörodejenerasyon mekanizmalarını kapsamlı bir şekilde incelemektedir. Patofizyolojik bir bağlamda, prenatal nörodejenerasyonun bu mekanizmalarının çözümlenmesi, terapötik hedeflerin keşfine katkıda bulunmaktadır.

1. INTRODUCTION

Most neurological diseases have a solid link to exposure to environmental agents during and after pregnancy. When the relationship between neurological diseases and prenatal conditions is examined, it is seen that prenatal exposure is the most important factor.The mother's surroundings impact the developing fetus since, throughout the in-utero stage, the fetus is solely exposed to the mother's body. Dopaminergic neurons are lost during pregnancy due to exposure to bacterial endotoxins, lipopolysaccharide (LPS), and heavy metals. (ii) Another factor is dietary exposure. Eating patterns significantly affect physiology and metabolism. High-iron-content foods cause neurodegeneration in the midbrain. Dopaminergic neurons appear to shrink. Metal exposure is also among the factors that cause neurological problems. Toxic contaminants called heavy metals are released into the environment. They are widespread in the environment and poison living systems as they build up. The blood-brain barrier (BBB), which prevents the function of metal-protein complexes that play a barrier role in transporting heavy metals from the systemic circulation to the brain and showing the toxic effects of metals, protects tissues from metal toxicity. Heavy metal lead (Pb) is renowned for its detrimental effects on the nervous system. Heavy metals with a strong potential for neurotoxicity are mercury (Hg). Inorganic arsenic (As) is a neurotoxic metalloid that negatively impacts neurodevelopment and cognitive function . Another harmful heavy metal linked to brain alterations, including memory loss and mental retardation, is cadmium (Cd). A neurotoxic substance called aluminum (Al) has a role in the pathogenesis of neurodegenerative diseases. An essential element that has critical physiological roles in maintaining cellular homeostasis is manganese (Mn). Parkinson's disease (PD) has been linked to Mn exposure. The transferrin and the divalent metal transporter 1 (DMT1) receptors allow the necessary element, iron (Fe), to be delivered to the brain. Epidemiological research has determined that Fe raises the possibility of developing Parkinson's. It is among the agents that cause Alzheimer's disease (AD) and PD, especially after exposure to pesticides.Insecticides, herbicides, and fungicides are essential examples of pesticides. More often, insecticides like organophosphates, organochlorines, and carbamates are utilized. A common herbicide called paraquat (PQ) can cause AD. PD is linked to the organophosphate pesticide rotenone. Lifestyle factors such as smoking, drinking, and drug abuse are important factors that negatively affect the health and well-being of the organism. The prevalence of neurodegenerative illnesses is rising along with a sedentary lifestyle and lack of exercise. Furthermore, it has been noted that substances like cocaine, heroin, and the commonly abused methamphetamine (MA)

decrease the formation of the myelin essential protein in the optic nerves and increase the number of malformed axons, mean optic fiber area, and lamellarity. Due to commercial uses of solvents in things like metal degreasing, dry cleaning, paint thinner, and detergent ingredients, solvents are widely used. Some solvents are readily absorbed by the peripheral and central nervous systems (CNS) because they are lipophilic. N-hexane and toluene exposure causes severe cases of Parkinsonism. One particular solvent whose capacity to promote neurodegeneration has been thoroughly investigated is trichloroethylene (TCE). (vii) Nanoparticles (NPs): With a diameter of approximately 100 nm are incredibly tiny molecules. NPs can cross the BBB because of their small size, allowing them to enter the body through various other entry points and eventually induce neurotoxicity, neuroinflammation, and neurodegeneration in the CNS. Mechanisms of environmental factors inducing neurodegeneration include oxidative stress formation, activation of glia-induced neuroinflammation by increasing reactive oxygen species (ROS) production and by releasing antioxidant enzymes and promoting the formation of protein aggregates such as β-Amyloid (Aβ), Tau, or α-syn.

1.1. Neurodegeneration

Neurodegenerative diseases are characterized by the progressive loss of structure or function of neurons, including neuron death, and spread to other organs in later stages. PD, AD, Batten disease, amyotrophic lateral sclerosis (ALS), multiple sclerosis (MS), and Huntington's disease (HD) are the most prevalent neurodegenerative diseases [1-6].

Several studies have suggested that the most crucial underlying cause of degenerative disorders is neurodegeneration [7-9]. The effects of neurodegeneration on patients can have serious adverse effects on mental and physical health. Environmental characteristics are considered significant factors contributing to neural dysfunction-related disorders. Exposure to environmental agents that cause neurological diseases develops as a result of prenatal and postnatal exposure. Some neurotoxic metals such as As, chromium (Cr), Pb, Cd, Hg, Mn, and Al as well as metal-based nanoparticles and pesticides, also play a role. These agents cause the production of neurofibrillary tangles (NFTs), amyloid, or senile plaques, which are hallmarks of neurological dysfunction. Additionally, exposure to solvents also has a significant contribution to the development of neurodegeneration [10,11].

1.2. Prenatal conditions

The connection between neurons and glial cells is extremely critical during pregnancy and after birth until the age of three. It has been proven that many epigenetic factors that are effective during pregnancy (including fetal hypoxia, premature birth, infection, inflammation, stress, malnutrition, developmental delay, low birth weight, hypertension, anemia, diabetes, and medications) play a role [12-14] (Figure 1). Data obtained from current literature show that stopping the spread of neurodegenerative diseases at the beginning of life should be defined as the only method to prevent neurodegeneration. The "two-hit" hypothesis, first proposed for PD and other human neurodegenerative pathologies, including AD, covers the intrauterine periods. The initial blow to the nervous system occurs early in life. It causes a risk of developing PD or AD later in life, depending on the resilience of the brain or the susceptibility to developing neurodegeneration later in life [7,8,12]. Prenatal exposure of the mother or the fetus to neurotoxic agents can affect the CNS, inhibiting normal neuronal development processes or preventing the brain from fully

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functioning [13]. Adverse effects on the CNS before birth also negatively impact cognitive functions in early childhood. Maternal use of cocaine or alcohol during pregnancy causes a decline in cognitive functions and an increase in psychopathologies in childhood. In addition, it has also been associated with substance use disorders in later life [15]. It has been suggested that even dementia is triggered by adverse prenatal conditions [16,17].

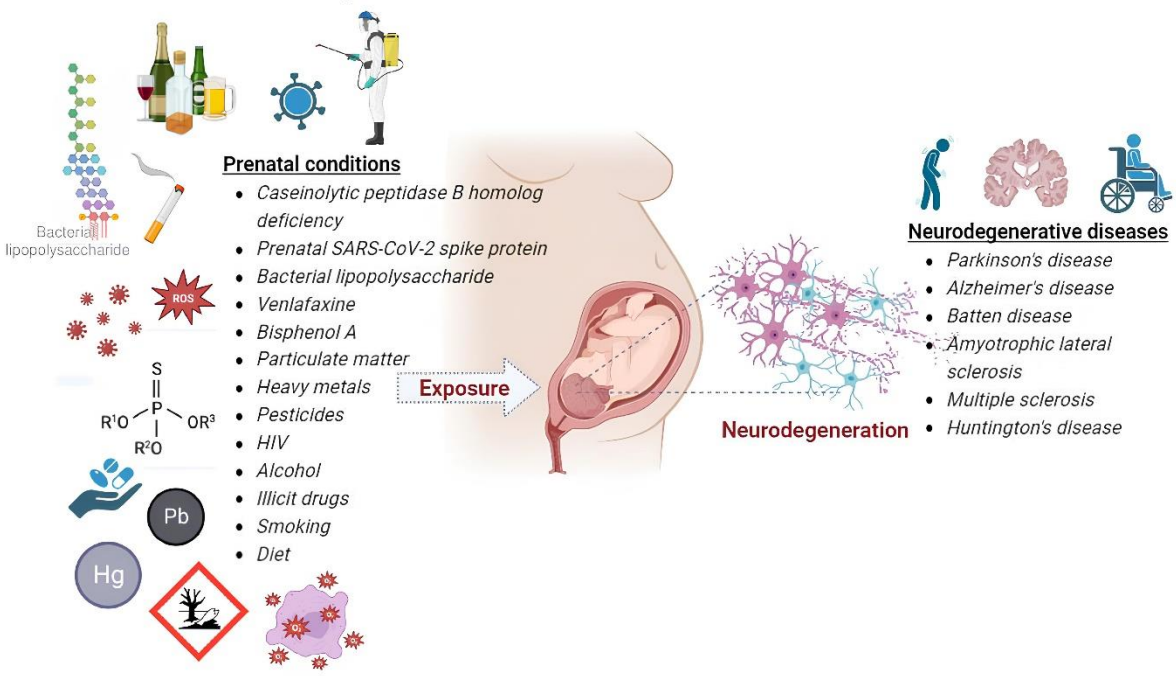

Figure 1. Environmental neurotoxic exposures on prenatal neurodegeneration.

1.3. Caseinolytic Peptidase B Homolog Deficiency

Caseinolytic peptidase B homolog (CLPB) is mitochondrial inner-membrane protein highly expressed in the brain. In 2015, a novel mitochondriopathy due to CLPB deficiency is identified as a cause of severe neonatal encephalopathy. . In other words, CLPB deficiency is a mitochondriopathy in which hyperlactatemia, white matter changes, and 3-methylglutaconic aciduria are observed. As a result of the mitochondrial localization of the CLPB protein, the enrichment of the CLPB protein in the mitochondrial proteome in various tissues, especially the brain, was supported by proteomic experiments. Therefore, in CLPB deficiency, severe brain damage is accompanied by multisystem involvement. Prenatal problems such as fetal growth restriction, polyhydramnios, and decreased or increased fetal movements occur in babies. They are born with severe neurological disorders with general hyperekplexia, hypertonia, respiratory failure, febrile seizures, and ventilator dependence. These neurological findings damage nerve pathways. The degeneration of the deep white matter protecting subcortical areas causes pathological findings. A strong presence of astrogliosis is observed in regions with degeneration. Reactive microgliosis consists of hyperbranched active microgliocytes that cluster widely in damaged areas. In brain regions where damage is intense, there is a significant decrease in chromatin condensation and marked white matter vacuolation, myelin density, strong astrogliosis, reactive microgliosis, and degradation of white matter due to death of oligodendrocytes are observed. White matter changes and a mitochondrial leukoencephalopathy-like condition were observed in a case with CLPB deficiency. Cortical and deep gray matter involvement (brainstem, globus pallidus, cerebellum, amygdala, dentate nucleus, and hippocampus),

cortical thinning, and significant decrease in neuron density were detected. CLPB deficiency triggers mitochondrial dysfunction and accumulation of protein aggregates, resulting in decreased mitochondrial energy production, apoptosis, vasoconstriction, and oxidative stress. The neurodevelopmental disorder associated with CLPB deficiency is characterized by facial dysmorphism. Dysmorphic faces include microcephaly, prominent eyes, ocular hypertelorism, broad nasal bridge, bulging nose, tented upper lip with microform cleft, micrognathia, and low-set backward-pointed ears. Interestingly, the absence of congenital cataracts supports that this anomaly is a variable feature unrelated to the severity of neurological involvement. In addition to all these effects, postmortem neuropathological examinations revealed widespread subependymal white matter loss, severe frontal cystic leukoencephalopathy, striate-thalamic neurodegeneration, and intense reactive astrogliosis observed in the fetus with CLPB deficiency [18].

1.4. SARS-CoV-2 Spike Protein

SARS-CoV-2 spike protein persisted in the skull-meninges-brain axis of COVID-19 patients. The presence of spike protein causes pathological and behavioral changes in the mouse brain. Spike protein exacerbates neurological damage in mice by increasing brain sensitivity [19]. Recent studies have found that the COVID-19 virus significantly affects embryonic organs, including the brain. It has been suggested that the SARS-CoV-2 spike protein is associated with neuroinflammation and that exposure to SARS-CoV-2 spike protein during pregnancy may mediate the development of autism in children. In a study conducted with male and female rats exposed to COVID-19 spike protein, it has been observed that male rats are more sensitive than female rats and have significant histological, biochemical, and behavioral changes in their brains. Neuroinflammatory changes are triggered, causing a progressive decrease in neuron numbers and cerebellum damage [20]. SARS-CoV-2 enters human cells by interacting with specific membrane cell receptors, such as the angiotensin-converting enzyme 2 transmembrane receptor, and activating the SARS-CoV-2 spike protein through cleavage of transmembrane serine protease 2. The first case of SARS-CoV-2-associated meningitis was reported, in which the virus was found in the nervous system [21].

1.5. Bacterial Lipopolysaccharide

SARS-CoV-2 spike protein and transcripts in the postmortem brain suggest that the virus directly causes CNS infection. SARS-CoV-2 spike protein can readily cross the BBB when injected intravenously. This indicates that the S1 protein translocating to the brain parenchyma may affect cognitive processes and brain functions, contributing to psychiatric and neurological COVID-19 symptoms [22]. In conclusion, prenatal exposure to SARS-CoV-2 negatively affects the neurodevelopmental process [23].

In pregnant rodents, maternal immune activation (MIA) has long-lasting effects on the offspring, with abnormalities in gene expression, histology, and behavior that are reminiscent of autism and schizophrenia [24]. In a study, STAT1 target genes were observed to be downregulated in MIA microglia exposed to systemic lipopolysaccharide (LPS) during adulthood [25]. Changes in microglial gene expressions in microglia with LPS-induced maternal immune activation have been associated with a decreased immune response, including interferon signaling and metabolic changes. Similar microglial changes have previously been reported in humans and animal models in neuropsychiatric and neurodegenerative conditions [26,27]. For example, activation of the prenatal immune system has been associated with the risk of developing schizophrenia in later life [28]. Environmental agents that affect microglia contribute to various

neurological diseases throughout life, depending on the week of pregnancy to which the fetus is exposed and whether the fetus is at a susceptible period of development [24].

1.6. Venlafaxine

Although venlafaxine appears to be a new antidepressant with better efficacy, safety, and tolerability in typically depressed individuals, it has been observed to cause fetal-developmental neurotoxicity when used by pregnant mothers in cases of prenatal depression. However, no obvious neurodevelopmental and neurobehavioral problems were observed in the baby [29]. A clinical study found no effect of venlafaxine on children's behavioral and intellectual outcomes [30]. However, opposite results were found in different research groups [31]. Administration of venlafaxine to rats during pregnancy has been shown to cause spontaneous abortions [32], premature birth, an increased number of stillbirths [33], and changes in the cardiovascular system [34]. A study in rats found that prenatal treatment with therapeutic doses of venlafaxine caused apoptotic neurodegeneration and neurocytoarchitectural damage in the hippocampus and striatum regions of the fetal brain during the sensitive periods of brain development of rats. It should not be overlooked that it causes mental and cognitive disorders as well as depressive-like behavior. Therefore, most antidepressants, including venlafaxine, have risks for both the fetus and the expectant mother [35].

1.7. Bisphenol A

Bisphenol-A (BPA) is used primarily in the plastic industry, and it is one of the agents that negatively affects human health and causes neurotoxicity. In animal studies, researchers have suggested that BPA reduces the differentiation and proliferation of neural stem cells (NSCs), disrupts mitochondrial protein transport and myelination, and causes cognitive problems and excessive mitochondrial fragmentation [36]. Low-level BPA exposure reduces hippocampal neurogenesis, leading to memory loss and learning disability [37]. Exposure to BPA triggers oxidative stress by inducing ROS formation, leading to neuron death [38-40]. A severe concern arises during pregnancy: BPA can pass through the placenta, negatively affecting brain development and fetal growth [41]. Exposure to environmental pollutants such as hazardous chemical compounds, harmful heavy metals, pesticides, and solvents causes deleterious changes in hippocampal neurogenesis [42-44]. For this reason, learning and memory functions related to the hippocampus are negatively affected. Exposure to BPA during developmental stages inhibits hippocampus-derived neurogenesis in the brain [36,45,46].

1.8. Particulate Matter

Air pollutants are believed to affect the CNS through several mechanisms. It affects directly through the transport of contaminants to the CNS or indirectly through systemic inflammation. After PM reaches the circulatory system, it negatively affects the CNS by creating a systemic inflammatory response. Proinflammatory mediators contributing to neuroinflammation and tissue loss have been detected in brain regions such as blood, cerebrospinal fluid, olfactory bulbus, frontal cortex, and hippocampus. Studies show that PM10 exposure triggers the expression of genes related to inflammation and oncogenesis in rat brains [47,48]. Polycyclic aromatic hydrocarbons (PAHs) are compounds with mutagenic, teratogenic, highly toxic, immunotoxicogenic, and carcinogenic effects that arise from incomplete combustion of organic compounds. PAHs enter the human body through the inhalation of air, drinking water, consumption of

contaminated foods, and cigarette smoke, causing mutations in DNA. Prenatal exposure to PAHs causes regression in neurocognitive development [49]. Neurodevelopmental deficits associated with prenatal PM exposure in children include attention and differences in cognition behavior [50]. Postulated mechanisms for PAH-induced neurotoxicity are related to the disruption of pathways that regulate synapse plasticity, formation, and neuronal differentiation [51]. The effect of prenatal PAH exposure on childhood IQ has been investigated in many studies [51-53]. Different results were found in the studies. Contrary to studies claiming it harms IQ [52], another study showed no relationship between prenatal PAHs measured in cord blood and the child's IQ [53]. The amount of PAH exposed through nutrition constitutes a significant portion (70-90%) of total PAH exposure. There is sufficient evidence that prenatal PAH exposure negatively affects memory, a child's intelligence, average overall development, mental development, and verbal IQ and causes anxious and depressive behavior [49].

The negative impact of polluted air on cognition in young children may occur due to exposure while still in the womb. Exposure to PAHs can cause decreased scores on the Bayley scale of infant development (BSID-II) and a decrease in verbal intelligence and IQ in children under five [53,54]. Another study reported that high-concentration PAH exposure caused a decline in verbal and non-verbal IQ scores [55].

In addition to PAH, in-utero exposure to motor vehicle traffic gases has been associated with decreased cognitive development [56,57]. Nitrogen dioxide (NO_2) exposure is associated with reduced IQ performance and lack of psychomotor development in young children [58]. Studies suggest that exposure to air pollution during pregnancy causes adverse effects on the cognitive performance of infants. A study found that increasing average NO_2 concentrations were associated with decreased gross motor skills in children [59]. Also, increased nitrogen dioxide levels are associated with reduced memory span among school children aged 9-11 [60]. It has been observed that as environmental levels of black carbon, whose concentration in the air increases as a result of the use of organic fuels, increase, intellectual performance such as visual vocabulary, compound intelligence, visual learning, and memory decrease in children [61,62].

1.9. Heavy Metals

Although some studies have suggested metal transfer to the fetus during pregnancy, the evidence is insufficient for all heavy metals [63]. Cd transfers to the fetüs during pregnancy, but it appears restricted [64]. Exposure to As at an early age affects brain weight and the neurotransmitter system, causing deficits in intelligence and memory [65]. Since Pb does not accumulate in the placenta, the Pb concentration in maternal blood is almost the same as in fetal blood [66]. Methyl mercury (MeHg) causes brain damage in the prenatal period [67,68]. Mn and As are the metals with the highest accumulation rates in the rat brain after a single exposure [69]. Pb and Cd exert synergistic effects to reduce the expression of glial fibrillary acidic protein, an essential macromolecule in the BBB [70,71].

An in vivo study in rats from prenatal to early life found that exposure to As produced an imbalance in the antioxidant defensive mechanism and neurotransmitter metabolism in the hippocampus region of the brain [72]. Here, As it reduces glutathione (GSH), glutathione peroxide (GPx) increases glutathione synthase (GS) activity and lipid peroxidation [73]. ROS and lipid peroxidation elements released due to oxidative stress increase superoxide dismutase (SOD) activity and decrease glutathione-related enzymes, leading to

changes in the cellular redox state [74]. It activates c-Jun N-terminal kinase 3 (JNK3) and p38 mitogen-activated protein kinase (p38 MAPK), which induces apoptotic factors in cerebral neurons. Causes brain cell damage and subsequent death and impairments in neurobehavioral function [75-78].

1.10. Organochlorine pesticides

Organochlorine pesticides (OCPs) have been extensively used, but their use has been reduced due to their environmental persistence and neurotoxic effects [79,80]. They pose a risk due to their excessive bioaccumulation [81]. Prenatal exposure to organochlorine compounds (OCs), among organochlorine pesticides, negatively affects neuropsychological development. In addition, maternal exposure to OCPs causes deterioration in cognitive and motor development and the occurrence of autism in the postnatal period. These environmental agents cause postnatal toxicity in infants not only through in-utero exposure but also through breastfeeding [82-84]. Considering the relationship between PD and exposure to organochlorines, it has been suggested that organochlorines lead to the accumulation of α-synuclein and depletion of dopaminergic neurons, thus predisposing to Parkinsonism [85]. In a study conducted in Finland, hexachlorobenzene (HCB) and dichloro diphenyl dichloroethylene (DDE) were detected in 75 cases of autism among 1.2 million newborns [86,8]. In an intrauterine study in pregnant mice and rats, even a single prenatal exposure to chlordecone was found to cause neurological disorders such as cerebral anomalies, decreased birth weight, stillbirth, tremors, memory impairment, and altered responses to stress [88]. Prenatal exposure to chlordecone has been observed to be associated with impairment in fine motor skills as scored by the BrunetLe'zine Psychomotor Development Scale in Early Childhood [89]. Chlordecone, which has acute and chronic toxic effects, causes neurotoxicity as well as immunotoxicity and reproductive, musculoskeletal, and liver toxicity. It has been suggested that motor dysfunctions and tremors observed in newborns due to chlordecone exposure are modulated by significant signaling pathways such as serotonergic, GABAergic, cholinergic, and dopaminergic systems in striatal neurons located in the basal ganglia and medial pontomedullary reticular formation [90,91]. It has been reported that the risk of autism is increased in children of mothers exposed to organochlorines in the first three months of pregnancy [92]. Prenatal exposure to PCBs and non-dioxin-like PCBs has been documented to increase the risk of impulsive behavior and attention-deficit/hyperactivity disorder [93]. Early exposure of the infant to PCBs through breastfeeding results in an increased prevalence of anxiety behavior with decreased exploratory behavior and locomotor activity [94]. Additionally, Forns et al. reported that exposure to PCBs in utero had adverse effects on cognition and psychomotor development [95]. The most important mechanism underlying the neurotoxicity observed due to PCB exposure is that it causes high sensitivity in the cerebellum, which provides motor control, including motor skills, cognitive development, attention, balance, and coordination [96]. Neurotoxicity is specifically associated with alterations in the cerebellum [97,98]. Disruption of myelination during the neurodevelopment process and changes in the concentrations of some monoamine neurotransmitters have been identified as the most important mechanisms underlying neuropsychological deficits caused by some organochlorine insecticides such as hexachlorobenzene [99].

Epidemiological studies have reported that PCBs show their neurotoxic effects through imbalances in inhibitory and excitatory neurotransmission that cause social, emotional, and cognitive development, language and speech development, and the primary symptoms and seizures of autism. Prenatal exposure to

PCBs and their hydroxylated polychlorinated biphenyls (OH-PCBs) has been found to cause decreases in mental and psychomotor developmental indices [100-103].

1.11. Human Immunodeficiency Virus

Maternal exposure to human immunodeficiency virüs (HIV) during pregnancy can cause impaired neurodevelopment of the child in the first years of life. During pregnancy, a woman's immune system becomes weaker than usual. With the known total immunosuppressive effect of HIV, it is thought that pregnancy may accelerate HIV infection and the development of acquired immune deficiency syndrome (AIDS) [104]. Different hypotheses have been proposed that exposure to HIV may affect pediatric brain development. Exposure of the fetus to inflammation in utero may weaken the developing brain's defenses against adverse postnatal conditions [105,106]. In the presence of HIV infection, chronic inflammation may continue despite antiretroviral therapy. Therefore, immune disorders may occur in pregnant women receiving HIV treatment [107].

1.12. Alcohol

Prenatal alcohol exposure (PAE) causes behavioral disorders and increases the risk of metabolic diseases [108]. Neuroinflammation is believed to contribute to ethanol-induced neurodegeneration; this suggests that anti-inflammatory therapeutics may provide a novel preventive approach for the treatment of fetal alcohol spectrum disorders [109]. Prenatal alcohol exposure also negatively affects the developing anatomical structures of the brain and body, leading to a variety of behavioral, cognitive, and physical effects [110].

1.13. Illicit drugs

Abuse of legal and illegal drugs is a worldwide problem of concern in pregnant women. The effects of illicit drugs depend on the duration of exposure, timing, dose, and degree of distribution of the drug. Teratogenic effects are observed as a result of fetal exposure to illicit substances. Long-term health problems triggered by oxidative stress and epigenetic changes in children of substance-addicted mothers play a role in the pathogenesis of neurodevelopmental disabilities. Disruption of redox homeostasis is an essential factor in the pathogenesis of illicit drug use-induced neurodegeneration, and new treatment alternatives are being investigated to prevent neurodegeneration through antioxidant and epigenetic modulator mechanisms. Important neurodegeneration targets must be identified to develop new neuroprotective strategies [111].

The most widely abused illicit drug during pregnancy is Cannabis sativa (marijuana) [112]. Studies conducted on experimental animals have shown that exposure to marijuana during the developmental period may pave the way for psychiatric diseases in children by affecting the endocannabinoid system in the brain. Endocannabinoids are secreted from postsynaptic neuron membranes in the brain, where cannabinoid receptor type 1 (CB1) receptors are activated; they spread rapidly by binding to the presynaptic neuron membrane from behind [113].

Additionally, exposure to the active ingredient in marijuana (T1-9-tetrahydrocannabinol/THC) in utero may alter responses in the mesolimbic dopamine pathway, leading to changes in the inhibitory and excitatory effects of dopamine on neurons [114]. As observed with many neurotoxic agents, the severity and

persistence of the impact of exposure to illicit drugs on the fetus's brain depend on the type of opioid, time of exposure, and dose. The opioids whose prenatal exposure has been most studied include methadone, morphine, oxycodone, and buprenorphine [115].

In utero, exposure to different types of opioids has been examined in studies in rat models. For example, buprenorphine and methadone cause changes in myelination and axon length, while morphine causes an increase in spine density and dendritic length [116].

Opioids show their effects by binding to opioid receptors in the brain and spinal cord, such as mu, delta, and kappa. The binding of opioids to these receptors prevents the release of neurotransmitters released by presynaptic neurons. Long-term use of opioids can lead to increased neuroinflammation. This can accelerate the progression of neurodegenerative diseases such as Alzheimer's disease. Some studies show that opioids can block neuroprotective mechanisms in the brain, which can negatively affect the course of diseases such as Alzheimer's disease. The effect of opioids on the dopamine system can improve some motor dysfunctions in Parkinson's patients. It has been shown that opioids can affect plasticity in the brain, changing the ability of neurons to connect and learn [117].

Methamphetamine is one of the most frequently used illegal drugs, like marijuana, heroin, and cocaine. Although factors such as the content of the substance used and the duration of use may vary in the findings observed in the newborn, all babies of mothers who use substances should be closely monitored for neonatal abstinence syndrome for 72-96 hours, and it should be taken into consideration that the findings may last longer. Generally, babies who develop neonatal abstinence syndrome are neurological (agitation, hyperactivity, increased muscle tone, tremor, jitteriness, myoclonic beats, high-pitched cry, hypotonia, sleep disorder, apnea, seizure), gastrointestinal (decrease in sucking, hyperphagia, vomiting, diarrhea), vasomotor symptoms such as sweating, hypothermia, and fever may be observed. Although these effects of methamphetamine are well known, its effects in early infancy and its long-term effects are not fully known. The most common recorded impacts in newborns include growth retardation, change in height and head circumference, and low birth weight [118,119].

Following prenatal exposure, amphetamine has been detected in the human umbilical cord tissue, plasma, and placenta during the first trimester. The cellular effects of amphetamine are nearly identical to methamphetamine, including increased levels of norepinephrine, dopamine, and 5-HT in the synaptic cleft through transporter reuptake inhibition. Using amphetamines during pregnancy also increases the risk of adverse effects such as placental bleeding [120]. Amphetamine negatively affects fetal brain growth and also reduces the amount of blood reaching the placenta by vasoconstriction. Depending on this situation, the embryo may not receive enough nutrients [121]. It has been observed that children exposed to amphetamines during the prenatal period are one year behind their peers in school success at the age of 14-15 [122].

In a study, delays in the motor skills of babies were observed up to two years after prenatal 3,4-methylenedioxymethamphetamine (MDMA), also called ecstasy exposure [123]. Behavioral studies conducted in animals have found significant adverse effects on learning, motor skills, and memory tests following exposure to MDMA. Although there was a decrease in motor activity and impairment in memory

and neuronal development in the offspring of rats exposed to prenatal MDMA and alcohol, it was not determined whether these effects were due only to MDMA [124].

1.14. Smoking

Smoking and exposure to nicotine during pregnancy have been associated with miscarriage, premature birth, sudden infant death syndrome, stillbirth, perinatal morbidity, and low birth weight. Recent data from clinical and preclinical studies suggest that exposure to nicotine during pregnancy may alter babies' brain circuits and responses, as well as increase the risk of adverse neurodevelopmental disabilities such as depression, anxiety, and attention deficit hyperactivity [125,126]. Some preclinical and clinical research focuses on neurodevelopmental and neurobehavioral complications. Nicotine binds to neuronal nicotinic acetylcholine receptors (nAChRs), which mediate rapid neurotransmission in the central and peripheral nervous system [127].

1.15. Diet

Mother's nutritional status and diet play an essential role in the neural development of the offspring. Changes in these early-life feeding schedules may have long-term effects on offspring health. A maternal diet rich in essential vitamins and nutrients significantly prevents various diseases, including stroke [128]. A study of the impact of maternal single-carbon diet deficiencies during pregnancy and lactation and their effects on stroke outcomes in offspring later in life found that maternal folic acid or choline deficiencies have a significant impact on ischemic damage, neurodegeneration, and neuroinflammation [129]. Prenatal nutritional deficiencies have detrimental effects on brain structure and functioning. Prenatal malnutrition has been associated with poor cognitive performance [130]. Gender-specific effects of malnutrition on brain volumes during early pregnancy have been reported. Study results show that malnutrition causes smaller brain volume in later life in men compared to women [131]. Therefore, the influence of the prenatal fetal environment on brain structure and cognitive functions may continue not only in early childhood but also throughout life. However, studies on the long-term neurological consequences of prenatal harmful factors later in life are limited.

1.16. Future Directions and Clinical Implications

Although neurogenetic disorders occur in the prenatal period, they can also happen in the future as widespread and early-onset brain damage. Examples include acute infantile neuronopathic disease (Gaucher type II) and mucopolysaccharidoses. Postpartum enzyme replacement therapy cannot be used widely due to BBB and neuron damage [132]. Effective and safe gene therapies are needed that can prevent early-onset neuropathy, correct the mutation, and protect the developing brain [133]. Neurodegeneration due to prenatal exposure to neurodegenerative agents is ideally treated in utero. A single dose of AAV1-HβH, which transduces human β-glucuronidase (GUSB), was effective in a mouse model. This lysosomal storage disease causes the spinal cord and brain degeneration, intellectual disability, skeletal abnormalities, and growth retardation [134]. One study found that prenatal and early postnatal treatment of pregnant mice with P021, a neurotrophic compound synthesized for the first time, reduced cognitive deficit and abnormal hyperphosphorylation, tau accumulation, and amyloid-β plaques. P021 competitively inhibits the leukemia inhibitory factor and increases brain-derived neurotrophic factor transcription, neuronal proliferation, and

differentiation. Oral administration of the compound increases neuronal plasticity and neurogenesis in rats [135].

Fetal gene therapy for inherited genetic diseases offers a prophylaxis option, especially against irreversible and fatal pathological changes. The acute childhood fatal form of neuronopathic Gaucher disease is not treatable. Because the enzyme cannot cross the BBB, these patients exhibit symptoms associated with hindbrain neurodegeneration, such as strabismus, neck hyperextension, and fatal apnea. After intravenous and intracerebroventricular injection of glucocerebrosidase (GCase), Gaucher cell infiltration was inhibited. As a result, it can be said that intracerebroventricular gene therapy may effectively treat fatal neuropathologies [136].

The presence of inflammation during fetal development and the fetus being affected by this inflammation increases the risk of neurological disorders in later life. Prenatal exposure to inflammation also affects the etiology of age-related neurodegenerative diseases. Gestational inflammation during pregnancy disrupts the formation of the fetal BBB through a cyclooxygenase-2 (COX2)-dependent mechanism. These functional and structural changes in the BBB, which continue after birth until later life, lead to excessive permeability. It has been suggested that microglia in the fetal brain's perivascular area increase due to COX2 activity [137].

HIV infection is known to be transmitted from mother to baby. Practical approaches to reducing perinatal transmission include mono/combined antiretroviral therapy applied to the mother or the baby, preferring cesarean delivery, and especially HIV-positive mothers not breastfeeding their babies. Among the theoretically practical approaches, washing the birth canal with disinfectants (5% chlorhexidine), avoiding artificial opening of the membrane, using immune therapy, and supplementing the mother with vitamin A can be listed. Antiretroviral drugs are not widely used, especially in developing countries, due to reasons such as the cost of antiretroviral treatment and the side effects of the drug. Requiring HIV tests from pregnant women, advising mothers not to breastfeed their babies, and testing drugs on experimental groups are approaches to reduce and prevent the transmission of HIV from mother to baby in health care [138].

Effective and implementable clinical approaches to protect prenatal neurological development and prevent neurodegenerative diseases can reduce the risk level. Early detection of exposure to environmental toxins during pregnancy may be critical to protecting fetal neurological development. Prenatal screening can be conducted more comprehensively to identify risks to pregnant women exposed to agents such as heavy metals, pesticides, and endocrine disruptors. These screenings can enable closer monitoring of high-risk groups and appropriate measures to be taken. Public health policies can be developed to reduce the effects of environmental factors on neurodegeneration. In particular, regulations can be introduced to minimize pregnant women's exposure to toxic chemicals, air pollution, and harmful industrial emissions. In addition, more investment should be made in public health research to understand the long-term consequences of harmful environmental exposures. More education and awareness should be created about environmental factors during pregnancy and the potential effects of these factors on the fetus. Pregnant women, family members and health professionals should be made more aware of environmental agents. In the future, educational campaigns can be organized at all levels of society about the effects of environmental exposures on neurological development. The interaction of genetic and environmental factors in the prenatal period should be investigated in more detail. Examining the effects of environmental toxins, especially in

individuals with genetic predisposition, observing this interaction and determining individual risk factors will provide important information for future treatment and prevention strategies for neurodegenerative diseases. Understanding the molecular mechanisms of prenatal neurodegeneration will contribute to the discovery of new therapeutic targets. These discoveries may guide the development of treatment methods that can stop or delay the neurodegeneration process.

1.17.Policies and Public Health Measures Implemented to Reduce Environmental Exposures

Air pollution is one of the factors that cause harmful effects on the neurological development of the fetus during pregnancy. Stricter emission control laws and regulations limiting industrial emissions can be implemented to improve air quality. These policies will reduce the effects of environmental toxins on the fetus and directly reduce the prevalence of neurological diseases. Strengthening laws against environmental toxins such as industrial chemicals and pesticides can significantly reduce the risk of exposure to these substances during pregnancy. Public health measures targeting pregnant women in particular will be an important step in reducing environmental exposure. Regulations aimed at reducing air and water pollution can help prevent the spread of many diseases, such as neurological diseases, by reducing the chemicals that will be exposed to the environment. Early detection of exposure to environmental toxins is important, especially for women in high-risk groups. Prenatal screening can help identify women exposed to toxic substances and take steps to reduce the risks. Early screening and monitoring can prevent possible adverse effects on fetal development by protecting pregnant women at high risk from environmental toxins. This can be effective in preventing neurological diseases by directly protecting brain development. Education programs can be organized to raise public awareness about environmental exposures during pregnancy and their negative effects on the fetus. Pregnant women can be guided to avoid exposure to toxins in particular. Such awareness studies will enable the public to become more aware of environmental risks and can take personal protective measures. Environmentally friendly agriculture and production methods should be encouraged, and organic farming practices should be expanded. Policies encouraging organic farming practices can be implemented to reduce the harms of pesticides and other chemicals used in agricultural production. By using environmental exposure monitoring systems, it becomes possible to monitor environmental toxins to which pregnant women are exposed and to determine exposure in advance. At the very least, the risk potential in risky areas can be determined and early warning can be provided. Systematic monitoring of exposure allows measures to be taken to protect public health. Understanding prenatal neurodegeneration at the molecular level can lead to the discovery of new treatment targets. Access to health services should be increased, and better access to health services should be provided in low-income and high-risk communities. Regular health checks for pregnant women can offer information and support regarding protection from environmental exposures**.**

2. CONCLUSION

Adverse prenatal exposures to the fetus are associated with changes in brain development later in life. The fetus is exposed to alcohol, addictive substances, and drugs in the womb. Exposure to cocaine, opioids, pathologies associated with placental dysfunction, nutritional deficiencies, and chronic diseases such as maternal anemia cause a decrease in brain size and changes in the temporal lobe and hippocampal volumes. The reduction in brain reserve paves the way for an increase in the risk of dementia. In addition to the acute adverse effects that develop due to fetal exposure to drugs of abuse and illicit substances during pregnancy,

it also causes permanent changes in brain function and structure. Additionally, the molecular targets of psychoactive drugs may be different in infants and children than in adults. Psychoactive drugs with synaptic modulatory roles in adults may have very different effects during early development. Surprisingly, research in this area is still in its infancy, and more precise and mechanistic studies are needed to characterize the extent of neurobehavioral changes. Its socioeconomic impact is also significant, given the costs of private education, long-term medical management, and lifetime loss of productivity.

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The Declaration of Ethics Committee Approval

This study does not require ethics committee permission or any special permission.

Declaration of research and publication ethics

The authors of the paper declare that we followed the scientific, ethical and citation rules of Environmental Toxicology and Ecology in all processes of the paper and that we did not make any falsification of the data collected. Furthermore, we declare that ETOXEC and its Editorial Board are not responsible for any ethical violations that may have occurred and that this study has not been evaluated in any other academic publication environment than ETOXEC.

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Investigation of Genotoxic Effect Potential of Water Pollution in Büyük Akgöl on *Scardinius erythrophthalmus* and *Perca fluviatilis*

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ABSTRACT

This study aims to evaluate heavy metal accumulation and related genotoxic effects on two species, the omnivorous *Scardinius erythrophthalmus* and the carnivorous *Perca fluviatilis*, living in Büyük Akgöl within the borders of Sakarya province. In this context, the genotoxic effect potential was evaluated using micronucleus (MN) and erythrocyte nuclear abnormalities methods in peripheral blood samples taken from fish. In addition, the levels of heavy metals such as Al, Fe, Zn, Cu, Mn, Pb, Cd, As, Ni, Co, Cr and Hg in muscle tissues were analyzed. According to the obtained data, significant genotoxic responses reflecting the effects of environmental pollutants were observed in both species. While the MN rate increased up to 16% in *S. erythrophthalmus* individuals, this rate remained at 1.80% in *P. fluviatilis*. In addition, nuclear abnormality frequencies also differed among species. Heavy metal analyses revealed that heavy metals including Zn, Pb, Fe, Cu, Al and Cr accumulated at relatively high levels in the muscle tissue in both species. These results suggest that pollution in Büyük Akgöl induces biological effects at genetic level and that heavy metal contamination may constitute a potential threat to fish health. In this context, it is recommended that use of agricultural chemical in agricultural areas surrounding Büyük Akgöl be limited and that domestic and industrial wastes be effectively regulated. Long-term, seasonal biomonitoring studies should be conducted on species at different trophic levels, such as *P. fluviatilis* and *S. erythrophthalmus*, in order to monitor genotoxic effects caused by heavy metals. In addition, considering that metals accumulated in fish can be transferred along the food chain from plankton to predatory fish, ecosystem-based protection strategies should be developed.

Büyük Akgöl'deki Su Kirliliğinin *Scardinius erythrophthalmus* ve *Perca fluviatilis* Üzerindeki Genotoksik Etki Potansiyelinin Araştırılması

ÖZET

Bu çalışma, Sakarya ili sınırlarında yer alan Büyük Akgöl'de yaşayan omnivor tür olan *Scardinius erythrophthalmus* ve karnivor tür olan *Perca fluviatilis* üzerinde ağır metal birikimi ve buna bağlı genotoksik etkileri değerlendirmeyi amaçlamaktadır. Bu kapsamda, balıklardan alınan periferik kan örneklerinde mikronukleus (MN) ve eritrosit çekirdek anomalileri yöntemleri kullanılarak genotoksik etki potansiyeli değerlendirilmiştir. Ayrıca, kas dokularında Al, Fe, Zn, Cu, Mn, Pb, Cd, As, Ni, Co, Cr ve Hg gibi ağır metallerin düzeyleri analiz edilmiştir. Elde edilen verilere göre, her iki türde de çevresel kirleticilerin

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etkilerini yansıtan anlamlı genotoksik yanıtlar gözlemlenmiştir. *S. erythrophthalmus* bireylerinde MN oranı %16'ya kadar çıkarken, *P. fluviatilis*'de bu oran %1,80 düzeyinde kalmıştır. Ayrıca çekirdek anomali frekansları da türler arasında farklılık göstermiştir. Ağır metal analizleri, Zn, Pb, Fe, Cu, Al ve Cr gibi ağır metallerin her iki türün kas dokusunda nispeten yüksek seviyelerde biriktiğini ortaya koymuştur. Bu sonuçlar, Büyük Akgöl'deki kirliliğin genetik düzeyde biyolojik etkilere neden olduğunu ve ağır metal kirliliğinin balık sağlığı açısından potansiyel bir tehdit oluşturabileceğini göstermektedir. Bu bağlamda, Büyük Akgöl çevresindeki tarım alanlarında kullanılan tarımsal kimyasalların kullanımının sınırlandırılması ve evsel ile endüstriyel atıkların etkin şekilde denetlenmesi önerilmektedir. Ağır metallerin neden olduğu genotoksik etkileri izleyebilmek amacıyla *P. fluviatilis* ve *S. erythrophthalmus* gibi farklı trofik seviyelerdeki türler üzerinde uzun vadeli, mevsimsel biyolojik izleme çalışmaları yapılmalıdır. Ayrıca, balıklarda biriken metallerin planktondan yırtıcı balıklara kadar olan besin zinciri boyunca taşınabildiği göz önünde bulundurularak, ekosistem temelli koruma stratejileri geliştirilmelidir.

1. INTRODUCTION

Ecotoxicology is a scientific discipline that analyzes the sources of environmental pollutants, the toxic effects of these pollutants on ecosystem components, and the ecological consequences of these effects at a multi-level. This field is not limited to examining biological responses at the species level, but also aims to evaluate the disruptions caused by these effects at the ecosystem level and to develop strategies for protecting ecosystem integrity [1]. Currently, chemical pollutants originated from expanding industrial, agricultural and urban activities pose serious threats to ecosystem health. In this context, ecotoxicology emerges as a multidisciplinary field that investigates the effects of toxic substances on individual organisms, population dynamics, natural communities, and entire ecosystems. Aquatic ecosystems, in particular, are extremely sensitive to environmental pollutants due to their high biodiversity and the fact that they form the basis of many food chains [2].

Among aquatic organisms, fish are particularly susceptible to the adverse effects of toxic pollutants, making them important indicators of environmental contamination [3]. The selection of model organisms is of great importance in biomonitoring studies. In particular, it is recommended to select fish species that occupy different ecological niches, belong to distinct taxonomic groups, and exhibit broad geographical distributions. Among these species, the omnivorous rudd (*Scardinius erythrophthalmus* L., 1758), which has an important place in the fish communities of many water resources in Eastern and Central Europe and is also found in Türkiye, and the carnivorous perch (*Perca fluviatilis*) stand out [3-4]. The ability of these fish species to adapt to different feeding habits, rapid adaptation to sudden changes in the environment [5], high resistance to diseases and rapid reproduction make them valuable organisms for toxicological research [6].

Perca fluviatilis belongs to the Percidae family and is an ecologically important predator, both commercially and in terms of fisheries [7]. Several studies have investigated the potential genotoxic effects of water pollution on *Perca fluviatilis* populations inhabiting rivers in various countries. In studies using micronucleus assay, nuclear damage levels were investigated in *P. fluviatilis* fish caught from the Odra River in the Czech Republic [8], rivers in the Rivne Region in Ukraine [9], the Desenka River [10], and the Nemunas River in Lithuania [11] and the sensitivity level of this species to environmental stress factors was reported. In addition, heavy metal accumulation levels were investigated by ICP-OES analysis of muscle tissue in samples collected from the Međuvršje Reservoir located in the West Morava River Basin in western Serbia [12].

Scardinius erythrophthalmus is one of the widespread species belonging to the Leuciscidae family [7]. As an omnivorous pelagic species, the rudd predominantly occupies macrophyte-rich zones in aquatic ecosystems, and its foraging behavior has been reported in some studies to exert adverse effects on macrophyte biomass [13]. These characteristics render it a suitable model organism for comprehensive ecotoxicological assessments of aquatic ecosystems, particularly in urban water bodies The tolerance of this species to variations in oxygen, salinity, and gas

conditions, along with its ability to adapt to atypical dietary sources, highlights its physiological adaptability [14]. Nuclear disorder levels were investigated in *S. erythrophthalmus* fish caught from the Desenka River in Ukraine [10]. In addition, several studies have focused on the effects of toxicant exposureon morphological, physiological and biochemical parameters in this species [3], on its histological and biochemical responses to environmental pollutants [15-17], and on the impacts of anthropogenic pollution on enzyme activity [14].

In ecotoxicological studies, micronucleus test [18] and erythrocyte nuclear abnormality test are among the most widely employed assays for the assessment of genotoxic effects. These tests are also biomarker tools used to determine whether xenobiotic agents cause DNA damage by causing changes in chromosomes or disruptions in the mitotic process [19]. By reflecting the genetic-level effects of environmental pollutants, these genotoxicity assays offer valuable insights for ecosystem health monitoring and are widely employed in both in vivo and in vitro settings across a range of taxa, particularly aquatic organisms [20].

The objective of this study was to assess the genotoxic effects of environmental pollutants in Lake Büyük Akgöl through the application of micronucleus and erythrocyte nuclear abnormality assays in the erythrocytes of *Scardinius erythrophthalmus* and *Perca fluviatilis*. Furthermore, heavy metal concentrations in the muscle tissues of these model species were quantified.

2. MATERIALS AND METHODS

2.1. Study Area and Fish Sampling

Büyük Akgöl is a shallow lake located in the Ferizli district of Sakarya province, in the north-western part of Türkiye's Marmara Region, at coordinates 41°01′ K and 30°33′ E. The lake covers an area of 3.6 km² with an average depth of 1.5 meters and has a drainage basin of 47 km². Field studies were carried out in November 2023. Fish samples were caught by local professional fishermen using gillnets with different mesh sizes. In order to perform micronucleus assay and heavy metal analyses, five fish samples were taken from each fish species.

2.2. Micronucleus Assay

The collected fish were anaesthetized using clove oil. Subsequently, peripheral blood was carefully drawn from the caudal venous sinus using heparinized syringes. The blood from each fish was spread across three separate slides and left to air dry for 24 hours. After this drying period, the slides were fixed with pure ethanol and stained with 5% Giemsa for 10 minutes. The samples were then observed under a light microscope, and the number of micronuclei was counted and recorded in 1,000 erythrocytes per slide. A total of 3,000 erythrocytes were randomly selected for analysis from each sample. For the MN assay micronuclei were identified according to the criteria established by Kirsch-Volders et al. [21].

2.3. Erythrocyte Nuclear Abnormality Assay

Blood samples were taken from preparations prepared for the MN assay. The other nuclear morphological alterations observed in erythrocytes were thoroughly examined using an Olympus® CX21 light microscope. Abnormalities in both cellular and nuclear structures were counted and visually documented. To evaluate nuclear deformations caused by environmental pollutants, the erythrocytes were categorized into specific morphological classes. These included segmented nuclei, kidney-shaped, lobed, notched nuclei, binucleated cells, and karyolysis. The classification criteria were based on the studies conducted by Canedo et al. [22] and Braham et al. [23].

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2.4.Heavy Metals Analysis of Fish Tissue

After fish were anesthetized using clove oil, muscle tissue samples were taken using a plastic knife. Tissue samples were stored at -20ºC until analyzed and heavy metal analyses were carried out at DUBIT (Scientific and Technological Research and Application Center)

For heavy metal analysis, 0.45–0.60 g of muscle tissue was weighed and placed into microwave digestion vessels. Initially, 1 mL of H_2O_2 was added to Teflon containers and allowed to react for 1 minute. Subsequently, 6 mL of HNO_3 was added, and the vessels were sealed. The samples were then subjected to digestion for 20 minutes using a Milestones Microwave Digestion System. Following digestion, the samples were allowed to cool within the device for 10–15 minutes. The digested fish tissue samples were filtered through 45 µm pore-size filters into 50 mL falcon tubes and diluted to a final volume of 13 mL with deionized water. The concentrations of heavy metals in the tissue samples were then measured in triplicate using a ThermoScientific X Series ICP-MS instrument. Concentrations of heavy metals such as aluminum (Al), iron (Fe), zinc (Zn), copper (Cu), manganese (Mn), lead (Pb), cadmium (Cd), arsenic (As), nickel (Ni), cobalt (Co), chromium (Cr), mercury (Hg) were determined in muscle tissues.

2.5.Statistical Analysis

The frequency of micronucleus and other nuclear abnormalities in erythrocytes of *Scardinius erythrophthalmus* and *Perca fluviatilis* were analyzed with the Kruskall-Wallis test, one of the non-parametric tests, using SPSS 20.0 software.

3. RESULTS

This study was conducted to evaluate the presence of possible genotoxic effects on the aquatic ecosystem in Büyük Akgöl located in the lower part of Sakarya River Basin. Micronucleus (MN) test and erythrocyte nuclear abnormalities analyses were applied to determine the genotoxic effects of environmental pollution around the lake on fish. Data obtained from two different fish species, *Scardinius erythrophthalmus* (rudd) and *Perca fluviatilis* (perch), were used in the study. MN frequencies were determined as a result of microscopic examinations and the findings are presented in Figure 1.

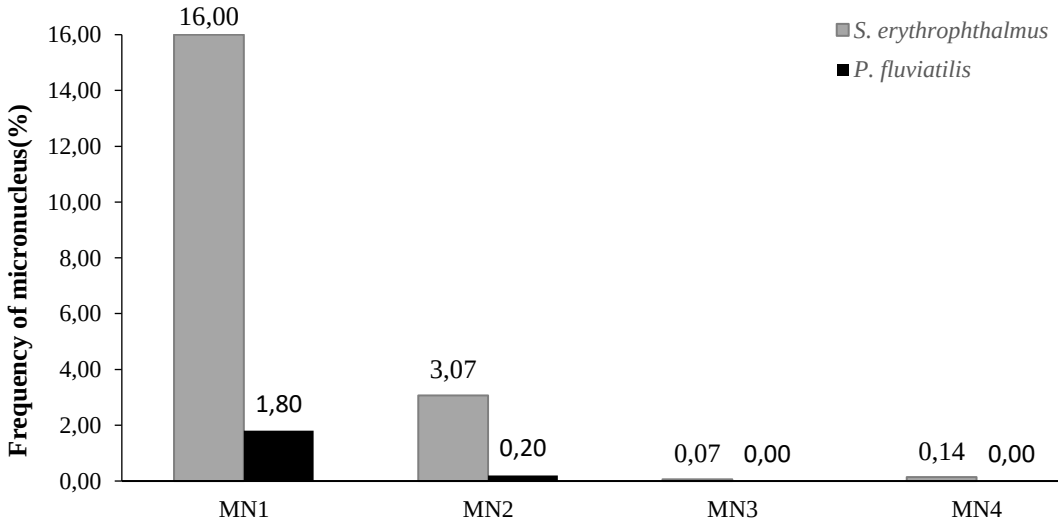

Figure 1. Frequency of different types of micronucleus abnormalities in *Scardinius erythrophthalmus* and *Perca fluviatilis*

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According to the data obtained from the micronucleus test showed significant differences between the two fish species. While the frequency of one micronucleus cell (MN1) was detected at 16.00% in *S. erythrophthalmus* erythrocytes, the same form was observed at only 1.80% in *P. fluviatilis* erythrocytes. Similarly, the frequency of two micronucleus cells (MN2) was found at 3.07% in rudd, and this rate remained at 0.20% in perch erythrocytes. The frequencies of three and four micronucleus cells (MN3 and MN4) were detected only at 0.07% and 0.14% in *S. erythrophthalmus* individuals, respectively, while these abnormalities were not observed in *P. fluviatilis* individuals. These data suggested that *S. erythrophthalmus* species carried nuclear abnormalities at higher frequencies compared to *P. fluviatilis* in all micronucleus frequencies.

The data obtained from the erythrocyte nuclear abnormality test were analyzed with statistical methods and the data related to the results are presented in Figure 2. The highest rate in *P. fluviatilis* erythrocyte cells was observed as "kidney shaped" nucleus form with 1.60%; this form was not detected at all in *S. erythrophthalmus* erythrocyte cells. "Lobed nuclei" abnormality was detected in 0.13% in *S. erythrophthalmus* and 0.40% in *P. fluviatilis*. "Notched nuclei" type was only seen in 0.27% in *S. erythrophthalmus* and was not found in perch individuals. Similarly, "Segmented nuclei" form was detected in 0.20% in rudd and was not observed in *P. fluviatilis*. "Binucleated" cells were detected only in *S. erythrophthalmus* individuals at a rate of 0.13%.

As a result, while nuclear abnormalities such as "notched", "segmented" and "binucleated" were observed in *S. erythrophthalmus* individuals, these forms were not detected in *P. fluviatilis*; on the other hand, a high rate of "kidney shaped" form was observed, which is specific to *P. fluviatilis*.

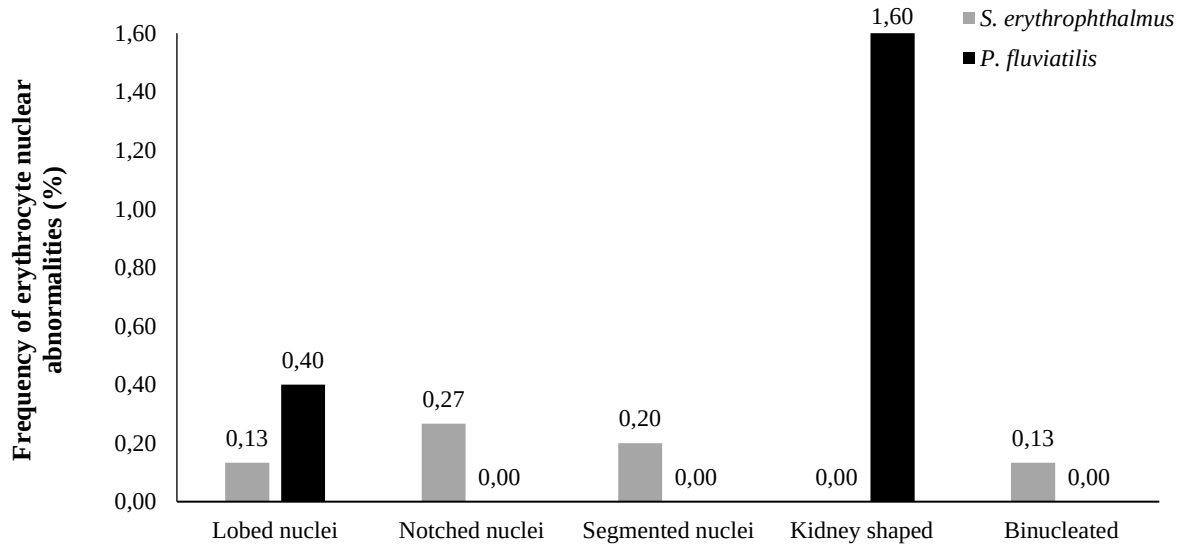

Figure 2. Frequency of erythrocytic nuclear abnormalities observed in *Scardinius erythrophthalmus* and *Perca fluviatilis*

The results of heavy metal analysis of muscle tissue are presented in Figure 3. As seen in the graph, the accumulation levels of some metals differ in both species. The highest concentration in *S. erythrophthalmus* muscle tissue was determined for zinc (Zn) with 6.03 mg/kg. This was followed by aluminum (Al) 3.47 mg/kg and iron (Fe) 2.39 mg/kg, respectively. On the other hand, zinc (Zn) showed the highest value in *P. fluviatilis* muscle tissue with 8.134 mg/kg, followed by copper (Cu) 5.002 mg/kg and iron (Fe) 2.334 mg/kg. These findings reveal that the heavy metals detected in muscle tissue accumulate at different rates according to species and that especially zinc, copper, lead, aluminum and iron elements were at remarkable levels.

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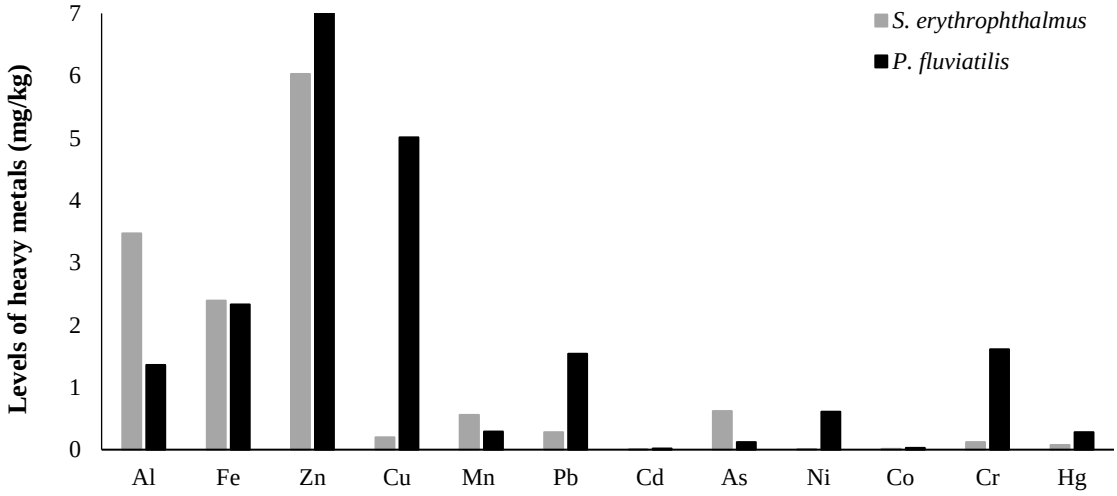

Figure 3. Concentrations (mg/kg) of heavy metals detected in the muscle tissues of *S. erythrophthalmus* and *P. fluviatilis*

4. DISCUSSION

In this study, the frequencies of nuclear abnormalities observed in erythrocytes of *S. erythrophthalmus* and *P. fluviatilis* species emerged as a significant biological indicator in assessing the potential genotoxic effects of environmental factors. The detection of nuclear abnormalities at varying frequencies in both species reveals species-specific differences in sensitivity to pollutants present in the aquatic environment. The combined use of nuclear abnormality analysis with micronucleus testing allows for a more comprehensive determination of the damage caused by environmental stress factors at the genetic level.

According to the micronucleus and erythrocyte nuclear abnormality analyses performed on peripheral blood cells taken from fish, it was revealed that the genotoxic effect levels between *S. erythrophthalmus* and *P. fluviatilis* species living in Büyük Akgöl differed significantly. As a result of the evaluation of micronucleus (MN) and nuclear abnormalities, it was understood that *S. erythrophthalmus* individuals were exposed to a higher level of genotoxic effect compared to *P. fluviatilis* individuals. The results of the present study were compared with other studies investigating the potential genotoxic effects of water pollution on *S. erythrophthalmus* and *P. fluviatilis* in rivers located in different countries.

Řehulka & Bradík, [8] conducted a study to evaluate the mutagenic effects of chemical substances on *P. fluviatilis* in three water reservoirs located in the Odra River basin in the Czech Republic and revealed that micronucleus and erythrocyte nuclear abnormalities showed differences in genotoxicity levels among species. Particularly, *P. fluviatilis* has a high MN frequency, which is consistent with the "kidney shaped" nuclear anomaly and general nuclear abnormality frequencies observed in *P. fluviatilis* individuals in our study, supporting that *P. fluviatilis* may be a potential biomarker against environmental stress factors. Biedunkova et al., [10] reported that nuclear anomalies observed in fish in the Desenka River were hidden below the spontaneous mutagenesis limit in all species. This situation indicates that genotoxic stress in the region is at a low level. Comparison rates between species show that *S. erythrophthalmus* has a low average nuclear abnormality (0.92±0.11‰), while *P. fluviatilis* species have a slightly higher average (1.69±0.29‰). Biedunkova et al., [10] suggest that this difference may be due to *S. erythrophthalmus* being more resistant to activity stress behaviors or lower exposure, while *P. fluviatilis* may be slightly more sensitive to discharge. In our study, it is seen that *P. fluviatilis* was more resistant to activity stress, while *S. erythrophthalmus* was more sensitive. Differences in diet, habitat use and ecological niche can be played consistently in this diversity [10]. Klimenko and Biedunkova [9] stated that *P. fluviatilis* living in small rivers showed higher nuclear damage and morphological deterioration compared to those living in medium-sized rivers. Bagdonas et al. [11] reported that *P.*

fluviatilis showed high sensitivity to micronucleus test. This situation is consistent with the detection of relatively high rates of specific abnormalities such as “kidney shaped” nucleus in *P. fluviatilis* individuals despite the low rates of general nuclear abnormalities in our study. These findings support that *P. fluviatilis* can be considered as a sensitive bioindicator species in biological monitoring of environmental genotoxic agents.

There are studies on heavy metal analysis in *S. erythrophthalmus* and *P. fluviatilis* muscle tissue. Tokatlı and Ustaoğlu [24] reported that estimated daily intake (EDI) values for Li, B, Cr, Co, Se, Tl, and Pb in edible muscles of *S. erythrophthalmus* and *P. fluviatilis* from the delta of Maritsa River (Türkiye) generally exceeded the permitted daily dose limit. Tokatlı et al. [25] stated that the levels of cadmium, lead, nickel, and chromium detected in muscle tissues of all fish, including *S. erythrophthalmus* and *P. fluviatilis* in Gala Lake and Meriç River (Türkiye), were well above human consumption limits. Çetin et al. [26] reported that heavy metals, especially Zn, Pb, and Cd, accumulated at high levels in *P. fluviatilis* in Altınyazı Reservoir; Pb reached 6.01 mg/kg and Cd reached 0.25 mg/kg. These findings indicate the presence of serious heavy metal pollution in the lake. Similarly, in this study conducted in Büyük Akgöl, it was observed that metals Zn was found at high levels in the analyses performed on *P. fluviatilis* and *S. erythrophthalmus* species, and toxic metals such as Cu (5.002 mg/kg), Fe (2.334 mg/kg) were prominent especially in *P. fluviatilis*. From this perspective, there are similarities in terms of different heavy metal accumulations in the studies. The accumulation of heavy metals in muscle tissue may increase depending on factors such as the feeding habits of the fish, the pollution level of the environment in which it lives and the chemical properties of the metal [27, 28]. In addition, the high accumulation of Zn and Al in *S. erythrophthalmus* reveals that heavy metal retention varies between species. In this study, micronucleus and erythrocyte nucleus abnormalities were determined in *S. erythrophthalmus* and *P. fluviatilis* erythrocytes. It is thought that these abnormalities may be due to the higher environmental pressure caused by local pollution sources (agricultural activities, pesticides, domestic waste, heavy metal accumulation) in Büyük Akgöl. In fact, in the study conducted by Durmaz [29] in Büyük Akgöl, water analyses conducted between 2016 and 2017 revealed that heavy metals such as arsenic, mercury, selenium and cadmium in the lake exceeded the regulation limits. The fact that the water is at the IV and V class quality level in most stations indicates that the lake ecosystem is exposed to serious pollutants. The pollution in question is based on sources such as intensive agricultural activities, arsenic-containing pesticide use, domestic waste discharges and atmospheric transport. In addition, uncontrolled fishing and direct discharge of lake water into the Sakarya River are also evaluated among other factors that increase the existing pollution load. Similarly, in the study of Aras and Arslan [30], the water quality of Büyük Akgöl was classified as "inappropriate" according to the water quality index (WQI). In the same study, it was reported that heavy metals such as Pb, Hg, Cd, Se and Al, as well as turbidity values, were above both WHO and national limits. Agricultural inputs, industrial wastes, pesticide use and pollutants carried by drainage waters come to the fore as pollution sources.

5. CONCLUSION

In this study, two different fish species (*Scardinius erythrophthalmus* and *Perca fluviatilis*) obtained from the Büyük Akgöl ecosystem were examined and heavy metal accumulation and nuclear abnormalities were evaluated. The findings obtained show that various heavy metals may accumulate in the muscle tissu in both species and this leads to genotoxic effects. In *P. fluviatilis* individuals, especially Zn, Cu, Fe and Cr accumulation was prominent; in *S. erythrophthalmus* individuals, Zn, Al and Fe accumulation was prominent. In micronucleus analyses, while the total micronucleus rate reached 19.07% in *S. erythrophthalmus* individuals, this rate was determined as 1.80% in *P. fluviatilis*. It is thought that these abnormalities may have occurred as a result of exposure to environmental pollutants such as heavy metals, pesticides and domestic waste rather than spontaneous mutations in the Büyük Akgöl environment.

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The Declaration of Conflict of Interest/ Common Interest

No conflict of interest or common interest has been declared by the authors.

Authors' Contribution

The first author contributed 45%, the second author 35%, the third author 20%.

The Declaration of Ethics Committee Approval

Since the fish used in this study were supplied by fishermen, they did not require ethics committee approval or any special permit.

The Declaration of Research and Publication Ethics

The authors of the paper declare that they comply with the scientific, ethical and quotation rules of ETOXEC in all processes of the paper and that they do not make any falsification on the data collected. In addition, they declare that Environmental Toxicology and Ecology and its editorial board have no responsibility for any ethical violations that may be encountered, and that this study has not been evaluated in any academic publication environment other than Environmental Toxicology and Ecology.

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Research Article

Benalaxyl-M maddesinin arılar üzerindeki toksik etkilerinin *in silico* değerlendirilmesi

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Article Info

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ÖZET

Bu çalışmada, fenilamid grubuna ait sistemik bir fungisit olan ve tarımda özellikle Phytophthora, Pythium ve Peronospora gibi oomycete mantarlarının neden olduğu hastalıklara karşı kullanılan Benalaxyl-M maddesinin bal arıları (Apis mellifera) üzerindeki akut toksik etkileri, makine öğrenimi tabanlı bir QSAR (Kantitatif Yapı-Aktivite İlişkisi) aracı olan BeeToxAI kullanılarak in silico yöntemlerle değerlendirilmiştir. Analiz sonuçları, Benalaxyl-M'nin bal arıları için akut oral ve kontakt toksisite açısından toksik olmadığını (%83-86 güvenilirlik) göstermiştir. Uygulanabilirlik Alanı analizi, tahminlerin güvenilir olduğunu doğrularken, katkı haritaları moleküldeki toksisiteye katkı yapan fonksiyonel grupları belirlemede kullanılmıştır. Elde edilen bulgular, Benalaxyl-M'nin bal arıları üzerindeki düşük akut riskini ortaya koysa da, sonuçların deneysel çalışmalarla desteklenmesi önerilmektedir. Bu çalışma, pestisitlerin ekotoksikolojik risk değerlendirmelerinde yapay zekâ tabanlı araçların potansiyelini vurgulamaktadır.

In silico evaluation of toxic effects of Benalaxyl-M on bees

ABSTRACT

This study evaluated the acute toxic effects of Benalaxyl-M on honey bees (*Apis mellifera*) using BeeToxAI, a machine learning-based QSAR (Quantitative Structure-Activity Relationship) tool, through *in silico* methods. The analysis results indicated that Benalaxyl-M is non-toxic in terms of acute oral and contact toxicity for honey bees (83-86% reliability). The Applicability Domain analysis confirmed the reliability of the predictions, while contribution maps identified functional groups contributing to toxicity. Although the findings suggest a low acute risk of Benalaxyl-M to honey bees, experimental validation is recommended. This study highlights the potential of AI-based tools in the ecotoxicological risk assessment of pesticides.

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1. GİRİŞ

Pestisitler, tarımsal üretimde verim kayıplarını önlemek amacıyla yaygın kullanılsa da, kontrolsüz kullanımları ekosistemlerde kalıcı kirliliğe yol açmaktadır. Özellikle fungisitler, hedef dışı organizmalar üzerinde toksik etkileri nedeniyle biyoçeşitlilik kaybı ve gıda zincirinde birikim gibi sorunlara neden olur [1], [2].

Pestisitlerin %95'inden fazlası hedef dışı alanlara yayılarak su, toprak ve havada birikir. Örneğin, DDT gibi organoklorlu bileşikler, onlarca yıl çevrede kalabilir ve besin zincirinde birikerek yüksek trofik seviyelerdeki canlıları etkiler [1], [4]. Benalaxyl-M'in yüksek stabilitesi, sucul ekosistemlerde uzun süreli kalıntılara ve biyoakümülasyona yol açabilir [3].

Pestisit kalıntıları, gıda ve su yoluyla insan vücuduna girerek akut zehirlenmeler, kanser, nörolojik bozukluklar ve üreme sorunlarına yol açabilir [3], [5]. Özellikle tarım işçileri ve kırsal kesimde yaşayanlar, yüksek maruziyet riski altındadır [6] ,[5]. Son çalışmalar, pestisitlerin bilişsel fonksiyonlarda azalmaya ve uyku bozukluklarına neden olduğunu ortaya koymuştur [6].

Benalaxyl-M, phenylamide grubuna ait bir fungusit olup, suda yüksek çözünürlüğü ve toprakta uzun yarılanma ömrü (~60 gün) nedeniyle yeraltı sularına sızma riski taşır [7]. EFSA (2010), Benalaxyl-M'in ADI (Kabul Edilebilir Günlük Alım) değerini 0.07 mg/kg vücut ağırlığı olarak belirlemiştir, ancak metaboliti olan benalaxyl asidin toksisitesi henüz tam olarak karakterize edilmemiştir [8]. Laboratuvar çalışmaları, bu fungisitin sucul organizmalarda endokrin sistem bozukluklarına neden olduğunu göstermektedir [3], [6]. Benalaxyl-M gibi sistemik fungisitlerin, sucul organizmalar üzerindeki ekotoksikolojik etkileri laboratuvar çalışmalarıyla belirlenmiş olsa da bal arıları gibi hedef dışı türlerdeki akut toksik etkileri hakkında sınırlı veri bulunmaktadır [9]. Özellikle bal arıları (Apis mellifera), polen taşıyıcılık rolleri nedeniyle ekosistem sürdürülebilirliği için hayati öneme sahip olmalarına rağmen, pestisit maruziyetine bağlı akut toksisite ve popülasyon kayıplarıyla karşı karşıyadır [10]. FAO (2021) verilerine göre, pestisitlerin kontrolsüz kullanımı, arı kolonilerinde %30-40'a varan azalmaya yol açmıştır. Bu durum, insan sağlığı ve gıda güvenliği açısından da küresel bir tehdit oluşturmaktadır [11]. Bal arıları, pestisitlerin sinir sistemi üzerinde inhibitör etki gösteren neonikotinoidler ve organofosfatlar gibi bileşiklere karşı özellikle savunmasızdır. Örneğin, imidacloprid gibi neonikotinoid pestisitlerin, arılarda yalnızca birkaç nanogramlık dozlarda bile (örneğin 3.7 ng/birey) ölümcül toksisiteye yol açtığı gösterilmiştir [12]. Arıların pestisitlere duyarlılığı, metabolizmalarının hızlı olması ve koloni dinamiklerinin karmaşıklığı nedeniyle deneysel çalışmalarla değerlendirilmesi zorlu bir süreçtir. Bu noktada, Niceliksel Yapı-Aktivite İlişkisi (QSAR) gibi in silico yöntemler, akut toksisite parametrelerinin hızlı ve düşük maliyetli tahmini için kritik bir rol oynamaktadır [13].

QSAR modelleri, kimyasal yapı ile toksisite arasındaki ilişkileri istatistiksel olarak analiz eder. Örneğin, OECD tarafından validasyonu yapılan modeller, Benalaxyl-M'in logP (oktanol-su dağılım katsayısı) değerini 3.2 olarak tahmin etmiş ve bu da biyoakümülasyon potansiyelini desteklemiştir [14]. Bununla birlikte, Benalaxyl-M'in bal arıları üzerindeki toksik etkilerine yönelik çalışmalar sınırlı sayıdadır ve bu tür organizmalarla laboratuvar çalışmaları, koloni davranışları ve çevresel değişkenler gibi etmenler nedeniyle oldukça karmaşıktır aynı zamanda oldukça maliyetlidir. Bu durum in silico çalışmaların pestisit toksisite değerlendirilmesinde önemini artırmaktadır.

Bu çalışma, Benalaxyl-M'in bal arıları üzerindeki akut toksik etkilerinin QSAR tabanlı modellemelerle değerlendirilmesini amaçlamaktadır.

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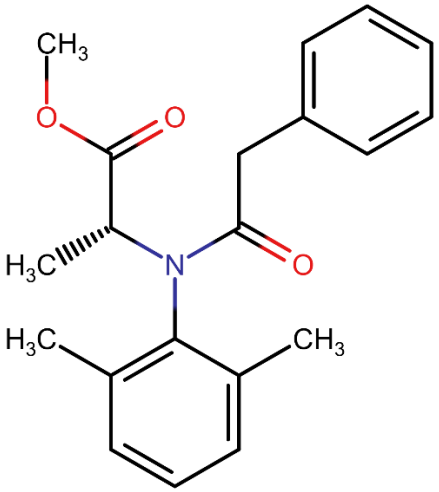
2. METOD

2.1. Benalaxyl-M maddesinin kimyasal yapısı

Benalaxyl-M için SMILES (Basitleştirilmiş Moleküler Giriş Satırı Giriş Spesifikasyonu) kodu Pubchem'den elde edildi. SMILES kodu CC1=C(C(=CC=C1)C)N([C@H](C)C(=O)OC)C(=O)CC2=CC=CC=C2'tür [15]. (Şekil 1)



Şekil 1. Benalaxyl-M maddesinin kimyasal yapısı

2.2. BeeToxAI

Bu çalışmada, kimyasalların bal arıları üzerindeki akut toksisitesini değerlendirmek için makine öğrenimi tabanlı, açık kaynaklı ve ücretsiz bir QSAR (Kantitatif Yapı-Aktivite İlişkisi) yapay zeka aracı olan BeeToxAI kullanılmıştır. Program, bal arılarında akut kontakt ve oral toksisitenin tahmini için, sıkı tahmin modelleme uygulamaları ve OECD yönergeleriyle tam uyumlu ilk web uygulamasıdır. Ekonomik İşbirliği ve Kalkınma Örgütü (OECD) ilkelerine ve Kimyasalların Kaydı, Değerlendirilmesi, İzni ve Kısıtlanması (REACH) düzenlemelerinin gerektirdiği testler listesine uygunluk göstermektedir. Bu özellikleri sayesinde, BeeToxAI regülasyonlara uygun ve güvenilir bir toksisite değerlendirmesi sağlamaktadır [16]. Ayrıca, QSAR modelleri tarafından üretilen katkı haritaları sayesinde, kimyasalların akut temas ve oral toksisitelerine katkıda bulunan atomlar ve fragmanlar belirlenebilmektedir. Bu yöntem, tahmin edilen sonuçların mekanistik yorumlanmasını kolaylaştırmakta ve kimyasalların toksisite potansiyelini azaltmak için yapısal modifikasyon önerileri geliştirilmesine yardımcı olmaktadır [17], [18]. BeeToxAI, yüksek tahmin gücüne sahip olup, doğruluk, hassasiyet ve özgüllük gibi sağlam validasyon metrikleri ile %83 ila %96 arasında başarı oranlarına ulaşmaktadır [19].

Akut oral toksisite için Random Forest algoritması (MACCS tanımlayıcıları ile), akut temas toksisitesi için ise Destek Vektör Makineleri (Support Vector Machine, SVM) algoritması (FeatMorgan FCFP2 tanımlayıcıları ile) uygulanmıştır.

Analizler, kullanıcı dostu arayüzü sayesinde üç basit adımda gerçekleştirilmiştir:

a) SMILES Girişi veya Moleküler Çizim

Kimyasalın SMILES gösterimi doğrudan ilgili alana yapıştırılabilir ya da modele entegre "Molecular Editor" (Moleküler Düzenleyici) aracı kullanılarak yapı çizilebilir.

b) Tahmin Süreci

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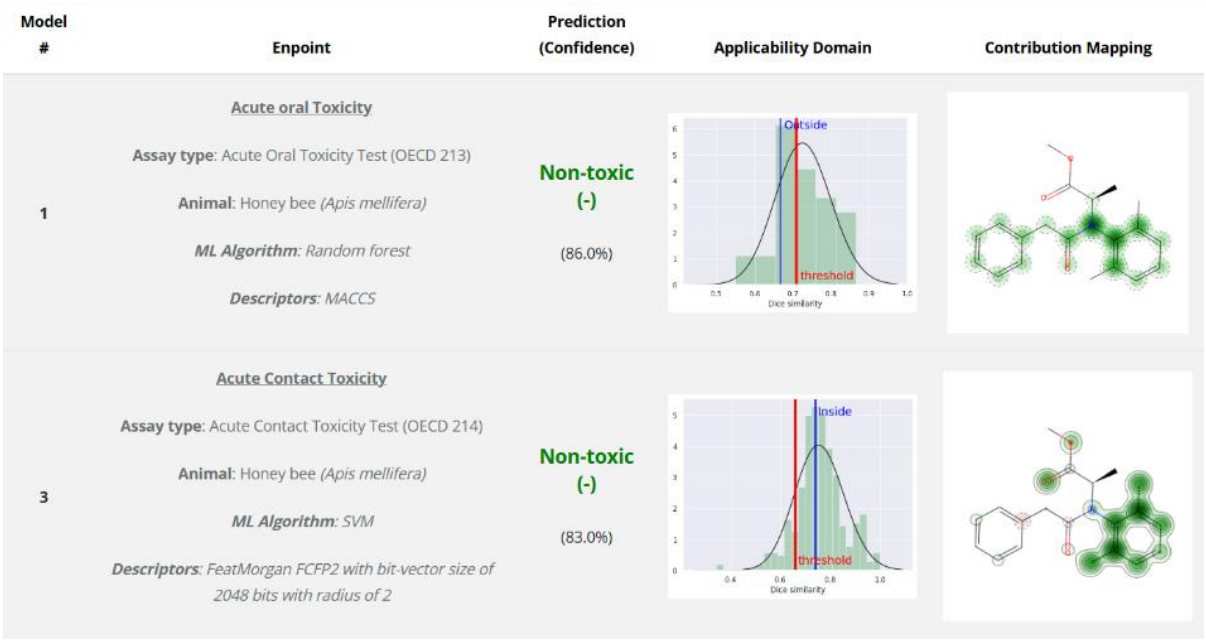
"Submit analysis" (Analiz Gönder) butonuna tıklanarak model tetiklenir. Tahminler, saniyeler içinde ekranda görüntülenir.

c) Sonuçların Yorumlanması

Model çıktıları, toksisite sınıflandırması, tahmin güvenilirliği, uygulanabilirlik alanı (AD) ve yapısal katkı haritalarını içermektedir. Katkı haritaları (contribution maps) aracılığıyla, moleküldeki toksisiteye katkı yapan atom/fragmanlar renk kodlu olarak vurgulanır. Bu haritalar, toksik etkiyi azaltacak yapısal modifikasyon önerileri için kullanılır.

3. SONUÇLAR

Benalaxyl-M maddesinin bal arıları (*Apis mellifera*) üzerindeki akut toksik etkileri, BeeToxAI platformu kullanılarak değerlendirilmiştir (Şekil 2). Programın sınıflandırma modellerine göre maddenin; akut oral toksisite açısından toksik olmadığı (%86 güvenilirlik), ve akut kontakt toksisite açısından da toksik olmadığı (%83 güvenilirlik) belirlenmiştir.



Şekil 2. BeeToxAI web sitesinden elde edilen analiz sonuçları

Uygulanabilirlik Alanı (Applicability Domain) analizi, maddenin modelin kimyasal uzayı içinde yer aldığını (Dice benzerlik skoru > eşik değer) ve tahminlerin güvenilir olduğunu göstermiştir. Katkı Haritaları (Contribution Maps) ise molekülün toksisiteye katkı yapan fonksiyonel gruplarını mekanistik olarak yorumlamaya olanak sağlamıştır. Akut oral ve kontakt toksisite açısından, toksisiteye katkı sağlayan belirgin bir yapısal motif tespit edilmemiştir.

Bu sonuçlar, Benalaxyl-M'in bal arıları için akut toksik risk oluşturmadığını göstermektedir.

4. TARTIŞMA ve ÖNERİLER

Pestisitler tarımda çok yaygın olarak kullanılan, çeşitli bitki hastalıklarını önlemek ve zararlı organizmalarla mücadele etmek amacıyla kimi zaman çiftçiler tarafından yanlış ve gereğinden fazla uygulanan kimyasal ajanlardır. Pestisitlerin toksik yapıları sebebiyle insanlar da dahil olmak üzere hedef olmayan organizmalar ve çevre üzerinde zararlı etkiler gösterebilir [20]. 2022 yılında Türkiye'de toplam tarım ilacı kullanımı, bir önceki yıla göre %4,5 artış göstererek 55.374 tona ulaşmıştır. Bu miktarın %35,1'i fungusitlerden, %26,3'ü herbisitlerden (yabancı ot öldürücüler), %22,0'si insektisitlerden (böcek öldürücüler), %4,5'i akarisitlerden (akar öldürücüler), %0,5'i

rodentisitlerden (kemirgen öldürücüler) ve %11,6'sı ise bitki aktivatörleri, gelişim düzenleyiciler, cezbediciler, fumigantlar, nematositler, kükürt ve madeni yağlar gibi diğer ürünlerden oluşmaktadır [21]. Pestisitlerin bir alt grubu olan fungusitler, tarımda ürün hasarına sebep olan funguslarla (mantarlar) mücadele atma amacıyla kullanılır. Fungusitlerin bazıları çok toksiktir ve zehirlenmelere yol açabilir [22].

Pestisitlerin geniş çapta kullanımı, böcek ve özellikle arı popülasyonlarının ve biyoçeşitliliğin önemli ölçüde azalmasına yol açmıştır. Avrupa ülkelerinde böcek biyokütlesinde %70, tarım alanlarındaki kuş sayısında ise %50 oranında azalma bildirilmiştir [23]. Benzer şekilde, Avrupa, Avustralya ve Kuzey Amerika'da tür zenginliğinde %42'lik bir düşüş gözlemlenmiştir [24]. Pestisitlerin, özellikle neonicotinoid türlerinin, arı popülasyonları üzerinde doğrudan bir azaltıcı etkisi olduğu belirtilmektedir [25]. Araştırmalar, arıların yaşam döngüsündeki bozulmaların yanı sıra, beslenme ve üreme gibi önemli davranışlarını da olumsuz etkilediğini göstermektedir [26], [27].

Pestisit kalıntıları arılar üzerinde zararlı etkiler yaratmakta, bu da onların ekosisteme sağladığı hizmetlerin azalmasına neden olmaktadır. Birleşmiş Milletler 2016 yılında, özellikle arılar ve kelebekler gibi omurgasız tozlayıcıların %40'ının yok olma riskiyle karşı karşıya olduğu konusunda uyarıda bulunmuştur [28]. Ayrıca, Amerika ve Avrupa'da bal arısı popülasyonunda pestisitlerin yoğun kullanımı nedeniyle yaklaşık %30 oranında azalma yaşandığı rapor edilmiştir [11].

Pestisitlerin arılar üzerindeki etkilerini öngörmek ve araştırmak, tarımın sürdürülebilirliği ve ekosistem hizmetlerinin devamlılığı açısından hayati öneme sahiptir. Arılar, tozlaşma aracılığıyla bitki üretkenliğini artıran temel tozlayıcılardır ve ekosistemde vazgeçilmez bir rol oynarlar. Pestisit kaynaklı popülasyon azalmaları, sadece arıların biyolojik çeşitliliğini tehdit etmekle kalmaz, aynı zamanda meyve, sebze ve yağlı tohum üretimi gibi doğrudan ekonomik çıktılara da zarar verir. Bu nedenle, pestisitlerin arılar üzerindeki akut ve kronik toksik etkilerinin, davranışsal bozulmaların ve koloniler üzerindeki uzun vadeli sonuçlarının detaylı biçimde araştırılması gerekmektedir. Bu tür çalışmalar, çevre dostu pestisitlerin geliştirilmesi ve mevcut tarım uygulamalarının daha güvenli hâle getirilmesi için bilimsel bir zemin oluşturur. Ayrıca, yöneticilerin ve pestisit üreticilerinin daha etkili düzenlemeler oluşturabilmeleri açısından da bu araştırmalar yol gösterici niteliktedir.

US EPA'nın 2024 verilerine göre, pestisit kaydı için gerekli çalışmalardan bal arısı akut toksisite testlerinin maliyeti 9.500 dolar ile 26.300 dolar arasında değişmektedir. Polinatörler için yarı-saha çalışmalarının maliyeti ise 118.200 dolar ile 330.100 dolar aralığındadır. Bu maliyetler, bal arısı temas toksisitesi (9.500 dolar), yetişkin oral toksisite (26.300 dolar), koloni besleme çalışması (118.200 dolar) ve tünel çalışması (330.100 dolar) gibi testleri kapsamaktadır [29]. Yüksek test maliyetleri, hayvan deneylerindeki etik kaygılar ve zaman alıcı deneysel süreçler gibi kısıtlamalar, *in silico* test yöntemlerinin son yıllarda hızla gelişmesinin temel nedenlerini oluşturmaktadır. Ayrıca *in silico* hesaplamalı yöntemler, hayvan deneylerinde etik yaklaşımın temelini oluşturan 3R ilkesine ('değiştirme, azaltma, iyileştirme') uyum sağlamaktadır.

Bu çalışmada, Benalaxyl-M fungusitinin bal arısı (*Apis mellifera*) üzerindeki potansiyel akut toksik etkileri BeeToxAI platformu aracılığıyla *in silico* olarak değerlendirilmiştir. BeeToxAI'nin sınıflandırma algoritmaları, hem akut oral (OECD 213) hem de akut kontakt toksisite (OECD 214) testleri açısından maddenin toksik olmadığını öngörmüştür. Bu sonuçlar sırasıyla %86 ve %83 güven düzeyleriyle desteklenmiştir.

ABD Çevre Koruma Ajansı (U.S. EPA) tarafından geliştirilen bal arısı toksisite test kılavuzlarına göre, bir pestisit aktif bileşeni 11 µg/bee eşiğinin altında LD_{50} değerine sahipse "yüksek ya da orta derecede toksik" olarak sınıflandırılmakta ve ek toksisite testlerine ihtiyaç duyulmaktadır [30]. BeeToxAI platformu da bu eşik değerini temel alarak bileşikleri toksik (≤11 µg/bee) ve toksik olmayan (>11 µg/bee) olarak kategorize etmektedir. Bu bağlamda, Benalaxyl-M'nin hem oral hem de kontakt yoldan uygulanmasında eşik değerin üzerinde bir güvenli aralıkta yer alması, bu maddenin akut toksisite açısından düşük riskli olduğunu göstermektedir.

Ayrıca, Uygulanabilirlik Alanı (Applicability Domain) analizleri, modelin verdiği tahminlerin kimyasal uzay açısından güvenilir olduğunu ortaya koymuştur. Dice benzerlik skorunun eşik değerin üzerinde olması, analizlerin Benalaxyl-M'ye benzer yapısal özellikler taşıyan bileşiklere dayandığını ve bu nedenle tahminlerin geçerli olduğunu göstermektedir. Molekülün toksisiteye katkı yapan ya da nötral kalan bölgelerini gösteren Katkı Haritaları (Contribution Maps), maddenin toksikolojik profiline dair mekanistik öngörüler sunarak hem risk değerlendirmesini derinleştirmiş hem de ileri çalışmalar için potansiyel modifikasyon alanlarını ortaya koymuştur.

Benalaxyl-M, fenilamid grubuna ait sistemik bir fungisit olup, Benalaxyl rasematının saf R(–)-enantiomeridir (SMILES: CC1=C(C(=CC=C1)C)N([C@H](C)C(=O)OC)C(=O)CC2=CC=CC=C2) [15]. Enantiyomerik saflaştırma, bileşiğin biyolojik aktivite ve toksisite profilini belirgin şekilde değiştirmektedir [32].

Kâğıt teması metoduyla gerçekleştirilen çalışmalar, 48 saatlik maruziyette R(–)-benalaxyl'in LC_{50} değerini 4,99 µg/cm², rasemat için 5,08 µg/cm² ve S(+)-enantiomer için 6,66 µg/cm² olarak tespit etmiştir. Maruziyet süresi 72 saate uzatıldığında ise sırasıyla 1,23 µg/cm², 1,73 µg/cm² ve 2,45 µg/cm² değerleri bulunmuştur [31]. Bu veriler, saf R(–)-formun hem rasemat hem de S(+)-formdan anlamlı derecede daha yüksek akut toksisite sergilediğini göstermektedir [31], [32].

96 saatlik EC_{50} testlerinde, rasemik karışım için 2,893 mg/L, R(–)-enantiomer için 3,867 mg/L ve S(+)-enantiomer için 8,441 mg/L değerleri belirlenmiştir. Bu sıralama (rasemat < R(–) < S(+)), R(–)-benalaxyl'in su alglerinde de rasemattan daha yüksek toksisiteye sahip olduğunu ortaya koymaktadır [32].

Hem toprak solucanı hem de su algi modellerinde saf R(–)-benalaxyl'in, rasemik ve S(+)-formlara kıyasla artmış toksisite göstermesi, ekotoksikolojik risk değerlendirmelerinde enantiomerik dağılımın göz önünde bulundurulmasının gerekliliğini vurgular [31], [32]. Stereoseçim mekanizmaları, maruziyet senaryoları ve metabolit birikimi bakımından yapılacak ayrıntılı çalışmalar, gerçek dünya koşullarında risk analizi doğruluğunu artıracaktır.

Sonuç olarak, Benalaxyl-M maddesinin bal arıları üzerindeki akut toksik etkilerinin mevcut *in silico* modellemelerle toksik olmayan bir profil sergilediği görülmektedir. Ancak, maddenin enantiyomerik yapısı, çevresel koşullar, metabolit etkileri ve birikimli toksisite gibi parametreler göz önünde bulundurularak *in vivo* testlerle desteklenmesi, toksikolojik değerlendirmenin bütüncüllüğü açısından önem arz etmektedir. Ayrıca *in silico* yöntemlerin geliştirilmesi ve gelecekte daha yaygın kullanımı için veri bankası oluşturmak için önem arz etmektedir. Bu bulgular, pestisitlerin ekotoksikolojik risk değerlendirmelerinde yapay zekâ tabanlı araçların destekleyici rolünü göz önüne sermektedir.

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Çıkar Çatışması/Ortak Çıkar Beyanı

Yazarlar tarafından herhangi bir çıkar çatışması veya ortak çıkar beyan edilmemiştir.

Yazarların Katkısı

Yazarlar çalışmaya eşit katkıda bulunmuştur.

Etik Kurul Onayı

Bu çalışma etik kurul izni veya herhangi bir özel izin gerektirmez.

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