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Determination of Metaphor Perceptions of Students Affected by Natural Disaster for Earthquake in the Context of Inclusive Education: Hatay (Antakya)

Hakan Şevki Ayvacı[1], Emine Bilge[1], Elif Sude Bilge[2]

Fen Bilgisi Eğitimi/ Science Education | Araştırma Makalesi / Research Article

Makale Bilgileri	Öz
	Bu çalışmanın amacı; okul öncesi, ilkokul ve ortaokul öğrencilerinin deprem olgusu ile ilgili metafor algılarını karşılaştırmaktır. Araştırmada nitel araştırma desenlerinden biri olan fenomenoloji araştırma yöntemi kullanılmıştır. Veri toplama sürecinde görüşme tekniği kullanılırken ayrıca modelleme ve çizim tekniği ile sürece katkı sağlanmıştır. Araştırmanın katılımcılarını Hatay iline bağlı Antakya ilçesinde depremden etkilenen 3-5 yaş aralığındaki okul öncesi, ilkokul 3 ve 4 ve ortaokul 5, 6, 7 ve 8. sınıf öğrencileri olmak üzere toplamda dokuz öğrenci oluşturmuştur. Araştırmadan elde edilen bulgulara göre okul öncesi öğrencilerinin deprem olgusuna yönelik metaforlarının bilimsel açıklamalardan uzak olduğu saptanırken, ortaokul öğrencilerinin deprem olgusuna yönelik metafor algılarında bilimsel ifadelerin daha fazla yer aldığı tespit edilmiştir. Depremin etkisi altında kalan öğrencilerin eğitim-öğretim ortamlarından ayrı kalmaları onların bulundukları sınıf seviyesinden daha alt grupta olgunluk göstermelerine sebep oluşturmuş ve buna bağlı kalarak da fen bilimleri dersini sadece oyunlaştırma ile sınırlandırdıkları ayrıca bulgu sonuçlarına yansımıştır. Araştırma kapsamında elde edilen ilgili sonuçlara bağlı kalarak birtakım önerilerde bulunulmuştur.

Article Info	Abstract
	The purpose of this study is to compare the metaphorical perceptions of earthquakes by preschool, primary and secondary school students. The phenomenology research method, one of the qualitative research designs, was used in the study. While the interview technique was used in the data collection process, modeling and drawing techniques were also used to contribute to the process. The participants of the study consisted of nine students in total, including preschool and primary school (3th, 4th grade), secondary (5th, 6th, 7th, and 8th grade) students between the ages of 3-5 who were affected by the earthquake in Antakya district of Hatay province. According to the findings obtained, it was determined that the metaphors of preschool students towards the earthquake phenomenon were far from scientific explanations. On the other hand, it was determined that scientific expressions were more common in the metaphor perceptions of secondary school students towards the earthquake phenomenon. The fact that the students who were under the influence of the earthquake were separated from their educational environment has caused them to show maturity in a lower group than the class level they were in. Accordingly, it was also determined that they limited the science course only to gamification. Based on the relevant results obtained within the scope of the research, some suggestions were made.

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1. **INTRODUCTION**

The world press referred to the recent 7.7 and 7.6 magnitude earthquakes that struck Turkey's Kahramanmaras province as the most horrific natural disaster of the 20th century, following the pandemic that severely impacted living conditions in the majority of the world's nations. The earthquake had the greatest impact on 11 provinces (Kahramanmaras, Osmaniye, Sanliurfa, Kilis, Adana, Diyarbakır, Adiyaman, Hatay, Malatya, Gaziantep, and Elazığ) in the Mediterranean, Southeastern, and Eastern Anatolia areas. According to information obtained through AFAD (2023), 50,500 people (April data) lost their lives in this earthquake process. The fact that there was a significant earthquake increase in the provinces of Ordu, Giresun, Trabzon, Rize, and Gumushane in the Eastern Black Sea Region, except for the base area of the earthquake, has proved how large and destructive the fracture in the fault line is which is the most important aspect of the earthquake (Bilge, 2023). Antakya was among the most affected provinces and around 23 thousand citizens lost their lives within the provincial borders. In addition, almost all of the city has come to a standstill due to power outages due to destroyed buildings.

People most needed shelter and safety in the early stages of the earthquake. In this situation, attempts have been made to mitigate issues to some extent by employing workable solutions like containers and tents. Nevertheless, the ongoing aftershocks, the demolishment of structures, and the multiple near fatalities have masked the earthquake process, and the ongoing trigger has constantly set off negative emotions in individuals (Demir-Yildiz & Demir-Ozturk, 2022). This led to both material and spiritual harm and impeded the solution of vital activities. This disaster in the region has also stopped the education system, and face-to-face training has become almost impossible. The process of adapting to face-to-face training after the earthquake has become traumatic as it has damaged various aspects of the school, such as the demolition of the school building and the loss of teaching staff and students (Karabulut & Bekler, 2019).

A World Bank research from 2019 states that every year, disasters prevent almost 175 million children from receiving an education in the globe. In the contents of the actions to be implemented in the sphere of education following the disaster, the relevance of science has been underlined because education is a vital and necessary service for a developing, safe, and especially sustainable society. First of all, it has been determined that approximately 130

million primary and secondary school students are out of school in countries affected by the crises, accounting for about half of all students in the world. In addition, the observation results of the students whose school building was changed due to disasters showed that their academic achievement decreased significantly. In particular, students with special needs and students who passively participate in educational processes suffer more from education. In this case, a strong and ready infrastructure for distance learning is required. Since disasters have strong emotional, behavioral, and academic effects on children and young people, it has been revealed that students need to learn about the effects of disasters and ways of coping, and psychosocial studies are needed. In fact, according to the VIII Training Workshop Report, the inclusion of this guiding role in the curriculum is a better choice.

It is clear that the earthquake, which is one of the most destructive natural events of the last century in our country, affected the students of our country the most. Due to both natural disaster (earthquake) and human-caused (pandemic) disasters in the country, the number of students with special needs has increased and demographic characteristics have changed. Due to internal migration caused by the natural disaster, 202.817 students were transferred to 71 provinces, mostly in Istanbul, Konya, Ankara, Kayseri, Bursa, Mugla, Aydin, Izmir, Mersin, and Antalya, resulting in the transfer of students to other provinces. The training flow was first included in the regional emergency plan, and then it caused differences for all regions. Therefore, students with special needs affected by the disaster were found in different parts of the country. Thus, recent events within the borders of the country have made inclusive educational practices for students affected by natural disasters more imperative than necessary.

Inclusive education is an approach that guides the educational needs of students according to their specific needs (Such as students with mental and physical disability, exposed to violence, temporarily protected, affected by migration, terror and natural disaster, who know Turkish as a foreign language, who have individual differences according to the diagnosis of special ability). Inclusive education is crucial in early childhood, the first step in education. Because as the foundation of children's attitudes, behaviors, and judgments is laid during this period, it is believed that the development of an inclusive understanding in the minds of students will help students develop as non-discriminatory individuals. Therefore, educational environments should be designed to help all children realize their potential (Ira & See, 2018).

It seems that system content needs to be changed, evaluated, and inclusive strategies developed according to disaster conditions and student demands to ensure the transmission and sustainability of education system understanding to future generations. Primary and secondary students in the concrete process stage can benefit from learning in an environment where an inclusive attitude is adopted. This can help them take a meaningful look at the events and situations around them and, more importantly, resume their lives from where they left off.

Due to the recent earthquake in the country, it is important for preschool and primary students educated in container and tent city conditions to hold on to their academic achievements and social lives. While each disciplinary area is crucial for students, science concepts help students better understand the natural events occurring in their environment. The field of science describes everyday nature events such as rain, sunrise, and setting, the phases of the moon, lightning, as well as electrical charge-discharge. They are also open to events that can evolve from natural events such as earthquakes, landslides, tsunamis, floods, global warming, greenhouse effect, pandemics to disasters (Bilge, 2023). Considering the feature of the field of science to focus on events and situations discussed in this context, preschool, primary and secondary students' mental perceptions of the earthquake can be accurately shaped.

Earthquakes are among the most important natural disasters affecting human life (Karakus, 2013; Rij, 2016). It is possible to associate the main cause of this condition with various reasons, such as the fact that the moment of the earthquake can not be predicted, the earthquake can not be prevented and it causes a large number of lives and property losses. Earthquakes are natural phenomena that originate from the depths of the earth and produce short-term ground shaking (Baytiyeh & Naja, 2013; Izbirak, 1991) or can be described as short-term ground shaking occurring in the earth's crust (Sahin & Sipahioglu, 2002). It is very important how the concept of an earthquake is perceived in the minds of the students who experience the earthquake. Metaphors provide students with the opportunity to explain the environment in which they live, events, phenomena, and objects according to which mental processes they make sense of and how they perceive them. Also, it is preferred in the field of science as it allows students to benefit from various simulating tools while doing so (Cerit, 2008). Metaphors are one of the techniques generally used to help individuals improve their

mental information processor and get rid of existing stereotyped information. This is especially important in the intervention processes after a traumatic event (Kuehlwein, 2000; Otto, 2000). The metaphor tool represents a concrete entity. Its purpose is to reveal people's mental model of the concept and to decipher the abstract domain, allowing information to leak out. Recent research emphasizes that metaphorical gestures can have an enhancing effect on the comprehension and learning process (Khatin-Zadeh, 2022; Khatin-Zadeh et al., 2022). Therefore, it is important to give feedback on students' developmental processes that can reveal their mental models to produce original works (Bilge, 2017).

Recent events relate to the possibility of a repeat of natural disasters, both in our country and around the world. For normalization to take place quickly in the processes thereafter and continuity in education, students need to be disaster-conscious and have sufficient knowledge of the content of education-training processes in line with teachers' disaster awareness. When the literature is examined; 9th-grade students experiencing the Van earthquake (Aksoy, 2013), 12th-grade students experiencing the Duzce earthquake (Karakus, 2013), middle school students experiencing the Sivrice earthquake (Dogan, Nacaroglu & Ablak, 2021), pre-school and middle school students experiencing the Kahramanmaras earthquake (Alptekin & Sarikaya, 2023) produced metaphors for the concept of earthquakes. In addition, some studies reach the metaphor information of candidate teachers and preschool students about earthquakes (Degirmenci, 2019; Demir-Yildiz & Demir-Ozturk, 2023; Yilmaz & Arslan, 2023). As a result, there has been little research in the literature that treats students as student echelons rather than as demographics (students affected by natural disasters). This has shown a gap in research in identifying what an inclusive learning environment should be to meet students' needs, interests, and values. Whereas, education is a crucial tool for creating a sustainable world and a necessary prerequisite for recognizing transformation and change. Thus, rather than evaluating students as level, this research purpose is to conceptualize students as demographics. More importantly, children and families in particular are considered the most vulnerable group affected by disasters (Ronan vd., 2015). In addition to this vulnerability, the educated child and family are the most powerful elements in ensuring that the society is self-sufficient in disaster situations (Takeuchi, Mulyasari & Shaw, 2011). For this reason, disaster awareness trainings should be started from a young age. Research often emphasizes that students in this level of education are in the age range best suited to gain

basic disaster awareness (Ozguven, 2006). Children do not know the cause of disasters, how to behave during disasters, and this feeling of not being ready causes their fears to increase. The crises experienced during the disaster reflect seriously on the education life of children (Grotberg, 2001). However, with the update of the Science Education Program (2024), the removal of the gains in the old program (MEB, 2018)- which refers to the ways of protection in destructive natural events- is contrary to the inclusive perspective. Whereas most countries are Bangladesh (Ahsan, Sharma, & Deppeler, 2012), Australia (Sharma, 2012; Woodcock, Hemmings, & Kay, 2012), Hon Kong, Indonesia, Canada and Australia (Loreman, Sharma, & Forlin, 2013), India (Yadav, Das, Sharma, & Tiwari, 2015), Italy (Aiello, Pace, Dimitrov, & Sibilio, 2017), Macau (Monteiro, Kuok, Correia, Forlin, & Teixeira, 2019), Spain (Cardona Moltó, Tichá, & Abery, 2017) and Finland (Saloviita, 2020) discussed inclusive education in different subject contexts in literature studies and revealed the extent of the importance that should be given to inclusive education. Under the inclusion principle, the Twelfth Development Plan (2023) seeks to create a people-centered understanding centered around the tenets of "qualified people, strong family, healthy society" to enhance the quality of life and encourage equitable access to opportunities for lifelong learning and qualified education. So this research was conducted to guide policy practitioners and researchers. Therefore, this study's purpose is to investigate how preschool, primary and secondary students affected by natural disasters are shaped by their age and class of metaphor perceptions regarding the concept of earthquakes.

2. MATERIAL AND METHOD

This section provided details on the design of the research, working group, data collection tools, and collection and analysis of the data.

2.1. Research Design

This study's purpose is to compare preschool, primary and secondary students' perceptions of metaphors for the concept of earthquakes. One of the qualitative research methods, research based on the pattern of factual science, is to find similarities and differences in students' thoughts about the phenomenon of earthquakes. Factual science (phenomenology) examines phenomenon concepts that people are aware of but fail to grasp in-depth and in detail. Events, experiences, perceptions, situations, and concepts in the world

experienced can arise in various ways called phenomena (Buyukkozturk et al., 2011; Yildirim & Simsek, 2018). The phenomenology provides favorable conditions for investigating random, unfamiliar, or not fully grasped phenomena as it does not show that human beings can fully understand everything despite developing around them (Yildirim & Simsek, 2018). Since the purpose of the study is to ascertain the metaphors that kids at all preschool, primary and secondary school levels use to explain earthquakes, as well as the part that demographic factors play in this process, it is believed that the research is in line with the pertinent research pattern.

2.2. Research Groups

The participants of this study were preschool, primary and secondary students in the Antakya district of Hatay province affected by the Kahramanmaras base earthquake on February 6, 2023 (n = 9). While participants in the study were selected, the maximum sampling method, one of the purposeful sampling methods, was preferred. The maximum diversity sampling method is to research similar or different situations or individuals for the research (Buyukozturk et al., 2016). The scope of the research involved volunteer preschool, primary and secondary pupils who were diverse in class level and age ranges. The students' demographic statistics are provided in Table 1' below.

Table 1. Participants' Demographic Characteristics

Educational Level	Class/ Level	Age	Quantity Of Students	Education-Training Continuity
Age range: Preschool 3-5 years	3 years old		n=1	Going on
	4 years old		n=1	Going on
	5 years old		n=1	Going on
Primary Stage	3rd grade		n=1	Unified class
	4th grade		n=1	Unified class
Secondary Stage	5th grade		n=1	Unified class
	6th grade		n=1	Unified class
	7th grade		n=1	Unified class
	8th grade		n=1	Unified class

Upon examining Table 1, it was observed that the three students fell into the preschool pupil level age range of three, four, and five. The accompanying content demonstrates that preschoolers were in education-training continuity. Primary and secondary students made up one student from each grade level (3, 4, 5, 6, 7, and 8) included in the tier, while primary and

secondary students were being continued to learn in unified classrooms. Nine students in all, comprising preschool, primary and secondary students, are displayed in the table. Since there were no first- and second-year students in the area where the research was conducted, they could not be included in the study.

2.3. Data Collection Process

The data was collected in two phases. Phase one took place in the area where pupils were attending preschool. Preschoolers were educated in a classroom within specially designed tent-container living conditions. Through this design, the process of collecting data has become easier. Because students were in preschool (assuming illiterate) it would be appropriate to use verbal expression and model with playdough to determine their metaphor for the earthquake phenomenon. Because the way children describe adverse conditions that affect them may differ from the way adults express them. Therefore, children's emotional change is difficult to understand from the outside. They prefer to describe situations that upset them with games and drawings (Danisman & Okay, 2017). In this case, it was considered that the drawing and modeling work and verbal expressions of preschool pupils would be in line with their age range. In the process, the science and psychology education specialist accompanied the students.

Data were gathered in the second stage in artificial classrooms designed for primary and secondary students within the tentative setting. A semi-structured interview form was employed to gather the metaphors that students used to explain the idea of earthquakes. Interview is the process of expressing opinions regarding a phenomena or event (Yildirim & Simsek, 2018). To determine students' perceptions of the earthquake, "Earthquake ... is like. Because ..." a form with the phrase has been created. Additionally, students can use blank portions of the form in multiple metaphor sentences to express their thoughts on the concept of an earthquake, it said. Therefore, all the ideas of the students about the concept of earthquakes were reached and not limited to one metaphor. At this stage, the students were accompanied by an educational specialist in science and psychology. In the first and second stages, a voice recorder was used to prevent data loss. Participants in the study were selected voluntarily, and data collected under ethical guidelines were deleted after the transcript.

2.4. Data Analysis

Data from this research was transcripted using the method of content analysis. The purpose of content analysis is to reach out to ideas and links that can explain the data collected (Yildirim & Simsek, 2011). There is also the identification and categorization of similarities between codes. In this two-step study, the drawing or modeling of preschool students, as well as the words expressed about these techniques, were instrumental in the theme-setting process. In this form, students created numerous metaphor sentences related to the earthquake. Two expert researchers were actively involved in the data collection process and took specific notes for the phrases expressed. Because modeling and drawing techniques support verbal expression, analysis has become easier. The metaphor phrase that first came to mind on the semi-structured interview form in the primary pupils' data collection process and the reasons for it were considered to apply to all themes. Students were asked to write various metaphor sentences. A careful approach was taken to the techniques of expressing students so that the justifications for the sentences indicating the metaphor were consistent. However, the researchers didn't offer any guidance that could shape students' ideas. During the analysis of the research data, the following was done.

2.5. Phase of Naming and Tagging

Metaphors generated from raw data collected during the research process were sequenced in alphabetical order at this step. The dataset included those that made sense of the content of the metaphors obtained, were easy to understand, and were in line with the metaphor's rationale. At this stage, metaphors that has not supported by a logical statement has removed from the data set along with the eliminations. It has also been determined that some participants wrote only a metaphor but not a justification for this metaphor because of its expression (e.g., "An earthquake is like a disaster because..."). Only the data in which the metaphor was written but the justification was not, are therefore excluded from the study. Furthermore, because some metaphors cannot be placed into any conceptual category, are not illogical or comprehensible, these metaphors are also excluded from the data set (e.g., "Earthquakes are like earthquakes because…"). At the end of this process, a total of 90 metaphors were obtained (See Tables 2 and 3).

2.6.   Classification Stage (Screening and Filtering)

The similarity or common characteristics between metaphors from raw data were examined at this stage. Each metaphor was divided into analyzable groups, and common characteristics were identified. The codes were created using metaphors grouped according to their common characteristics. As a result, the forms in which 90 appropriate metaphors were written were subjected to a stage of reorganization and compilation (See Table 4).

2.7.   Reorganization and Compilation Phase

Sample phrases that best represent each metaphor were set at this stage. The concept of earthquakes has been transformed into a theme based on students' mental perceptions. Metaphor themes, on the other hand, were distributed in five different category titles. Throughout this process, the researchers worked independently, which gave them different perspectives on various issues (See Table 5).

2.8.   Validity and Reliability

Credibility, transferability, consistency, and verifiability strategies have been used for the reliability and validity of the research (Shenton, 2004). Expert opinions were taken into account in questions to be asked to students as part of research into credibility that depends on internal validity. To improve credibility, students' views have been passed directly to the findings department. Regarding transferability, which depends on the external validity of the research, details have been provided on the collection and analysis of the data. For the reliability and repeatability of the research (Lincoln & Guba, 2013), the data was first transcribed separately by the two researchers and then together by the two researchers. The credibility formula between coders is used to calculate the reliability of qualitative research (Reliability = Agreement / Agreement + Disagreement x 100). The reliability level of coders 80% and above is considered good (Miles & Huberman, 1994). In the research, metaphorical encodings of both coders were compared, and different views were obtained about the eight metaphors. Based on the calculation result, a value of 92% was found (Reliability = 90/90 + 8 = 0.92). The analysis result showed that coders reached the desired level of reliability.

3. FINDINGS

Under the title of the findings of the study, metaphors containing the thoughts of preschool, primary and secondary tier students about the earthquake were presented through the table and figure.

Table 2. Information On Metaphors Used By Preschoolers

Age Level	Metaphor	Frequency
3 years old	Walking Giant, Swinging, Death, Tent, Toy	5
4 years old	Swinging, Death, Tent, Ghost, Parent	5
5 years old	Swinging, Death, Tent, Lost, Ghost, Parent	6

When examined in Table 1; students in three age groups appear to produce 5 metaphors, including walking giant, swinging, death, tent, and toy. It is understood that the four-year-old students formed 5 metaphors, including swinging, death, tent, ghost, and parent. The metaphors produced by students in the five-year age group showed distribution in the form of rocking, death, tent, loss, ghost, and parent. Preschoolers produced a total of 16 metaphors. Preschoolers explained their thoughts on the concept of earthquakes as follows. Preschoolers expressed their views on the earthquake as follows:

The figure on the side shows a metaphor modeling of a third-year student related to an earthquake. For student modeling, "*People die when earthquake becomes a sway. The dough contains parts of the house inside the soil. Everything stops in the soil*" he said.

Figure 1. Visual Concerning Mystery Power

Three age-group students who believe the quake may have been made by several hidden forces have been involved in the theme of the walking giant metaphor. The student's statement is below:

Three years old: *An earthquake is like mud because the earthquake swallowed our house. The destruction of houses is like an earthquake. Concrete can then move and the earthquake can end.*

The figure on the side shows a metaphor modeling of a fourth-year student related to an earthquake. For student modeling, "*Smoke is streaming from the house, blood is streaming from the roof, homes are being destroyed and people can't stand.*" he said.

Figure 2. Visual Concerning Chaos

The four-year-old student who thought that the earthquake existed because of the awareness of individuals who remained between the conflicts was involved in the theme of parental metaphor. The student's explanation is below:

Four years old: *Big buildings like in a cartoon don't want small buildings and they fight and her mother gets angry. An earthquake is like the demolition of homes and people can fall among the stones.*

The figure on the side shows a metaphor modeling of a fifth-year student related to an earthquake. For student modeling "*The building leans over the girl and she's surprised because the girl's phone stays at home so she can't call anyone.*" he said.

Figure 3. Visual Concerning Desperation and Bewilderment

The five-year-old student who attributes the earthquake to the willingness of supernatural beings to change the location of homes is present in the ghost metaphor. The student's explanation is below:

Five years old: *The earthquake is like a ghost because the land walks. The houses collide with each other and fall. The earthquake is like swinging because you're scared.*

Table 3. Information On Metaphors Used By Primary and Secondary Tier Students

Class Level	Metaphor	Frequency
3rd grade	Homeless, Swinging, Death, Tent, Disaster, Sadness, Wreckage, Lost, Grave, Mobile Toilet, Winter, Teacher	12
4th grade	Bad ground, Swinging, Death, Tent, Disaster, Sadness, Wreckage, Grave, Mobile toilet, Friend, Winter, Teacher	12
5th grade	Climate, Energy, Swinging, Kizilay, AFAD, Sadness, Wreckage, Ambulance sound, Grave, Mobile toilet, Friend, Winter, School building	13
6th grade	Sheet, Energy, Swinging, Global warming, Kizilay, AFAD, Distance education, Internet, Solidarity, Chaos, Friend, School building	12
7th grade	Crack, Energy, Swinging, Global warming, Kizilay, AFAD, Distance Education, Internet, Solidarity, Desk, School building, Friend	12
8th grade	Fault fracture, Energy, Swinging, Awareness, Global warming, Kizilay, AFAD, Distance education, Internet, Solidarity, Desk, School building, Friend	13

As outlined in Table 3 it is seen how students in the primary and secondary tier are grouped into metaphors based on the topic of the earthquake. Twelve metaphors were utilized by 3rd-grade students in the primary group, including " *Homeless, Swinging, Death, Tent, Disaster, Sadness, Wreckage, Lost, Grave, Mobile Toilet, Winter, and Teacher*". 4th-grade students used twelve metaphors, such as " *Bad ground, Swinging, Death, Tent, Disaster, Sadness, Wreckage, Grave, Mobile toilet, Friend, Winter, and Teacher*". Thirteen metaphors were utilized by 5th-grade students in the secondary group, including "*Climate, Energy, Swinging, Kizilay, AFAD, Sadness, Wreckage, Ambulance sound, Grave, Mobile toilet, Friend, Winter, and School building*". Twelve metaphors were utilized by 6th-grade students, including "*Sheet, Energy, Swinging, Global warming, Kizilay, AFAD, Distance education, Internet, Solidarity, Chaos, Friend, School building*". Twelve metaphors were utilized by 7th-grade students, including "*Crack, Energy, Swinging, Global warming, Kizilay, AFAD, Distance Education, Internet, Solidarity, Desk, School building, Friend*". Thirteen metaphors were utilized by 8th-grade students, including "*Fault fracture, Energy, Swinging, Awareness, Global warming, Kizilay, AFAD, Distance education, Internet, Solidarity, Desk, School building, Friend*". Primary and secondary school students produced a total of 74 metaphors. Primary and secondary students explained their thoughts on the concept of earthquakes as follows. Primary and secondary students expressed their views on the earthquake as follows:

The third-year student, who relates the idea of the earthquake to the fatigue of the world due to the unconscious use of all the beings around him, has described the idea about the earthquake as follows under the name of the metaphor of disaster:

3rd-grade student: *The earthquake is like the end of the world because the trees are being cut down. And I think the concrete is getting bigger...*

Associating the formation of the earthquake with the soundness of the basic structure of the house, the fourth-year student, 's thoughts on the bad-ground metaphor are as follows:

4th-grade student: *The earthquake is like solidity because if the ground were solid, we would not be homeless…*

The climate metaphor-oriented expressions of the fifth-year student who associated the earthquake with global warming as it did not continue in the normal course of the seasons are as follows:

5th-grade student: *An earthquake is like vanishing. Global warming seems to be destroying everything.*

The sixth-year student, who described the earthquake with the reversible movement of the layers of the earth, expressed the concept of the earthquake under the name of the plate metaphor:

6th-grade student: The earthquake is like a swing because the sheets in the layers of the soil are moving in the opposite direction.

Arguing that the earthquake could be caused by the transformation of energy, the seventh-year student's thoughts on the crack metaphor are as follows:

7th-grade student: *Earthquake is energy. Because the potential energy is converted into kinetic energy and cracks in the earth's crust.*

Addressing the earthquake within the framework of disaster awareness and attributing it to the limited awareness of the subunits that make up the society, the eighth grader explained the perception of the earthquake as follows:

8th-grade student: *An earthquake is negligence. Since there is no social consciousness, cities are built on fractures in the fault line.*

Table 4. Frequency Distributions Of All Metaphors

Metaphor Codes	Frequency	Metaphor Codes	Frequency	Metaphor Codes	Frequency
Swinging	9	Solidarity	3	Toy	1
Death	5	Remote training	3	Homeless	1
Tent	5	Global warming	3	Awareness	1
Friend	5	Internet	3	Chaos	1
School building	4	Winter	3	Ambulance sound	1
AFAD	4	Disaster	2	Walking Giant	1
Kizilay	4	Parent	2	Sheet	1
Energy	4	Teacher	2	Bad ground	1
Grave	3	Desk	2	Climate	1
Wreckage	3	Ghost	2	Fault fracture	1
Mobile toilet	3	Lost	1	Crack	1
Sadness	3				

When Table 4 is examined, it is seen that frequencies of all metaphors are included. According to the table, metaphors are collected in 34 different code headings. The most frequently repeated codes include swinging, death, tent, and friend codes, while the least repeated codes include sheet, bad ground, climate, fault fracture, and crack codes.

Table 5. Category Distributions Involving Codes of All Metaphors

Level of education	Metaphor Categories				
	*Tragic	*Emotional	* Psychological	*Fictional	*Scientific
	Death (5), Tent (5), Grave (3), Wreckage (3), Mobile toilet (3), Winter (3), Disaster (2), Lost (1), Toy (1), Homeless (1)	Friend (5), Sadness (3), Parent (2), Teacher (2)	School building (4), Solidarity (3), Distance Education (3), Working Desk (2), Awareness (1), Chaos (1), Ambulance voice (1)	Ghost (2), Walking Giant (1)	Swinging (9), Energy (4), AFAD (4), Kizilay (4), Global warming (3), Internet (3) Sheet (1), Bad ground (1), Climate (1), Fault fracture (1), Crack (1)
3 years old	☺☺☺			☺	☺
4 years old	☺☺	☺		☺	☺
5 years old	☺☺☺	☺		☺	☺
3th grade	☺☺☺☺☺☺☺☺	☺☺			☺
4th grade	☺☺☺☺☺☺☺	☺☺☺			☺☺
5th grade	☺☺☺☺	☺☺	☺☺		☺☺☺☺☺
6th grade		☺	☺☺☺		☺☺☺☺☺☺☺
7th grade		☺	☺☺☺☺☺		☺☺☺☺☺☺☺
8th grade		☺	☺☺☺☺☺		☺☺☺☺☺☺☺☺
Total (N=90)	N=27	N=13	N=15	N=3	N=32

Table 5 reviews show that a total of 90 metaphors -tragic, emotional, psychological, fictional, and scientific- are collected in 5 category titles. Metaphors are in the scientific (n=32) category, while other categories are ranked in the form of tragic (n=27), psychological (n=15), emotional (n=13), and fictional (n=3) categories. According to the table, it is understood that preschool students are not frequently (n=3) in the scientific category and that elementary second-tier students are more (n=24).

Figure 4. Frequently Used Metaphor Distributions As illustrated in Figure 4, it is seen that among the most commonly used metaphors by frequency values are death (n=5), tent (n=5), friend (n=5), school building (n=4), swinging (n=9), energy (n=4), AFAD (n=4) and Kizilay (n=4).

4. CONCLUSION AND DISCUSSION

The purpose of this research is to compare preschool, primary and secondary students' perceptions of earthquake-related metaphors. A total of ninety metaphors were obtained, according to findings from the study. The metaphors obtained were found to fall into five categories - tragic, emotional, psychological, fictional, and scientific.

When the metaphor categories were examined, they were reflected in the research findings that preschool, primary and secondary students often take place in certain categories (See Table 5). The fact that its scientific category contained 32 metaphors, more than any other, was among the most remarkable results. These results show that students scientifically grasp the concept of earthquakes. However, although there is a group of students who study

the phenomenon of earthquakes scientifically, the fact that the students identified were only in the secondary school and the measures needed to be taken in the aftermath of the earthquake led to different views (such as the tent, AFAD, Kizilay). So the fact that students in this age group scientifically express the phenomenon of earthquakes may also be an indication that students have gained knowledge by heart. This is because the prevalence of social networks in the digital age can make a person who has experienced an earthquake aware of the aftermath of an earthquake. A study in the literature (Simsek, 2007) stated that scientific statements are learned from television and supported this aspect of the study. Preschoolers and primary students in the 3 to 5-year-olds who are not often included in the scientific category confirm this interpretation. Whereas students should be informed about the scientific reality of each concept, starting with preschool. But the unscientific fictional expressions of students in the 3-5 age range are far from earthquake reality (ghosts, like the walking giant). However, the use of similar phrases by primary students requires a reconsideration of the science teaching program, along with early childhood science and math course-related attainments included in the preschool teaching program. However, it can be said that the extraction of the gains in the content of the updated science curriculum (refers to ways of protection in destructive natural events) negatively affects the importance given to the natural disaster issue. As well as, the lack of inclusive expression in the content of the science curriculum also showed one of the missing aspects of the program and resulted in the students staying away from scientific expressions. The inclusion of inclusive expressions in the preschool curriculum will support the next generation scientifically.

Like the carriages of a train, the training process progresses on the condition that each locomotive pulls each other (Bilge & Ayvaci, 2018). As noted in the literature study, it is clear that missing one factor at one grade level can negatively affect another grade level. The use of terms like "fault fracture," "sheet," and "crust" in scientific metaphor content reveals students' deficiencies in foundational knowledge (See Table 5). On the other hand, metaphors such as the tent, AFAD, and Kizilay include measures that can be taken following an earthquake, needs, and institutions that can get help. This has been interpreted as students not knowing the precautionary steps that should have been taken before the earthquake but knowing the precautionary steps that should have been taken in the aftermath of the earthquake. However, some students' use of scientific facts such as distorted urbanization,

building damage detection, earthquake bag preparation, measures that can be taken at the time of an earthquake, and the proliferation of high-rise buildings have contributed positively to the results of the research. However, to put it again, students have little awareness. Similar statements exist in the literature. It is known how difficult it is for every person on the street to be made aware of disasters. However, education is an important element in order for the society to take an effective role in reducing disaster damages (Altay, 2008; Kirikkaya, Unver & Cakin, 2011). In all levels of education, especially in preschool, earthquake awareness studies, which are enriched with drama, art and game works, should be done regularly (Tuncer, Sozen, Sakar, 2021). Therefore, in order to raise public awareness, inclusive education activities should be continued from pre-school.

Numerous natural disasters occurring in the country, such as a pandemic and an earthquake, have changed student demographics. According to this research finding, the needs of preschool, primary and secondary students post-earthquake have been differentiated. When these findings were examined more extensively, it was notable that preschoolers often featured in the tragic metaphor category. The fact that preschool students spelled out more deaths suggested that it was linked to death (presence or absence) as a concrete output of the earthquake. While primary students characterized the emotional extent of the earthquake as to lack of friends, primary second-tier pupils showed that the psychological effects of the earthquake prevented them from adapting to their lives as time went on because they could not adapt to studying due to a lack of factors such as school building and work desk. This suggests that students have a different attachment to concepts during the transition period from concrete to abstract thinking phase, and this commitment is in line with age group characteristics. It is clear that during the last childhood period (ages six to twelve), also known as middle childhood, children were more affected than their peers. In addition to supporting this finding, literature studies have emphasized that basic emotions such as fear, surprise and insecurity come to the fore in the preschool period (Erden & Gurdil, 2009; Erden, Erman & Oztan, 2011). Many of the studies examining the psychological effects of natural disasters on children have focused on symptoms of anxiety and depression (Cheng Liang, Fu & Liu 2018; Felix et al. 2011, Hansel, Osofsky & Osofsky, 2015). In order to see disasters as comprehensible situations at the level of society and individuals and to teach their vital requirements effectively to all individuals, inclusive education activities should be carried

out on the basis of society and individual (Tuncer, Sozen & Sakar, 2021). For this, it is time to develop fairer education and economic policies and more inclusive education programs (Karacaoglu et al. 2024).

Another remarkable finding, has been the resulting statements on remote education. As the finding suggests, there have been power outages in almost the entire earthquake zone, preventing remote education on internet networks from being an alternative option. However, in most of the literature research conducted during pandemic periods (Almaghaslah & Almayari, 2020; Alpaslan, 2020; Andoh et al, 2020; Chan et al., 2007; De Paepe et al., 2018; Fidan, 2020; Horspol & Lange, 2012; Kaden, 2020; Kocayigit & Usun, 2020; Coach, 2020; Yolcu, 2020) contrary to this finding, there are views that training can continue online. However, according to research results, this earthquake suggests that remote training cannot be used in every case of a natural disaster. Furthermore, beyond the inequality mentioned in the literature and issues such as the countryside (Ramos-Morcillo vd., 2020; Sercemeli & Kurnaz, 2020; Keskin & Ozer-Kaya, 2020), it has not been possible to find a building and instantly a tent city to provide electricity due to the complete demolition of the area due to the natural disaster. In one of the literature studies (Telli-Yamamoto & Altun, 2023), it was stated that remote education is vital for students in Turkey after the Kahramanmaraş base earthquake. This perspective has destroyed the existence of individuals with special requirements. Every child who goes on to education in the country does not need academic achievement alone, but children who are among the 1 310 605 special needs children and need to learn simple behaviors just to maintain their daily living activities, need lessons for practice and a teacher by adhering to it. Overall, individuals with autism, a vision disability, or a mental disability cannot be expected to complete remote education. Therefore, when it comes to the features that make remote education an alternative during natural disasters, the importance of developing lasting solutions for special-needs students should be remembered. While it is known in the literature that socioeconomically disadvantaged children do not have access to computers, tablets, internet or even television, it is also stated that they are excluded from the distance education process because they do not have a TV or tablets to access the content offered on EBA TV (TEDMEM, 2022). For this, it is essential that education environments and processes are inclusive for all children at every step taken (Tuzun, 2023). On the other hand,

it is essential to adopt the necessary education policies for education to be inclusive, qualified, supportive and democratic (Erbil, 2023).

The continued education training of students in combined classrooms in the container-tent environment has led to some negative consequences depending on the conditions of the district. Due to the continued importance of academic achievement in earthquake conditions, more importance was given to LGS students, while students at other levels were deprived of many courses such as science. The findings of the study concluded that students who stayed away from the school building limited the course of science to gamification due to their maturity below the level of the tier. There is a problem with the education system in Turkey as students preparing for LGS carry more exam anxiety than the destruction caused by the earthquake. A literature study by Karakus (2013) put forward the idea that being successful could help students build a tight bond in their lives. The interpretation of the literature study is similar.

This study showed that because student demographics are a rapidly changing country, there is a need for inclusive educational environments that adopt differentiated teaching based on the level of interest, need, and age of special needs students. That's why there needs to be inclusive printed material with a guidance qualification for teachers in unusual situations.

5. RECOMMENDATIONS

1. Since the metaphors obtained within the scope of the research move away from scientific expression, educators should prefer sentences that do not move away from academic language when explaining the events and phenomena that develop in daily life to their preschool students.

2. Since it is understood that the deficiencies of the primary school students related to the natural disaster are caused by the gains in the science program, it can be said that the science teaching program should be handled with an understanding of inclusiveness just like the preschool program.

3. Providing awareness services for all educators involved in both the in-service and pre-service period for students whose demographics are rapidly changing due to natural disasters will help ensure that the understanding of disaster awareness contained in targeted development policy content is sustainable.

4. Adverse conditions in the aftermath of the earthquake have significantly affected the alternative contribution of the transition to remote education. This has eliminated equality of opportunity in education for special-requirement students affected by natural disasters. Therefore, teaching modules that support inclusive education need to be presented with printed materials.

5. In the content of both preschool, primary and secondary all-tier pupil attainments, there is a need to develop activities where they can practice concerning the steps that need to be taken in the event of a disaster. It is proposed to develop guidance studies for improving and improving the psychology of students during and after an earthquake provided that their socio-psychological status is identified in advance for disaster-conscious educators to conduct these activities.

6. In the content of the educational program, which includes the subject of natural disasters, some recommendations may be offered for researchers who will provide literature support for interdisciplinary areas such as science, life knowledge, and social studies. Various studies will contribute to the literature to support the modification of teaching programs in line with the level of age, need, and interest of demographics addressed within the research.

Conflict of Interest

There is no conflict of interest with any institution or person in the study.

Author(s) Contribution

All authors were involved in the concept, design, collection of data, interpretation, writing, and critical revising of the article. Therefore, the contribution rate of the authors is first author 35%, second author 35% third author 30%.

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IYTE VIII. Eğitim Çalıştay Raporu (2023). Afet durumlarında sürdürebilir eğitim.

Laurus nobilis L. Ekstraktlarının Bazı Patojen Bakteriler Üzerindeki Antibakteriyel Etkileri ve Bileşenlerinin MreC Proteini ile Etkileşimlerinin Moleküler Kenetlenme Yöntemi ile Değerlendirilmesi

Investigation of Antibacterial Effects of *Laurus nobilis* Extracts on Some Pathogenic Bacteria and the Interactions of Components with MreC Protein by Molecular Docking Method

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Biyoloji / Biology

Araştırma Makalesi / Research Article

Makale Bilgileri	Öz
Geliş Tarihi 01.09.2024 **Kabul Tarihi** 26.12.2024 **Anahtar Kelimeler** Patojen Antibakteriyel MreC *Escherichia coli* *Laurus nobilis*	Patojen mikroorganizmaların çoklu antibiyotik direnci geliştirmesi tedavi sürelerinin uzaması, ölüm oranlarının artması ve ekonomik kayıplara neden olmaktadır. Bu çalışmada, geleneksel antibiyotiklere karşı gelişen direnç nedeniyle alternatif antibakteriyel ajanlar geliştirilmesi ihtiyacı ele alınmıştır. *Laurus nobilis* yapraklarından elde edilen ekstraktların *Escherichia coli* ve *Proteus mirabilis* patojenlerine karşı antibakteriyel etkileri araştırılmıştır. Araştırmada, *L. nobilis* yaprak ekstraktlarının, özellikle yüksek dozlarda (50 µL), her iki patojen üzerinde de inhibisyon sağladığı belirlenmiştir. Ayrıca, moleküler kenetlenme yöntemi ile defne yaprağının uçucu yağ bileşenlerinin bakteri hücre duvarı oluşumunu kontrol eden proteinlerle etkileşim potansiyeli incelenmiştir. Sonuçlar, *L. nobilis* ekstraktlarının, özellikle çoklu antibiyotik direnci geliştiren patojenlere karşı, etkili bir alternatif olabileceğini göstermektedir.

Article Info	Abstract
Received 01.09.2024 **Accepted** 26.12.2024 **Keywords** Pathogen Antibacterial MreC *Escherichia coli* *Laurus nobilis*	The development of multiple antibiotic resistance by pathogenic microorganisms causes prolongation of treatment periods, increased mortality rates, and economic losses. In this study, the need to develop alternative antibacterial agents due to the development of resistance to conventional antibiotics was addressed. The antibacterial effects of extracts obtained from Laurus nobilis leaves against *Escherichia coli* and *Proteus mirabilis* pathogens were investigated. In the study, it was determined that *L. nobilis* leaf extracts, especially at high doses (50 µL), provided inhibition on both pathogens. In addition, the interaction potential of the volatile oil components of bay leaf with proteins that control bacterial cell wall formation was investigated by molecular docking method. The results show that *L. nobilis* extracts may be an effective alternative, especially against pathogens that develop multiple antibiotic resistance.

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1. GİRİŞ

Son yıllarda klinik patojen bakterilerin geleneksel antibiyotiklere karşı geliştirdikleri direnç, halk sağlığı açısından önemli sorunlardan biri haline gelmiştir (Iwu ve ark., 2020; Pal ve ark., 2020; Sugeçti 2021 a,b). Patojen mikroorganizmaların çoklu antibiyotik direnci geliştirmesi tedavi sürelerinin uzaması, ölüm oranlarının artması ve ekonomik kayıplara neden olmaktadır. Antibiyotik direncinin en önemli nedenleri yoğun ve bilinçsiz kullanımdan kaynaklanmaktadır. Enfeksiyona neden olan patojenin tedavisinde kullanılan antibiyotiklerin konsantrasyonları dikkatle belirlenmeli ve tedavi için etkili minimum dozlar kullanılmalıdır. Son yıllarda çoklu antibiyotik direncine karşı geleneksel antibiyotikler tedavilerde yetersiz kalmıştır (Sertçelik ve ark., 2018; Church and McKillip 2021). Bu nedenle geleneksel antibiyotiklere alternatif yeni kimyasalların ve bitkisel özütlerin geliştirilmesi oldukça önemlidir. Düşük toksisiteye sahip bazı metal bileşiklerin patojen bakteriler üzerinde antibakteriyel etki yaptıkları belirlenmiştir (Sertçelik ve ark., 2018; Hashem ve ark., 2022). Ayrıca bazı bitkilerden elde edilen uçucu yağların ve ekstraktların insan patojenlerinin neden olduğu enfeksiyonların tedavisinde kullanılabileceği bildirilmiştir (Sugeçti and Koçer 2015; Nabila ve ark., 2022).

Laurus nobilis antibakteriyel (Aumeeruddy-Elalfi ve ark., 2015), antifungal (Houicher ve ark., 2016), anti-inflamatuar (Mazzio ve ark., 2016) gibi etkilere sahip ekonomik olarak önemi bir bitkidir. Bitkinin yapraklarından elde edilen uçucu yağlar kozmetik, tıp, eczacılık ve veterinerlik gibi birçok alanda kullanılmaktadır (Yazıcı ve ark., 2024). Bitkinin yaprağından elde edilen ana bileşenler 1,8-sineol, sabinen ve linalooldür (Ekren ve ark., 2013; Snuossi ve ark., 2016). Defne yaprağının bileşenlerinin antibakteriyel özelikleri olduğu daha önceki çalışmalarda bildirilmiştir (Goudjil ve ark., 2015; Caputo ve ark., 2017).

Bakterilerin hayatta kalması ve morfogenezi, hücre duvarının temel bir bileşeni olan peptidoglikana (PG) önemli derecede bağlıdır. PG, çoğu bakteri hücresini çevreleyen, ozmotik lizise karşı koruma sağlayan temel bir heteropolimerdir. PG biyosentezinde yer alan proteinler, hücre bölünmesini ve hücre duvarı uzamasını (elongasom veya Rod sistemi) düzenler. Bu yapıların inhibisyonu veya düzensizliği hücre şekli kusurlarına, bozulmuş büyümeye ve en sonunda bakterinin ölümüne yol açabilir (Typas ve ark., 2012). Elongasom veya Rod sistemi, çubuk şeklindeki bakterilerde hücre duvarı oluşumunu kontrol eden bir protein kompleksidir. MreC, hücre duvarı biyosentez enzimlerinin aktivitesini düzenler (Van Den Ent ve ark., 2006; Lovering and Strynadka 2007; Contreras-Martel ve ark., 2017).

Bu çalışmada, *L. nobilis* yapraklarından elde edilen ekstraktların patojen mikroorganizmalar *E. coli* ve *P. mirabilis* üzerindeki antibakteriyel etkileri araştırıldı. Ayrıca defne yaprağının uçucu yağ bileşenlerinin hücre duvar oluşumunu kontrol eden proteinler ile etkileşimleri moleküler kenetlenme yöntemi kullanılarak incelendi.

2. MATERYAL VE YÖNTEM

2.1. *L. nobilis* ekstraktlarının hazırlanması

L. nobilis yaprakları Zonguldak bölgesinden toplandı ve laboratuvarda kuramaya bırakıldı. Kurutulmuş ve toz haline getirilmiş bitki yaprakları tartıldı (10 g) ve üzerine 100 mL etil alkol eklendi. Erlen 24 saat 50 °C'de bekletildi. Süre sonunda karışım filtre kağıdından süzülerek süzüntü ayrıldı. Fitre kâğıdı üzerinde kalan katı partiküller tekrar erlene alınıp üzerine 100 mL etil alkol eklendi. Bu şekilde bu işlem 3 kez tekrar edildi. Elde edilen süzüntüler birleştirilerek döner buharlaştırıcıda etil alkol uzaklaştırıldı ve deney için gerekli ekstraktlar elde edildi.

2.2. Mikroorganizmaların Hazırlanması ve Uygulanması

Mikroorganizma suşları, Mueller Hinton Broth besiyerlerine ekildi. Bakterilerin üremesi için 37 ± 2 °C'ye ayarlı bir inkübatörde 24 saat bekletildi. Elde edilen bakteri suşlarının tanımlanmasında yarı otomatize antibiyogram cihazı kullanıldı. Antibiyogram testleri için Mueller Hinton Agar kullanıldı. Patojen mikroorganizmalar 0.5 McFarland standart bulanıklığına eş olarak hazırlandı ve ekimi yapıldı. Besiyerler 37 ± 2 °C'ye ayarlı bir inkübatörde 24 saat bekletildi. Defne ağacının yapraklarından elde edilen ekstraktlar antibakteriyel aktivitesi disk difüzyon yöntemi ile belirlendi. Defne ağacının yapraklarından elde edilen ekstraktlar boş steril antibiyotik disklerine 25 μL ve 50 μL emdirildi. Daha sonra ekstrakt uygulanmış diskler belirli aralıklarla besiyerlerine hafifçe üzerine bastırılarak yerleştirildi. Petri kapları 24 saat 37 ± 2 °C'ye ayarlı bir inkübatörde bekletildi ve oluşan inhibisyon zonları ölçüldü.

2.3. Moleküler Kenetlenme Analizi

Bu çalışmada, bitkisel bileşenlerin MreC ile bağlanma afinitesini hesaplamak için Autodock Vina programı (Trott & Olson, 2009) kullanıldı. MreC yapısal proteinin (PDB kodu:

6ZLV (Martins ve ark., 2021) kimyasal yapısı RCSB Protein Veri Bankasından alındı (https ://www.rcsb.org). Polar hidrojenler ve Kollman yükleri eklenmeden önce protein yapılarından su molekülleri uzaklaştırıldı. Her ligand molekülünün kökü otomatik olarak algılandı ve burulmaların seçilip dönmesine izin verildi. Görselleştirme işlemleri BIOVA Discovery Studio Visualizer 2021 (BIOVIA, 2021) kullanılarak gerçekleştirilmiştir.

3. SONUÇLAR

3.1. *L. nobilis* ekstraktlarının antibakteriyel etkileri

L. nobilis yapraklarından edilen ekstraktların *E. coli* ve *P. mirabilis* patojenleri üzerindeki antibakteriyel etkileri tablo 1'de gösterilmektedir. Yapılan çalışmada 25 µL uygulanan ekstrakt *E. coli* üzerindeki antibakteriyel etki gösterirdi. *P. mirabilis* üzerinde ise antimikrobiyal etki göstermediği belirlendi. Yapılan çalışmada 50 µL uygulanan *L. nobilis* ekstraktlarında *E. coli* ve *P. mirabilis* üzerinde antibakteriyel etkileri olduğu belirlendi. Sırasıyla 11 mm ve 7 mm inhibisyon zonları ölçüldü (Tablo 1).

Tablo 1. *L. nobilis* ekstraktlarının antibakteriyel etkileri

Bakteri	İnhibisyon Zonları (mm)	
	L. nobilis ekstraktları	
	25 µL	50 µL
E. coli	6 mm	11 mm
P. mirabilis	-	7 mm

3.2. Moleküler kenetlenme sonuçları

Bu çalışmada, *L. nobilis* yaprağından elde edilen bileşiklerin, patojen bakterilerin hücre duvarı oluşumunu kontrol eden MreC proteini ile olan etkileşimleri moleküler kenetlenme yöntemi ile incelenmiştir. Sabinen ve 1,8-sinanol bileşikleri üzerine yapılan analizler, bu bileşiklerin MreC proteini ile çeşitli etkileşimlere girdiğini ortaya koymuştur. Sabinen bileşiği, -7.6 kcal/mol'lik bağlanma enerjisi ile MreC proteini üzerinde güçlü bir bağlanma göstermiştir. Bu etkileşimler arasında PHE183 amino asidi ile π-sigma etkileşimi, VAL102, ILE197, PRO193 ve VAL252 amino asitleri ile alkil etkileşimler, ayrıca PHE225, PHE226 ve TYR44 amino asitleri ile π-alkil etkileşimler yer almıştır. Bu etkileşimler, Sabinen'in MreC proteini ile güçlü ve kararlı

bir bağlanma gerçekleştirdiğini göstermektedir (Tablo 2 ve Şekil 1). Diğer yandan, 1,8-sineol bileşiği, -6.0 kcal/mol'lik bir bağlanma enerjisi ile Sabinen'e kıyasla daha düşük bir bağlanma affinitesi sergilemiştir. Bu bileşik de PHE183 amino asidi ile π-sigma etkileşimi göstermiştir. Ayrıca, ILE197, PRO193, VAL252 ve VAL102 amino asitleri ile alkil, TYR44, PHE183, PHE226 ve TRP43 amino asitleri ile π-alkil etkileşimler gerçekleştirmiştir (Tablo 2 ve Şekil 2).

Tablo 2. *L. nobilis'in* bazı bileşenlerinin MreC ile etkileşiminin moleküler kenetlenme yöntemi ile incelenmesi

Protein	Bileşik	Bağlanma Enerjisi (kcal/mol)	Etkileşim Türü	Amino asit
MreC	Sabinen	-7.6	π- Sigma	PHE183
			Alkil	VAL102; ILE197; PRO193; VAL252
			π-Alkil	PHE225; PHE226; TYR44
	1,8-sineol	-6.0	π- Sigma	PHE183
			Alkil	ILE197; PRO193; VAL252; VAL102
			π-Alkil	TYR44; PHE183; PHE226; TRP43

4. TARTIŞMA

Patojen bakterilerin geliştirdikleri antibiyotik direnci önemli bir insan sağlığı sorunudur. Geleneksel antibiyotiklere direncin artması enfeksiyonların tedavi süresinin uzamasına neden olmaktadır. Bazı dirençli patojenlerin tedavileri ise oldukça zordur. Bu durum ciddi bulaşıcı hastalıkların yayılmasını ve ölüm riskini arttırmaktadır. Bu çalışmada *E. coli* ve *P. mirabilis* patojen bakterilerinin karşı defne ağacının yapraklarından elde edilen ekstraktların

antibakteriyel etkileri araştırılmıştır. Ayrıca moleküler kenetlenme yöntemi kullanılarak *L. nobilis* yaprağındaki bazı bileşiklerin patojen bakteriler üzerindeki etkileri araştırılmıştır.

(a) (b)
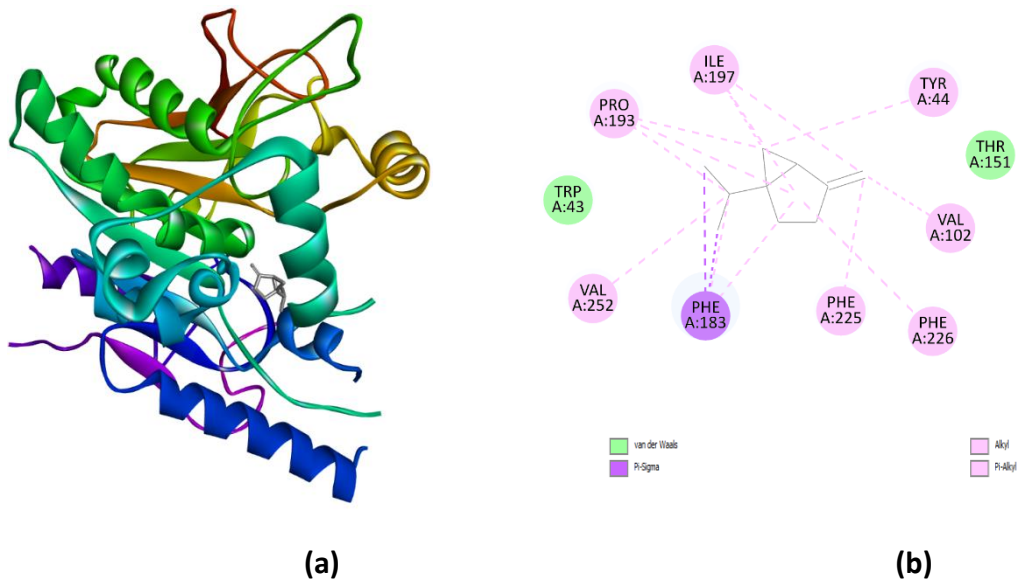
Şekil 1. Sabinen bileşiğinin MreC proteini ile moleküler kenetlenme modeli (a: Üç boyutlu yapısı, b: iki boyutlu yapısı)

Yapılan çalışmada, defne ağacının yapraklarından elde edilen ekstraktların klinik patojen bakteriler *E. coli ve P. mirabilis* suşları üzerinde antibakteriyel etkilere sahip olduğu belirlenmiştir. Ökmen ve ark. (2014) yaptığı çalışmada *Cyclamen mirabile* kök özütlerinin gıda patojeni bakteriler üzerinde antibakteriyel etkiler olduğu görülmüştür. Rezene ve ada çayının antibakteriyel etkisinin araştırıldığı bir çalışmada, klinik patojen bakteri *Staphylococcus aureus* üzerinde bitkilerden elde edilen uçucu yağların antibakteriyel etkileri olduğu bildirilmiştir (Haşimi ve ark., 2015). Yapılan başka bir çalışmada, *Psidium guajava, Salvia officinalis, Ziziphusspina christi, Morusalba L., Oleaeuropaea L. bitkilerinin antibakteriyel etkileri araştırılmıştır. Bu çalışmanın sonucunda bitki ekstralarının E. coli ve S. aureus* üzerinde ekili olduğu fakat *Psidium guajava* dışında hiçbir ekstraktın *Mycoplasma galliceptum* üzerinde antibakteriyel etkisinin olmadığı bildirilmiştir (Hemeq ve ark., 2020). Başka bir çalışmada, biyolojik olarak sentezlenen gümüş nanopartiküllerinin yüksek antibakteriyel etkiye sahip olduğu bildirilmiştir (Said ve ark., 2024). Yapılan başka bir çalışmada Cezayir defne esansiyel yağının *E. coli* ve *Pseudomonas aeruginosa* suşlarına karşı önemli antibakteriyel etki gösterdiği bildirilmiştir (Goudjil ve ark., 2015).

(a)   **(b)**
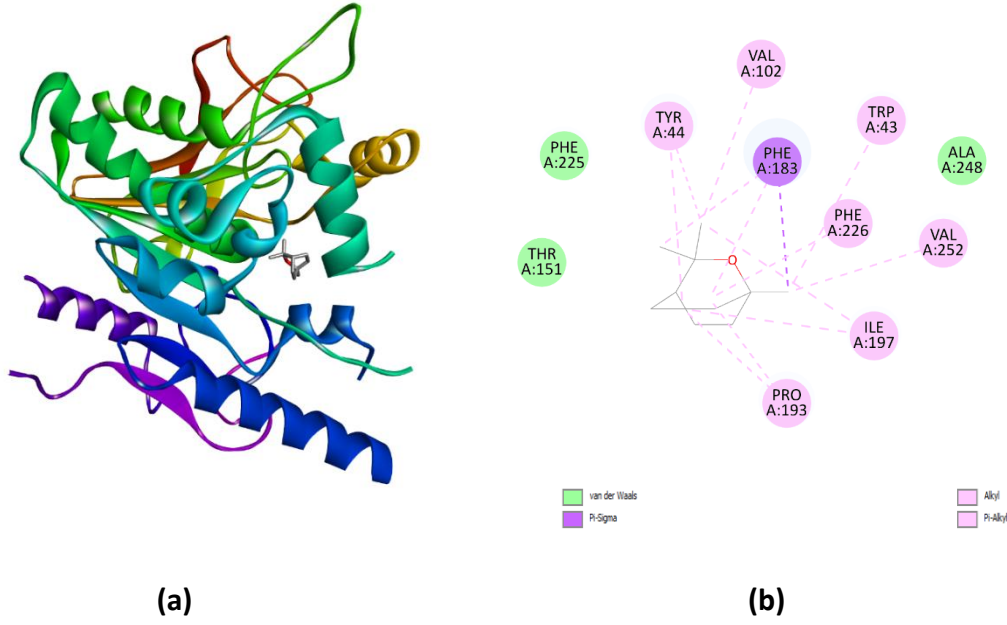
Şekil 2. 1,8-cineole bileşiğinin MreC proteini ile moleküler kenetlenme modeli (a: Üç boyutlu yapısı, b: iki boyutlu yapısı)

Bu çalışmada, *L. nobilis* yaprağından elde edilen sabinen ve 1,8-sineol bileşiklerinin, patojen bakterilerin hücre duvarı biyosentezini düzenleyen MreC proteini ile olan etkileşimleri moleküler kenetlenme yöntemi ile incelenmiştir. Moleküler kenetlenme sonuçları, her iki bileşiğin de MreC proteini ile belirgin etkileşimler gösterdiğini, ancak bağlanma enerjileri ve etkileşim türleri açısından farklılıklar sergilediğini ortaya koymuştur. Sabinen bileşiği, -7.6 kcal/mol gibi düşük bir bağlanma enerjisi ile MreC proteini üzerinde güçlü bir bağlanma affinitesi göstermiştir. Bu düşük bağlanma enerjisi, sabinenin proteine sıkı bir şekilde bağlandığını ve bu bağlanmanın kararlı olduğunu işaret etmektedir. Sabinen'in PHE183 amino asidi ile π-sigma etkileşimi ve bir dizi alkil ve π-alkil etkileşimler gerçekleştirdiği gözlenmiştir. Bu etkileşimler, sabinenin MreC proteinine bağlanma mekanizmasını desteklemekte ve bu bileşiğin hücre duvarı biyosentezini etkileyebileceğini düşündürmektedir. Hücre duvarı sentezinin bozulması, bakteriyel hücrelerin morfolojisini, bölünmesini ve hayatta kalmasını olumsuz etkileyebilir, bu da sabinenin antibakteriyel etkisini açıklayabilir. Yapılan başka bir çalışmada, patojen bakteri *Salmonella typhimurium* üzerinde okaliptol, sabinen ve sinamaldehit bileşiklerinin in silico etkileri araştırılmıştır. Elde edilen sonuçlarda, en düşük yerleştirme puanı sabinen için elde edilmiştir (Vimal ve ark., 2017).

Bu çalışmada, *L. nobilis* yapraklarından elde edilen ekstraktların antibakteriyel etkileri, klinik patojen bakteriler *E. coli* ve *P. mirabilis* üzerinde değerlendirilmiştir. Yapılan deneyler, yüksek konsantrasyonlardaki *L. nobilis* ekstraktlarının, her iki bakteriyel patojen üzerinde belirgin inhibisyon zonları oluşturduğunu göstermiştir. Moleküler kenetlenme analizleri ise, defne yaprağındaki bileşenlerin bakterilerin hücre duvarı oluşumunu kontrol eden proteinlerle potansiyel olarak etkileşimde bulunabileceğini ortaya koymuştur. Bu çalışmadan elde edilen sonuçlar, *L. nobilis* yaprak ekstraktlarının, antibiyotik direncine sahip bakterilere karşı alternatif bir tedavi seçeneği olabileceğini düşündürmektedir. Ancak, bu bulguların klinik uygulamalarda doğrulanabilmesi için daha fazla araştırmaya ihtiyaç vardır. Sonuç olarak, defne yaprağı ve bileşenlerinin antibakteriyel potansiyeli, antibiyotik direncinin neden olduğu ciddi halk sağlığı sorunlarına karşı umut verici bir çözüm sunmaktadır.

Çıkar Çatışması

Makale yazarları aralarında herhangi bir çıkar çatışması olmadığını beyan ederler.

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UV Mutagenez ve Akış Sitometrisi Taraması ile *Schizochytrium* sp. S31'de Dokosahekzaenoik Asit Üretiminin Artırılması

Enhancement of Docosahexaenoic Acid Production by UV Mutagenesis Coupled with Flow Cytometry Screening in *Schizochytrium* sp. S31

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Biyoloji / Biology   Araştırma Makalesi / Research Article

Öz

Mikroalgler, omega-3 yağ asitleri de dahil olmak üzere zengin biyoaktif bileşikleri nedeniyle terapötik ve farmakolojik uygulamalardaki potansiyelleri nedeniyle büyük ilgi görmüştür. Bunlar arasında, *Schizochytrium* sp. bu değerli lipitleri yüksek seviyelerde üretme kabiliyeti nedeniyle kapsamlı bir şekilde incelenmiştir. Bu çalışmanın amacı, genomda UV kaynaklı rastgele mutasyonlar oluşturarak *Schizochytrium* sp. S31 mutant kütüphanesi oluşturmak ve ardından akış sitometrisi tabanlı teknoloji kullanarak yüksek lipid birikimine sahip mutantları taramaktır. *Schizochytrium* sp. S31'in yüksek verimli lipid biriktiren mutantlarını izole etmek için rastgele mutagenez ve akış sitometrisi tabanlı seçimin bir kombinasyonu kullanılmıştır. Sonuçlar, Mutant 1'in toplam lipid içeriğinde %28,4'lük bir artış sergilediğini, Mutant 2'nin ise yabani tipe göre %10,8'lik bir artış gösterdiğini ortaya koymuştur. Sonuçlar, UV ışığı (30 saniye boyunca) ile muamele edilen kültürlerin, muamele edilmeyen kontrollerden daha yüksek DHA seviyeleri sergilediğini gösteren gaz kromatografisi-kütle spektrometresi ile desteklenmiştir. DHA yüzdesi, yabani tipe göre iki farklı mutantta %17,9 ve %12,1 oranında artmıştır.

Abstract

Microalgae have garnered significant attention for their potential in therapeutic and pharmacological applications due to their rich bioactive compounds, including omega-3 fatty acids. Among these, *Schizochytrium* sp. has been extensively studied for its ability to produce high levels of these valuable lipids. The aim of this study was to create a *Schizochytrium* sp. S31 mutant library by generating UV-induced random mutations in the genome and then screening for mutants with high lipid accumulation using flow cytometry-based technology. A combination of random mutagenesis and flow cytometry-based selection was employed to isolate high-yield lipid-accumulating mutants of *Schizochytrium* sp. S31. The results revealed that Mutant 1 exhibited a 28.4% increase in total lipid content, while Mutant 2 demonstrated a 10.8% increase relative to the wild type. The results were corroborated by gas chromatography-mass spectrometry, which indicated that the cultures treated with UV light (for 30 seconds) exhibited higher levels of DHA than the untreated controls. The percentage of DHA increased by 17.9% and 12.1% in two distinct mutants relative to the wild type.

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1. INTRODUCTION

Microalgae represent a highly diverse unicellular group found in various ecosystems across the Earth, primarily in oceans and freshwater bodies. It is estimated that there are over 50,000 species of microalgae, yet only a limited number have undergone analysis. One such species is *Schizochytrium sp. S31*, a marine thraustochytrid known for its lipid-rich content, particularly in polyunsaturated fatty acids (PUFAs) such as docosahexaenoic acid (DHA) (Yokochi et al., 1998). This microalga contains a range of bioactive compounds with high-value applications, including their use as pharmaceutical raw materials, food additives, and supplements (Koyande et al., 2019; Sathasivam et al., 2017; Kay & Barton, 1991; Pulz & Gross, 2004; Garcia et al., 2017). Moreover, microalgae serve a vital role in biofuel production, offering an alternative energy source, and supporting aquaculture practices (Spolaore et al., 2006; Chisti, 2007; Brennan & Owende, 2010). Extensive research has been conducted to characterize the lipid content of various algal species. Notably, microalgal lipid content exhibits substantial variation, ranging from 5% to 70% of dry weight biomass across different species (Mata et al., 2010; Brown et al., 1997; Han et al., 2011). The major fatty acids in most microalgae cells are palmitic acid (C16:0), oleic acid (C18:1), and linoleic acid (C18:2) or α-linolenic acid (C18:3) (Zhukova & Aizdaicher, 1995; Servel et al., 1994; Viso & Marty, 1993). Certain microalgal species possess the capability to synthesize long-chain polyunsaturated fatty acids (LC-PUFAs), including omega-3 and omega-6 fatty acids (Adarme-Vega et al., 2014; Khozin-Goldberg et al., 2011; Ryckebosch et al., 2012; Lenihan-Geels et al., 2013; Gupta et al., 2012; Raghukumar, 2008). These species hold significance due to their nutritional advantages (Ryckebosch et al., 2012; Unkefer et al., 2017; Doughman et al., 2007; Deckelbaum & Torrejon, 2012; Adarme-Vega et al., 2012). Research has demonstrated variations in lipid composition among microalgal species, with microalgae generally exhibiting a higher content of neutral lipids compared to other species (Lv et al., 2010). The composition and fatty acid profile of lipids, both saturated and polyunsaturated, have been influenced by factors including the life cycle of microalgae and various physicochemical conditions. These conditions encompass parameters such as temperature, pH, aeration rate, medium composition, stress conditions, and illumination intensity (Guzman et al., 2010; Rao et al., 2007; Wu et al., 2013). Numerous studies have reported the capacity of UV mutagenesis to enhance lipid content in microalgae (Bougaran et al., 2012; Liu et al., 2015; Manandhar-Shrestha & Hildebrand, 2013; Lim et al., 2015; Lian et al., 2010). In one particular study, UV mutagenesis was applied to augment

neutral lipid productivity in *Microalgae Isochrysis Affinis Galbana* (T-Iso) through a mutation-selection procedure (Bougaran et al., 2012). Another notable study achieved a 33% increase in both EPA and DHA levels in *Pavlova lutheri* through the implementation of UV mutagenesis (Meireles et al., 2003). Additionally, the EPA content of *Phaeodactylum tricornutum* experienced a 37% increase through the same technique (Alonso et al., 1996). The combination of UV mutagenesis with fluorescence-activated cell sorting (FACS) has emerged as a widely employed method for isolating potential mutants with enhanced lipid content in microalgae, without compromising cellular growth. This integrated approach enables high-throughput screening to identify the cells exhibiting the highest lipid production, utilizing Nile red luminescence as a basis for selection (Dempster & Sommerfeld, 1998). The use of UV mutagenesis in conjunction with FACS has been documented in several studies, encompassing high lipid-producing *I.galbana* strains and carotenoid hyperproducing *D. salina* strains (Bougaran et al., 2012; Mendoza et al., 2008).

2. MATERIALS AND METHODS

2.1. Microorganism and growth medium

A stock culture of Schizochytrium sp. S31 (ATCC 20888) was maintained using F/2 medium. Large-scale growth experiments were conducted employing both complex and fermenter media.

2.2. Death curve analysis

The experiment's initial phase involved inducing mutations in a wild-type Schizochytrium sp. culture using UV radiation from a lamp emitting a wavelength of 250 nm, positioned 7 cm above the culture. The samples consisted of 5 mL cultures with a cell density of 106 cells/mL. To assess cell viability, the culture was divided into five separate petri dishes, each subjected to UV exposure for 5, 15, 30, 45, and 60 seconds, respectively. To prevent photoreactivation, the samples were kept in darkness for 24 hours. After appropriate dilution, the treated cultures were plated, and the resulting colonies were collected and counted. The UV exposure duration that resulted in a 50% survival rate was determined to be optimal for mutant development, with untreated cells serving as a control.

2.3. Construction of mutant library

The mutant library was constructed following the methodology detailed by Manandhar-Shrestha and Hildebrand (2013). The Schizochytrium sp. S31 cell culture was grown to the early exponential phase before being harvested. The resulting cell pellet was washed and resuspended in F/2 medium to achieve a concentration of 3×10^6 cells/mL. These cells were then placed in a sterile 60 mm petri dish, covered with two layers of Kimwipe, and exposed to UV light (UVP CX-2000) for 30 seconds at maximum intensity (1 joule/cm2) from a distance of 7 cm. To prevent photoreactivation, the samples were kept in darkness overnight following exposure. The mutant cultures were subsequently transferred into both liquid and agar growth media and incubated for 2-3 days at 25°C. The purified cell pellet was then inoculated into NP medium and cultured for 24 hours at a temperature range of 20-25°C to induce lipid production.

2.4. Staining by fluorescent dye BODIPY

The lipophilic fluorescent dye BODIPY (4,4-difluoro-3a,4a-diaza-s-indacene), sourced from Invitrogen, was employed for the detection of intracellular lipids in Schizochytrium sp. The cell culture was first washed and then resuspended in a 0.1 M potassium phosphate buffer at pH 7. Subsequently, the cells were stained with BODIPY at a concentration of 2.6 μg/mL for 20 minutes at room temperature, followed by placement on ice. The constructed and induced mutant library was then screened using fluorescence-activated cell sorting (FACS) to identify and isolate the desired mutants.

2.5. Fluorescent activated cell sorting (FACS)

The mutant library was screened using high-throughput Fluorescence Activated Cell Sorting (FACS). Cells were labeled with BODIPY at a concentration of approximately 10^6 cells/mL. The sorting process was carried out using a Becton Dickinson Influx flow cytometer, excited by a 488-nm laser. Cells exhibiting elevated BODIPY fluorescence, indicative of high lipid content, were selectively collected from the upper fraction of the population. A total of 100,000 cells were sorted and then transferred to a liquid medium containing antibiotics for further growth.

2.6. Image Stream analysis

The quantification of TAG accumulation was conducted by means of BODIPY fluorescence using an ImageStreamX imaging flow cytometer (Amnis Corp., Seattle, WA). A total of $1x10^7$ cells were extracted from the culture at various time points during the incubation process and subjected to staining with BODIPY for lipid analysis. Cell classifier settings were configured to capture images with an area falling within the range of 50 µm2 to 300 µm2. Specifically, the bright-field image was recorded in channel 4, the side scatter image in channel 6 (with an excitation wavelength of 785 nm and emission range of 745-800 nm), BODIPY fluorescence in channel 2 (with an excitation wavelength of 488 nm and emission range of 470-560 nm), and chlorophyll autofluorescence in channel 5 (with an excitation wavelength of 488 nm and emission range of 660-720 nm).The 488 nm laser was adjusted to a power of 15 mW, while the bright-field 785 nm laser was set to 2 mW. The magnification level was established at 40X. For data analysis, the core velocity value was configured at 66, and the core track was adjusted to accommodate bead fluids. Data collection encompassed 5,000 to 10,000 cells for each analysis.

2.7. Gas Chromatography Analysis

The FAME (Fatty Acid Methyl Ester) analysis was conducted following the established procedure as outlined by Schlechtriem et al. (2008). To initiate the process, the total lipid content was suspended in a solution consisting of 5 mL of 0.5 N methanolic NaOH and subjected to heating at 100°C for a duration of 10 minutes. Subsequently, 5 mL of BF3-methanol was introduced for methylation purposes. The resulting FAMEs were extracted from the upper phase using 5 mL of n-heptane, followed by solvent evaporation and the addition of NaCl. To remove any residual moisture, the FAMEs were transferred to a vial containing Na2SO4 and heptane. These prepared samples were then subjected to analysis utilizing an Agilent 6850 Gas Chromatograph (GC) equipped with an Agilent HP 88 column (0.25 mm, 100 m, 0.2 µm). The detection and quantification of FAMEs were accomplished through chromatographic comparison with established standards provided by Sigma Chemical Co. in the USA.

3. RESULTS

3.1. Cellular Growth and Lipid Accumulation

The study demonstrated a time-dependent relationship in cell survival rates. Cell enumeration on agar plates revealed that a 30-second exposure to 1 joule of UV radiation resulted in a 50% survival rate, as shown in Figure 1. To evaluate differences in lipid accumulation, 100,000 cells were screened using FACS. Phosphorus and nitrogen deficiencies led to a significant rise in the mean BODIPY fluorescence value (530/40) [488] in 48 hours of incubation (Figure 2).
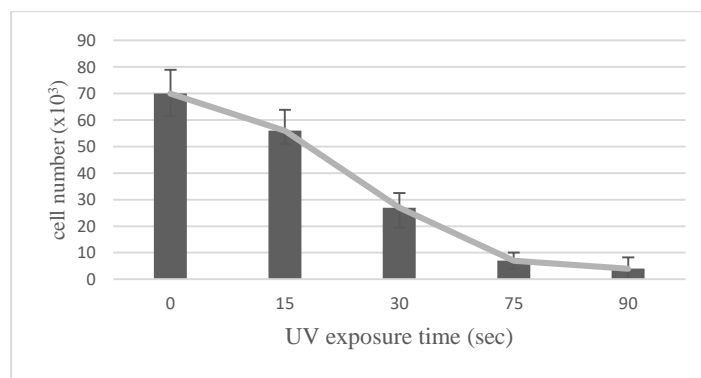
Figure 1. Schizochytrium sp. death curve under different UV exposure time
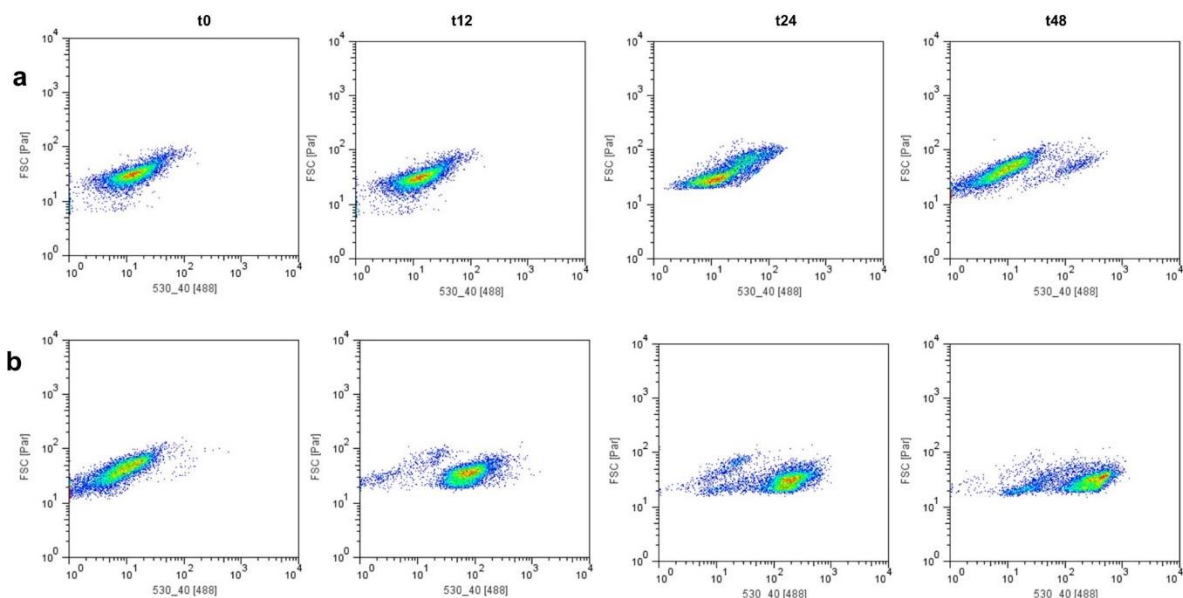
Figure 2. Flow cytometry analysis of the Schizochytrium sp. cell during the time under normal and limited conditions. a: Growth medium; b: Phosphorus and nitrogen limited medium

3.2. UV Mutagenesis and Screening

After three rounds of sorting and plating on agar, six mutant colonies which showed high lipid ratio signal in FACS results and one wild-type colony were selected for ImageStream (IS) analysis, with 50,000 cells sorted per treatment. IS analysis showed that two mutants (M1 and M2) exposed to 30 seconds of UV radiation had higher lipid content than the wild type.

Further IS analysis was performed on two mutants and the wild type, each with two replicates, to assess variations in fluorescent intensity over time. Mutant 1 and Mutant 2 exhibited significantly higher fluorescence levels than the wild type, with increases of 63.2% and 52.1%, respectively, as shown in Figure 3. A total of 5,000 cells were analyzed to compare the mutants with the wild type, revealing marked differences in fluorescent intensity.
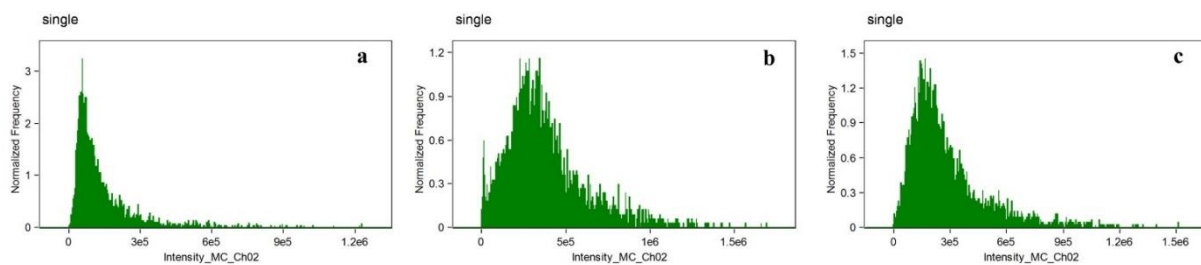
Figure 3. Light intensity difference between mutants and wild type on IS analysis a: Control; b: Mutant 1; c: Mutant 2 (30 sec UV exposure)

3.3. Characterization of Selected Mutants

The selected mutants were characterized by comparing parameters such as cell dry weight (CDW), total lipid content, DHA percentage, biomass productivity, and lipid productivity to the wild type, as detailed in Table 1. All tests were conducted in 1-liter baffled Erlenmeyer flasks under consistent conditions and were replicated twice. The wild-type CDW was 34.1 g/L, while Mutant 1 and Mutant 2 had CDWs of 28.5 g/L and 31.4 g/L, respectively. The total lipid content for the mutants consistently exceeded that of the wild type, with values of 25.6% for Mutant 1, 22.1% for Mutant 2, and 19.93% for the wild type.

GC-MS analysis was performed to assess fatty acid composition, revealing DHA concentrations of 156 mg/L in the wild type, 198 mg/L in Mutant 1, and 178 mg/L in Mutant 2, as shown in Table 1. This analysis confirmed the flow cytometry results, indicating a 17.9% increase in DHA for Mutant 1 and a 12.1% increase for Mutant 2 compared to the wild type

Table 1. Comparative results of the Mutant 1, Mutant 2 and Wt *Schizochytrium* sp.

	Mutant1	Mutant2	Wild type
%lipid	25,6	22,1	19,93
lipid productivity (g/l.h)	0,0729	0,0693	0,0679
%DHA	27,13	25,79	23
DHA productivity (g/l.h)	0,0198	0,0179	0,0156

4. DISCUSSION

UV mutagenesis is a well-established method for inducing lipid accumulation in various microalgal species (Tale et al., 2018; Trovão et al., 2022; Banerjee et al., 2018). Previous studies, such as Bougaran et al. (2012), demonstrated that combining UV mutagenesis with fluorescence-activated cell sorting (FACS) in Isochrysis galbana resulted in an 80% increase in lipid productivity (Bougaran et al., 2012). Similarly, the present study employs UV mutagenesis coupled with high-throughput selection using ImageStream to enhance lipid production in Schizochytrium sp. microalgae. Under nutrient-deprived conditions, microalgal cells typically shift their lipid biosynthesis pathways, accumulating neutral lipids—mainly in the form of triacylglycerol (TAG)—as a protective mechanism to endure environmental stress (Minhas et al., 2016). Studies have shown that temperature and nitrogen limitation maximize lipid content in microalgal cultures (Rios et al., 2015; Jiang & Chen, 2000a; Jiang & Chen, 2000b).

In this study, nitrogen (N) and phosphorus (P) starvation increased the mean BODIPY fluorescence value (530/40 [488]) from 78 to 290, indicating enhanced lipid accumulation. UV treatment of Schizochytrium sp. cells for 30 seconds led to faster lipid accumulation in mutants compared to the wild type (WT), as confirmed by time-course experiments. The total lipid content was 19.93%, 25.6%, and 22.1% for WT, Mutant 1, and Mutant 2, respectively. This corresponds to a 28.4% increase in Mutant 1 and a 10.8% increase in Mutant 2 relative to the wild type.

Further analysis via GC-MS revealed that UV-treated mutants also showed higher levels of docosahexaenoic acid (DHA), with Mutant 1 and Mutant 2 displaying increases of 17.9% and 12.1%, respectively, compared to the WT. These results are consistent with findings from Meireles et al. (2003), who observed a 33% increase in both eicosapentaenoic acid (EPA)

and DHA in Pavlova lutheri following UV mutagenesis (Meireles et al., 2003). Similar techniques have been successfully applied to enhance EPA content in Phaeodactylum tricornutum by 37% (Alonso et al., 1996). However, the effects of UV mutagenesis can vary across species, as differential stress tolerance mechanisms influence lipid accumulation outcomes (Gao et al., 2009). In the current study, further UV-induced mutations did not result in additional lipid content increases beyond the initial mutation trials, suggesting that the limits of lipid productivity under these specific stress conditions had been reached. These findings emphasize the importance of optimizing mutagenesis parameters and stress conditions for each species.

5. CONCLUSION

This study demonstrates the efficacy of UV mutagenesis combined with high-throughput selection techniques in enhancing lipid productivity in *Schizochytrium* sp. microalgae. Mutant 1 exhibited a 28.4% increase in total lipid content, while Mutant 2 showed a 10.8% increase compared to the wild type. GC-MS analysis corroborated the flow cytometry results, revealing significant elevations in DHA levels in UV-treated cultures, with a 17.9% and 12.1% increase in Mutant 1 and Mutant 2, respectively. These results suggest that nutrient deprivation and UV exposure act synergistically to boost lipid accumulation, likely as a cellular adaptation to stress. However, additional mutations beyond the initial trials did not yield further improvements, indicating that lipid enhancement through mutagenesis may have species-specific limitations

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3-Aril-4-(benzensulfoniloksi)-benzilidenamino-4,5-dihiro-1*H*-1,2,4-triazol-5-on Bileşiklerinin Antimikrobiyal Aktiviteleri

Antimicrobial Activities of 3-Aryl-4-(benzenesulfonyloxy)-benzylideneamino-4,5-dihyro-1*H*-1,2,4-triazol-5-one Compounds

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Biyoloji / Biology

Araştırma Makalesi / Research Article

Öz

Çalışmanın sentez bölümünde, ilk olarak 3 adet 3-aril-4-amino-4,5-dihidro-1*H*-1,2,4-triazol-5-on bileşiğinin bir benzaldehid türevi olan 3-etoksi-4-hidroksibenzaldehidin trietilamin varlığında benzensulfonil klorür ile muamelesinden sentezlenen 3-etoksi-4-(benzensulfoniloksi)-benzaldehid ile ayrı ayrı muamelesinden karşın olan 3 adet 3-aril-4-(benzensulfoniloksi)-benzilidenamino-4,5-dihiro-1*H*-1,2,4-triazol-5-on bileşiği elde edilmiştir. 3 Adet 1,2,4-triazol türevli Schiff bazı bileşikleri olan 3-aril-4-[3-etoksi-4-(benzensulfoniloksi)-benzilidenamino]-4,5-dihidro-1*H*-1,2,4-triazol-5-on'ların *Klebsiella pneumoniae, Pseudomonas aeruginosa, Echerichia coli, Staphylococcus aureus, Entercoccus faecalis* bakterileri üzerindeki antimikrobiyal etkileri disk difüzyon yöntemi ile araştırılmıştır. Yapılan araştırma sonucunda 3-*p*-klorobenzil-4-[3-etoksi-4-(benzensulfoniloksi)-benzilidenamino]-4,5-dihidro-1*H*-1,2,4-triazol-5-on'un *E. coli ve K. pneumoniae* üzerinde antimikrobiyal etki gösterdiği, 3-*m*-klorobenzil-4-[3-etoksi-4-(benzensulfoniloksi) benzilidenamino]-4,5-dihidro-1*H*-1,2,4-triazol-5-on'un da yalnızca *K. pneumoniae* üzerinde etkili olduğu belirlenmiştir.

Abstract

In the synthesis section, three 3-aril-4-amino-4,5-dihydro-1*H*-1,2,4-triazol-5-one compounds were obtained through the reaction of 3-ethoxy-4-hydroxybenzaldehyde, a derivative of benzaldehyde, with benzenesulfonyl chloride in the presence of triethylamine. Subsequent reactions with these compounds yielded three distinct 3-aril-4-(benzenesulfonoxy)-benzylideneamino-4,5-dihydro-1*H*-1,2,4-triazol-5-one derivatives. The antimicrobial effects of three 1,2,4-triazole derivative Schiff base compounds, which are 3-aryl-4-[3-ethoxy-4-(benzenesulfonyloxy)benzylideneamino]-4,5-dihydro-1*H*-1,2,4-triazol-5-ones, were investigated against *Klebsiella pneumoniae, Pseudomonas aeruginosa, Escherichia coli, Staphylococcus aureus*, and *Enterococcus faecalis* using the disk diffusion method. Results revealed that 3-p-chlorobenzyl-4-[3-ethoxy-4-(benzenesulfonyloxy)benzylideneamino]-4,5-dihydro-1*H*-1,2,4-triazol-5-one displayed antimicrobial activity against *E. coli* and *K. pneumoniae*, while 3-m-chlorobenzyl-4-[3-ethoxy-4-(benzenesulfonyloxy) benzylideneamino]-4,5-dihydro-1*H*-1,2,4-triazol-5-one was effective only against *K. pneumoniae*.

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1. INTRODUCTION

Schiff bases were first synthesized by the German chemist Hugo Schiff, who was awarded the Nobel Prize in the 1860s. Due to their stability and ease of synthesis, Schiff bases have become widely used and studied compounds (Blagus et al., 2010; Karaca, 2018; Aydınlı Esen, 2006). There are numerous areas where Schiff bases are effectively utilized. In the paint industry, Schiff base metal complexes are employed (Aydınlı Esen, 2006). Compounds containing aromatic rings and metal complexes are also used as catalysts (Pişkin, 2011). Schiff bases have been utilized as ligands to coordinate metal ions and to coordinate anions (Blagus et al., 2010). They have been observed to play a regulatory role in plant growth and to affect growth hormones in roots (Pişkin, 2011). Additionally, they exhibit herbicidal properties (Karaca, 2018) and have been found to show toxicity against insects (Pişkin, 2011). Schiff bases are used in the treatment of diabetes and AIDS (Karaca, 2018). Similar to important coordination compounds in the human body such as hemoglobin and in plants such as chlorophyll, Schiff bases are used as ligands and play a role in the oxidation of biologically significant molecules such as free oxygen and ascorbic acid (Aydınlı Esen, 2006). Due to their antitumor and anticancer properties, Schiff bases hold an important place in cancer drugs (Özsen, 2010; Değirmencioğlu, 2010). In recent years, numerous *in silico, in vitro,* and *in vivo* studies regarding Schiff bases have gained significant importance (Koç et al., 2020; Beytur and Avinca, 2021; Beytur and Uğurlu, 2020; Kardaş et al., 2016; Yüksek et al., 2018; Turhan Irak and Beytur, 2019; Gürsoy Kol et al., 2020). Many studies on Schiff bases have observed their antiviral, antifungal, and antibacterial properties (Bahçeci et al., 2017; Beytur et al., 2019; Karaca, 2018; Aydınlı Esen, 2006; Özsen, 2010; Değirmencioğlu, 2010; Pişkin, 2011; Kayapa, 2018; Zeydan, 2009).

Microorganisms have numerous beneficial applications in foods, such as kefir, yogurt, vinegar, bread fermentation, alcohol and acetone production, as well as biological wastewater treatment and use as biological fertilizer. However, they can also cause diseases and fatalities in humans, animals, and plants, in addition to economic losses resulting from food spoilage. The emergence of antibiotic resistance among bacteria has become a significant problem in modern times, increasing the importance of alternative treatment methods to antibiotics. Therefore, the production of effective drugs against harmful microorganisms is important and valuable (Arda, 2006).

S. aureus is a staphylococcal bacterium capable of causing food poisoning and pyogenic infections in humans and warm-blooded animals. It is commonly found in nature and is a significant cause of mastitis. It can contaminate milk and dairy products from carrier cows. Infections caused by S. aureus typically manifest as skin and mucosal infections, such as abscesses, furuncles, impetigo, carbuncles, panaris, hidradenitis, blepharitis, tonsillitis, pharyngitis, and peritonsillar abscess. S. aureus can also lead to conditions like sepsis, endocarditis, pneumonia, food poisoning, and enteritis. (Akan, 2006). *E. faecalis*, on the other hand, is a fecal bacterium found in human and animal feces. Enterococci can cause diseases such as peritonitis, bacteremia, and endocarditis. The presence of a high level of enterococci in water samples indicates the presence of fecal contamination (Akan, 2006; Murray vd.,2016). *P. aeruginosa* is commonly found in nature and can survive for long periods in organic matter and water. It is a component of the intestinal flora in humans and animals and typically causes suppurative and sometimes acute systemic infections. Diseases caused by *P. aeruginosa* include respiratory tract infections, urinary tract infections, skin and soft tissue infections, ear and eye infections, bacteremia, and endocarditis (Tuncer ve Akova, 1997; Murray vd., 2016; Esendal, 2006). *E. coli*, a normal flora of the intestine, becomes an opportunistic pathogen when the host's defense mechanisms are compromised. It can cause diseases such as urinary tract infections, gallbladder and bile duct infections, meningitis, peritonitis, sepsis, sinusitis, and wound infections (Murray vd., 2016). *E. coli* is a major cause of gastroenteritis and can also lead to intestinal diseases, urogenital infections, mastitis, lung inflammations, and wound infections in domestic animals (İzgür, 2006). Klebsiella species, including *K. pneumoniae* and *K. oxytoca*, can cause community or hospital-acquired primary lobar pneumonia (Murray vd., 2016). *K. pneumoniae* is an opportunistic pathogen found in the upper respiratory tract and fecal flora, including in domestic animals. It synergistically causes metritis in horses with *Streptococcus zooepidemicus*. Isolates from dogs have been associated with cystitis, mastitis, and metritis. In pigs, they are responsible for agalactia syndrome (Akan, 2006).

The increasing prevalence of resistance in bacteria has led to a growing importance of anti-infective models in modern medicine and biotechnology. Numerous studies have demonstrated that Schiff bases, which exhibit antimicrobial activity, are among the prominent options in this field. The utilization of Schiff bases as therapeutic agents is considered a

potential treatment approach for the control of pathogenic bacteria. In this study, the efficacy of newly synthesized Schiff bases in controlling certain pathogens crucial for human and animal health has been investigated.

2. MATERIALS AND METHODS

The chemicals utilized in the synthesis of the Schiff bases were procured from Merck, Fluka, and Aldrich companies. The necessary solvents were obtained from domestic or international sources. The melting points of the synthesized compounds were determined using a Stuart SMP30 brand melting point determination apparatus within the scope of the study. The IR spectra of newly synthesized compounds were recorded at the Department of Organic Chemistry Research Laboratory, Faculty of Science and Literature, Kafkas University, using the ALPHA-P BRUKER FT-IR spectrometer. The ^{1}H-NMR and ^{13}C-NMR spectra were acquired at Mersin University Advanced Technology Education, Research, and Application Center, using a BRUKER ULTRASHIELD PLUS BIOSPIN 400 MHz NMR spectrometer.

2.1. Method

In the synthesis section of the study, three new 3-aryl-4-amino-4,5-dihydro-1*H*-1,2,4-triazol-5-one compounds were obtained from the treatment of a benzaldehyde derivative, namely 3-ethoxy-4-hydroxybenzaldehyde, with benzene sulfonyl chloride in the presence of triethylamine. These compounds were synthesized separately from the treatment of 3-ethoxy-4-(benzenesulfonyloxy)benzaldehyde, which was obtained through the aforementioned reaction, resulting in three novel 3-aryl-4-(benzenesulfonyloxy)benzylideneamino-4,5-dihydro-1*H*-1,2,4-triazol-5-one compounds. (Özdemir, 2016).
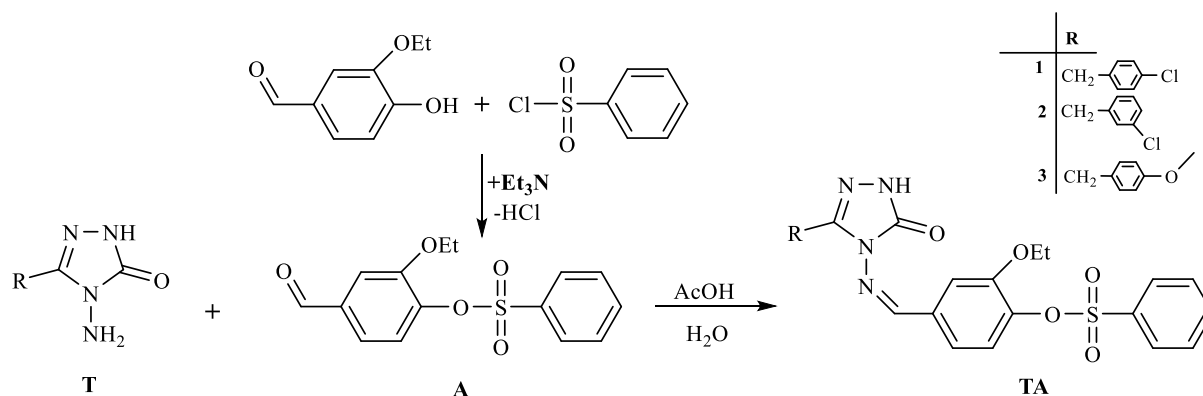
Scheme 1. Synthesis of Schiff Base Compounds (Özdemir, 2016)

2.2. Synthesis of 3-Aryl-4-[3-ethoxy-4-(benzenesulfonyloxy)benzylideneamino]-4,5-dihydro-1*H*-1,2,4-triazol-5-one (TA) Compounds

After dissolving three 3-aryl-4-amino-4,5-dihydro-1*H*-1,2,4-triazol-5-one (T) compounds in acetic acid, 0.01 mol of 3-ethoxy-4-(benzenesulfonyloxy)benzaldehyde (A) was added, and the mixture was refluxed under a cooling condenser for 1.5 hours. The resulting mixtures were left overnight in a refrigerator, and the precipitated crude product was filtered, washed with distilled water, dried under vacuum over $CaCl_2$ in a desiccator, and recrystallized from ethanol.

2.2.1. 3-p-Chlorobenzyl-4-[3-ethoxy-4-(benzenesulfonyloxy) benzylidene-amino]-4,5-dihydro-1H-1,2,4-triazol-5-one (TA1): Productivity 96%, mp. 172°C. IR (KBr) cm^{-1}: 3169 (NH), 1704 (C=O), 1587 (C=N), 1370 ve 1174 (SO_2), 845 (1,4- Disubstitute benzenoid ring), 752 ve 687 (monosubstitue benzenoid ring) ^{1}H NMR (200 MHz, DMSO-d_6): δ 11.12 (t, 3H, OCH$_2$CH$_3$; J=7.20 Hz), 3.79 (q, 2H, OCH$_2$CH$_3$; J=7.20 Hz), 4.07 (s, 2H, CH$_2$Ph), 7.29 (d, 1H, ArH), 7.34-7.40 (m, 6H, ArH), 7.67 (t, 2H, ArH; J=8.00 Hz), 7.81-7.85 (m, 3H, ArH), 9.64 (s, 1H, N=CH), 12.03 (s, 1H, NH). ^{13}C NMR (50Mz, DMSO-d_6): δ 14.04 (OCH$_2$<u>CH$_3$</u>), 30.48 (CH$_2$Ph), 63.98 (O<u>CH$_2$</u>CH$_3$), 111.99, 120.53, 124.26, 128.14 (2C), <u>128.36 (2C),</u> 129.46 (2C), <u>130.56 (2C),</u> <u>131.37,</u> 133.68, 134.82 (2C), 135.12, 139.53, 151.12 (ArC), 145.80 (Triazole C$_3$), 150.82 (N=CH), 151.97 (Triazole C$_5$).

2.2.2. 3-m-Chlorobenzyl-4-[3-ethoxy-4-(benzenesulfonyloxy) benzylidene-amino]-4,5-dihydro-1H-1,2,4-triazol-5-one (TA2) Productivity 97%, mp. 179°C. IR (KBr) cm^{-1}: 3166 (NH), 1705 (C=O), 1576 (C=N), 1350 ve 1194 (SO$_2$), 817 ve 701 (1,3-disubstitue benzenoid ring), 754 ve 691 (monosubstitue benzenoid ring). ^{1}H NMR (200 MHz, DMSO-*d$_6$*): δ 1.11 (t, 3H, OCH$_2$CH$_3$; *J*=7.20 Hz), 3.81 (q, 2H, OCH$_2$CH$_3$; *J*=6.80 Hz), 4.09 (s, 2H, CH$_2$Ph), 7.25-7.33 (m, 4H, ArH), 7.37-7,39 (m, 2H, ArH), 7.66 (t, 2H, ArH; *J*=8.00 Hz), 7.80-7.84 (m, 3H, ArH), 9.64 (s, 1H, N=CH), 12.04 (s, 1H, NH). ^{13}C NMR (50Mz, DMSO-*d$_6$*): δ 14.04 (OCH$_2$CH$_3$), 30.70 (CH$_2$Ph), 64.00 (OCH$_2$CH$_3$), 111.84, 120.68, 124.23, 126.74, 127.41, 128.14 (2C), 128.73, 129.47 (2C), 130.26, 132.94, 133.67, 134.85, 135.10, 138.26, 139.55, 151.09 (ArC), 145.65 (Triazole C$_3$), 150.85 (N=CH), 151.97 (Triazole C$_5$).

2.2.3. 3-p-Methoxybenzyl-4-[3-ethoxy-4-(benzenesulfonyloxy) benzylidene-amino]-4,5-dihydro-1H-1,2,4-triazol-5-one (TA3): Productivity 95%, mp. 174°C. IR (KBr) cm^{-1}: 3209 (NH), 1696 (C=O), 1595 (C=N), 1366 ve 1174 (SO$_2$), 856 (1,4-disubstitue benzenoid ring), 755 ve 685 (monosubstitue benzenoid ring). ^{1}H NMR (200 MHz, DMSO-*d$_6$*): δ 1.12 (t, 3H, OCH$_2$CH$_3$; *J*=7.20 Hz), 3.71 (s, 3H, OCH$_3$), 3.82 (q, 2H, OCH$_2$CH$_3$; *J*=7.20 Hz), 3.98 (s, 2H, CH$_2$Ph), 6.85 (d, 2H, ArH; *J*=8.80 Hz), 7.22 (d, 2H, ArH; *J*=8.80 Hz), 7.30 (d, 1H, ArH; *J*=8.00 Hz), 7.39 (s, 1H, ArH), 7.42 (m, 1H, ArH), 7.66 (t, 2H, ArH; *J*=8.00 Hz), 7.81-7.85 (m, 3H, ArH), 9.64 (s, 1H, N=CH), 11.98 (s, 1H, NH). ^{13}C NMR (50Mz, DMSO-*d$_6$*): δ 14.03 (OCH$_2$CH$_3$), 30.29 (CH$_2$Ph), 55.01 (OCH$_3$), 63.99 (OCH$_2$CH$_3$), 112.05, 113.85 (2C), 120.47, 124.27, 127.57, 128.14 (2C), 129.47 (2C), 129.72 (2C), 133.76, 134.83, 135.14, 139.50, 151.15, 158.06 (ArC), 146.46 (Triazole C$_3$), 150.83 (N=CH), 151.87 (Triazole C$_5$).

2.3. Test Bacteria

The bacterial strains *S. aureus* (ATCC 25923), *E. faecalis* (ATCC 29212), *K. pneumoniae* (ATCC 700603), *E. coli* (ATCC 25922), and *P. aeruginosa* (ATCC 27853) utilized in the study were obtained from the Department of Microbiology, Faculty of Medicine, Kafkas University.

2.2.1. Bacterial Culture Media

To activate the bacteria, Nutrient Broth (Oxoid) was utilized, while for antimicrobial studies, Muller Hinton Agar (Oxoid) was employed.

2.4. *Preparation of Schiff Base Solutions*

The stock solutions of Schiff bases, whose antimicrobial effect will be determined, were prepared by dissolving them in DMSO at concentrations of 10 mg/ml and 4 mg/ml. Subsequently, the 4 mg/ml stock solution was diluted each time to prepare dilutions of 2 mg/ml, 1 mg/ml, and 0.5 mg/ml.

2.5. *Disc Diffusion Method*

The test bacteria were inoculated into Nutrient Broth and left to incubate overnight. Following incubation, the turbidity was adjusted to 0.5 McFarland. Subsequently, 100 µl of each bacterial solution was spread onto Muller Hinton Agar using a Drigalski spatula. After inoculation, the petri dishes were allowed to air dry at room temperature for 10 minutes. Three 6mm sterile discs were impregnated with dilutions of Schiff base at concentrations of 10 µl/ml, 4 µl/ml, 2 µl/ml, 1 µl/ml, and 0.5 µl/ml, and then placed onto the surface of the dried petri dishes. Dimethyl sulfoxide (DMSO) was used as the negative control, while ampicillin/sulbactam, tigecycline, and amoxicillin/clavulanic acid were used as positive controls. The petri dishes were then incubated at 37°C for 24 hours. By measuring the inhibition zone diameters formed after incubation, Schiff bases that formed a zone diameter of 5 mm and above were evaluated as effective (Aydınlı Esen, 2006).

3. RESULTS AND DISCUSSION

The antimicrobial effect of Schiff bases was evaluated, revealing that TA1 (3-p-Chlorobenzyl-4-[3-ethoxy-4-(benzenesulfonyloxy)benzylideneamino]-4,5-dihydro-1*H*-1,2,4-triazol-5-one) Schiff base exhibited antimicrobial activity against both *E. coli* and *K. pneumoniae*, while TA2 (3-m-Chlorobenzyl-4-[3-ethoxy-4-(benzenesulfonyloxy)benzylideneamino]-4,5-dihydro-1*H*-1,2,4-triazol-5-one) Schiff base showed antimicrobial activity only against *K. pneumoniae*. Both TA1 and TA2 Schiff bases exhibited inhibitory effects at a concentration of 10 mg/ml. However, TA3 (3-p-Methoxybenzyl-4-[3-ethoxy-4-(benzenesulfonyloxy)benzylideneamino]-4,5-dihydro-1*H*-1,2,4-triazol-5-one) Schiff base showed no significant effect on the test bacteria at any concentration tested. Additionally, concentrations below 10 mg/ml of the Schiff bases did not exhibit any inhibitory effects on *E. coli, S. aureus, E. faecalis, K.*

pneumoniae, or *P. aeruginosa*. Figure 2 shows the inhibition zones of Schiff bases against the test bacteria.
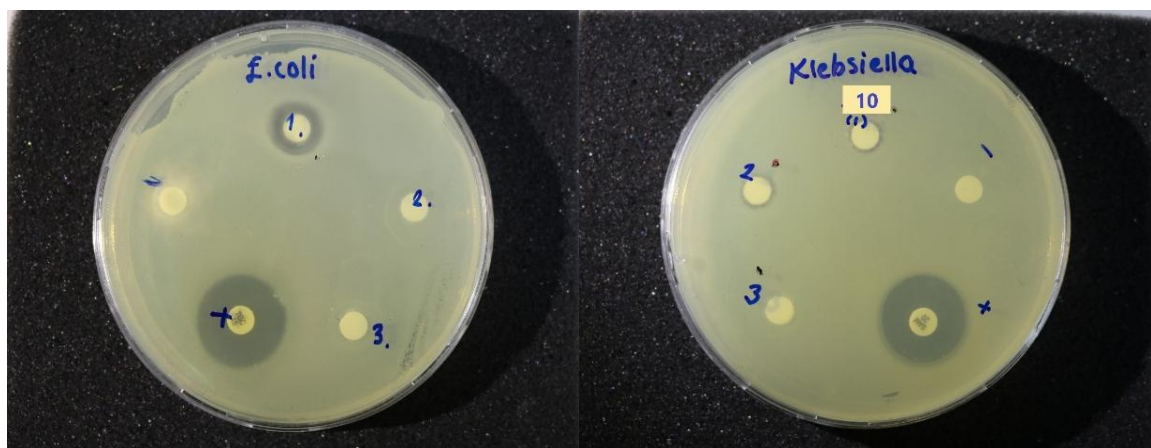
Figure 1. The inhibition zones formed by Schiff base No.1 against *E.coli* at a dose of 10 mg/ml, and the inhibition zones formed by Schiff base No.2 against *K.pneumoniae* at a dose of 10 mg/ml

Table 1. Inhibition zone diameters of utilized Schiff bases

No	Schiff base	Inhibition zone (mm)				
		S. aureus	*E. faecalis*	*K.pneumoniae*	*E.coli*	*P. aeruginosa*
1	TA1	≤6	≤6	8	11	≤6
2	TA2	≤6	≤6	8	≤6	≤6
3	TA3	≤6	≤6	≤6	≤6	≤6

Tablo 2. Effect Dosages of Schiff Bases

No	Schiff base	Testing Bacteria				
		S. aureus	*E. faecalis*	*K.pneumoniae*	*E.coli*	*P. aeruginosa*
1	TA1	-	-	10	10*	-
2	TA2	-	-	10	-	-
3	TA3	-	-	-	-	-

*mg/ml

When reviewing studies similar to the research conducted with triazole derivative Schiff bases, similarities in results have been observed. Generally, it has been reported that simple Schiff bases exhibit insignificant activity in these studies, whereas substances coordinated with vanadium (IV) metal demonstrate higher activity (Chohan vd., 2010). In the study conducted by Bagihalli and colleagues, it was determined that triazole derivatives of

Schiff bases exhibited varying degrees of effectiveness against *E. coli, S. aureus, S. pyogenes, P. aeruginosa,* and *S. typhi* at various doses (Bagihalli vd., 2008). Bayrak and colleagues have determined that newly synthesized triazole derivative Schiff bases exhibit either good or moderate inhibitory effects on test bacteria (Bayrak vd., 2009). In another study, the effects of 1,2,4-triazole-3-thione-imidazole 1 derivative on *E. coli, S. aureus, B. subtilis, S. typhi,* and *S. dysentrae* bacteria were investigated. It was observed that compounds 1b, 1d, and 1f exhibited similar characteristics to tetracycline (inhibition zone: 14-17 mm) (Ghasemzadeh vd., 2018). Yüksek et al. investigated the effects of 9 synthesized triazole-derived Schiff bases on various bacterial strains including *B. subtilis, B. cereus, Y. enterocolitica, S. aureus, E. coli, P. multocida*, and *K. pneumoniae* using the agar well diffusion method. The study revealed that all tested Schiff bases were effective against all investigated bacteria except for *P. multocida* and *K. pneumoniae*. Specifically, it was observed that 4 out of the 9 Schiff bases did not exhibit efficacy against *P. multocida*, while 2 of them were ineffective against *K. pneumoniae* (Yüksek vd., 2017). Zeydan determined the Minimum Inhibitory Concentration (MIC) values of 10 Triazol Schiff bases against *S. aureus, S. epidermidis*, *E. coli, K. pneumoniae*, and *P. aeruginosa*. While none of the substances were effective against *K. pneumoniae*, nine of them exhibited effectiveness against *S. aureus*, with MIC values ranging between 156 mg/ml and 612 mg/ml (Zeydan, 2009). In some studies, 1,2,4-triazole-pyrazole derivatives have shown weak to moderate antibacterial activity (MIC: 62.5-250µg/mL) against various bacteria including *E. coli, S. typhi, S. pneumoniae, B. subtilis,* and *C. tetani* (Prasad et al., 2018). Similarly, 1,2,4-triazole-thiazole derivatives exhibited antimicrobial activity (MIC: 1.95–62.5µg/mL) against two Gram-positive bacteria (*L. monocytogenes* and *S. aureus*) and three Gram-negative bacteria (*E.coli, S. typhimurium*, and *P. aeruginosa*) (Nastasa et al., 2018). Additionally, it has been observed that some triazolothiadiazole-pyrimidone derivatives exhibited inhibitory properties ranging from 44% to 92% at 25µg/mL against *B. amiloliquefaciens, S. aureus,* and *B. subtilis* (Cui et al., 2017).

In numerous studies, the antimicrobial, antifungal, and antiviral effects of Schiff bases have been evaluated. The majority of obtained results support the potential of Schiff bases as alternatives to antibiotics. Some studies have even found Schiff bases to exhibit more effective activity than antibiotics. In our conducted research, it was observed that two newly

synthesized Schiff bases demonstrated antimicrobial activity. It is anticipated that these effective Schiff bases could be utilized in combating sensitive bacterial species

4. CONCLUSION

This study investigated the antimicrobial properties of Schiff base derivatives of 3-aryl-4-(benzenesulfonyloxy) benzylideneamino-4,5-dihydro-1*H*-1,2,4-triazol-5-one. The findings of our experiments revealed that one specific compound, namely 3-p-chlorobenzyl-4-[3-ethoxy-4-(benzenesulfonyloxy) benzylideneamino]-4,5-dihydro-1*H*-1,2,4-triazol-5-one, exhibited remarkable efficacy against both *E. coli* and *K. pneumoniae*.

This promising antimicrobial activity underscores the potential of these synthesized compounds as effective agents in combating bacterial infections. The observed effectiveness against such clinically relevant bacteria highlights the importance of further investigations, including *in vivo* studies and toxicity assessments, to validate their therapeutic potential and safety profile. Additionally, exploring the mechanisms underlying their antimicrobial action could provide valuable insights for the development of novel antimicrobial agents to address the growing challenge of antibiotic resistance.

In conclusion, the results of this study suggest that Schiff base derivatives of 3-aryl-4-(benzenesulfonyloxy) benzylideneamino-4,5-dihydro-1*H*-1,2,4-triazol-5-one hold promise as potential candidates for the development of new antimicrobial drugs, emphasizing the need for continued research in this area to address the pressing global health threat posed by antibiotic-resistant bacteria.

Conflict of Interest

There are no conflicts of interest among the authors.

Author Contributions

The research was conceived and written by E. Koc, antimicrobial analyses and data evaluation were conducted by S.G. Talay, N. Mutlu, synthesis of chemicals used in the study was performed by G. Özdemir Toraman, M. Beytur and characterization was carried out by H. Yuksek.

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Kars Çayı (Kars) Sediment ve Su Örneklerinin Mutajenik Etkilerinin Ames Testi ile Araştırılması

Investigation of the Mutagenic Effects of Sediment and Water Samples from the Kars River (Kars) Using the Ames Test

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Biyoloji / Biology

Araştırma Makalesi / Research Article

Makale Bilgileri	Öz
	Bu çalışmada, Kars Çayı'nın merkeze yakın olan bölgelerinden 3 ayrı istasyon belirlendi. Bu istasyonlar; 1. istasyon; Yolaçan Köyü mevkisi, 2. istasyon; Kafkas Üniversitesi kampüs mevkisi, 3. istasyon; aktif yerleşimin bittiği yer olan Kars müze mevkisidir. İstasyonlar arasında mesafe gözetilerek, belirlenen noktalardan 2022 Eylül ayında su ve sediment örnekleri alındı. Kısa zamanlı mutajenite test sistemlerinden biri olan Ames testi ile su ve sediment örneklerinin olası mutajenik özellikleri belirlendi. 3 ayrı istasyondan alınan su örneklerinden ekstraktlar (hekzan ve kloroform) ve sediment örnekleri hazırlandı. Çalışmada su ve sediment örneklerinin 10^0, 10^{-1}, 10^{-2} ve 10^{-3} seri dilüsyonları kullanıldı. Deneyler, *Salmonella typhimurium* TA98 ve TA100 mutant suşları ile metabolik aktivasyon (S9) yokluğunda gerçekleştirildi. Deney sonuçları; spontan kontrol, negatif kontrol dimetil sülfoksit (DMSO) ve pozitif kontrol grupları ile birlikte değerlendirildi. Pozitif kontrol olarak, metabolik aktivasyon (S9) yokluğunda TA98 suşu için 4-Nitro-o-fenilendiamin ve TA100 suşu için ise sodyum azid kullanıldı. Mutajenite testleri sonucunda, su örneklerinin hekzan ve kloroform ekstraktları ve sediment örneklerinde, çevresel kirliliğin daha az olduğu 1. istasyondan alınan örneklerde potansiyel mutajenik etki gözlemlenmezken, 2. istasyonda yalnızca çerçeve kayması değişimine yol açan mutajenik etki gözlemlendi. Çevresel kirlenmenin en fazla olduğu düşünülen 3. istasyonda ise hem çerçeve kayması, hemde baz çifti değişimine neden olan mutajenik etki görüldü.

Article Info	Abstract
	In this study, three different stations were determined from the regions of Kars River that are close to the center. These stations are; 1st station; Yolaçan Village location, 2nd station; Kafkas University campus location, 3rd Station; Kars museum location where the active settlement ends. Water and sediment samples were taken from the determined points by considering the distance between the stations in September 2022. Possible mutagenic properties of water and sediment samples were determined with the Ames test, which is one of the short-term mutagenicity test systems. Extracts (hexane and chloroform) of water samples and sediment samples were prepared from samples taken from three different stations. Serial dilutions of 10^0, 10^{-1}, 10^{-2} and 10^{-3} of water and sediment samples were used in the study. Experiments were performed with TA98 and TA100 mutant strains of *Salmonella typhimurium* in the absence of metabolic activation (S9). Experiment results were evaluated together with spontaneous control, negative control dimethyl sulfoxide (DMSO) and positive control groups. 4-Nitro-ophenylenediamine for strain TA98 in the absence of metabolic activation (S9) and sodium azide for strain TA100 were used as positive controls. As a

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result of the mutagenicity tests of the hexane and chloroform extracts of the water samples and sediment samples, no potential mutagenic effect was observed in the samples taken from the 1st station where environmental pollution was less, while a mutagenic effect caused to only a frameshift change was observed in the 2nd station. Both frameshift and mutagenic effect causing base pair change were observed in the 3rd station, which is thought to have the highest environmental pollution.

1. INTRODUCTION

The increasing environmental pollution worldwide has been negatively affecting ecosystems, particularly aquatic ecosystems. This has led to significant social and economic challenges while contributing to the gradual disruption of the ecological balance. Environmental pollution in aquatic environments, unplanned population growth, and unregulated agricultural activities, particularly contamination caused by mutagenic and carcinogenic substances, have become significant issues affecting human health. While the technology required to meet the needs of the growing population brings innovations and conveniences to human life, it has also exacerbated the problem of environmental pollution. Moreover, interventions such as transferring water through closed pipelines for residential use or the misuse of water resources have contributed to the emergence of water pollution issues. Additionally, with technological advancements, the number of chemical substances used by humans has been steadily increasing over time (Öncül, 2009; Tomatis, 1979).

Numerous substances contribute to environmental pollution, such as various pharmaceuticals used by living organisms, industrial waste, food additives, and a wide range of chemicals employed in pest control. Investigating the mutagenic and carcinogenic effects of these natural or synthetic substances, which may cause environmental pollution, is crucial for the health of living organisms. Furthermore, assessing the impact of these substances on all living organisms and the importance of implementing preventive measures is a significant concern. The detection of organic chemical substances in aquatic environments is particularly challenging. Investigating these pollutants, especially those that have already been identified, in specific environments helps develop practical methods for determining the toxicity, pollution levels, and other characteristics of the studied areas. Therefore, combining molecular chemical analysis methods with short-term biological research techniques enables the development of a rapid, reliable, and practical method for identifying toxic substances in various environmental samples (Schuetzle & Lewtas, 1986; Akyıl, 2006; Uysal, 2006; Yüksel, 2005).

Water pollution occurs when the bacteriological, chemical, physical, and ecological characteristics of a water source are adversely affected, either directly or indirectly (Uslu & Türkman, 1987). It can take various forms, including inorganic substance pollution, organic substance pollution, solid waste pollution, thermal pollution, and radioactive contamination (Göksu, 2003). The chemicals and pollutants mentioned above can cause mutations in the genetic material of plants, animals, and humans, thereby exhibiting genotoxic effects (Gesamp, 1991; Galli & Schiestl, 1996).

Since there is a connection between mutagenic and carcinogenic effects, mutagenic tests play a crucial role in the examination of various substances with carcinogenic effects (Temizkan, 1994; Mortelmans & Zeiger, 2000). The Ames test, developed by Dr. Bruce Ames in 1972, is a reliable bacterial test that provides rapid results and is widely used to detect the presence or absence of mutagenic effects in various substances consumed or used by humans, such as drugs, extracts, cosmetic products, and food additives (Ames et al., 1975; Choy, 2001). In the Ames test, bacterial mutants are used to examine the mutagenic effects of various substances. Several strains of *Salmonella typhimurium*, such as TA98, TA100, TA1535, and TA1537, are used. When the necessary amino acid (histidine) is not synthesized, bacterial growth does not occur, and no colony formation takes place. However, after exposure to the tested chemical, the strains may regain the ability to synthesize histidine and form colonies (Maron & Ames, 1983; Mortelmans & Zeiger, 2000). The Ames test is widely used to evaluate chemical substances and environmental samples. In many studies, the Ames test has been extensively employed to determine mutagenic effects in samples such as surface waters (37%), sediments (41%), and soils (38%) (Beceren et al., 2017).

This study determined that no prior mutagenicity studies had been conducted in the Kars River, located in Kars province. For the first time, this study aims to investigate the presence or absence of mutagenicity in water and sediment samples from the Kars River using the Ames mutagenicity test and to emphasize the importance of taking preventive measures against water pollution.

2. MATERIALS AND METHODS

In this study, three separate stations were established in the central and near-central areas of the Kars River. Water and sediment samples were collected in September 2022, with careful consideration given to the distances between the stations.
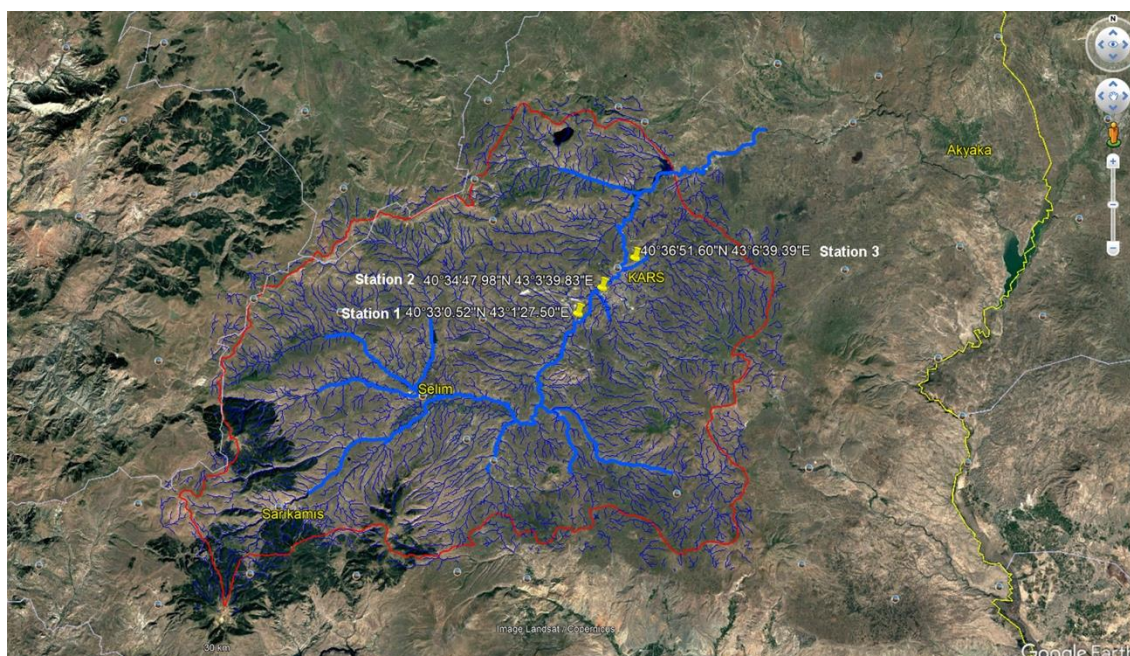
Figure 1. Stations of the Water and Sediment Samples on the General Map

The samples were collected from three specific locations along the Kars River. The coordinates of the sampling stations are as follows: Station 1 (40°33'0.52"N, 43°1'27.50"E), Station 2 (40°34'47.98"N, 43°3'39.83"E), located within the campus of Kafkas University, and Station 3 (40°36'51.60"N, 43°6'39.39"E). The stations where water and sediment samples were taken are shown in Figure 1.

Station 1, where water and sediment samples were collected, is located in the Yolaçan Village area along the Kars-Erzurum road. This station is at the confluence of the Kars River tributaries originating from Sarıkamış and Selim. The region surrounding this station includes residential areas, livestock farming activities, and agricultural lands. Station 2 is situated near the underpass within the campus of Kafkas University. This station is primarily influenced by nearby residential areas. Station 3 is located near the Kars Museum, marking the endpoint of active settlement. At this station, the Kars River, which flows through the city center, is contaminated by substantial amounts of domestic and industrial waste, along with other pollutants. Additionally, livestock farming activities are also observed in this area.

2.1. Extraction of Water and Sediment Samples

In this study, 5 liters of water samples collected from the Kars River were filtered using filter paper. Subsequently, 50 mL of hexane was added to 1000 mL of the filtered water sample, which was then stirred vigorously for a specific period. After the addition of hexane, phase separation was observed, and the samples were transferred into separate bottles. Water samples collected from the designated stations were initially extracted with hexane, followed by three successive extractions using chloroform. The solvents were removed using a rotary evaporator. The obtained water extracts were transferred into 1.5 mL Eppendorf tubes and prepared by dissolving them in dimethyl sulfoxide (DMSO). These extracts were stored at +4 °C (Singh et al., 1987; Güzey, 2013).

For sediment samples, surface-layer sediments were collected using a Van Veen Grab sampler and a metal spatula to a depth of approximately 2 cm. The samples were placed in single-use petri dishes and dried in an oven at 60 °C. The dried sediment samples were ground into a fine powder using a porcelain mortar. From the powdered sediment, 0.1 g was weighed and transferred to Eppendorf tubes. Each tube was then treated with 1 mL of chloroform and hexane, followed by vortexing. The tubes were centrifuged at 7000 rpm for 10 minutes. The supernatant obtained during centrifugation was transferred to separate tubes, and this process was repeated three times. The collected supernatants were evaporated at +40 °C to remove the organic solvents. The resulting extracts were dissolved in DMSO (100%) and stored at +4 °C for further use in the study (Keijzer et al., 2000; Güzey, 2013).

The study utilized the TA98 and TA100 strains of *Salmonella typhimurium*, which were developed from the ancestral strain through in vitro mutagenesis studies conducted by Prof. Dr. Ames and Dr. Maron in 1971. The preparation of master plates and stock cultures of the *Salmonella typhimurium* TA98 and TA100 strains, verification of their genetic properties, and mutagenicity studies were performed using the plate incorporation method developed by Maron and Ames (1983).

When calculating the mutagenic effect, the number of revertant colonies was compared to the revertant colonies in the negative control. If the observed value was at least twice as high, the sample was considered mutagenic. Additionally, if an increase in the number of revertant colonies was proportional to the concentration, the sample was classified as

weakly mutagenic (Mortelmans & Zeiger, 2000). The control of histidine amino acid requirements, *uvrB* mutations, *rfa* mutations, R-resistance factor (RF), spontaneous reverse mutation frequencies, and negative controls were also carried out as part of the study.

Hexane and chloroform extracts were prepared from the water and sediment samples collected from the Kars River. Four different dilutions (10^0, 10^{-1}, 10^{-2}, and 10^{-3}) were prepared for both water and sediment extracts. These dilutions were tested separately at four different concentrations, with each concentration repeated independently three times.

Experiments were performed with *S. typhimurium* TA98 and TA100 mutant strains in the absence of metabolic activation (S9). Spontaneous control, negative control, and positive control groups were included. In the absence of S9, 4-nitro-o-phenylenediamine (NPD) was used as the positive control for the TA98 strain, while sodium azide (SA) was used for the TA100 strain. NPD was applied at 10 µg/petri, and SA was applied at 1 µg/petri in the positive control group. Dimethyl sulfoxide (DMSO), which was used to dissolve the water and sediment extracts, was applied to the negative control group.

3. RESULTS

Water and sediment samples were collected from three designated stations along the Kars River. In the experiments, the averages and standard deviation values of revertant colony counts for the negative control, spontaneous control, and positive control groups were calculated for water and sediment extracts tested on *S. typhimurium* TA98 and TA100 strains. The results are presented graphically in Figures 2, 3 and 4.

When calculating the mutagenic effect value, the number of revertant colonies was compared to the number of revertant colonies in the negative control (NC). If the result was twice as high, it was considered mutagenic. Additionally, when an increase in the number of revertant colonies occurred in a concentration-dependent manner, it was evaluated as weak mutagenicity.
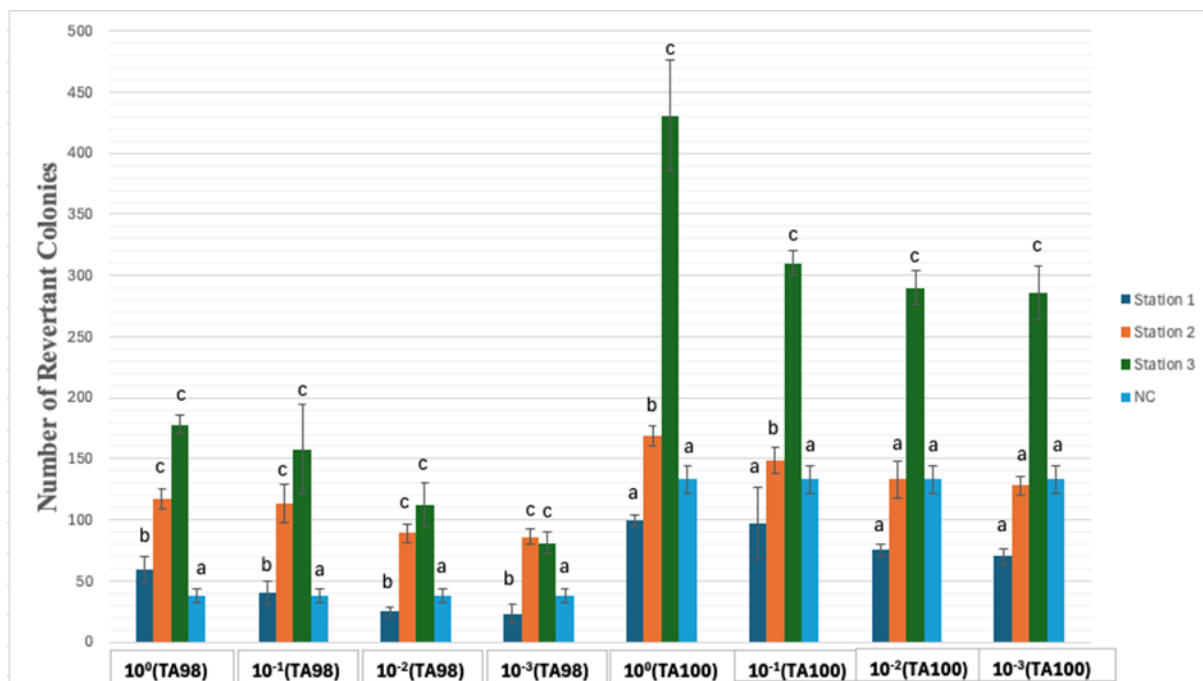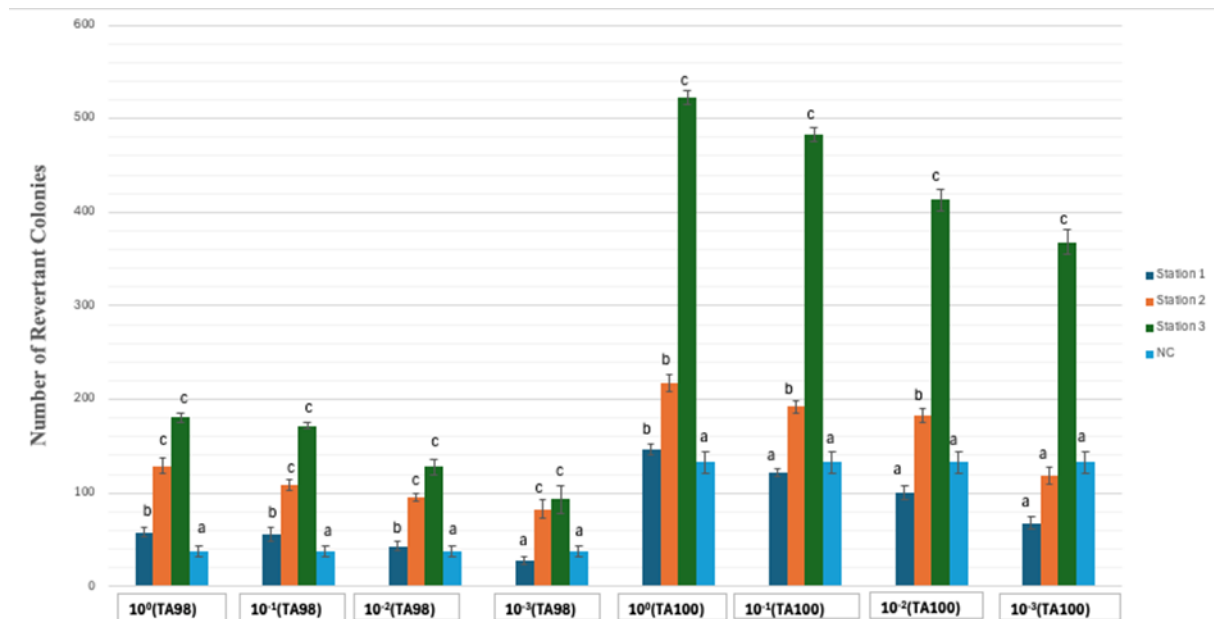
*(a: no mutagenic effect, b: weak mutagen, c: mutagen)

Figure 2. Effects of Hexane Extracts at Concentrations of 10^0, 10^{-1}, 10^{-2}, and 10^{-3} from Water Samples Collected from Stations 1, 2, and 3 of Kars River on *S. Typhimurium* TA98-TA100 Strain in the Absence of S9.

The effects of hexane extract obtained from water samples collected from the 1., 2., and 3. stations of the Kars River on *S. Typhimurium* TA98 strain were analyzed, and the results are presented in Figure 2. At the 1. station, all concentrations of the hexane extract exhibited weak mutagenic activity (b) compared to the negative control values. However, at the 2. and 3. stations, mutagenic activity (c) was observed at all concentrations.

In the TA100 strain, no mutagenic activity was observed at the 1. station. In contrast, the 3. station showed mutagenic activity (c) across all concentrations. At the 2. station, weak mutagenic activity (b) was detected only at 10^0 and 10^{-1} concentrations.
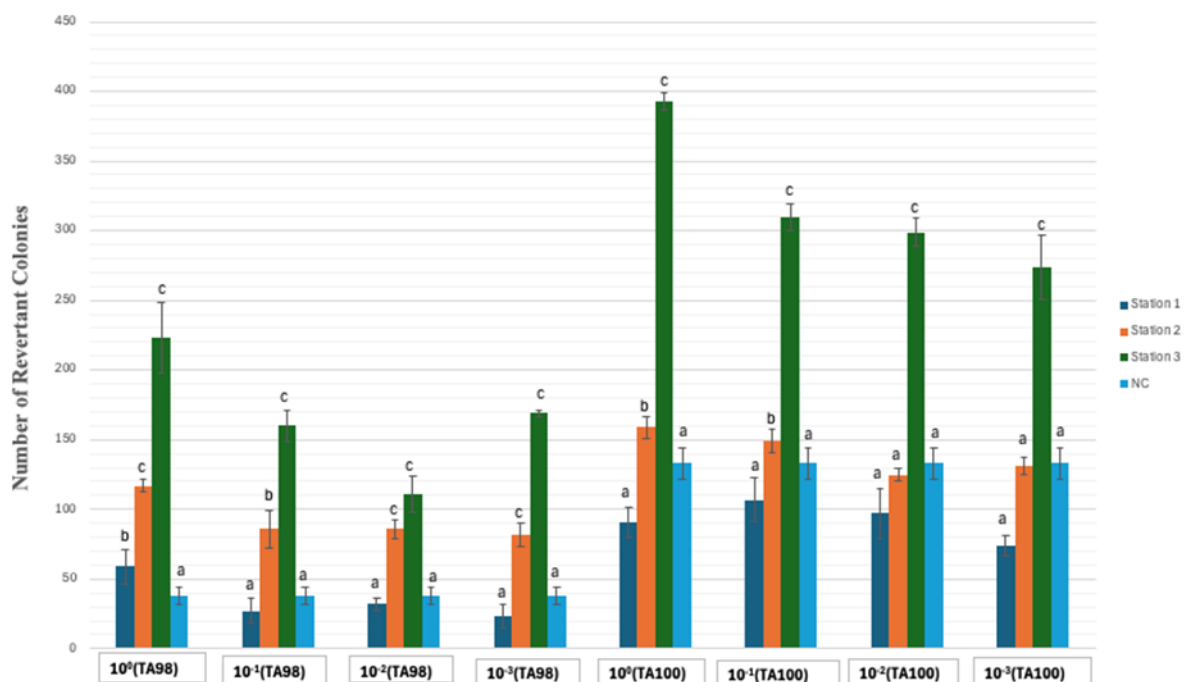
*(a: no mutagenic effect, b: weak mutagen, c: mutagen)

Figure 3. The Effects of Chloroform Extracts from Water Samples Collected at Stations 1, 2, and 3 of the Kars River at Concentrations of 10^0, 10^{-1}, 10^{-2}, and 10^{-3} on *S. Typhimurium* TA98-TA100 Strain in the Absence of S9.

The effects of chloroform extract obtained from water samples collected from the 1., 2., and 3. stations of the Kars River on *S. Typhimurium* TA98 strain were analyzed, and the results are presented in Figure 3. At the 1. station, weak mutagenic activity (b) was observed at the concentrations of 10^0, 10^{-1}, and 10^{-2}. However, at the 2. and 3. stations, mutagenic activity (c) was detected at all concentrations.

In the TA100 strain, weak mutagenic activity (b) was observed only at the 10^0 concentration at the 1. station, while no mutagenic activity (a) was detected at other concentrations. At the 3. station, mutagenic activity (c) was observed across all concentrations. At the 2. station, weak mutagenic activity (b) was detected only at the concentrations of 10^0, 10^{-1}, and 10^{-2}.

*(a: no mutagenic effect, b: weak mutagen, c: mutagen)

Figure 4. The Effects of Extracts from Sediment Samples Collected at Stations 1, 2, and 3 of the Kars River at Concentrations of 10^0, 10^{-1}, 10^{-2}, and 10^{-3} on *S. Typhimurium* TA98-TA100 Strain in the Absence of S9.

The effects of sediment samples collected from the 1., 2. and 3. stations on the *S. Typhimurium* TA98 strain were analyzed, and the results are presented in Figure 4. At the 1. station, weak mutagenic activity (b) was observed only at the 10^0 concentration. At the 2. station, mutagenic activity (c) was detected at concentrations of 10^0, 10^{-2}, and 10^{-3}. At the 3. station, mutagenic activity (c) was observed at all concentrations.

In the TA100 strain, no mutagenic activity was observed at the 1. station. At the 3. station, mutagenic activity (c) was reported at all concentrations, while at the 2. station, weak mutagenic activity (b) was detected only at the concentrations of 10^0 and 10^{-1}.

Sediment samples, particularly those from the 3. station, demonstrated strong mutagenic activity at all concentrations in both strains. This finding indicates that the contaminants accumulated in the sediment create a more intense mutagenic effect compared to water samples.

In conclusion, there is significant variation in environmental pollution levels among the stations, and this pollution plays a critical role in influencing mutagenic effects.

As the dilution factor decreased, a reduction in the number of revertant colonies was noted. This observation clearly demonstrates that the mutagenic effect is concentration-dependent.

Substances exhibiting concentration-dependent mutagenicity are commonly observed among environmental pollutants or chemicals.

The mutagenicity tests revealed no potential mutagenic effects in the hexane and chloroform extracts of water or sediment samples from the 1. Station. However, at the 2. station, mutagenic effects leading to frameshift mutations were detected. At Station 3, mutagenic effects causing both frameshift mutations and base pair substitutions were observed.

4. DISCUSSION AND CONCLUSION

This study aimed to assess the pollution level of the Kars River by determining the presence of mutagenic materials stored in sediments and dissolved in water. The investigation of mutagenicity was conducted using the Salmonella/Ames test with TA98 and TA100 strains in an environment lacking metabolic enzymes (without S9). Water and sediment samples collected from three different stations were subjected to doses of 10^0, 10^{-1}, 10^{-2}, and 10^{-3}. The experiments were independently repeated three times for each of the four concentration doses. As a result of the mutagenicity tests, no potential mutagenic effects were observed in the water samples (hexane and chloroform extracts) and sediment samples collected from Station 1. However, at Station 2, a mutagenic effect causing only frameshift mutations was detected. At Station 3, mutagenic effects causing both frameshift mutations and base pair substitutions were observed.

When the results are evaluated, it is evident that a weak mutagenic effect was detected at Station 1. The mutagenic effect increased at Station 2 compared to Station 1, and the highest mutagenic effect was observed at Station 3. The mutagenic potential of water and sediment samples shows similarities across the stations. The low mutagenic effect at Station 1 and the highest mutagenic effect at Station 3 can likely be attributed to the passage of the Kars River through the city center of Kars Province. Active residential areas, shopping centers, and livestock waste contribute to the pollution of the Kars River. Since Station 3 is located on

the outskirts of the city, the high mutagenic effect observed there can be linked to these factors.

In the experiments conducted, the number of revertant colonies observed in the spontaneous, negative, and positive control groups was found to be consistent with previously reported studies in the literature (Maron & Ames, 1983; Mortelmans & Zeiger, 2000; Güzey, 2013; Çakmak, 2013).

In a study investigating the mutagenic effects of the Nagara River and its sediments using the Ames test system, samples were subjected to extractions with different solvents. The study focused on mutagenic activity using the *S. typhimurium* TA100 strain in the presence of S9 (+). The highest mutagenic effect was observed in the isooctane-benzene group (Sato et al., 1983).

Another study revealed that surface waters associated with industrial activities exhibited mutagenic effects. Additionally, the mutagenicity of water entering and exiting treatment plants was higher compared to surface waters, emphasizing the need to evaluate the efficiency of treatment plants (Vargas et al., 1995).

The mutagenicity of drinking water was tested in another study, and positive results were reported for both strains (TA98-TA100). This study indicated that chemical agents capable of causing both base pair substitutions and frameshift mutations might be present in drinking water (Tortora, 1992).

Sediment samples collected from the Adriatic Sea were extracted using petroleum ether and methanol and analyzed with the Ames test using the *S. typhimurium* TA98 strain in the presence of S9 (+). Mutagenic activity was reported in five out of the seven extracts tested (Picer et al., 2001).

A study conducted by Boyacıoğlu investigated sediment samples from İzmir Bay (inner, middle, and outer regions) using the Ames test system with TA98 and TA100 strains. The study reported various forms of pollution across the regions and compared their mutagenic effects (Boyacıoğlu, 2004).

Further studies on water and sediment samples from other rivers, including the Karasu Stream in Bilecik (Ateş, 2011), the Tunca, Meriç, Arda, and Ergene Rivers (Soylu, 2012), and

the Tunca River (Güzey, 2013), have shown varying levels of mutagenic effects, emphasizing the importance of studying pollution in aquatic ecosystems.

The increase in the human population, continuous industrial growth, urbanization, and agricultural activities are contributing to ecosystem degradation. Polluted wetlands directly threaten human and environmental health.

In this study, water and sediment samples from the Kars River revealed no potential mutagenic effect in water samples from Station 1, while a mutagenic effect causing frameshift mutations was observed at Station 2. At Station 3, both frameshift mutations and base pair substitutions were observed. In particular, the samples from Station 3 indicated a high level of pollution, suggesting a potential mutagenic risk.

Considering these findings, it is essential to implement measures to minimize aquatic pollution, which causes genotoxic effects in living organisms. Controlling the use of pesticides in agricultural areas, filtering domestic and industrial waste before it is released into water sources, reducing plastic usage, and avoiding products that cannot decompose naturally are critical steps toward preventing pollution.

The method used in this study (Ames test) is a preliminary step in determining mutagenic activity. To ensure the reliability of the findings, in vivo and in vitro genotoxicity tests should be conducted, and the results should corroborate those of the Ames test.

Statement

This article is derived from the Master's thesis of Beyza SEZEN.

Conflict of Interest

The authors declare that there is no conflict of interest among them.

Author Contributions

The planning of the research was carried out by P. Aksu-Kılıçle, data collection was conducted by B. Sezen, and data analysis was performed by P. Aksu-Kılıçle and B. Sezen.

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