# NATIONAL & INTERNATIONAL SCIENTIFIC EVENTS

IEEE 40th International Conference on Consumer Electronics

Venue: Tuscany Suites and Casino
Location: Las Vegas, USA

Begins: January 07, 2022
Ends: January 09, 2022

12nd International Conference on Power Energy and Electrical Engineering (CPEEE 2022)

Venue: Biwako-Kusatsu Campus of Ritsumeikan University
Location: Shiga, Japan

Begins: February 25, 2022
Ends: February 27, 2022

16th European Conference on Antennas and Propagation (EuCAP 2022)

Venue: IFEMA Palacio Municipal
Location: Madrid, Spain

Begins: March 27, 2022
Ends: April 01, 2022

20th International Conference on Soil Mechanics and Geotechnical Engineering (ICSMGE 2022)

Venue: International Convention Centre Sydney
Location: Sydney, Australia

Begins: May 01, 2022
Ends: May 05, 2022

8th European Congress on Computational Methods in Applied Sciences and Engineering

Venue: Norway Convention Center
Location: Oslo, Norway

Begins: June 05, 2022
Ends: June 09, 2022

The 75th IIW Annual Assembly and International Conference

Venue: Grand Nikko Tokyo Daiba
Location: Tokyo, Japan

Begins: July 17, 2022
Ends: July 22, 2022

The 35th International Conference on Micro Electro Mechanical Systems

Venue: Tokyo International Forum
Location: Tokyo, Japan

Begins: January 09, 2022
Ends: January 13, 2022

16th International Conference on Martensitic Transformation (ICOMAT 2022)

Venue: Ramada Plaza Jeju Hotel
Location: Jeju, Korea

Begins: March 13, 2022
Ends: March 18, 2022

27th International Conference on Database Systems for Advanced Applications (DASFAA-2022)

Venue: Online Conference
Location: Hyderabad, India

Begins: April 11, 2022
Ends: April 14, 2022

The 31st IEEE International Symposium on Industrial Electronics (IEEE ISIE 2022)

Venue: Dena'ina Center/Egan Center
Location: Alaska, USA

Begins: June 01, 2022
Ends: June 03, 2022

39th IAHR World Congress

Venue: IAHR World Congress
Location: Granada, Spain

Begins: June 19, 2022
Ends: June 24, 2022

26th International Conference on Pattern Recognition

Venue: Palais des congrès de Montréal
Location: Montréal, Canada

Begins: August 21, 2022
Ends: August 25, 2022

In this study, a machine learning based liquid control system that can be used in airports, railway stations and shopping malls as well as in places with high fire probability is proposed.

In this research, some physical and chemical properties of the biocomposite obtained from synthesized epoxy modified palm oil (MPO) and epoxy resin have been characterized.
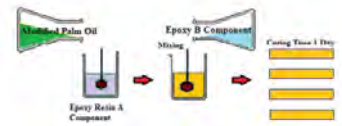
In the present study, we aimed to investigate exogenous effect bFGF supplementation on serum TBARS, RSH and NOx levels in hyperglysemic rats.

In this study, ethanol, ethyl acetate and methanol extracts of Tribulus terrestris L. plant, which is used by local people as one of these medicinal plants in Kars province (Turkey), were obtained by ultrasound assisted extraction method.

This study aimed to remove Hg (II) ions from wastewater by using ground rice grains as adsorbents.

In the present study, it was determined that COMT gene was significantly less expressed in PCPG than in normal tissue and COMT gene showed a remarkable relationship between differential expression with shorter overall survival among the individuals with PCPG (HR=1, p=0.011).

# HITTITE
JOURNAL OF SCIENCE & ENGINEERING

This new issue of Hittite Journal of Science and Engineering contains ten manuscripts from the disciplines of chemistry, molecular biology and genetics, bioengineering, chemical engineering, computer engineering, geological engineering and food engineering. These manuscripts was first screened by Section Editors using plagiarism prevention software and then reviewed and corrected according to the reviewer's comments.

I would like to express my gratitude to all our authors and contributing reviewers of this issue. I would like to thank to the new President of Hitit University, Prof. Dr. Ali Osman Öztürk, for his support and interest in HJSE and also to the Section Editors of HJSE, namely Prof. Dr. Dursun Ali Kose and Assoc. Prof. Dr. Oncu Akyildiz, as well as our Production Editors Assoc. Prof. Dr. Kazim Kose, Mustafa Reşit Haboğlu, Dr. Erhan Çetin, Tugrul Yıldırım, Harun Emre Kıran and Ömer Faruk Tozlu for their invaluable efforts in making of the journal.

It's my pleasure to invite the researchers and scientists from all branches of science and engineering to join us by sending their best papers for publication in Hittite Journal of Science and Engineering.

Prof. Dr. Ali Kilicarslan

Editor-in-Chief

# A New Approach for Liquid Scanners to Determine Flammable Liquid Concentration in Solutions

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A B S T R A C T

Strong liquid explosives were obtained by mixing some chemical liquids and these explosives were used in many terrorist attacks in crowded places such as airports, railway stations and shopping malls. They were also used to cause sabotage to facilities that produce, store or use hazardous chemicals in their processes. For this reason, it is very important to take the necessary measures to prevent sabotage and terrorist attacks that may occur in such places in order to ensure public and environmental safety. In this study, a machine learning based liquid control system that can be used in airports, railway stations and shopping malls as well as in places with high fire probability is proposed. The difference of the proposed system from traditional liquid scanner systems is that it can detect the hazardous liquid concentration in the solutions as well as the detection of pure flammable liquids. Linear Discriminant Analysis and Quadratic Discriminant Analysis are used as classifiers and the performances of these techniques are compared. The results show that Quadratic Discriminant Analysis offers higher accuracy and lower error rates compared to Linear Discriminant Analysis.

**Keywords:**
Security; Liquid classification; Scattering parameter; Linear discriminant analysis; Quadratic discriminant analysis; Accuracy; Performance metrics

## INTRODUCTION

Fire pools can be formed by mixing hazardous chemical liquids; on the other hand, mixing incompatible chemicals can cause exothermic oxidation [1]. Fires involving self-incineration may accelerate depending on the nature of the first spilled liquid and its proximity to the surrounding material. Hazardous chemical reactivity events have been conducted and lessons learned from these cases as presented in [2]. In most cases, oxidisers have caused these fires to start or have contributed to the increasing coverage of the fires [3]. The mixing of incompatible liquids during the use of chemical liquids found in small containers with open covers showed that accidents and fires occurred as a result of accidental spillage and contamination [4]. Therefore, classification of liquids plays an important role in ensuring fire safety [5]. Considering this, fire and explosion hazards of some flammable liquid mixtures were estimated [6] .

Most of the deaths in fires are caused by the inhalation of toxic gases produced during combustion, since it creates a complex toxic environment that includes fire, flame, heat, oxygen depletion, smoke and toxic gases [7].

Researchers have provided methods for assessing life safety hazards in fires and understanding the effects of smoke, heat and toxic fire wastes on humans [8]. The use of machine learning techniques for process safety has been heavily investigated. For example, by considering aerosolisation liquid flammability levels were predicted using machine learning techniques [9]. Performance estimation of suspension freezing crystallization was made for the treatment of hazardous liquid wastes with machine learning methods [10]. In addition, machine learning was used to predict flammability leading properties for liquid aerosol safety [11], to predict hazardous properties of chemical mixtures [12], to set a hazard index for logistics warehouses [13]. Classification of diesel and biodiesel mixtures was carried out using the electronic nose and Linear Discriminant Analysis (LDA) and Quadratic Discriminant Analysis (QDA) techniques [14]. A combustion risk index was developed for flammable liquids based on unsupervised clustering algorithms [15]. Microwave measurement method is fast and is not sensitive to environmental conditions [16] and it is generally used to determine the relative perme-

ability values of liquids. Microwave measurement methods were used to measure the permeability of thin-layer materials [17] and to measure parameters of silicon [18]. Although there are many microwave measurement methods, the most frequently used one is the open-ended coaxial probe (OECP) method. In this method, liquid measurement is carried out by immersing a probe in the liquid. The complex permeability of glucose / water and water / fluoride solutions was estimated using OECP and artificial neural networks [19],[20].

In the past, X-ray safety systems were used for the detection of hazardous liquids [21] and low energy X-ray transmission technique was one of the techniques employed for this purpose [22]. As well as the low energy X-ray transmission technique, spectral droplet analysis was used for the same purpose [23]. However, since these methods cannot accurately detect some flammable liquids, in other words, the false alarm rate is high, two-stage control consisting of the combination of electronic nasal odour recognition technology and x-ray method has been proposed [24]. In this study, unlike the literature, a liquid identification system with high accuracy, fast and cheaper than other systems is proposed. This proposed system is capable of detecting even a mixture containing 10% hazardous liquid. Also, thanks to this system, unlike other systems, the hazardous liquid concentration in the mixtures can be determined. The measurement system presented in the study can analyse the liquid remotely without any intervention to the liquid and without opening the lid of the bottle / container filled with liquid, as well as measuring by immersion in the liquid.

## MATERIALS AND METHODS

### Discriminant Analysis Methods
In the following subsections LDA and QDA are reviewed.
### LDA
LDA is a simple and useful classification technique that gives good results in solving complex problems. It performs the separation of classes by searching for the linear combination of variables. The discriminant function is the weighted average of the values of the independent variables. These weights are chosen to divide observations into groups. The discriminant function (L) is given in Eq. (1).

$$L = a_1 x_1 + a_2 x_2 + \ldots + a_n x_n \tag{1}$$

In (1), $x_1, x_2 \ldots \ldots x_n$ represent the variables and $a_1, a_2 \ldots \ldots a_n$ represent the weights, model coefficients. The weights are calculated using (2).

$$a = c^{(-1)} (b_1 - b_2) \tag{2}$$

where c represents covariance matrix, $b_1$ and $b_2$ are mean vectors.

The discriminant function is obtained from previously known units of group membership, and then this function is used to determine which group will be assigned to new units with unknown group membership. Using a score function defined by the algorithm, linear coefficients that give the highest values in the function are found (3). The aggregated covariance matrix is given in (4).

$$S(a) = \frac{a^T b_1 - a^T b_2}{a^T c a} \tag{3}$$

$$c = \frac{1}{n_1 + n_1} (n_1 c_1 + n_2 c_2) \tag{4}$$

The Mahalanobis distance is used to determine the best discriminate. The probability that the algorithm has classified correctly determines the value of this distance. A value less than 3 means that the probability of correct classification is high. The Mahalanobis distance between the two groups is given in (5).

$$d^2 = a^T (b_1 - b_2) \tag{5}$$

In order to end the classification process, the condition given in (6) must be met.

$$a^T \left( x \left( \frac{b_1 + b_2}{2} \right) \right) > log \left( \frac{P(c_1)}{P(c_2)} \right) \tag{6}$$

Here p shows class probabilities.

### QDA
QDA has quadratic decision limits. Using it, data can be classified into two or more class datasets. It is generally used when the data show normal distribution and the variance-covariance matrices between groups are different. While applying this technique, it should be taken into consideration that the number of observations in each group should be more than the number of variables. The difference of QDA from LDA is that it estimates the covariance matrix for each class. The function specified in (7) is used.

$$L_k(x) = -\frac{1}{2}(x - b_k)^T c_k^{-1} (x - b_k) - \frac{1}{2} ln|c_k| + lnP(c_k) \tag{7}$$

where $c_k$ is the covariance matrix for class k, $c_k^{-1}$ is the inverse of the covariance matrix, and $|c_k|$ is the determinant of the covariance matrix, $P(c_k)$ is the previous probability of class k. Here, the aim is to find the class with the highest L value.

**Experimental Setup and Methodology**

The schematic representation of the single-port measurement system with the test setup is shown in Fig. 1. The arrangement consists of a 10 cm x 10 cm square-shaped antenna, vector network analyser (VNA) and 50 Ohm SubMiniature version A (SMA) coaxial cable to feed the system. Electromagnetic waves are transmitted by the transmitting antenna and reflected signals are collected after the electromagnetic radiation interacts with the liquid. These signals collected by a single-port measuring system are called scattering parameters (S11-parameter). The antenna patch was designed to be 55 mm in diameter and the antenna resonant frequency is calculated using (8) and (9). The design of the antenna was constructed on a FR4 based dielectric substrate with 1.6 mm height, 4.4 relative permittivity.

$$F = \frac{8,791x10^9}{f_r\sqrt{\varepsilon_r}} \tag{8}$$

$$a = \frac{F}{\left\{1 + \dfrac{2h}{\pi\varepsilon_r F\left[\ln\left(\dfrac{\pi F}{2h}\right) + 1,7726\right]^{1/2}}\right\}} \tag{9}$$

where $\varepsilon_r$ is relative permittivity of the substrate, $f_r$ is the resonant frequency, h is the height of the substrate, a is the radius of the patch.

The implementation of this method is easy and fast. Since there is an air gap between the measured liquid and the antenna, measurements should be performed by keeping the antenna as close as possible to the liquid container in order to make more accurate measurements. Liquid measurements can be carried out in closed containers, without opening the lid, non-destructively and by approaching the container filled with liquid 4-5mm. In the study, measurements were made between 1-3 GHz and the S-parameter of each liquid in this frequency band was measured. The measurements were made at room temperature and in the same type of containers. Antenna-liquid distance was 5 mm. Flammable liquids should not be stored in the same place
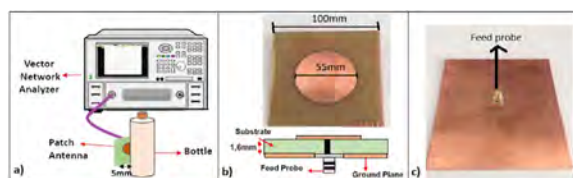
with oxidising liquids, and their mixing as a result of any impact should be prevented. Because oxidising liquids enter into exothermic reactions with flammable liquids and cause fires and explosions. For this reason, the hazardous liquid recognition system we recommend consists of 3 stages. It makes the S-parameter measurement using a liquid measurement system of unknown type and at the first stage, from this measurement, it decides whether there is a hazardous liquid such as flammable or oxidant in the liquid. It tells you that the liquid is safe if there is no hazardous liquid concentration in the liquid. In step 2, if there is a hazardous liquid concentration in the liquid content, it decides the type of the liquid, i.e. whether it is flammable or oxidant (oxidiser). In the third stage, if the liquid is a flammable liquid, it finds the type of liquid (Methanol, ethanol, 1-propanol and Isopropanol) and % of the flammable concentration in the liquid. The steps of the algorithm are given in Fig. 2.
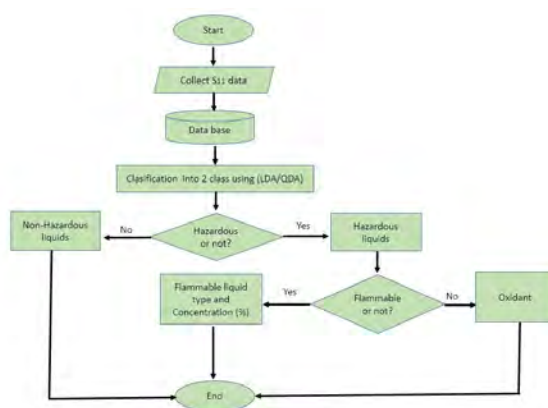


**Figure 2.** Flowchart of the classification phase

For liquid identification, predictions were made for a total of 49 liquids, 41 hazardous and 8 non-hazardous liquids. In this prediction, 2 different algorithms were used to select the most successful algorithm and the performances of the algorithms were compared. The types of the liquids tested are given in Table 1.

**Table 1.** Liquid types.

| Hazardous liquids | | | Non-hazardous liquids |
|---|---|---|---|
| Inflammable | | Oxidant | |
| Pure | Impure | | |
| Ethanol | Ethanol-water (10%-90%) | Hydrogen peroxide | Cola |
| Methanol | Methanol-water (10%-90%) | | Liquid Soap |
| 1-propanol | 1-propanol-water (10%-90%) | | Shampoo |
| Isopropanol | Isopropanol-water (10%-90%) | | Milk |
| | | | Body lotion |
| | | | Buttermilk |
| | | | Ice-tea |
| | | | Cherry juice |



**Figure 1.** Experimental setup a) The measurement system b) Front view and structure of the antenna c) Back view of the antenna.
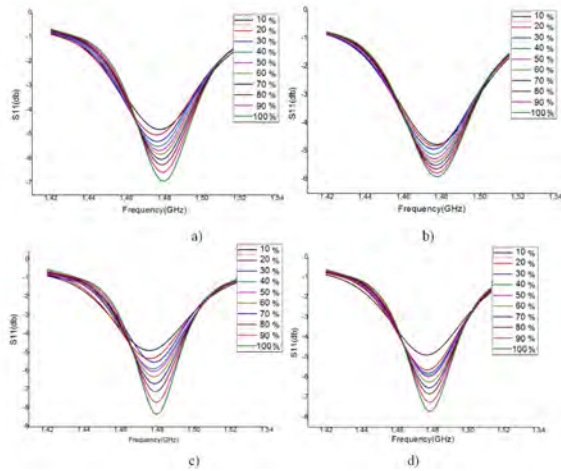
**Figure 3.** $S_{11}$ parameters of the water- flammable liquids solutions of different flammable liquid concentrations a) Ethanol b) Methanol c) 1-Propanol, d) Isopropanol.

## RESULTS AND DISCUSSION

The proposed microwave measurement system was used to measure all liquids. S parameter measurement of pure (100% flammable liquids) and their aqueous solutions containing 10-90% flammable liquid by volume are given in Fig. 3 and the measurement of other liquids are given in Fig. 4.
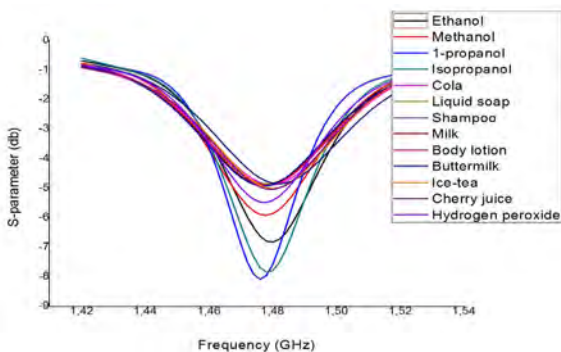


**Figure 4.** S-parameter measurement of different liquids

### Performance Metrics

There are some performance metrics used to compare the classification performance of algorithms. These metrics indicate which classification algorithm performs better in the given setting. One of the performance metrics used in the study is the accuracy criterion, which gives the total sample rate of correctly classified samples. The accuracy rate of the classification algorithm is calculated using (10).

$$\text{Accuracy} = \frac{TP + TN}{TP + TN + FP + FN} \tag{10}$$

where, TP (True positive) is positive test result when actual state is positive, FP (False positive) is positive test result when actual state is negative, TN (True negative) is negative test result when actual state is negative, and FN (False negative) is negative test result when actual state is positive.

Precision and Recall values are also among the performance metrics used and it is accepted that a classifier with high Precision and high Recall values makes a good classification. Precision (P) is the number of TP over the number of TP plus the number of FP (11).

$$P = \frac{TP}{TP + FP} \tag{11}$$

Recall (R) is the number of TPs over the number of TP plus the number of FN (12).

$$R = \frac{TP}{TP + FN} \tag{12}$$

Another commonly used performance metric is Kappa and is calculated using (13). P(a) represents the algorithm's accuracy, P(e) represents the weighted average of the expected accuracy of the algorithm, which makes random predictions in the same dataset. If a classification is successful, precision and recall values become close to 1.

$$K = \frac{P(a) - P(e)}{1 - P(e)} \tag{13}$$

One of the indicators of how many errors occurred during classification is Root Mean Square Error (RMSE) value and it is calculated using (14).

$$RMSE = \sqrt{\frac{(a_1 - b_1)^2 + \dots + (a_n - b_{1n})^2}{n}} \tag{14}$$

where a represents the estimated values and b represents the actual values. Confusion matrices are used to measure the success of the algorithm and contain the most descriptive information about the classification results.

### Overall Results

The recall precision, Kappa and RMSE values obtained from the classification made using LDA and QDA are given in Fig. 5(a) and (b) in order to compare their performances. As can be seen in Fig. 5(a) the RMSE of QDA was 0.008 but the RMSE of LDA was 0.075. The low RMSE value indicates that QDA classified with fewer errors. While the accuracy of LDA was 92%, the accuracy of QDA was 98%. Recall, Precision and Kappa value also indicate the success of QDA. The Precision value of LDA in classifications was 0.97, the Recall and Kappa values were 0.96 and 0.91, respectively. The higher of these values (precision 1, recall 0.99 and Kappa 0.99) indicates that

**Table 2.** Accuracy of the proposed approach for different liquids

| | | LDA | | QDA | |
|---|---|---|---|---|---|
| *Type of liquids* | *Tested liquids* | *Correctly predicted liquids* | Incorrectly predicted liquids | Correctly predicted liquids | Incorrectly predicted liquids |
| **1st Step** Non-hazardous liquids | *Cola Soap, Shampoo Milk Body lotion Buttermilk Ice-tea (peach) Cherry juice* | *Cola Soap, Shampoo Milk Body lotion Ice-tea (peach) Cherry juice* | *Buttermilk* | *Cola Soap, Shampoo Milk Body lotion Buttermilk Ice-tea (peach) Cherry juice* | --- --- --- --- --- --- --- --- |
| Hazardous Liquids | *Ethanol (10, 20, 30, 40, 50, 60, 70, 80, 90, 100)% Methanol (10, 20, 30, 40, 50, 60, 70, 80, 90, 100)% 1-Propanol (10, 20, 30, 40, 50, 60, 70, 80, 90, 100)% Isopropanol (10, 20, 30, 50, 60, 70, 80, 90, 100)%* | *Ethanol (20, 30, 40, 50, 60, 70, 80, 90, 100)% Methanol (20, 30, 40, 50, 60, 70, 80, 90, 100)% 1-Propanol (10, 20, 30, 40, 50, 60, 70, 80, 90, 100)% Isopropanol (10, 20,30, 40, 50, 60, 70, 80, 90, 100)%* | *Ethanol %10 Methanol %10* | *Ethanol (10, 20, 30, 40, 50, 60, 70, 80, 90, 100)% Methanol (10, 20, 30, 40, 50, 60, 70, 80, 90, 100)% 1-Propanol (10, 20, 30, 40, 50, 60, 70, 80, 90, 100)% Isopropanol (10, 20, 30, 50, 60, 70, 80, 90, 100)%* | ---- ---- ---- ---- ---- ---- ---- ---- ---- ---- ---- ---- ---- ---- ---- ---- ---- ---- ---- |
| **2nd Step** Oxidising liquid | Hydrogen peroxide | Hydrogen peroxide | --- | Hydrogen peroxide | --- |
| **3rd Step** Detect flammable liquids concentration | Ethanol (10, 20, 30, 40, 50, 60, 70, 80, 90, 100)% Methanol (10, 20, 30, 40, 50, 60, 70, 80, 90, 100)% 1-Propanol (10, 20, 30, 40, 50, 60, 70, 80, 90, 100)% Isopropanol (10, 20, 30, 50, 60, 70, 80, 90, 100)% | Ethanol (10, 20, 30, 40, 50, 60, 70, 80, 90, 100)% Methanol (10, 20, 30, 40, 50, 60, 70, 80, 90, 100)% 1-Propanol (10, 20, 30, 40, 50, 60, 70, 80, 90, 100)% Isopropanol (10, 20, 30, 50, 60, 70, 80, 90, 100)% | Isopropanol 40% | Ethanol (10, 20, 30, 40, 50, 60, 70, 80, 90, 100)% Methanol (10, 20, 30, 40, 50, 60, 70, 80, 90, 100)% 1-Propanol (10, 20, 30, 40, 50, 60, 70, 80, 90, 100)% Isopropanol (10, 20, 30, 50, 60, 70, 80, 90, 100)% | ---- ---- ---- ---- ---- ---- ---- ---- ---- ---- ---- ---- *Isopropanol 40%* |

QDA was more successful.

Information including all liquid recognition experiments and techniques used are given in Table 2. In liquid recognition, the LDA algorithm predicted that buttermilk, which is actually a non-hazardous liquid, was a hazardous liquid, and the hazardous liquid 10% Ethanol and 10% Methanol aqueous solutions were predicted as nonhazardous liquids. It could not accurately predict a total of 3 types of liquids. While the algorithm was estimating the liquid concentration, it predicted the isopropanolwater solution with an isopropanol concentration of 40% as a hazardous liquid, but could not accurately predict its concentration. It can be seen that QDA correctly predicted all liquid types. On the other hand, although it correctly predicted the type of isopropanol-water solution with an isopropanol concentration of 40%, it could not predict the concentration correctly.

## CONCLUSION

Fire safety and liquid controls play a key role in preventing loss of life and property that may occur as a result of terrorist attacks and sabotage. In this study, a system for liquid classification using S-parameter measurements and discriminant analysis of liquids in the microwave frequency band is proposed. The false alarm rate of the system is very low, it is a system with a high accuracy rate and can detect even a hazardous liquid with a concentration of 10% in its content. Another advantage of this proposed system is that while other systems only detect liquid, this system can determine both the type of hazardous liquid and the proportion of the hazardous liquid concentration in the liquid. Moreover, this quick identification system is cheaper than other systems. LDA and QDA algorithms were used to select the best algorithm for liquid recognition on the data set obtai-
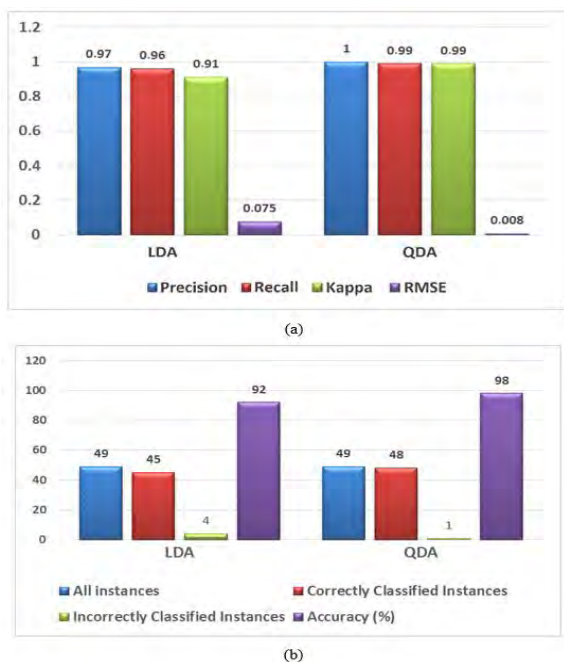
**Figure 5.** Performance metrics of LDA and QDA algorithms (a) Accuracy related metrics, (b) accuracy rate of LDA and QDA.

ned from microwave measurements and the performances of these algorithms were compared. The results show that QDA can detect liquids with lower RMSE values and higher accuracy rates compared to LDA. A prototype system that integrates the overall process proposed in this study and the experimental setup and uses QDA for liquid classification is under development. After a group of field tests, it can be used for liquid classification at security checkpoints.

## CONFLICT OF INTEREST

Authors approve that to the best of their knowledge, there is not any conflict of interest or common interest with an institution/organization or a person that may affect the review process of the paper.

## AUTHOR CONTRIBUTION

All the work in this study were performed equally by the authors.

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# Production and Characterization of Palm Oil Based Epoxy Biocomposite by Response Surface Methodology Design

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## ABSTRACT

In this research, some physical and chemical properties of the biocomposite obtained from synthesized epoxy modified palm oil (MPO) and epoxy resin have been characterized. The experimental study plan is made according to Response Surface Methodology (RSM) and biocomposites with different MPO rates are obtained. The chemical bond structure of MPO and epoxy biocomposite has been evaluated with Fourier Transform Infrared Spektrofotometre (FTIR). The experimental and RSM model results obtained, the density of the biocomposite rise as the MPO rate increases. It is determined that the Shore D hardness of the biocomposite is inversely proportional to the MPO rate by mass. As the MPO ratio (wt.%) increases in the biocomposite, the thermal conductivity coefficient and thermal stability also raise. In the thermal decomposition experiments of the obtained biocomposite, it is observed that the thermal stability of the composite goes up as the MPO rate rises. Activation energies are calculated using the Flynn Wall Ozawa, Kissinger, and Coats Redfern models. The activation energies calculated for the 9th, 2nd, and 13th experiments according to the Flynn Wall Ozawa method are approximately 139.65, 143.56, and 145.28 kJ/mol, respectively. The function $(f = (1-\alpha)^{1.273})$ with the highest $R^2$ value has been determined according to the Coats Redfern method, and the deviation in Flynn Wall Ozawa and Kissinger model results was below 7%.

**Keywords:**
Biocomposite; Modified palm oil; Sulfonamide; Characterization; Thermal decomposition.

## INTRODUCTION

Today, social awareness of the environmental impacts of plastics is increasing, and therefore environmentally friendly materials are sought for the plastics industry [1–4]. For this reason, in addition to being environmentally friendly, the tendency towards natural fibers is increasing due to their low cost, easy processing, low density, good corrosion resistance, and high strength in wide industrial applications [5-8, 9-14]. Moreover, natural fibers become an alternative to glass fibers by being applied to reinforced polymer composites and natural-based resins, as they contain hydrogen bonds and other bonds that reinforce the structure [15,16]. The use of bio-fibers as substitutes for synthetic fibers (carbon, and glass) as fillers in the development of polymer matrix composites has attracted much attention [17].

There is increased awareness about the properties of natural fiber-based epoxy composites to meet engineering requirements [18]. The use of epoxy composite materials reinforced with natural fibers is increasing strongly in many industrial areas, especially in the automotive sector [19-23], in civil construction [20], and marine production [21] due to their low cost of processing. German carmakers, soon followed by other manufacturers, took the lead in introducing natural fiber composites for interior and exterior applications; parcel shelves, door panels, mirror casing, backrests, voltage stabilizer cover, seat cushions, dashboard parts, projector cover helmet, roof linings, etc. In the civil construction area, they can be used for; beams, building panels, roofing products, autoclaved cement composite, and water tanks. For shipbuilding, the adoption of green composites can potentially represent a valid substitute for fiberglass. These include both purposely grown and harvested fibers, as well as those recovered from agricultural waste. Thanks to their recyclability and renewability, biocomposites allow them to comply with more and more

stringent environmental protection regulations [24,25] improving also the cost-effectiveness [26]. Increasing the mechanical performance of these materials is a mandatory task to spread their use not only in non-structural applications but also in semi and proper structural applications limited by their failure mechanisms [27].

Vijaya Ramnath et al. [28] conducted a study on the evaluation of mechanical properties of abaca–jute–glass fiber reinforced epoxy composite and revealed that abaca fiber had the highest flexural strength compared to jute fiber, with the values of 12.5 and 11.9 MPa, respectively, since its strength increased with improved interfacial adhesion. Besides that, Abaca exhibited more strength when it absorbed moisture.

Szolnoki et al. [29] reinforced twill woven hemp fabric with epoxy composites and discovered that the modification of the fabrics led to decreased flammability of the reference matrix composites, characterized with increased limiting oxygen index values and reduced heat release rate by 25%. Moreover, composites of modified fabric showed improvements in static and dynamic mechanical properties.

Pickering et al. [30] experimented on aligned short harakeke fiber (New Zealand flax) mats impregnated with epoxy resin. The result showed that these composites were found to possess significantly higher tensile properties at 46% fiber loading, than planar random-oriented short fiber composites, with the values of 136 MPa and 76.2 MPa, respectively. The epoxy resin is a feasible polymer, which has effective strength, good toughness, and appreciable resilience. It has good resistance to moisture and chemical attack. It also has great electrical insulating properties and is devoid of volatile matter [31].

Abu Bakar et al. [32], through their study, reported that one of the flaws of natural fibers is poor compatibility with its matrix. Moreover, studies done by Hassan et al. [33] showed that the recyclability of natural fiber within the automotive component had reduced the automotive weight. The use of biocomposite helped in a 25% reduction of vehicle weight, which consequently contributed to saving 39.45 trillion of crude oil [33]. Besides that, this material can be used for the composite frame in electromobility vehicles, as it will reduce energy consumption [9]. Currently, natural fibers are used as fillers to replace glass fiber in polymer composites [34].

The incompatibility and poor adhesion of natural fiber in a polymer matrix are usually addressed by fiber treatment and modification to enhance effective wetting and uniform dispersion. The primary techniques used for fiber treatment and modification can be grouped into fiber pretreat-

ment, surface coating modified with coupling agents, and in situ compatibilization during processing depending on the practical applications. Mercerization, a chemical treatment using alkali, is widely used to fibrillate and purify fibers (partially removing oil, wax, pectin, hemicellulose, and lignin) before composite fabrication.

For example, Rihayat et al. [35] and Wang et al. [36] have reported that biocomposites treated with alkaline solutions and silane fusing agents have twice the tensile strength of composites without temporary treatment. Mahmoud et al. [37], Kang and Kim [38] reported an increase in tensile strength, modulus of elasticity, and moisture resistance of biocomposites with the coupling agent in the composite matrix. Alkalisation refers to the treatment of fibers in an alkaline solution by dissolving some unstable fiber components such as hemicellulose, lignin, pectin, and other impurities so that the surface of the fiber becomes cleaner and rougher, which results in better mechanical interlocking between the fiber and the polymer. Lee and Wang [39], and Fan [40] have found that bamboo fibers treated at 6% NaOH produce the highest tensile properties of single fibers and matrix adhesion strength. Similar studies on other natural fibers using 5% NaOH concentration revealed comparable results, with higher NaOH concentrations causing a decrease in mechanical properties.

In many studies in the literature, vegetable oils have been modified and used in the synthesis of composites. Especially, biocomposite production can be made as a result of the epoxidation of triglyceride structures found in vegetable oils [41]. For example, when palm oil is modified by various processes, a biopolymer can be easily obtained by a chemical reaction [42]. It is known that the synthesized biocomposite, in which palm oil is used in the production of polyester composites, improves some of the thermophysical properties [43]. Evaluation of such similar sources in the production of biocomposites with epoxy resin is becoming more and more common. Because the epoxidation of triglyceride structures in vegetable oils easily offers many options for the development of bio-epoxy composites as raw materials. The advantages of the synthesized bio-epoxy composites such as being environmentally friendly, more thermally stable, and easy to process make such studies important [44].

The original aspect of this research is the synthesis of biocomposite using the modified epoxy palm oil. Unlike studies in the literature, palm oil has been modified that functional epoxy and hydroxyl structures are bonded for the production of biocomposite.

This study aims to treatment fiber with alkali where had effects on the mechanical properties improvement of natural fiber such as increased cellulose content and the

degree of crystallinity, which is indicative of higher fiber strength and stiffness; increased surface roughness topography for better mechanical interlocking between the fiber and matrix; increased cellulose exposure for increased bonding/reaction sites on the fiber surface; and increased surface energy for better wetting and compatibility. Treatment with a mild alkaline condition is typically sufficient to remove fiber impurities with minimal impact on the fiber texture and structure whereas higher alkaline concentration can lead to excessive removal of lignin and fiber damage. Furthermore, according to the study, the use of cellulosic fiber as reinforcement can reduce the material cost and at the same time raising strength to weight ratios [28].

## MATERIALS AND METHODS

### Materials

All chemicals used for the biocomposite production and analysis have been supplied from Merck and used without purifications. Epoxy raw material and hardener components are procured from Polisan (Turkey). Experimental studies use ethyl acetate (99.5%), ortho-phosphoric acid (85%), ethanol (99.5%), hydrochloric acid (37%), methanol (99.5%), and hydrogen peroxide (35%). Palm oil with a density of 904 kg/m$^3$ and a viscosity of 77 cP is supplied from commercial companies.

### Methods Used in The Experimental Study

In the experimental study, firstly palm oil (100 g) is mixed with acetic acid (45 g), ethyl acetate (10 g), and hydrogen peroxide (90 g). Phosphoric acid (0.02 g) is added to the mixture and stirred at 500 rpm and 60°C for 6 hours. After this process is completed, the light phase is recovered using a vacuum rotary evaporator. Then methanol (70 ml), ethanol (30 ml), and distilled water (100 ml) are added to the system. HCl (0.02 g) is dropped into the mixture and the aqueous and organic phases are separated after being mixed at 500 rpm and 70°C for 12 hours. The oil phase is washed with warm water until the pH is neutral. The modified epoxy palm-oil-based raw material (MPO) is obtained by removing impurities (methanol, ethanol, and water) with a vacuum rotary evaporator.

In the second stage, 5 g of MPO and 0.5 g of 4-(2-aminoethyl)benzene sulfonamide were placed in a flask. It was reacted in reflux state for 2 hours by adding 10 mL of ethanol. Then, the reaction temperature was brought to 60°C and the reaction was continued for 72 hours. At the end of the period, the second stage was passed. MPO was added to commercial epoxy medium according to the ratios indicated in 0-10 wt.% and mixed at 500 rpm for 15 minutes
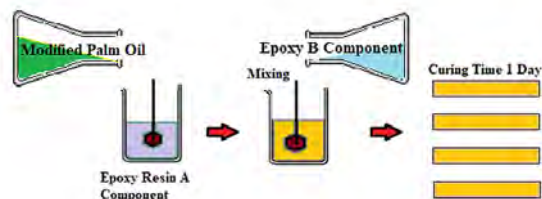


**Figure 1.** Experimental working schema for the biocomposite production.

to ensure the homogenization of the composite. Then, the hardener part was added to the medium and mixed at the same mixing speed for 15 minutes. At the end of the period, the solvent was removed with the evaporator and quickly transferred to the standard molds. It was left to cure for 24 hours at room conditions. Commercial epoxy resin has approximately 5/8 main components and 3/8 hardener components by mass. Biocomposite production was carried out according to the order in the schema in Fig. 1.

The chemical bond structure of the bio-epoxy composite has been analyzed with an FTIR spectrophotometer. The FTIR spectrum of each sample has been determined as transmittance (%) in the wavelength range of 600 cm$^{-1}$ to 4000 cm$^{-1}$. FTIR measurements have been made using Shımadzu QATR-S (IR Spirit S1102SC). The FTIR spectrum of biocomposites has been investigated by ATR method directly in solid powder form without making potassium bromide (KBr) pellets. Besides, the thermal decomposition of the biocomposite with the proportional integral derivative (PID) system, its hardness with the Shore D test, and its thermal conductivity coefficient (Thermtest TLS-100) with the thermal conductivity measuring device have been determined.

Thermal decomposition kinetics of biocomposites in an inert environment (nitrogen) have been investigated with the PID-controlled system. In this system, the temperature increase of 10 K/min from 25°C to 605°C is studied in non-isothermal conditions. The PID system has a total diameter of 19.5 cm, and a height of 21.5 cm. It is made of perlite-reinforced insulated mortar (5 cm thick), rock wool (1.5 cm thick), and aluminum plate (1 mm thick). The drying cell was placed in the cylindrical space (approximately inner diameter 6 cm) in the inner center of the reactor. The cylindrical drying cell has a diameter of 3.5 cm, a height of 3 cm, and a porous side surface area (nearly porous diameter 1-3 mm). The drying cell made of galvanized steel plate (1 mm thick) is placed in the center of the PID system. In this system, temperatures can be controlled very precisely with the help of thermocouples. A certain amount of sample (1 gram) can be taken and the temperature can be easily distributed on all surfaces to the conductive and porous cell.

## Thermal Decomposition Kinetics and Modeling

In model equations: $M_t$ is the mass at time $t$, $M_i$ is the initial mass and $M_f$ is the final mass. $\alpha$ is the conversion ratio, $\beta$ is the temperature rise ratio, $k(T)$ is the temperature-dependent function and $f(\alpha)$ is the conversion-dependent function. $k(T)$ expresses the thermal decomposition rate constant, it is also a function that changes depending on the temperature. In experiments, the temperature increase in non-isothermal conditions changes the thermal decomposition rate constant over time. $f(\alpha)$ is a function depending on the conversion ratio and is defined as a special mathematical function that expresses the variation of the conversion ratio with time or temperature. The $g(x)$ function corresponds to the function found by integrating $d\alpha/f(\alpha)$ [41-44].

$$\alpha = \frac{M_t - M_f}{M_i - M_f} \tag{1}$$

$$k(T) = A\exp(-\frac{E}{RT}) \tag{2}$$

$$\frac{d\alpha}{dt} = k(T)f(\alpha) \tag{3}$$

$$f(\alpha) = (1-\alpha)^n \tag{4}$$

$$ln(\frac{g(\alpha)}{T^2}) = \ln\frac{AR}{E\beta} - \frac{E}{RT} \tag{5}$$

$$\ln(\beta) = \ln(\frac{AE}{g(\alpha)R}) - 5.3305 - 1.052(\frac{E}{RT}) \tag{6}$$

$$\ln(\frac{\beta}{T^2}) = \ln\frac{AR}{g(\alpha)E} - \frac{E}{RT} \tag{7}$$

The activation energy $(E)$, Arrhenius constant $(A)$, and R (8.314 J/mol·K) values are expressed from the model equations. In Eq. 3, it can be solved by writing Arrhenius Equation instead of $k(T)$. Coats Redfern (Eq. 5), Flynn Wall Ozawa (Eq. 6), and Kissinger (Eq. 7) models are shown in the above equations. Here, the activation energy values of the biocomposite have been calculated using Coats Redfern, Flynn Wall Ozawa, and Kissinger methods. According to Coats Redfern method, if the activation energy is plotted $1/T$ versus $\ln(g(\alpha)/T^2)$, the slope gives $-E/R$. In the Flynn Wall Ozawa method, if $1/T$ versus $\ln(\beta)$ is plotted on the graph, the slope is found
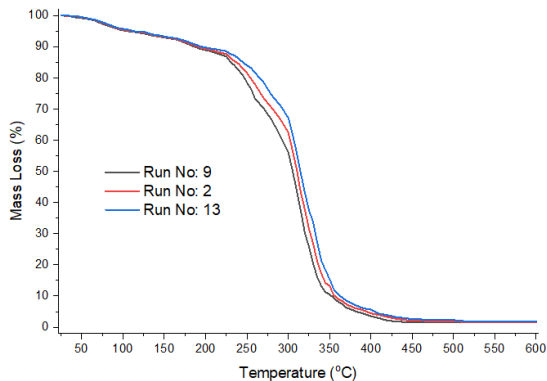


**Figure 2.** The effect of MPO content in the biocomposite for the thermal decomposition.

by the expression $-1.052E/R$. In the Kissinger method, if $1/T$ versus $\ln(\beta/T^2)$ is plotted, the slope is found as $-E/R$ [45-50].

## RESULTS AND DISCUSSIONS

### Proximate Analysis of The Biocomposites

Thermal degradation of biocomposites results is interpreted for specific experiments in Fig. 2 and Table 1. In thermal degradation experiments, physical decompositions (such as water, moisture) occurred in the first region, while chemical decompositions occurred in the second and third regions. It is possible to divide the chemical degradation of biocomposites into two in general. Weak structures that can chemically decompose at low temperatures degrade primarily in the composite, while groups with stronger thermal strength decompose slowly at higher temperatures [43-48].

### RSM Results for The Biocomposite

According to the RSM experimental study plan, the rate of epoxy components (wt.%) was kept constant in the production of the bio-epoxy composite. The mass percent values of epoxy resin and MPO have been entered into the program as the input data of RSM. RSM outputs are determined by the density of the obtained biocomposite, Shore D hardness, and thermal conductivity results. In Table 2, the experimental plan and response values arranged according to the RSM design are given.

In experimental studies, the results have been evaluated using analysis of variance (ANOVA) and RSM. When the obtained model equations are checked in ANOVA analysis, it is seen that the Quadratic Power model is suitable for this design. Also, $R^2$ values are found to be quite high and other error functions are also low.

According to the RSM results in Fig. 3, it is aimed to obtain maximum efficiency with the minimum economy

**Table 1.** Proximate analyses result of the experimental samples.

| Experiments | Moisture (%) | Ash (%) | Others (%) |
|---|---|---|---|
| Run No: 9 | 5.87 | 1.56 | 92.57 |
| Run No: 2 | 6.12 | 1.75 | 92.13 |
| Run No: 13 | 6.59 | 1.92 | 91.49 |

**Table 2.** The experimental study and RSM of the bio-epoxy composites.

| Run No | Epoxy Resin (wt.%) | MPO (wt.%) | Density (kg/m³) | Shore D Hardness | k (W/m·K) |
|--------|-------------------|------------|-----------------|------------------|-----------|
| 1 | 94.74 | 5.26 | 1164.27 | 74.39 | 0.097 |
| 2 | 95.00 | 5.00 | 1161.96 | 74.46 | 0.096 |
| 3 | 95.00 | 5.00 | 1161.97 | 74.47 | 0.096 |
| 4 | 95.00 | 5.00 | 1161.98 | 74.48 | 0.096 |
| 5 | 95.00 | 5.00 | 1161.96 | 74.46 | 0.096 |
| 6 | 95.24 | 4.76 | 1159.89 | 74.55 | 0.095 |
| 7 | 90.48 | 9.52 | 1201.47 | 73.02 | 0.111 |
| 8 | 92.03 | 7.97 | 1187.92 | 73.52 | 0.106 |
| 9 | 100.00 | 0.00 | 1118.30 | 76.07 | 0.079 |
| 10 | 98.42 | 1.58 | 1132.07 | 75.57 | 0.084 |
| 11 | 98.54 | 1.46 | 1131.09 | 75.60 | 0.084 |
| 12 | 95.00 | 5.00 | 1161.97 | 74.47 | 0.096 |
| 13 | 91.46 | 8.54 | 1192.84 | 73.34 | 0.108 |
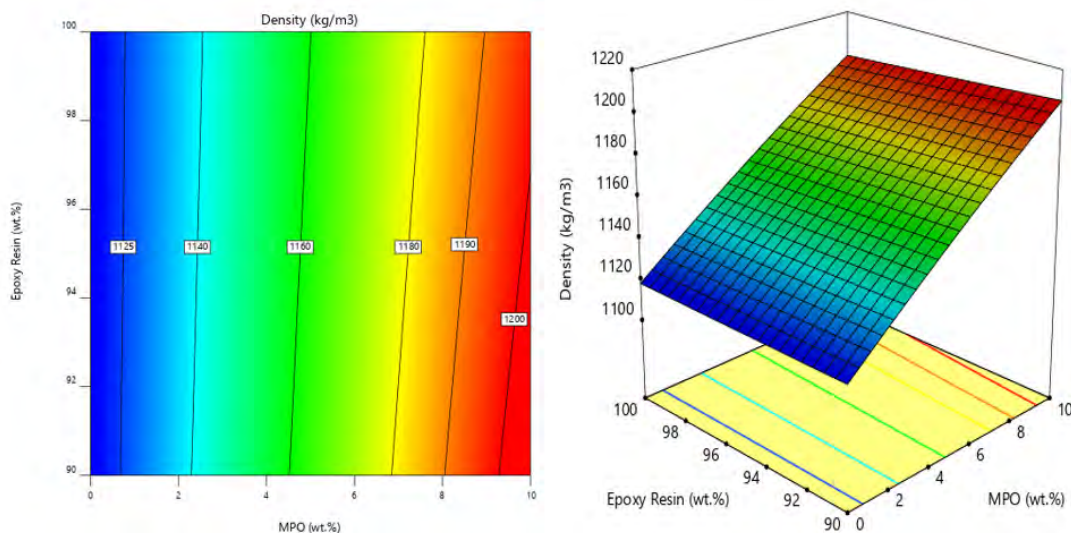


**Figure 3.** Effect of MPO (wt.%) rate on the density of biocomposite.

under optimum conditions in experimental studies. This method will save both time and raw material spent. With this method, 13 experiments are performed in the experimental study plan of RSM and theoretically, at least 100 compatible results are found. In Fig. 3, it can be stated that the density of the biocomposite increases depending on the MPO rate. The density of the composite can vary according to the polymer matrix structure, pore distribution, additives, and fillers [51,52].

In Fig. 4, experimental data and RSM model results have been evaluated by statistical analysis. The distribution of the actual values and the predicted data within the 95%

confidence interval is compared. In Eq. 8, the RSM polynomial function for density is expressed (A: MPO wt.%, and B: Epoxy Resin wt.%).

$$\rho = +1160.36113 + 16.65252 \cdot A \\ - 0.853011 \cdot B - 0.078989 \cdot A \cdot B \\ - 0.083227 \cdot A^2 + 0.004319 \cdot B^2 \qquad (8)$$

It is seen in Fig. 5 that the Shore D hardness decreases as the rate of MPO by mass increases in the biocomposite. Fig. 6 shows the agreement between statistical analysis and experimental data and RSM model values. According to the power model in the RSM central composite method, the Shore D polynomial function is expressed in Eq. 9.
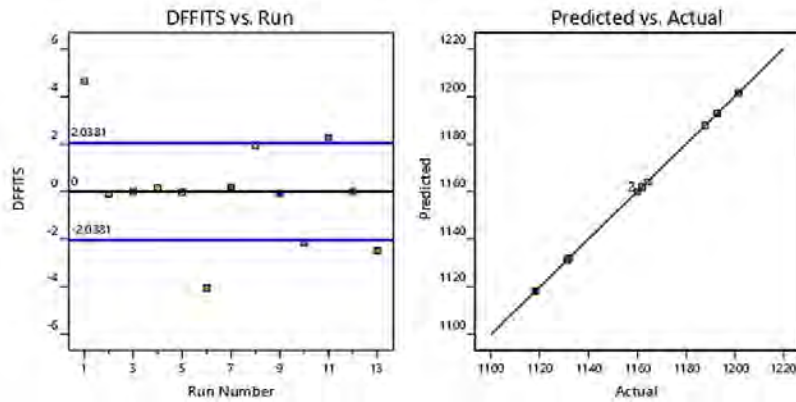
**Figure 4.** Comparison of experimental data and RSM model for the density of biocomposite.
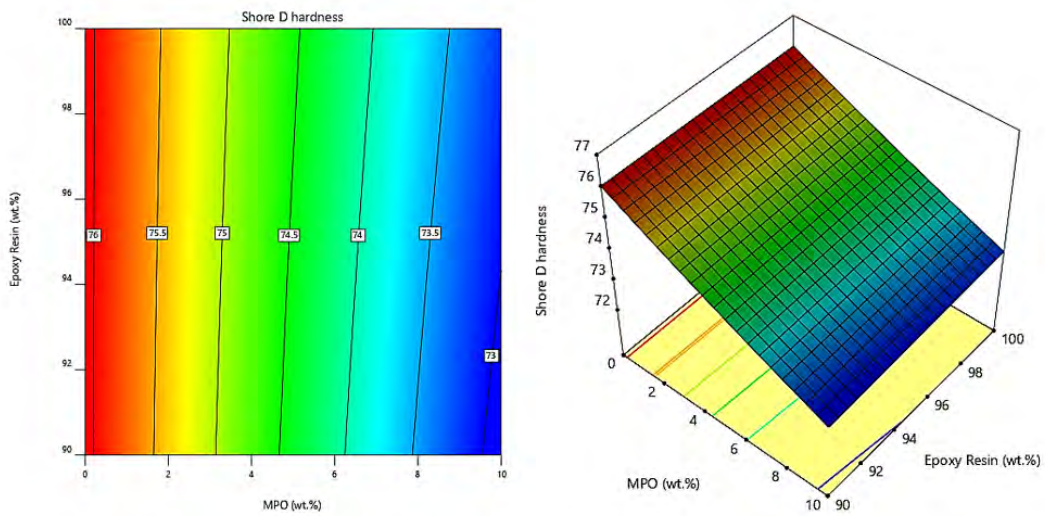


**Figure 5.** Effect of MPO (wt.%) rate on Shore D hardness of the biocomposite.
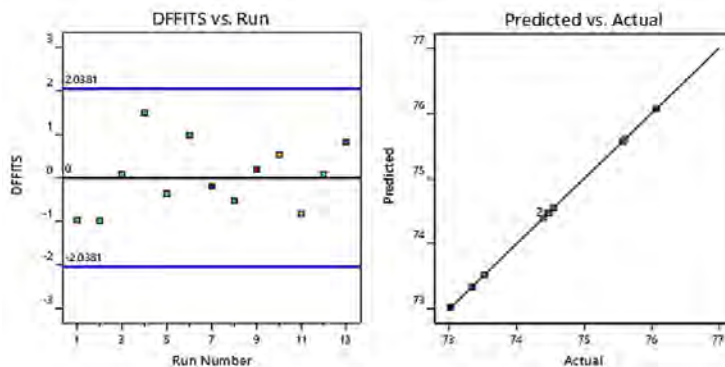


**Figure 6.** Comparison of experimental data and RSM model for Shore D hardness.

$$ShoreD = +76.39007 - 0.620282 \cdot A$$
$$- 0.007175 \cdot B + 0.003000 \cdot A \cdot B \qquad (9)$$
$$+ 0.003040 \cdot A^2 + 0.000040 \cdot B^2$$

The effect of the MPO ratio on the thermal conductivity coefficient of the biocomposite is compared in Fig. 7, and the compatibility of the experimental data with the theoretical model is evaluated in Fig. 8. The polynomial function expression of the thermal conductivity coefficient according to the RSM power model is expressed in Eq. 10.

$$k = +0.098413 + 0.006392 \cdot A$$
$$- 0.000399 \cdot B - 0.000030 \cdot A \cdot B \qquad (10)$$
$$- 0.000032 \cdot A^2$$
$$2.03223 \cdot 10^{-6} \cdot B^2$$
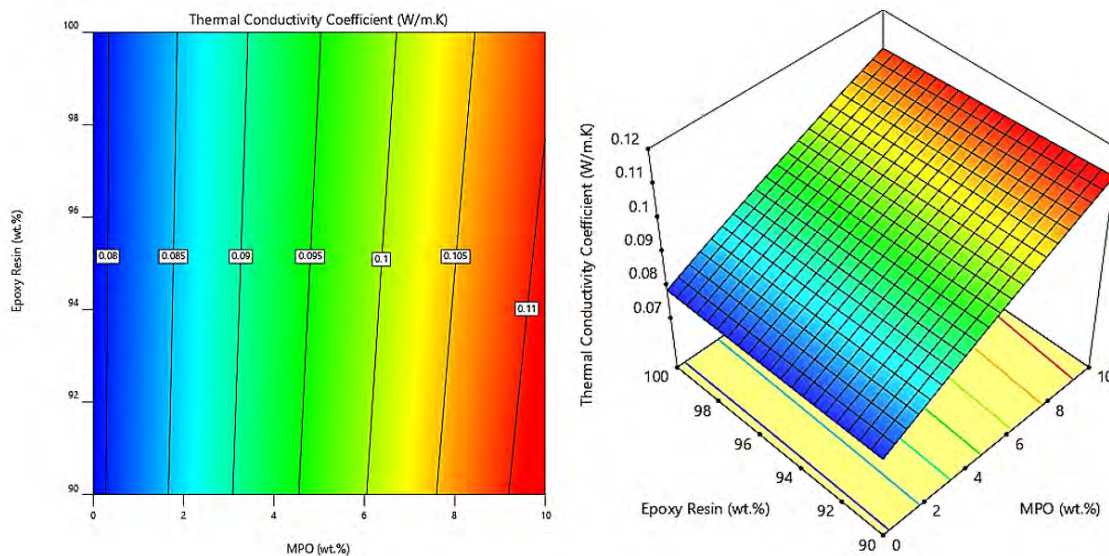
**Figure 7.** Effect of MPO (wt.%) rate on the thermal conductivity of the biocomposite.
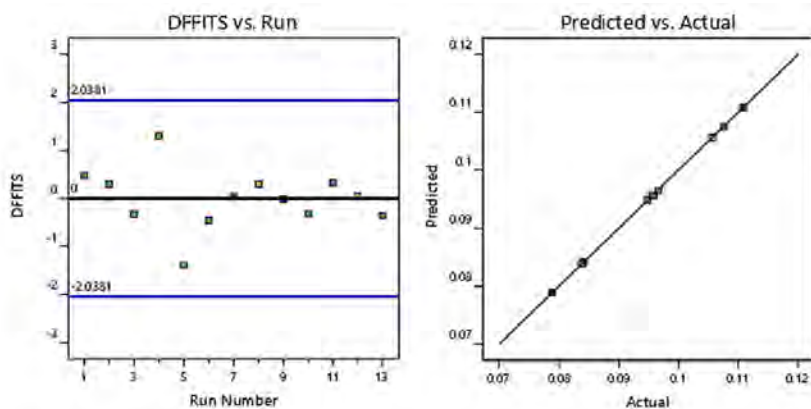


**Figure 8.** Comparison of experimental data and RSM model for the thermal conductivity.

**Table 3.** Statistical (ANOVA) evaluation of RSM results for the biocomposites.

| Parameters | Source | P-value | SST | Std. Dev. | C.V.% | R² | Adj. R² |
|---|---|---|---|---|---|---|---|
| ρ (kg/m³) | Quadratic | < 0.01 | 7.78 | 0.0399 | 0.0134 | 0.9989 | 0.9976 |
| Shore D | Quadratic | < 0.01 | 1.13 | 0.0165 | 0.0120 | 0.9991 | 0.9985 |
| k (W/m·K) | Quadratic | < 0.01 | 0.64 | 0.0087 | 0.0901 | 0.9982 | 0.9973 |

Statistical (ANOVA) evaluation for density, Shore D hardness, and coefficient of thermal conductivity is given in Table 3. High $R^2$ and adjusted $R^2$ values, good RSM model significance values (P-value<0.01), low error function (SST), and low standard deviation indicate that the theoretical model is successful according to the experimental results [53-57].

## FTIR Spectrophotometer

FTIR spectrum of extracted oil samples attained in the wavenumber region between 4000 and 600 cm⁻¹. The result from FTIR is tabulated in Table 4. At the vibra-tion at 3005 cm⁻¹, a stretch of =C-H, corresponding to an alkene, is observed. Symmetrical and asymmetrical stretching of C-H is observed in vibration at 2922 and 2852 cm⁻¹. The intense band observed at 1743 cm⁻¹ is the result of C=O vibrations indicating the presence of saturated aliphatic esters. This group is also known as triglyceride, the predominant component in fats and oils [58]. The peak at 1510 cm⁻¹ indicates stretching of the C=C of the alkene group. The peak at 1460 cm⁻¹ is observed due to C-C stretching in the aromatics group [59]. The peak at 3478 cm⁻¹, which occurs with the modification of palm oil, belongs to the hydroxyl group. It shows that the structure which is expected to disappear after the

**Table 4.** Results from FTIR analysis of the components.

|  | Palm oil peak (cm⁻¹) | Epoxy Palm oil peak (cm⁻¹) | MPO (cm⁻¹) |
|---|---|---|---|
| -OH | ---- | 3478 | 3538-3500 |
| -SO₂-N-H | ---- | ---- | 3357-3269 |
| Stretching of =C-H | 3005 | ---- | ---- |
| Symmetric and Asymmetric stretching of C-H | 2922-2852 | 2929-2875 | 2945-2858 |
| Triglyceride (TGA) | 1743 | 1628 | 1721 |
| Stretching of C=C | 1510 | ----- | |
| Stretching of C-C | 1460 | 1455 | |
| Asymmetric stretching -SO₂ | ---- | ---- | 1367-1228 |
| Oxirane group | ----- | 833 | ---- |
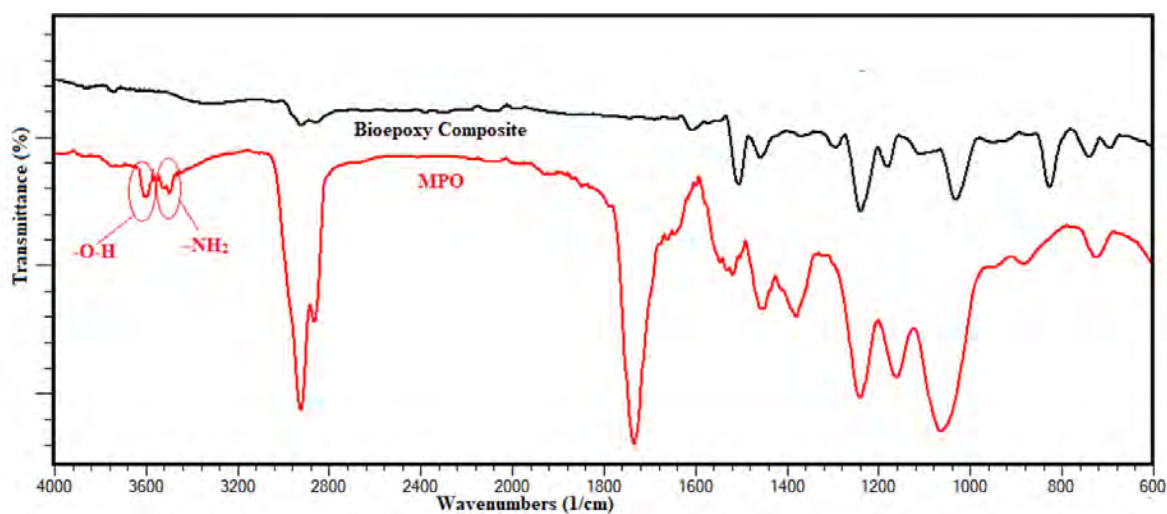


**Figure 9.** FTIR spectrum of the biocomposite and MPO.

modification of =C-H and C=C stresses corresponding to an alkene in vibration at 3005 cm⁻¹ and 1510 cm⁻¹ is formed. However, the peak formation of the oxirane group at 833 cm⁻¹ supports the expected structure [60]. The peaks at 3357 and 3269 cm⁻¹ observed in MPO spectrum belong to the -SO₂-N-H group [61-63]. The observation of 1367 and 1228 cm⁻¹ asymmetric stretching peaks of -SO₂ confirms the binding [64]. Epoxide ring-opening is generally observed with an increasing peak of hydroxyl peak. It is known that the shift of the hydroxyl characteristic peak from high to low wave number represents increased hydrogen bonding in the network. However, the disappearance of the oxirane peaks at 833 cm⁻¹ and the increase in the hydroxyl peak intensity confirm this information.

The FTIR spectrum in Fig. 9 shows the chemical bonds found in MPO and biocomposite. The disappearance of both -O-H groups and –NH₂ groups in MPO in the biocomposite indicates that these groups are lost by entering the chemical reaction.

## CONCLUSION

According to the results obtained, although the increase in the MPO ratio by mass increased the density, thermal conductivity, and thermal stability of the bio-epoxy composite, it decreased the Shore D hardness.

As the MPO rate (wt.%) in the produced biocomposite goes up, the thermal stability of the composite also rises. Calculated activation energies of the biocomposite according to the Flynn Wall Ozawa method are found to be 139.65 kJ/mol (Run No: 9), 143.56 kJ/mol (Run No: 2), and 145.28 kJ/mol (Run No: 13). According to the Coats Redfern method, the best model is determined by the function $(f = (1-\alpha)^{1.273})$. Coats Redfern, Flynn Wall Ozawa, and Kissinger model results, the deviation in the calculated activation energy values of the biocomposites are found below 7%. Experimental studies are carried out under non-isothermal conditions, and kinetic parameters are calculated with the best model approach. The model results are found in the

statistical analysis with minimum $R^2$ error functions and maximum efficiency values.

## CONFLICT OF INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## AUTHOR CONTRIBUTION

All the work in this study were performed equally by the authors.

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# Effect of Fibroblast Growth Factor (FGF) on Some Serum Oxidative Parameters in Hyperglycemic Rats

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## ABSTRACT

Wound healing is a complex and dynamic process that includes multiple biological pathways and has some successive healing periods. Most growth factor is responsible for wound healing. Fibroblast growth factor has a positive effect on wound healing problems that can be caused by diabetes. In the present study, we aimed to investigate the effect of bFGF applied to dorsolateral incision wounds in hyperglycemic rats on time-dependent serum oxidative events by measuring serum TBARS, NOx and RSH. Experiments were performed on 30 male Wistar albino rats (weight range:170-250 g ). Rats were hyperglycemic with streptozotocin (ip, 60 mg/kg). Experimental groups were divided into subgroups with and without treatment according to the days. bGF was applied locally to the dorsalateral wounds of rats (10 ng/ml). After these administrations, on the 3th and 7th days of wound healing, the animals were sacrificed. Serum TBARS, RSH and NOx levels were recorded spectrophotometrically. The results were expressed as mean ± Standard deviation and the mean differences were compared by Anova Variance Analysis (p<0,05). When compared with the treatment group, on the 7th day and the 3rd day, it was found that the serum TBARS levels increased statistically in hyperglycemic rats(p<0,05). Both in the 3rd day of the untreated and 3rd day of the rats treated with bFGF may significant decrease in the serum RSH levels. bFGF application was found both enhancing and reducing effects on oxidative stres. In subsequent studies, the effect of bFGF, which has positive effects on diabetic wound healing, on oxidative events can be investigated in detail using different doses and different treatment periods.

**Keywords:**
bFGF; Diabetic wound healing; Free radicals; Oxidative stress; Diabetes mellitus

## INTRODUCTION

Repair of tissue damage, loss or deterioration in tissue continuity is one of the most important functions of the organism. The purpose of this repair is to ensure tissue integrity and the function of the damaged organ [1]. The healing of damaged tissue is provided by a regular, in-line of cellular and biochemical chain of events [2, 3].

Wound healing is an evolutionarily preserved complex process aimed at tissue restoration. The wound healing process includes separate but intertwined stages of inflammation, cellular proliferation and maturation and remodeling[4, 5]. Each step; It takes place through the integration of a series of events controlled by endogenous and exogenous factors. This process is regulated by growth factors, cytokines and chemokines [6].

Growth factors are molecules with polypeptide structure that stimulate the growth, differentiation and proliferation of cells [7, 8]. Studies conducted in recent years have shown that Fibroblast Growth Factor is a large family consisting of 28 members. FGFs produced by keratinocytes, fibroblasts, endothelium, smooth muscle ant mast cells have characteristically high binding affinity for both heparin ant fibroblast growth factor receptors. Endothelial cells have the ability to both express ant respond to FGF [9-11] . bFGF is often used clinically; it has been emphasized that it has a significant effect on wound healing in the skin, cornea, eardrum ant salivary glands[12, 13].

Diabetes mellitus (DM) is a metabolic disease that occurs as a result of disorders in insulin secretion as a result of the interaction of genetic, environmental factors, lifestyle changes and events caused by the immune system. It is characterized by hyperglycemia[14, 15]. Inflammation, angiogenesis and collagen synthesis are impaired in diabetes. Blood circulation in the wound area

is negatively affected by the angiopathy and neuropathic effects of diabetes. Diabetes is a disease involving peripheral tissue injuries caused by oxidative stress caused by chronic hyperglycemia. Diabetes is known to cause an increase in oxidative stress and inflammatory response. Delayed wound healing is associated with an inflammatory response induced by hyperglycemia. This suggests that the effect of oxidative stress ant inflammatory response on wound healing may be positively affected by antioxidant supplementation. Diabetic scar; characterized by a disturbance in the wound healing process, according to animal experiments, a significant decrease in tensile strength was observed, particularly in inflammatory and proliferative phases, pathological angiogenesis and wound healing. Diabetes increases apoptosis of lymphocytes and increases the production of reactive oxygen species, also stimulating the signaling pathway of apoptosis [15-17].

Free radicals affect cellular structures and affect important components such as lipids, proteins, carbohydrates, DNA and enzymes[18]. There are many studies emphasizing the importance of reactive oxygen species in diabetic vascular pathogenesis and showing that diabetic complications are associated with oxidative stress[19].

Oxidative stress, which is one of the causes of diabetes, causes biological problems in wound healing. Considering that the mechanism of action of free radicals on wound healing is not clear, reducing the resulting products will affect the healing process. Many growth factors are effective against free radicals in wound healing.There are studies in which bFGF plays an important role in accelerating healing and biochemically.It is known that bFGF activity is high in the first 3 days of the healing process. However, studies showing the time-dependent effects of bFGF are insufficient. We aimed to measure the positive effect of bFGF, which is used in wound healing and whose doses are tried to be determined, against oxidative stress in the healing process in diabetic rats with biochemical parameters. Therefore, serum TBARS, RSH and NOx levels were measured spectrophotometrically in this study.

## MATERIAL AND METHODS

The studies were initiated with the permission of Gazi University Experimental Animals Ethics Committee (G.Ü. ET-15.032) and all stages until the tissue samples were taken in Gazi University Laboratory Animal Breeding and Experimental Research Center (GÜDAM) laboratory. In the experiments, 30 Wistar albino male rats, 170-250 grams, obtained from GÜDAM were used. Before animals fed with free feed and water and during the experiment were looked after in individual cages during the experiment, in an environment illuminated in pa-

rallel with the daylight cycle. Tissue samples taken were analyzed in Gazi University Faculty of Science, Physiology-Biochemistry research laboratory.

### bFGF Preparation

In previous studies conduced in our laboratory, the dosage was determined as 10ng / ml, since it was determined histologically that the contribution of bFGF to wound healing at a dosage of 10ng / ml was high. bFGF (10 ng / ml) was applied locally to the wounds of rats in the bFGF applied groups once a day at approximately the same time[20-22].

### Diabetes Model

In groups formed to compare wound healing in healthy and diabetic animals, streptozotocin (STZ)(ip, 60 mg / kg) dissolved in 0.1 M citrate buffer (pH 4.5) buffer was administered to diabetic animals[23]. One week later, blood glucose levels were measured with a glucometer and those above 300 mg / dl were considered as having diabetes. In contrast to the experimental animals that were resistant to diabetes, STZ administration was made in a second dose and made diabetic.

### Wound Model

In order to prevent infection before the experiment, the dorsal parts of the animals were made ready by applying batticon before the wound was created.

General anesthesia was provided by weighing the experimental animals on a standard scale and injecting ketamine (Alfamanine 50mg / kg) and xylazine (Alfazyne 5mg / kg) intramuscularly according to their weight.

Dorsolateral excisional incision wounds were made on the dorsal of the animals, approximately 4 cm long on both sides of the spine [24-26]. Later, the wound lips were adapted with suture. In order to provide postoperative analgesia, paracetamol, a pharmacological agent, was used as 2 mg / ml into drinking water.

### Diabetic Experimental Groups

5 separate experimental groups were formed, 6 in each group, and the following procedures were applied to the groups.

Hyperglycemic control group: In order to compare the values of the groups formed, only STZ and diabetic control group (n = 6)
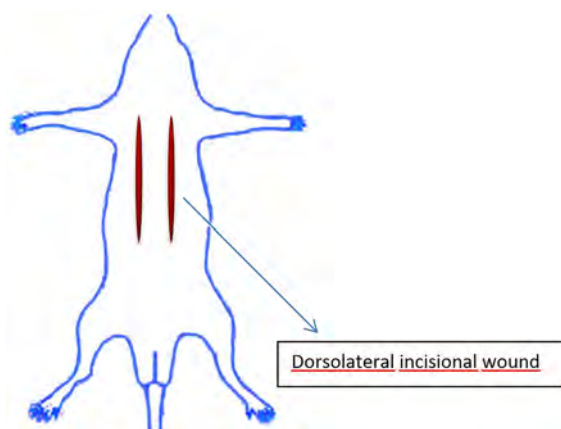
**Figure 1.** Incisional wound model

Hyperglycemic untreated groups: After being diabetic with STZ, only the wounded group (3rd day of healing was sacrificed) (n = 6) After being diabetic with STZ, only the wounded group (7th day of healing was sacrificed) (n = 6)

Hyperglycemic bFGF treated groups: The group that was wounded after being diabetic with STZ and applied bFGF (3rd day of recovery was sacrificed) (n = 6) The group that was wounded after being diabetic with STZ and applied bFGF (7th day of recovery was sacrificed) (n = 6)

After the applications, the rats were sacrificed by taking blood from their hearts on the 3rd and 7th days in accordance with the chronobiological order. The blood samples taken were centrifuged at 3000 rpm for 15 minutes and the serums were taken into ependof tubes and stored at -30℃ until analyzed.

## Biochemical Analyses

### Determination of TBARs Levels

The determination of MDA, which is the indicator of lipid peroxidation, was made by the method of thiobarbituric acid reactive substance (TBARS) formation using Kurtel method [27]. The solutions used are 15% TCA, 0.02% BHT (in 95% ethanol), 0.375% TBA and 0.25 N HCl. 400 µL of TBA-TCA-HCl mixture (in equal amounts) was added onto 200 µL of plasma and vortexed. After being kept at room temperature for 5 minutes, it was centrifuged at 10000 rpm for 5 minutes. The entire supernatant obtained was placed in glass tubes. After adding 4 µL of BHT to the supernatants, the tubes were boiled for 15 minutes. After cooling in tap water, it was placed in an ELISA plate and read at a wavelength of 532 nm.

### Determination of RSH Levels

Determination of plasma total sulfhydryl groups (RSH), which is an indicator of antioxidant capacity, was made

by spectrophotometric method [27]. The solutions used are the mixture containing 10 m M DTNB, 100 mM Tris-HCl (pH 8.2), 1% SDS-2 mM EDTA in 0.1 M potassium phosphate buffer (pH: 7). 1 mL of Tris-SDS-EDTA mixture was added to 500 µL sample. It was incubated for 5 minutes at room temperature, and then centrifuged at 10000 rpm for 5 minutes. 40 µL DTNB was added to the obtained supernatant. After being kept at 37 ° C for 20 minutes, it was read at 412 nm wavelength on the ELISA reader.

### Determination of Total NOx Levels

The modified Griess method was used for the determination of NOx, which is the sum of plasma nitrite and nitrate[28]. 0-50 µM dilutions of sodium nitrate (NaNO₃) prepared with deionized water were used as standard. 0.3 N NaOH was completed to 100 ml by adding deionized water to 1.2 g of NaOH. 10% ZnSO₄ is completed to 100 ml by adding deionized water on 10 g of ZnSO₄. VaCl₃ 4.2 ml HCl was added to 0.4 g VaCl₃ and this mixture was completed to 50 ml with deionized water. Sulfanolamide 2 gr sulfanolamide 13.6 ml HCl was added and this mixture was completed to 100 ml with deionized water. -NEDD (N- (1-naphtyl) ethylenediamide dihyrochloride) was completed to 100 ml by adding deionized water on 0.1 gr NEDD. 100 µL 0.3 M NaOH was added to 100 µL plasma and incubated for 5 minutes at room temperature. 100 µL of 10% ZnSO₄ was added to this mixture and vortexed. The resulting mixture was centrifuged at +4ºC at 14000 rpm. 100 µL of each of the supernatants obtained after centrifugation was placed on a 96-well ELISA plate. The samples were placed on the plate in duplicate with the same volume of standard solutions. 100 µL of VaCl₃ was added to all samples and standard solutions, followed by 50 µL of sulfanolamide and 50 µL of NEDD. The plate was incubated at 37℃ for 30 minutes. At the end of the incubation it was read on an ELISA reader at a wavelength of 540 nm.

### Statistical Evaluation

All values are expressed as arithmetic mean ± standard error. The values obtained were evaluated using Anova analysis of variance (one-way ANOVA) and Tukey multiple comparison test (SPSS 16.0 for Windows (SPSS, Inc., Chicago, USA). P value of <0.05 was considered statistically significant.

## RESULTS

Morphological images of the wounds belonging to the untreated and treated groups of hyperglycemic rats and their changes depending on the days, blood samples ta-

ken are shown in Figures 2, 3, 4 and 5.
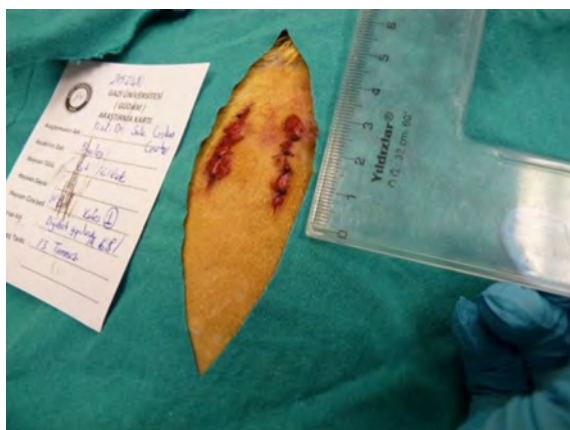


**Figure 2.** Dorsolateral incisional wound



**Figure 3.** Morphological image of hyperglycemic 3-day untreated and bFGF 3-day treated wounds

Findings of the oxidative parameters of TBARS, RSH and NOx in the control group, treated and untreated groups in hyperglycemic rats are shown in Table 1.

**Serum TBARS Levels in Hyperglycemic Rats**

TBARS level is measured to determine malondialdehyde, the final product of lipid peroxidation. When the 7 days untreated and treated groups were compared with the control group, a significant increase was observed ($p < 0.05$) (Table 1 and Figure 6). Serum TBARS levels statistically increased on the 7th day compared to the 3rd day in hyperglycemic rats treated with bFGF ($p < 0.05$) (Table 1 and Figure 6).



**Figure 4.** Morphological image of hyperglycemic 7-day untreated and 7-day bFGF-treated wounds



**Figure 5.** Taking blood samples

**Serum RSH Levels in Hyperglycemic Rats**

When the 7th day of the bFGF applied rats and the 7th day of the untreated group were compared, a significant decrease was detected ($p < 0.05$) (Table 1 and Figure 7)

It was observed that the RSH levels in rats treated with bFGF decreased compared to the 3rd and 7th days. ($p < 0.05$) (Table 1 and Figure 7). Both in the 3rd day of the untreated

**Table 1.** Serum TBARS, RSH and NOx levels in hyperglycemic rats

| Groups | | TBARS (nmol/mL) | RSH (nmol/mL) | NOx (µmol/L) |
|---|---|---|---|---|
| **Control (n=6)** | | 3,12 ± 0,56 | 135,43 ± 28,28 | 180,24 ± 9,95 |
| **U n t - reated (n=12)** | **3 Days (n=6)** | 3,4 ± 0,07 | 183,21 ± 3,4 | 121,92 ± 14,21* |
| | **7 days (n=6)** | 5,03 ± 1,15 *,a | 152,88 ± 14,38a | 92,68 ± 6,42* |
| **b F G F t r e a - ted (n=12)** | **3 Days (n=6)** | 4,35 ± 0,12 | 128,65 ± 6,06 | 173,87 ± 6,02* |
| | **7 days (n=6)** | 8,84 ± 1,15 *,b | 123,79 ± 7,76b,c | 151,22 ± 23,95* |

*: p <0,05: Compared with the control group
a: p <0,05: Comparison of 3 days untreated group and 7 days untreated group
b: p <0,05: 3 days treatment group compared to 7 days treatment group
c: p <0,05: Comparison of 7 days untreated group and 7 days treated group
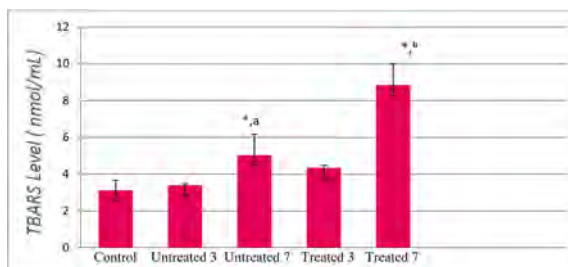


*p <0.05 When compared with the control group
a p <0.05 3-day untreated group compared
b p <0.05 3-day treatment group compared
c p <0.05 7 days without treatment group compared

**Figure 6.** Serum TBARS (nmol / mL) levels in hyperglycemic rats
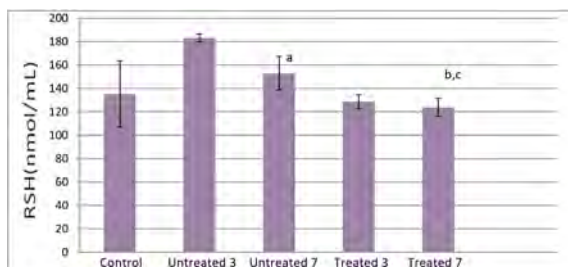


*p <0.05 When compared with the control group
a p <0.05 3-day untreated group compared
b p <0.05 3-day treatment group compared
c p <0.05 7 days without treatment group compared

**Figure 7.** Serum RSH (nmol / mL) levels in hyperglycemic rats

and 3rd day of the rats treated with bFGF may significant decrease in the serum RSH levels.

### Serum NOx Levels in Hyperglycemic Rats

A statistically significant increase was found in rats treated with hyperglycemic bFGF when compared with the NOx levels of the 3rd day of wound healing and the same day of the untreated group (p <0.05). Nitric oxide levels increased with the bFGF treatment we applied. When all groups were compared with the control, a statistically significant difference was found (p <0.05) (Table 1 and Figure 8).
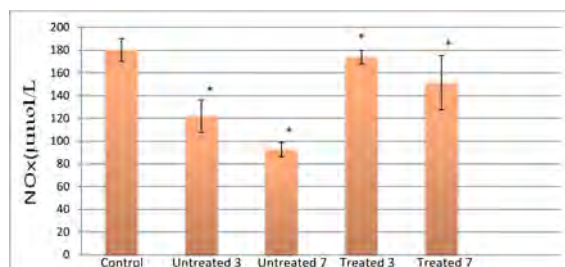


*p <0.05 When compared with the control group
a p <0.05 3-day untreated group compared
b p <0.05 3-day treatment group compared
c p <0.05 7 days without treatment group compared

**Figure 8.** Serum levels of NOx (µmol / L) Levels in hyperglycemic rats

### DISCUSSION

Free radicals affect organic compounds as a result of oxidative reactions and cause various biological problems [29] . The oxidative balance is disturbed when an increase in the rate of radical formation or a decrease in the rate of their removal is observed. Oxidative stress may reduce the effect of antioxidants and cause tissue damage. With the oxidative stress that occurs, the effect of antioxidants may decrease, resulting in tissue damage [30].

It can be said that bFGF significantly reduces oxidative stress and plays an important role in suppressing this damage.

The research is limited to the bFGF treatment applied to rats by the researchers and the data obtained biochemically. Çetin et al. (2004) pointed out that bFGF has a scavenging effect on free radicals that occur with respiratory burst, which is effective in the inflammatory phase of wound healing [31]. The production of $H_2O_2$ and ROS in the inflammatory process in the wound area causes increases in the TBARS level. Inflammatory responses are required for wound repair following injury [32].In our current findings, the negative effect of diabetes, lipid peroxidation in hyperglycemic rats with bFGF application could not be eliminated. The reason for this can be shown as increased oxidative stress due to diabetes.bFGF has a very short biological half-life (2-3 min). It can be rapidly removed from the blood when injected. For this reason, suitable systems such as controlled release systems should be used to increase the release time and eliminate its undesirable effects[31].Wei et al. (2009) emphasized in their research that the FGF family shows antioxidant properties in conditions related to oxidative stress [33]. In our experiment, the high TBARS levels in the treated 7-day group indicate that bFGF in serum did not have an antioxidant effect. Due to the strong link between diabetes and free radicals in the studies, it can be said that bFGF application against free radicals should be done at a more appropriate dose. In addition, it is necessary to provide long-term release of bFGF to increase the in vivo efficacy.

There are antioxidant systems that protect the body against free radical damage at many stages. Antioxidants are negatively affected by the oxidative stress caused of free radicals. Antioxidants used to prevent the effect of free radicals, especially thiol groups, can't protect the plasma and tissue levels due to interactions [34]. All plasma -SH groups are associated with proteins. With the increasing oxidative stress, –SH groups found in plasma and membranes are oxidized by free radical effect and a decrease in their reduced form is detected. GSH is required for the oxidized thiol groups to become reduced again and to be used in oxidative damage. While GSH neutralizes thiol groups affected by oxidative stress, it falls short[34, 35]. Both in the 3rd day of the untreated and 3rd day of the rats treated with bFGF may significant decrease in the serum RSH levels. The decrease in RSH levels of treated diabetes groups compared to untreated diabetes groups is that plasma proteins may be sensitive to bFGF, especially due to free sulfhydryl groups in the serum albumin structure. The molecular structure of bFGF could not positively affect the oxidative stress that increases with diabetes.

Nitric oxide levels increased with the bFGF treatment we applied. Topical application of bFGF decreased NOx levels and nitrite oxide synthase enzyme was suppressed. In the light of this information, bFGF application in diabetic rats contributed to wound healing systemically. NO is an endothelium-derived vasodilator agent and plays an active role in lowering blood pressure. It is one of the important molecules in the wound healing process. While it has a protective effect at physiological doses, it can show a cytotoxic effect at high levels [30]. NO is rapidly metabolized to nitrite and nitrate by interacting with the Hem group of hemoglobin in the blood. NO also reacts with heme and other iron-containing molecules, and with thiol (- SH) groups of proteins [36]. A statistically significant increase was found in rats treated with hyperglycemic bFGF when compared with the NOx levels of the 3rd day of wound healing and the same day of the untreated group (p <0.05). In hyperglycemic rats, there was an increase in NO production due to the increased leukocyte activation during the inflammation phase of wound healing and its effect preventing leukocyte outflow. The decrease in nitrite oxide production in the endothelium has been blamed for the disorders of endothelial functions seen in diabetes, although controversial [37]. However, there are also studies showing that NO is increased in diabetes [38].

## CONCLUSION

It can be said that bFGF significantly reduces oxidative stress and plays an important role in suppressing this damage. In this study, information about how bFGF, which is known to have an effect on wound healing, mana-

ges oxidative events was tried to be clarified. When all the information is evaluated, our study has shown that bFGF applied to dorsolateral incision wounds created in hyperglycemic rats has a regulatory effect on serum levels of oxidative events. It has been shown that this effect may vary depending on the days, the stages of wound healing and the dose administered. In subsequent studies, the effect of bFGF, which has positive effects on diabetic wound healing, on oxidative events can be investigated in detail by using different doses and different treatment times.

## CONFLICT OF INTEREST

Esra Oguz confirms, on behalf of all authors, that the information provided is accurate.

## AUTHOR CONTRIBUTION

Esra Oğuz and Sule Coskun Cevher designed the study. EO performed all experiments. EO and SCC analysed the data. EO and SCC wrote the paper.

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# Evaluation of the Cytotoxic Effects of Ultrasonic Extracts of *Tribulus Terrestris* L. on MCF-7 Cell Line by MTT Assay

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## ABSTRACT

People, especially those living in rural environments and those who have recently been dissatisfied with traditional medicine, use medicinal plants for their therapeutic effects. In this study, ethanol, ethyl acetate and methanol extracts of *Tribulus terrestris* L. plant, which is used by local people as one of these medicinal plants in Kars province (Turkey), were obtained by ultrasound assisted extraction method. The cytotoxic effects of the obtained extracts on MCF-7 breast cancer cells in the concentration range of 10-1000 ppm at 24 h of exposure were investigated by the MTT method, which is a colorimetric method. *T. terrestris L.* ethanol extracts at 1000 ppm caused a moderate cytotoxic effect on MCF-7 breast cancer cells. It was determined that ethanolic extracts in the concentration range of 10-500 ppm and methanol and ethyl acetate extracts in the concentration of 10-1000 ppm caused cell proliferation.

Keywords:
*Tribulus Terrestris L.*; MCF-7; Ultrasound assisted extraction; Cytotoxicity; MTT Assay

## INTRODUCTION

It is known that human beings used only natural medicines until 1800 in the treatment of diseases. Compounds obtained through isolation from natural sources since the beginning of 1800 have been synthesized in the laboratory environment since 1830 [1]. In recent years, the belief that natural remedies are safer than synthetic drugs have led to an increase in people's use of natural products such as herbs, phytotherapeutics and phytopharmaceuticals. Therefore, researchers conduct various studies on the biological significance of plant extracts [2, 3]. However, there is no regulatory system that determines the safety and required dose of natural products, or even commercially sells them. The most widely used of these natural products are herbs. Unfortunately, most of those who use these herbs therapeutically do not have adequate knowledge or training in the safe use of the products. For these reasons, many studies are required to standardize natural plant products and evaluate possible risks such as undesirable side effects, overdose and toxicity [4].

*Tribulus terrestris* L. is known in Turkey as "deve çökerten", "çoban çökerten" and "çarık dikeni". These names were given to the plant because its fruit has horn-shaped spikes (Figure 1). The plant is of medicinal and pharmaceutical interest due to its steroidal saponin content [5, 6]. *Tribulus terrestris* L., in addition to its hypolipidemic and hypoglycemic effects, is also used for the treatment of vitiligo, urological infections, prostatic hypertrophy, eye, abdomen and cardiovascular system diseases [6-8]. There are limited in vitro studies on the effect of *T. terrestris* L. extracts [7], which have low cytotoxicity on normal cells, on different cancer cells [7, 10-12]. In this context, in this study, the cytotoxic effects of ethanol, ethyl acetate and methanol extracts of *Tribulus terrestris* L. on MCF-7 human breast cancer cell line for 24 hours were investigated by MTT Assay.

## MATERIAL AND METHODS

### Collection of Chemicals and Plant Material

Ethanol, ethyl acetate, methanol, DMSO, MTT, DMEM, Penicillin-Streptomycin, Fetal Bovine Serum (FBS), Phosphate Salt Buffer (PBS) and Trypsin EDTA (Sigma Aldrich, Germany) were commercially available. *Tribulus terrestris* L. aerial parts (leaf, fruit and stem) were collected from Kars, Turkey at the altitude of 1410 m at 40°29'46'' North and 43°33'40'' East coordinates. Map views related to these coordinates were obtained from Google Earth online and were gi-

**Figure 1.** *Tribulus terrestris* L. plant

ven in Figure 2. The collected plant was described by Prof. Dr. Fatma Güneş. The collected plant samples were dried in the dark and ground using a grinder.


**Figure 2.** Map view of 40°29'46" North, 43°33'40" East coordinates where *Tribulus terrestris* L. samples were collected

### Instruments

In this study, Bandelin Sonorex RK 106 at 35 kHz frequency, Panasonic MCO-170AICUVH-PE CO2 Incubator, Hed Lab X BIO MSC CLASS II Biosafety cabinet, Thermo Fisher EVOS FL Inverted Microscope and BioTek Epoch UV-Vis Spectrophotometer were used.

### Plant Extraction

For ultrasound assisted extraction, 10 g from the milled plant sample was weighed and placed in the round-bot-

tomed flask, then added in a 30 mL the extraction solvent (ethanol, ethyl acetate and methanol). Extraction was continued 60 min. Temperature was measured at 15 minutes intervals. The obtained extracts were filtered with blue band filter paper and the liquid part was dried in a rotary evaporator. For the MTT test, a stock solution of 1000 ppm was prepared and other solutions (500, 250, 100, 10 ppm) were prepared from this stock solution.

### MTT Assay

100 µL (~5000 cells/well) of MCF-7 cell suspension prepared according to protocols [13] was placed in each well of 96-well plates and incubated for 24 h. After incubation, 100 µL aliquots from prepared solution (1000, 500, 250, 100 and 10 ppm) were added to the wells (100 µL of medium + 100 µL of cell suspension was added to the control wells) and allowed to incubate again for 24 h. Then, 10 µL of MTT (5 mg/mL) solution prepared in PBS was added to each well and incubated for 4 h. In order to dissolve the formed purple colored formazan crystals, 100 µL of DMSO was added to all wells and incubated at 18 h. After this period, absorbances were measured at a wavelength of 570 nm with a microplate spectrophotometer. Cell viability percentages were calculated with the following equation 1. All experiments were performed in triplicate.

$$Cell\ Viability\ (\%) = \frac{Absorbance\ of\ test\ well}{Absorbance\ of\ control\ well} x100 \quad (1)$$

## RESULTS AND DISCUSSION

### Extraction

Ultrasound assisted extraction is an important technology for green chemistry. The extraction process, which takes hours or days with traditional methods, can be completed in a short time thanks to ultrasound wave-assisted extraction. The most important advantages of this extraction method are short duration, high reproducibility, low solvent consumption and high purity of the final product [14, 15]. The temperatures were recorded as 24 °C, 29 °C, 34 °C and 38 °C (for ethanol); 25 °C, 36 °C, 42 °C and 44 °C (for ethyl acetate) and 26 °C, 32 °C, 38 °C and 39 °C (for methanol) at 15, 30, 45 and 60 minutes, respectively. The yields of extraction were found to be 45.25 % for ethanol, 52.39 % for ethyl acetate and 62.64 % for methanol.

### FT-IR Spectroscopy

In many studies, it has been reported that ethanol, ethyl acetate and methanol extracts of the *Tribulus terrestris*

L. plant are quite rich in terms of phenolic acids, flavo-noids and saponins [16, 17]. The biological activity of the plant is also attributed to these components. Dinchev et al. reported that there are saponins such as protodios-cin, prototribestin, pseudoprotodioscin, dioscin, tribes-tin, tribulosin, rutin in the ethanolic extracts of the TT plant collected from Turkey [17]. Li et al also determined that the plant contains tigogenin, gitogenin, hecogenin and neohecogenin [18]. Reshma et al. attributed the an-tioxidant activity of ethyl acetate extracts of the plant to the total phenolic substance content and reported that it contains caffeic acid, chlorogenic and 4-hydroxyben-zoic acid phenolic acids [19]. Phenolic compounds such as α-Amyrin as the major component and 3,7,11,1–tet-ramethyl 2-hexadecen 1-01, n-hexadecanoic acid, hexa-decanoicacid, ethyl ester, phytol, 1,2-octadecadienoic acid, 9,12,15-octadecanic acid, benzenedicarboxylic acid and diisooctyl ester as the minor component have been reported in the methanolic extracts of the plant [20]. In another study, it was determined that the methanolic extracts of the plant contain flavanoids such as narin-gin, rutin, hyperoside, quercetrin, quercetin, naringenin, hesperetin, campferol and apigenin, as well as phenolic acids such as pyrogallol, gallic acid, protocatechuic acid, catechin, catechol, chlorogenic acid, p-hydroxybenzoic acid, caffeic acid, vanillic acid, ferulic acid, salicylic acid, ellagic acid, coumaric acid and cinnamic acid [21]. The-se compounds generally have functional groups such as O-H, C-H (aromatic), C-H (aliphatic), C=O, C=C, C-H bending and C-H belonging to substituted benzene rings vibrations. When the FT-IR spectrums of the obtained extracts are examined, the vibrations of the O-H groups are seen in the range of 3500-3200 cm$^{-1}$. C-H stretching (aromatic), C-H bending (aliphatic) and C-H bending (substitue benzenes) vibrations are observed in the 3100-2900 cm$^{-1}$, 2900-2800 cm$^{-1}$ and 850-700 cm$^{-1}$ regions, res-pectively. The vibrations of the C=O group are observed at 1750-1650 cm$^{-1}$ (Figure 3-5).
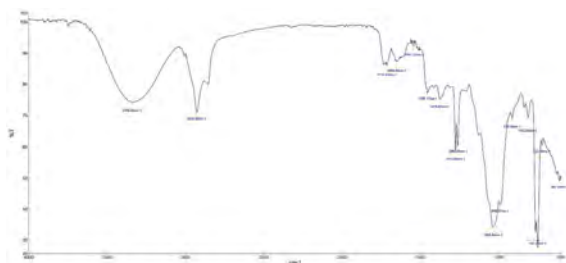


**Figure 3.** FT-IR spectra of ethanol extract of *Tribulus Terrestis* L.

## MTT Assay

In this study, compared with the cell control group, it was determined that *Tribulus terrestis* L.'s methanol extracts caused proliferation of MCF-7 cells at all concentrations.
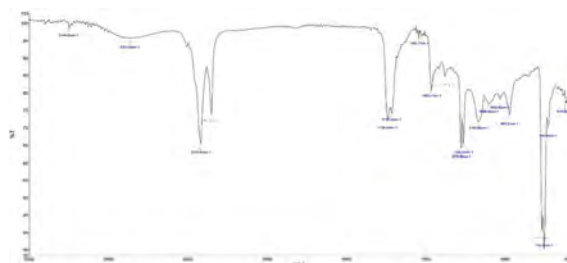


**Figure 4.** FT-IR spectra of ethyl acetate extract of *Tribulus Terrestis* L.
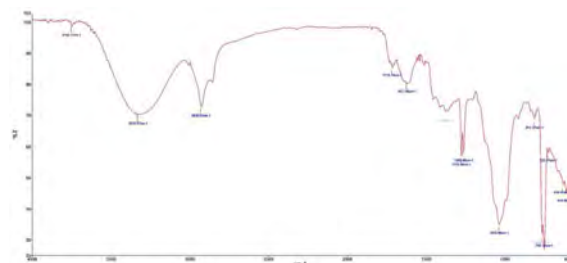


**Figure 5.** FT-IR spectra of methanol extract of *Tribulus Terrestis* L.

It was determined that cell proliferation increased with decreasing concentration. While 12 % cell proliferation occurred at 1000 ppm concentration, cell viability doub-led (210 %) at 10 ppm concentration. Methanol used as a solvent caused 5 % cytotoxicity on MCF-7 cells (Figure 6).
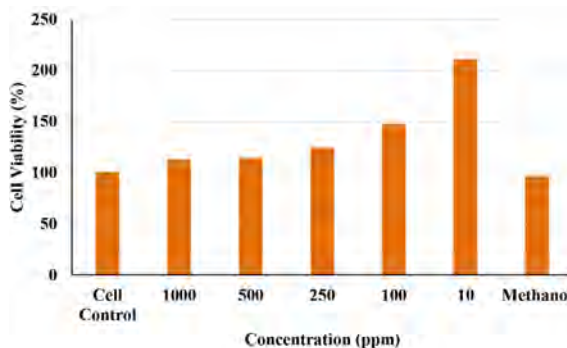


**Figure 6.** Effect of *Tribulus terrestris* L.'s methanol extract on MCF-7 cell line

The ethanolic extract of *Tribulus terrestris* L. at 1000 ppm concentration caused 19.9 % cytotoxicity. *Tribulus ter-restis* L. extracts at 500 ppm, 250 ppm, 100 ppm and 10 ppm concentrations caused proliferation in MCF-7 cells. While proliferation increased with decreasing concentration, more cell viability was found from the methanolic extracts of the plant. Ethanol used as a solvent also increased cell viability by 10 % (Figure 7).

The effects of *Tribulus terrestis* ethyl acetate extracts on MCF-7 are similar to its methanol extracts. That is, the cell viability percentages are similar. 10 % cell viability was found at 1000 ppm concentration and 230 % cell viability at 10 ppm concentration. Ethyl acetate, which was used as a solvent, caused 9.3% moderately cytotoxic [33] on MCF-7 cells (Figure 8). Comparison of effect of the all extracts on

MCF-7 cell viability was given in Figure 9.
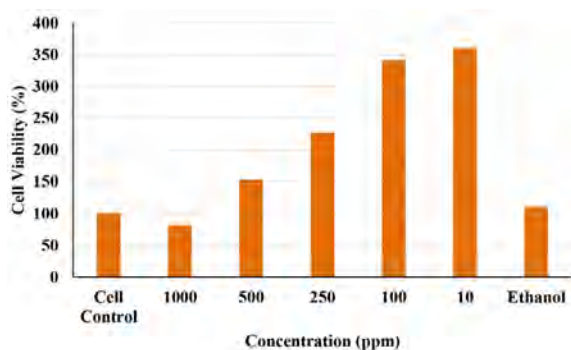


**Figure 7.** Effect of *Tribulus terrestris* L.'s ethanol extract on MCF-7 cell line
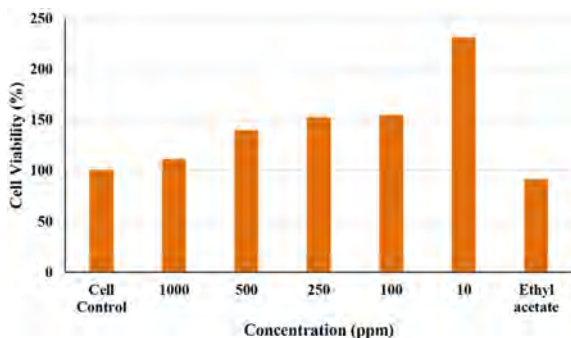


**Figure 8.** Effect of *Tribulus terrestris* L.'s ethyl acetate extract on MCF-7 cell line

Ammar et al (2018) reported that methanol and petroleum ether extracts of *T. terrestris* L. were cytotoxic to human hepatocellular carcinoma cells (HepG2) at 16.1 µg.mL⁻¹ and 21.5 µg.mL⁻¹ concentrations [21]. Angelova et al. (2013) examined the cytotoxic effects of *T. terrestris* extract on cell viability in human breast cancer (MCF-7) and normal (MCF10A) cell lines. It was determined that this plant extract inhibited the viability of breast cancer cells in a concentration-dependent manner (IC50 =15 mg/mL) [7]. Similarly, Neychev et al. (2007) found that the obtained saponins from *T. terrestris* exhibited low toxicity to normal human fibroblasts than some cancer cells [9]. Sun et al. reported that saponins isolated from the of *T. terrestris* L.'s ethanolic extract inhibited the proliferation of Bcap-37 breast cancer cell line and showed a cytotoxic effect on BEL-7402 cell line by causing apoptosis [10, 12]. Wang et al. reported that steroidal saponins isolated from the *T. Terrestris* L.'s fruits exhibited antitumor property on NCI-H460, SF-268, MCF-7 and HepG2 tumor cells [22]. Abudayyak et al. examined the cytotoxicity of NRK-52E cells exposed to *T. terrestris*'s methanol extracts for 24 hours by MTT assay. It was found that cell viability was 68.5 % at 500 mg/mL concentration. The IC50 value was determined as 160 mg/mL. It was determined that water and chloroform extracts of this plant did not show cytotoxic effects in the concentration range of 62.5-500 ppm [23]. Chauhan et al. obtained the extracts of T. Terrestis fruits by mixing method in ethanol:water (1:1) solvent
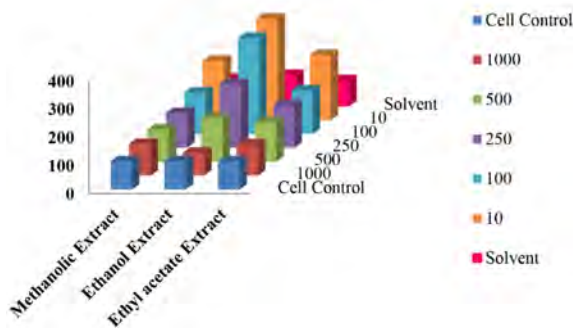


**Figure 9.** Comparison of effect of the all extracts on MCF-7 cell viability

mixture for 24 h. Researchers examined the effects of the extracts on HCT-15 cells in the concentration range of 10-70 ppm and found that the cells proliferated at 10 and 20 ppm. At 40-70 ppm, the cell viability was found as 25 %. They determined that cytotoxicity increased with increasing concentration [24]. In another study, the water:methanol (1:4) extracts of the fruits of the plant were obtained by the Soxhlet method. The effects of plant extracts on colon cancer cells (HT29) and prostate cancer cells (LNCaP-FGC-10) cells were investigated by MTT method. While colon cancer cell viability was 10 % at 0.5 ppm, no cytotoxicity was observed on prostate cancer. At 12 ppm, the viability of prostate and cancer cells was reported as 10 % and 30 %, respectively [25]. In addition, the aqueous extracts of this plant obtained by ultrasonic wave assisted extraction and these extract's cytotoxicity was investigated on Hepa 1c1c7 and Ovcar 3 cells. Apart from our study, this study is the only study in which ultrasonic wave assisted extraction method was used. Plant samples were purchased commercially and water was used as the extraction solvent. In these samples, only one caused severe cytotoxicity at 250 ppm and 500 ppm concentrations, while notable cytotoxicity was observed in the others [26]. Naz et al. evaluated the cytotoxicity of extracts of leaves and fruits of *Tribulus Terrestis* on brine shrimps. They used methanol as the extraction solvent. Leaf and fruit extracts at 1000 ppm concentrations exhibited mortality of 3.50 % and 5.20 %, respectively. Mortality percentages at 10 ppm were reported as 1.55 % and 2.65 %, respectively [27]. In a study by Menon et al., the effects of methanolic extracts of *Tribulus Terrestis* on DLA and EAC cells in the concentration range of 100-500 ppm were investigated by trypan blue exclusion method. Cell viability rates decreased below 50 % at 380 and 420 ppm [28]. Gacche et al. obtained extracts of *Tribulus Terrestis* fruits with different solvents by Soxhlet extraction. Water extract, ethanol extract and chloroform extract caused cytotoxicity of 50 %, 45 % and 25 %, respectively, at 1000 ppm concentrations on HeLa cells [29]. The cell line we used in our study is MCF-7 breast cancer cell line. Patel et al. first extracted the plant with n-hexane and then re-extracted the defatted extracts with ethanol. It was determined that the seeds of the plant caused 47.02 % cell vi-

ability at 100 ppm concentration and 80.54% cell viability at 12.50 ppm on MCF-7 cell line. It had been reported that the leaves of the plant cause 98.46 % and 69.51 % cell viability on MCF-7 cells at 100 ppm and 12.50 ppm concentrations, respectively [30]. The biological activity of a plant sample varies according to many conditions such as the solvent used in the isolation of its phenolic compounds, extraction technique, climate, coordinates, altitude, soil quality and fertilizer used [31, 32]. The results of previous studies are also very different from each other as they are heavily influenced by these factors.

## CONCLUSION

In conclusion, according to the findings of previous studies, it was determined that saponins isolated from methanol and petroleum ether extracts and ethanolic extracts of *T. terrestris* L. cause cytotoxicity. In this study, the effects of the extracts of the aerial parts of the plant obtained by ultrasound assisted extraction using methanol, ethanol and ethyl acetate solvents on the viability of MCF-7 cells were investigated by MTT method. It was determined that only 1000 ppm ethanol extract from the obtained extracts caused moderate cytotoxicity and cell viability was found approximately 80 %. In general, it was determined that *T. terrestris* L. ethanol, ethyl acetate methanol and extracts caused cell proliferation at all other concentrations. Also, breast cancer cells are strong cells compared to normal cells. The fact that the plant extract used in this study did not caused cytotoxicity is attributed to these reasons. Therefore, the use of *Tribulus Terrestis* L. plant collected for this study as an anticarcinogenic agent against breast cancer is not recommended. In addition, it is recommended that more studies be carried out for the use of plants for alternative treatment, especially in patients.

## ACKNOWLEDGEMENT

## CONFLICT OF INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## AUTHOR CONTRIBUTION

Giray Bugra Akbaba: Performing cytotoxicity experiments and Evaluation of Results; Füreya Elif Ozturkkan: Planning of the study, Performing all experiments Writing-Reviewing and Editing and Evaluation of Results; Mustafa Sertcelik: Performing extraction experiments

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# Bioadsorbent (Rice Grains) Efficiency in Mercury II Removal from Aqueous Solutions: Adsorption Kinetics, Isotherm and Thermodynamics

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## ABSTRACT

Heavy metals are major pollutants in marine, soil, industrial, and even treated wastewater. These metals are transported by flowing waters and polluted water sources downstream of the industrial site. Mercury is a highly toxically heavy metal. Mercury, on the other hand, is a highly toxically heavy metal. Mercury spillage is hazardous for it destroys the tissue, lungs, brain, and can affect the nervous systems and kidneys, causing some diseases. Therefore, removing Hg (II) from drinking water, aqueous solutions is essential in wastewater treatment and hydrometallurgical. A diverse process has been suggested to eliminate Hg (II) ions from wastewater. The adsorption method is used as a low-cost, efficient, and effective technique for removing toxically heavy metals from wastewater. Researchers have turned to inexpensive adsorbents such as vegetable wastes. This study aimed to remove Hg (II) ions from wastewater by using ground rice grains as adsorbents. The suitability of different isotherm and kinetic models for the adsorption process was researched. It was determined that the Langmuir isotherm best describes the adsorption equilibrium process, and the pseudo-second-order kinetic model is the most suitable model for adsorption. As a result of the analysis of thermodynamic parameters, it was concluded that the adsorption mechanism proceeds spontaneously and has an endothermic character. The data obtained show that rice grains can be considered a cheap, practical, and effective adsorbent for Hg (II) adsorption from wastewater.

**Keywords:**
Adsorption; Contamination; Isotherm; Kinetics; Mercury II; Thermodynamics

## INTRODUCTION

The rapid increase in the world population, the excessive increase in industrialization, but the lack of environmental awareness in parallel with this rapidly consumes usable water resources. In addition to the advantages arising from the rapid rise in automation, it poses significant threats to the environment and living things due to the wastes produced. Harmful wastes produced by various industrial establishments can be divided into two categories as organic and inorganic wastes. Of these, organic wastes may be more unstable in the environment than inorganic wastes. However, inorganic wastes can remain undegraded for a long time, especially in aqueous environments, and can cause accumulations. When it comes to inorganic wastes, heavy metals usually come to mind [1]. Various definitions can be made for heavy metals. These metals are called "heavy metals" because their density is more than five g.cm³ in terms of their physical properties. More than 60 heavy metals can be identified in this way, including iron, cop-

per, lead, zinc, nickel, cobalt, mercury, and chromium [2]. Heavy metals are among the pollutants that need to be removed due to their toxic and carcinogenic effects on human and aquatic environments through many sources. These dangerous pollutants are formed because of industrial, agricultural, waste disposal, and military activities. Industrial wastewater is the leading source of heavy metal pollution [3]. Some heavy metal salts are easily mixed in aqueous environments due to their excellent solubility in water. Since most of them dissolve in water as colorless, it is impossible to detect water contaminated with heavy metals easily. Heavy metal ions mixed with aqueous environments from various industrial establishments form precipitates as slightly soluble salts, especially in the sediments of seas, rivers, and lakes. Thus, when the heavy metal dissolved in the water decreases, the deposits release heavy metal ions into the water. This state shows how dangerous heavy metal pollution can be. In this way, living creatures living in waters pol-

luted with heavy metal ions take heavy metals into their bodies. Thus, heavy metals in aquatic environments pass from the most minor living thing to the body of other living things with an increasing concentration through the food chain and threaten [4].

Some conventional techniques of dealing with aqueous contamination include adsorption, coagulation, precipitation, membrane separation, reduction, photocatalysis, ion exchange, and so forth [5, 6]. Amongst these techniques, the adsorption process is easy, low-cost to and an effective way to handle water pollution [7]. Therefore, the quality and quantity necessities of adsorbents are rising. Recently, there are many kinds of commercially present adsorbents for different practices. The improvement of environmentally more efficient, cheap, and friendly adsorbents is a very active investigative issue. Therefore, alternative low-cost adsorbents such as chitin [8], coffee [9], tea waste [10], rice husk [11], orange peel [12], bark [13], and coir pith [14] have been studied.

A widely sourced and low-cost adsorbent with high adsorption capacity should be used to remove mercury ions, a toxic heavy metal, from wastewater. Grown rice is the second most grown cereal plant globally and significant fundamental food for more than half of the world's population [15]. Rice contains some unique ingredients that have proven benefits for human health. Rice grains contain phenolic compounds with antioxidant activity. The most widespread shapes of phenolic compounds in rice are hydroxybenzoic and hydroxycinnamic acids. Other compounds defined contain protocatechuic acid, sinapic, and p-hydroxybenzoic acid, which are benzoic acids. Additionally, the two primary groups of compounds influential, aldehyde analogs such as vanillin are also called phenolics. [16].

This research aims to specify the optimum parameters for the maximum adsorption of Hg (II) ions from aqueous solutions using rice grains, which are widely used and contain different compounds in their structure as adsorbents. In addition, using the obtained data, adsorption kinetics, isotherm models, and thermodynamic properties were evaluated.

## MATERIAL AND METHODS

### Chemicals and instruments

The rice grains used were purchased from local markets. The HgCl$_2$ salt, 1,5-diphenylcarbazide, Acetic acid (CH$_3$COOH), and Sodium hydroxide (NaOH) were obtained from Merck and were of analytical grade. A stock solution of mercury chloride salt was prepared (500 mg/L) and diluted to desired concentrations.

WiseStir multiple mechanical stirrer heater, NUVE FN 400 oven, Thermo Scientific ultrapure water device, 620 Lab pH Meter, Optizen POP UV spectrophotometer were used in the experiments.

### Adsorption Experiments

Rice obtained from local markets was ground into particles of approximately 100-150 mesh and dried in an oven at 50°C for 12 hours to remove moisture. Dried rice grains were stored in a desiccator for use in experiments without any further modification. Mercury ions adsorption studies were carried out with bioadsorbent (rice grains) in Hg (II) solution (10 mL). The pH of the Hg (II) solution is adjusted between 3 and 10 using diluted 0.1 M CH$_3$COOH or 0.1 M NaOH solutions. A known mass of bioadsorbent was then added to the mercury solution and shaked at 500 rpm and room temperature. After the adsorption process, the bioadsorbent was removed from mercury solutions by filtration. The concentration of Hg (II) in the solutions was determined at 532 nm with a UV spectrophotometer using 1,5-diphenylcarbazide [17]. The effects of process variables like contact time, temperature, pH, and initial concentration on the mercury removal efficiency were investigated.

Equations for determining the amount of Hg (II) adsorbed using bioadsorbent are given below:

$$q_t = \frac{(C_0 - C_t)}{m} x V \tag{1}$$

$$q_e = \frac{(C_0 - C_e)}{m} x V \tag{2}$$

$$\%R = \frac{(C_0 - C_t)}{C_0} x 100 \tag{3}$$

Where, q$_e$ and q$_t$ are the adsorption capacity (mg/g) at equilibrium and t, respectively; C$_o$, C$_e$ and C$_t$, initial concentration at time t, mercury equilibrium concentration (mg/L) and liquid phase concentration, respectively; the volume (L); m is the amount of adsorbent (g); and R is removed yield (%) [18].

## RESULTS AND DISCUSSION

### Infrared Spectrum of Bioadsorbent

FT-IR spectra of the bioadsorbent before and after adsorption were recorded between 4000 and 400 cm$^{-1}$ using Bio-Rad-Win-IR spectrophotometer. When the FT-IR graphs before and after Hg (II) adsorption are examined (Fig. 1); stretching vibration of hydroxyl (O-H) and N-H bonds around 3600 cm$^{-1}$; at 2970 cm$^{-1}$ the asymmetric stretching of the −CH− groups; asymmetrical carboxy-
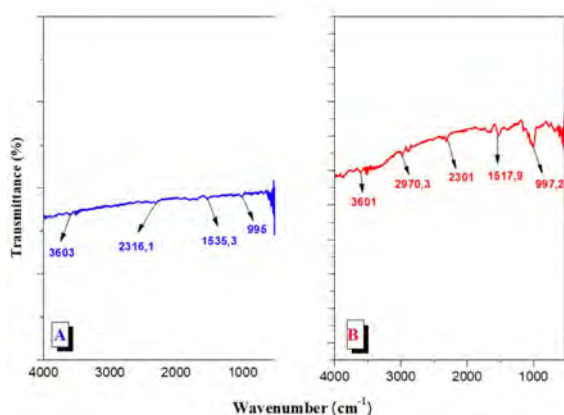
**Figure 1.** FTIR plots of bioadsorbent (a) after and (b) before Mercury II adsorption



**Figure 2.** The impact of pH on Mercury II adsorption (25ºC, 60 min, 50 mg/L, pH: 4-10).



**Figure 3.** The impact of adsorbent amount on Mercury II adsorption (25ºC, pH: 6, adsorbent: 25-150 mg, 50 mg/L).

late (-COO-) stretching vibration at 1517 cm$^{-1}$; -C–O stretching vibration of alcohol, phenol and carboxylic groups is observed at 997 cm$^{-1}$. It was observed that the peaks of the functional groups of the bioadsorbent determined before the adsorption process changed because of Hg (II) adsorption. It was observed that some of the previously determined peaks disappeared after Hg (II) adsorption, and there was a shift in frequency values at some peaks. This proves that physical or chemical bonds are formed between the surface functional groups of the bioadsorbent and Hg ions and that adsorption takes place.

## pH Effect

The effect of initial pH on Hg (II) removal efficiency was investigated in the pH range of 4-10. Maximum adsorption (84.58%) was obtained at pH=6 (Fig. 2). Depending on the pH range, three main types of HgCl$_2$, HgO, and Hg(OH)Cl can be found in the solution. Under acidic conditions, HgCl$_2$ is present, and protons compete with mercury ions to occupy active sites. Thus, the uptake of HgCl$_2$, mercury ions is reduced. In the pH (4-6) range, Hg (OH)Cl is formed, a species that increases mercury uptake. Under primary conditions, red mercuric oxide precipitates with the most thermodynamically steady kinds obtained at high pH [19]. In a study on the adsorption of mercury on granular activated carbon in aqueous solutions containing nitrates and chlorides, the optimum pH value was found to be 5 [20].

### Effect of Adsorbent Amount

In the experiments carried out to examine the effect of the adsorbent dose on the adsorption of mercury ions; Varying amounts of adsorbent in the range of 25-150 mg were used. According to the results obtained, maximum adsorbance was obtained when 100 mg of adsorbent was used and this amount was also used in subsequent studies. The results are shown in Figure 3.
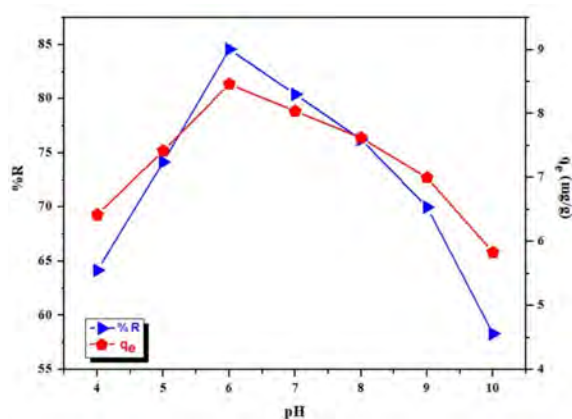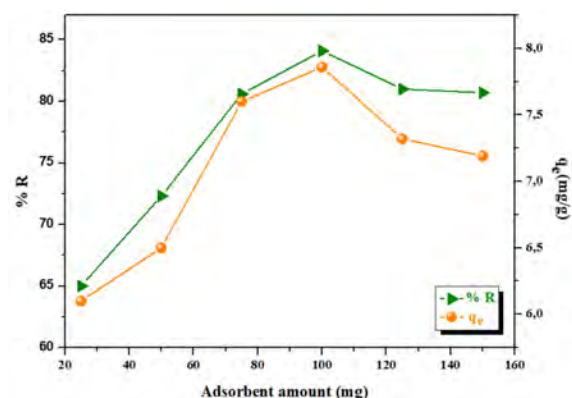
## Effect of Contact Time and Kinetic Studies

One of the parameters effecting adsorption is contact time. As a result of adsorption of 100 mg bioadsorbent and 50 mg/L HgCl$_2$ solutions at pH 6.0 at 25ºC and mixing at 500 rpm for 5-180 minutes, the maximum contact time was found to be 30 minutes (Fig. 4). This time was also used in subsequent parameter studies.
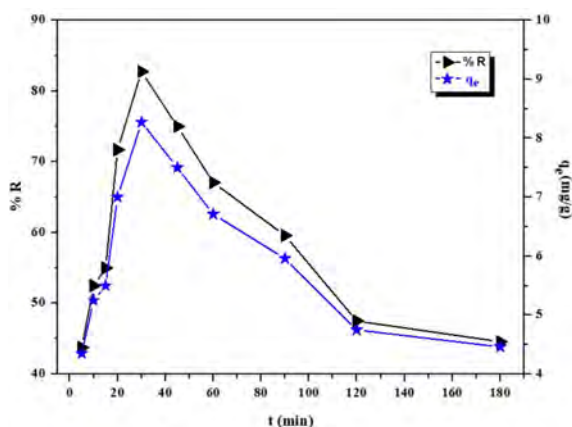


**Figure 4.** The impact of contact time on Mercury II adsorption (pH: 6, 25ºC, 100 mg adsorbent, 50 mg/L).

The kinetics of Hg (II) adsorption were investigated with Pseudo first order (Fig. 5a) [21] and Pseudo second order (Fig. 5b) [22], and intra-particle diffusion (Fig. 5c) [23] models according to the following equations and the calculated parameters are shown in Table 1.

$$\ln(q_e - q_t) = \ln q_e - k_1.t \qquad (4)$$

$$\frac{t}{q_t} = \frac{1}{k_2 q_e^2} + \frac{1}{q_e}.t \qquad (5)$$

$$q_t = K_{id}.t^{1/2} + I \qquad (6)$$

Where, $q_t$ and $q_e$ are the adsorption capacity (mg/g) at t and equilibrium, respectively. $k_1$ (1/min) and $k_2$ (g/mg.dk), the ratio constant of the pseudo-first-order and pseudo-second-order model, $K_{id}$ particle inside diffusion rate regular (mg/g.min$^{1/2}$). The value of I gives an idea of the thickness of the boundary layer. That is to say, the greater the intersection degree, the greater the boundary layer effect.

**Table 1.** Kinetic parameters of Mercury II adsorption on bioadsorbent.

| Models | Parameters | Hg (II) |
|---|---|---|
| Pseudo-first order | $q_e$ (mg/g) | 6.36 |
| | $k_1$ (1/min) | 0.07 |
| | $R^2$ | 0.8527 |
| Pseudo-second order | $q_e$ (mg/g) | 8.19 |
| | $k_2$ (g/mg.min) | 0.02 |
| | $R^2$ | 0.9935 |
| Weber-Morris intra-particle diffusion | $q_{exp}$ | 8.27 |
| | k (mg/gmin$^{1/2}$) | 1.08 |
| | I (mg/g) | 1.8 |
| | $R^2$ | 0.8937 |

When Table 1 is examined, the pseudo-second-order kinetic model appears to be the most appropriate for Hg (II) adsorption. This equality is used to explain the adsorption behaviors at lower concentrations. In the table, it is seen that
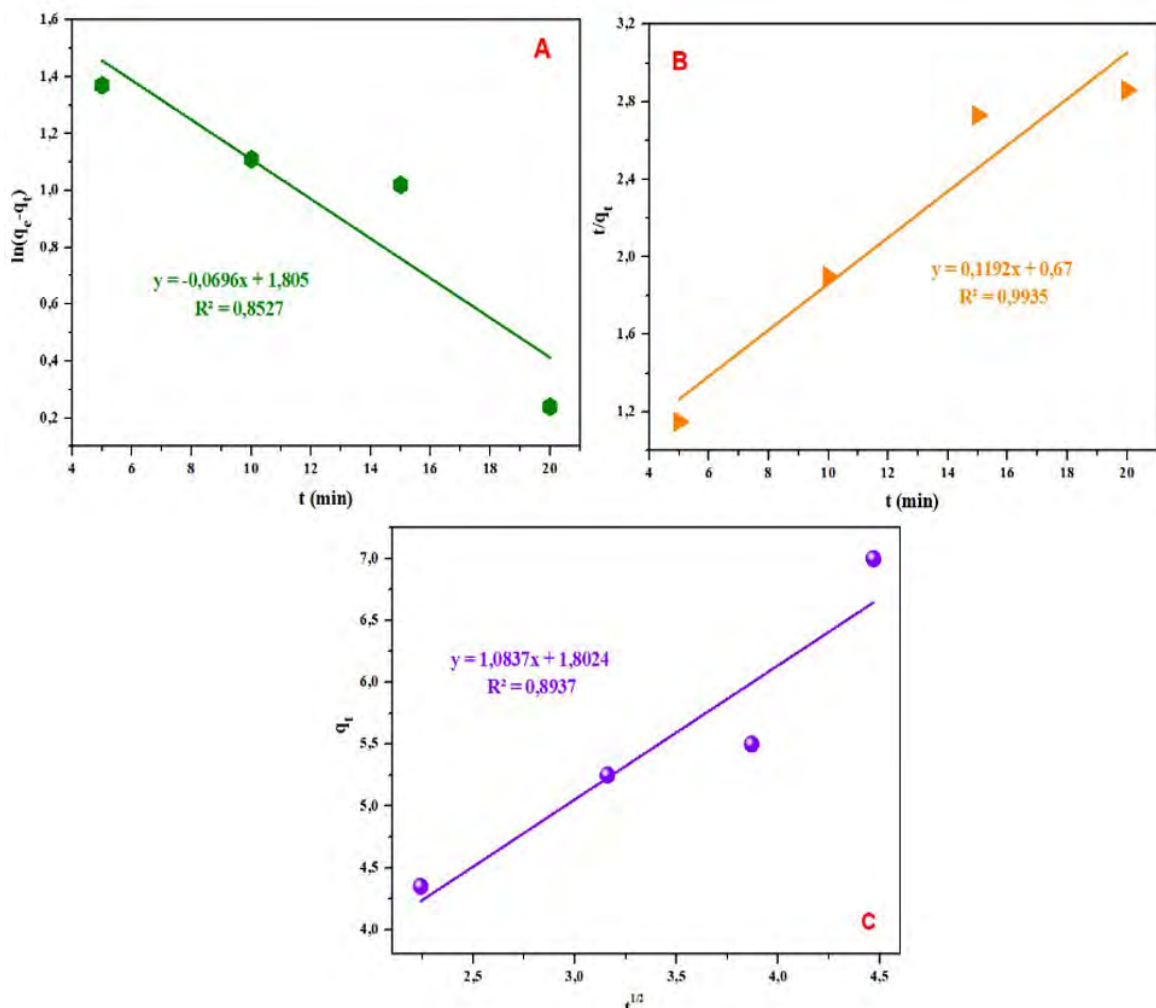


**Figure 5.** Adsorption kinetics of Mercury II adsorption on bioadsorbent according to (a) pseudo-first-order, (b) pseudo-second-order, (c) intraparticle diffusion kinetic models.

the correlation coefficient $R^2$ of the pseudo-second-order equation is close to 1 (0.9935), and the $q_e$ value (8.19) calculated from the equation gives a result closer to the $q_{exp}$ (8.27) value. The inadequacy of the pseudo-first-order rate equation to fit the kinetic data is due to the boundary layer that controls the initiation of the adsorption process. In a study in which toxic mercury was removed from petroleum oil using a molecularly imprinted polymer, it was stated that the adsorption process was suitable with the pseudo-second-order kinetic model [24].

## Initial Concentration Effect and Adsorption Equilibrium Isotherms

The effect of the initial mercury concentration on the adsorption efficiency was investigated using $HgCl_2$ solutions with concentrations ranging from 10 to 250 mg/L. In the given concentration range at pH 6.0, mercury solutions were shaken with 100 mg bioadsorbent at 500 rpm

for 30 minutes at 25°C. Optimum adsorption efficiency (85.8%) was obtained at 50 mg/L concentration.

Experimental data analyzes were performed in Langmuir, Dubinin-Radushkevich, Temkin, and Freundlich isotherm models to evaluate the adsorption equilibrium process [25, 26, 27, 28]. Isotherm graphs are given in Figure 6, and isotherm parameters are given in Table 2.

The compatibility of Langmuir, Dubinin-Radushkevich (D-R), Temkin, and Freundlich isotherm models to the mercury ion adsorption mechanism was investigated and it was determined that Langmuir isotherm was more compatible. The correlation coefficient calculated for the Langmuir isotherm was $R^2 = 0.9964$, which was higher than the other isotherms. Accordingly, in the Langmuir equilibrium isotherm, the adsorption process is adsorption in which mercury ions bind to functional groups and binding sites homogeneously distributed on the adsorbent surface in a monolayer. At the
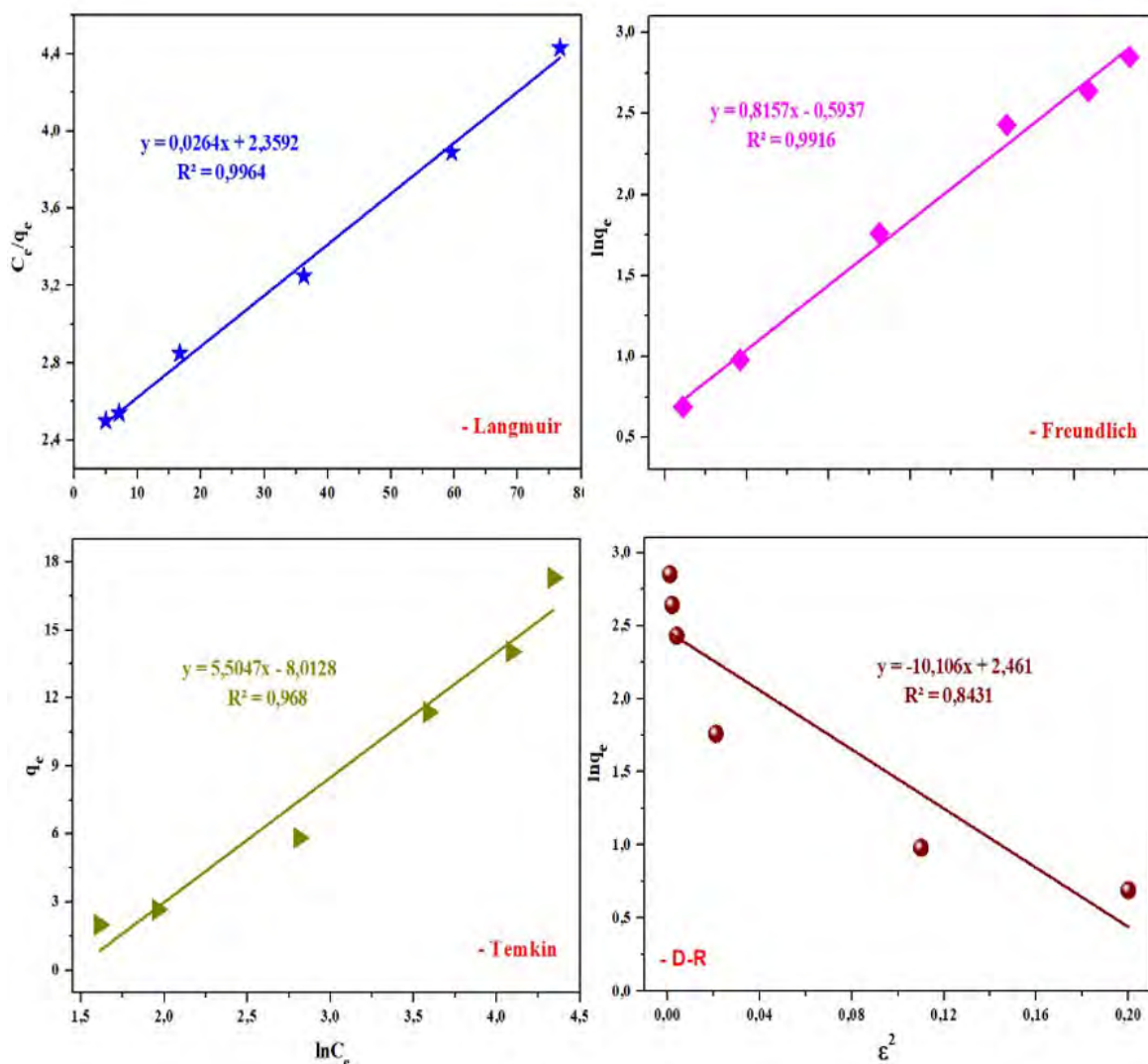


**Figure 6.** Isotherm graphics for the Mercury II adsorption process.

**Table 2.** Isotherm parameters for adsorption process.

| Models | Parameters | Hg (II) |
|---|---|---|
| Langmuir | $q_{max}$ (mg/g) | 37.88 |
| | b (L/mg) | 0.01 |
| | $R_L$ | 0.2 |
| | $R^2$ | 0.9964 |
| Freundlich | Kf [(mg/g) (L/mg)$^{1/n}$] | 0.55 |
| | n | 1.23 |
| | $R^2$ | 0.9916 |
| Temkin | bT | 0.23 |
| | $K_T$ (L/g) | 5.5 |
| | $R^2$ | 0.968 |
| Dubinin-Radushke-vich | $q_m$ (mg/g) | 11.72 |
| | β (1/mol$^2$.J$^2$) | 10.1 |
| | E (J/mol) | 0.22 |
| | R2 | 0.8431 |

same time, in this adsorption, there is a certain number of active sites on the adsorbent surface and the molecules bind to these active ends. Accordingly, mercury ions are attached to the functional groups and binding sites on the surface as a single layer. The maximum monolayer adsorption coefficient ($q_{max}$) value was found to be 37.88 mg/g. Using the b value from the calculated Langmuir parameters, the $R_L$ values (dimensionless separation factor) were found to be as 0.2. The fact that these values are in the range of $0<R_L<1$ indicates that the adsorption mechanism is suitable for mercury ions. In a study in which mercury adsorption was performed by rice husk ash, it was stated that Langmuir isotherm, one of the isotherm models, was more compatible with the adsorption system and the maximum adsorption capacity ($q_{max}$) was 9.32 mg/g [29].

Then value defined in the Freundlich isotherm model was calculated as 1.23. The calculated n value in the range of $1<n<10$ means that the adsorption of mercury ions on the bioadsorbent is favored and positive.

The mean adsorption energy (E) value defined in the Dubinin-Radushkevich isotherm delivers an opinion concerning the reaction mechanism that is efficient in the adsorption process. Since the calculated E value is calculated to be 0.22 kJ/mol (E<8 kJ/mol), physical forces are efficient in adsorption. Due to physical adsorption, it can be mentioned that there are weak Van der Waals attraction forces between bioadsorbent and mercury ions.

## Temperature Effect and Thermodynamic Studies

To examine the impact of ambient temperature on the adsorption of 50 mg/L solutions of mercury ions with 100 mg bioadsorbent; It was studied for 30 minutes at pH 6.0, at a stirring speed of 500 rpm, at temperatures ranging from 20-50°C. The optimum temperature for the adsorption process was found to be 50°C (Fig. 7). The rising in
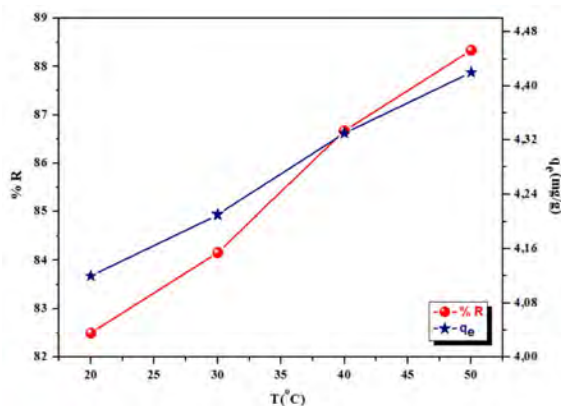


**Figure 7.** The effect of temperature on Mercury II adsorption (pH: 6, 30 min, 100 mg adsorbent, 50 mg/L).

adsorption yield with temperature indicates that the process is endothermic.

The thermodynamic parameters enthalpy change (ΔH°), free energy (ΔG°), and entropy change (ΔS°) are geted from the following equations [30].

$$\ln K_d = \frac{\Delta S^o}{R} - \frac{\Delta H^o}{RT} \tag{7}$$

$$\Delta G^o = \Delta H^o - T\Delta S^o = -RT \ln K_d \tag{8}$$

$$K_d = \frac{C_{ad}}{C_e} \tag{9}$$

Inequality, $C_e$ is the equilibrium concentration of mercury in the solution; $K_d$ is the equilibrium constant; $C_{ad}$, the concentration of mercury adsorbed to the adsorbents at equilibrium; R (8.314 (J/mol.K) is the gas constant; T (K) is temperature. ΔH° and ΔS° are obtained from the incline and intersection of the Van't Hoff plot of $\ln K_d$ for 1/T (Fig. 8), values are obtained from the slope and intersection of the graph, and these results are shown in Table 3.

The enthalpy of adsorption (ΔH°) value calculated from the incline of the line in the graph in Figure 8 was favorable for the adsorption of mercury ions. It showed that the adsorption had an endothermic nature. At the same time, the fact that the adsorption enthalpy values are lower than 40 kJ/
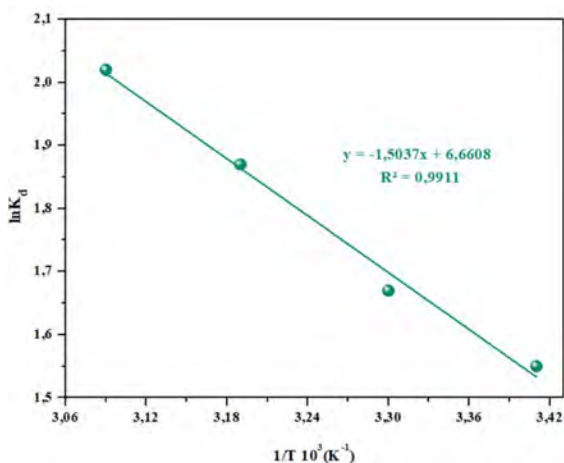
**Figure 8.** Change of ln $K_d$ with 1/T in Mercury II adsorption.

**Table 3.** Thermodynamical parameters for Mercury II adsorption.

| | T (K) | $\Delta H^0$ (J/mol) | $\Delta S^0$ (J/mol K) | $T\Delta S^0$ (kJ/mol) | $\Delta G^0$ (kJ/mol) |
|---|---|---|---|---|---|
| | | | **Thermodynamic parameters** | | |
| | 293 | | | 16.4 | -3.77 |
| | 303 | | | 16.78 | -4.21 |
| Hg (II) | | 12.5 | 55.38 | | |
| | 313 | | | 17.33 | -4.87 |
| | 323 | | | 17.89 | -5.42 |

mol shows that the adsorption processes can be explained by physical adsorption. The adsorption entropy ($\Delta S°$) value calculated from the shift of the graph in Figure 8 was also found to be positive. This result shows that the adsorption occurs spontaneously and that the bioadsorbent has an affinity for mercury ions. The Gibbs free energy change ($\Delta G°$) was calculated as an average of -4.57 kJ/mol. The increase in the negative values of $\Delta G°$ with rising temperature indicates that adsorption at high temperatures is even more convenient and applicable. At the same time, the negative value of Gibbs free energy ($\Delta G°$) shows that adsorption consists spontaneously. In a study in which mercury adsorption was carried out on a carbon sorbent obtained from fruit peel; It was stated that the positive value of $\Delta H°$ indicates the endothermic adsorption of Hg ions and the positive value of $\Delta S°$ indicates the increasing randomness at the solid-solution interface during Hg (II) adsorption. It was also stated that the negative values of $\Delta G°$ and the decrease in $\Delta G°$ with temperature increase indicate the spontaneous nature of its adsorption [31].

## CONCLUSION

The adsorption method is a very effective method among the various methods used for the removal of heavy metals in wastewater, nowadays, where the prevention of environmental pollution is becoming increasingly important.

In the study, rice grains containing different compounds in their structure were used as adsorbents to remove toxic metal pollutants.

The suitability of adsorption with isotherm models, kinetic models, and thermodynamic expressions was investigated with the data obtained due to the experiments for the adsorption of mercury ions from aqueous solutions on bioadsorbent.

Pseudo-first-order kinetic model, pseudo-second-order kinetic model, and intraparticle diffusion models were evaluated for mercury adsorption processes. The most suitable kinetic model was determined to be the pseudo-second-order kinetic model. Freundlich, Langmuir, Dubinin-Radushkevich, and Temkin's isothermal models were examined for their suitability for adsorption processes. It was stated that the equilibrium data of adsorption isotherms were quite compatible for the Langmuir model in the concentration range studied, and the qmax value was calculated as 37.88 mg/g. As a result of the analysis of thermodynamic parameters, it was determined that the adsorption processes were spontaneous and endothermic. Also, entropy with a positive value shows the affinity of mercury ions to the bioadsorbent.

According to the results, it is stated that rice grains used without any chemical treatment to remove mercury from aqueous solutions are low cost and effective adsorbents. It is thought that this bioadsorbent can be used as an alternative to adsorbents prepared with costly and complex processes, and this study will contribute to environmental studies.

## CONFLICT OF INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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# Computational Analysis of Potential Key Genes Associated with Dopamine Neurotransmission in Pheochromocytoma and Paraganglioma

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## ABSTRACT

Pheochromocytoma and Paraganglioma (PCPG) are rare and potentially lethal neuroendocrine tumors. PCPG that predominantly or exclusively produce and secrete DA is rarely seen, and it has been known that exclusively dopamine-secreting PCPG is related to advanced malignant features and metastases. Up to the present, little has been known about the role of dopamine neurotransmission and the dopaminergic system in the initiation and progression of PCPG. The genes with significant expression differences between normal tissue and pheochromocytoma and paraganglioma, survival and correlation analysis, CpG islands prediction, and miRNA-target enrichment analysis were performed by several bioinformatics tools. In the present study, it was determined that the COMT gene was significantly less expressed in PCPG than in normal tissue and the COMT gene showed a remarkable relationship between differential expression with shorter overall survival among the individuals with PCPG (HR=1, p=0.011). MAOA and COMT gene pair was significantly correlated with PCPG (p=0.012; R=0.19), and hsa-miR-5000-5p regulates the expression of both COMT and MAOA genes (p=0.00215, FDR=0.127). Our findings suppose that COMT may potentially be implicated in tumor suppressive mechanism. The expression values of COMT and MAOA genes, and hsa-miR-5000-5p may have the potential to be used in the genetic evaluation of the pathogenesis and prognosis of PCPG. Further *in vitro* and *in vivo* studies are required to clarify the molecular mechanism of the dopaminergic system in the pathogenesis and prognosis of PCPG.

**Keywords:**
Dopamine; Pheochromocytoma and Paraganglioma (PCPG); Dopaminergic system; Gene expression; Neurotransmission

## INTRODUCTION

Pheochromocytoma and Paraganglioma (PCPG) are rare chromaffin cell tumors that produce catecholamines (CA) and present a therapeutic and diagnostic challenge, and are associated with severe morbidity when non-diagnostic [1, 2]. Pheochromocytomas and Paragangliomas are originated from adrenal tissues or extra-adrenal parasympathetic or sympathetic paraganglia, respectively [2]. PCPG produce and secrete CA in the neuroendocrine system. PCPG may lead to the elevation of blood pressure and subsequently damage of target organs such as the brain, heart, and kidney. The symptoms of PCPG include palpitations, hypertension, headache, anxiety, and profuse sweating. Furthermore, PCPG may lead to metabolic disorders that are associated with insulin and blood sugar, therefore endanger the life of patients [3, 4].

Circulating catecholamines are composed of norepinephrine (NE), epinephrine (EPI), and dopamine (DA) that are mainly synthesized and released from the adrenal medulla (chromaffin cells) [5]. Specifically, DA acts as a neurotransmitter and neurohormone that modulates cognition, motivation, food intake, appetite, sexual behavior, emotion, reward system, motor activity, and prolactin secretion in the central nervous system [6, 7]. Moreover, DA participates in the regulation of inhibition of aldosterone secretion, vasodilation, inhibition of insulin secretion, diuresis, phagocytic inhibition, IgM and IgG secretion, and natriuresis in the periphery [6].

Increased generation of catecholamines is the distinctive biochemical feature of PCPG. Until recently, the biochemical prediction of PCPG was based primarily on detecting NE, EPI, or their metabolites (no interest in DA or its metabolites). PCPG that exclusively produce and secrete DA are rarely seen, and it has been known that exclusively dopamine-secreting PCPG are related

to advanced malignant features. Lack of clinical symptoms and the rarity of the tumor make it difficult to be detected and may cause to delay of diagnosis and poorer prognosis [8, 9]. Delayed diagnosis might be associated with the increased incidence of malignancy and metastasis. Up to the present, little has been known about the role of dopamine neurotransmission and the dopaminergic system in the initiation and progression of PCPG. Furthermore, differential diagnosis might be achieved by analyzing the expression levels of dopamine neurotransmission-related genes. Therefore, the particular relationship between dopamine neurotransmission and PCPG behavior needs further elucidating. In the present study, we aimed to determine the relationship between the genes associated with dopamine neurotransmission and PCPG by computational methods.

## MATERIALS AND METHODS

### Expression and Survival Analysis of Target Genes in PCPG and Normal Tissue

The sequence information and the functions of the target genes that are associated with dopamine neurotransmission were analyzed and extracted from National Center for Biotechnology Information (NCBI) database [10]. The genes with remarkable expression differences between normal tissue and pheochromocytoma and paraganglioma were designated via Gene Expression Profiling Interactive Analysis (GEPIA) database. GEPIA is an online tool and based on The Genotype-Tissue Expression (GTEx) and The Cancer Genome Atlas (TCGA) that delivers cancer and normal tissue gene expression and interactive analysis data. Furthermore, the 95% confidence interval (95% CI) and the Cox proportional hazard ratio (HR) of the survival plot are provided by the database [11]. Analysis of differential gene expression ensures to find the tumor-specific genes by comparing normal and tumor groups.

### Correlation Analysis of Some Target Genes in PCPG

The statistical analysis of the relationship between MAOA and COMT genes and PCPG; TH and DDC genes and PCPG were verified by the use of the Spearman correlation test.

### Estimation of Protein-Protein Interactions

The Search Tool for the Retrieval of Interacting Genes/Proteins (STRING) web tool was used to predict the functional partners of the proteins that are encoded by the target genes. The significant integration and assessment of protein-protein interactions with functional (in-

direct) and physical (direct) associations are provided by the STRING database [12].

### Prediction of CpG Islands of Target Genes

In this study, the CpG islands in some target genes were analyzed by the MethPrimer bioinformatics tool to predict the effect of methylation on the expression of target genes. The values of "observed/expected CpG ratio"= 0.60, "island size" > 100 nucleotide, and "percentage of G plus C" = 50.0 were set in the MethPrimer program as standard values. MethPrimer is a program that finds the potential CpG islands based on DNA sequence and designs the primers for bisulfite sequencing PCR and methylation specific PCR [13].

### miRNA-Target Enrichment Analysis

microRNAs are implicated in the regulation of gene expression. Multiple genes can be regulated by one miRNA and one gene can be targeted by several miRNAs in a simultaneous way. MicroRNA ENrichment TURned NETwork (MIENTURNET) program was used for the miRNA-target enrichment analysis. miRNA-target enrichment analysis is a standard method to clarify the hierarchical effects of microRNAs in the regulatory networks of genes [14, 15].

## RESULTS AND DISCUSSION

### Expression and Survival Analysis of Dopamine Neurotransmission-Related Genes in PCPG and Normal Tissue by *in silico* Methods

The expression values of TH, DDC, DBH, PNMT, MAOA, MAOB, COMT, ALDH2, DRD1, DRD2, DRD3, DRD4, DRD5, SLC6A3, SLC18A1 and SLC18A2 genes were analyzed and expression differences between PCPG and normal tissue were compared. The genes with remarkable expression differences between PCPG and normal tissue were identified. According to the analysis, MAOA, MAOB, COMT, ALDH2, and DRD1 genes were significantly less expressed in PCPG than in normal tissue. On the other hand, expression of TH, DDC, DBH, PNMT, DRD2, SLC18A1, and SLC18A2 genes were significantly higher in PCPG compared to normal tissue (Table 1). The median, log2, and percentage values of the over-expressed genes in PCPG were shown in Table 2. The comparison of the expression values of the genes in PCPG and normal tissue was illustrated in Figure 1, Figure 2, and Figure 3. The relationship between gene expressions with overall survival of PCPG patients were evaluated to determine the role of the genes associated with the dopaminergic system in PCPG prognosis by dividing the

population into low expression and high expression, only one gene showed a remarkable relationship between differential expression with shorter overall survival among the individuals with PCPG. Log rank ($p<0.05$) is accepted as statistically significant. The gene-COMT (HR=1, p=0.011) may serve as a molecular biomarker for PCPG (Figure 4).

## Correlation Analysis of MAOA and COMT Genes and TH and DDC Genes in PCPG

The statistical analysis of the relationship between MAOA and COMT genes (encode the enzymes that degrade dopamine) and PCPG was performed via the GEPIA database. It was determined that MAOA and COMT gene pair was significantly correlated with PCPG by the Spearman correlation analysis (p=0.012; R=0.19) (Figure 5). Furthermore, Spearman correlation analysis has determined that the TH and DDC gene (encode the enzymes that are involved in the synthesis of dopamine) pair was significantly associated with PCPG (p=0.043; R=0.15) (Figure 6).

## Prediction of Protein-Protein Interactions

Protein-protein interactions were estimated by STRING web tool to elucidate the functional partners of the enzymes degrading DA (MAOA and COMT) and the enzymes catalyzing the synthesis of DA (TH and DDC). Based on the findings of STRING for MAOA protein, functional interactants with high confidence were determined as: COMT (0.989), DDC (0.971), DBH (0.962), ALDH2 (0.961), CYP2D6 (0.955), AOC2 (0.951), ALDH3B2 (0.945), HNMT (0.943), ADH1B (0.942), PNMT (0.940) (Figure 7). Functional interactants of COMT protein were predicted as follows: MAOA (0.989), MAOB (0.987), DDC (0.980), DBH (0.971), CYP1B1 (0.971), CYP1A1 (0.968), PNMT (0.963), ALDH2 (0.944), ALDH1B (0.944), DRD2 (0.940) (Figure 8). Functional partners of TH protein were determined as follows: SNCA (0.992), DDC (0.992), SPR (0.968), PCBD1 (0.953), QDPR (0.951), DBH (0.945), TYR (0.943), SLC18A2 (0.937), AKR1B1 (0.927), GOT1 (0.926) (Figure 9). The functional interactants of DDC protein with high confidence were determined as: TH (0.992), DBH (0.987), TPH1 (0.984), COMT (0.980), MAOB (0.975), MAOA (0.971), TPH2 (0.969), PAH (0.966), AOC1 (0.964), AANAT (0.962) (Figure 10). According to the predictions of STRING web tool, it was demonstrated that MAOA and TH interact with COMT protein, and DDC, respectively.

## Analysis of CpG Islands

Methylation is involved in the regulation of gene expression [16]. In the present study, CpG islands were analy-

zed to determine the potential role of methylation in the regulation of TH, DDC, MAOA, and COMT genes. According to the findings of the study, the number of CpG islands (CIG) were indicated as follows: TH-4 CIG; DDC-1 CIG; MAOA-0 CIG; COMT-4 CIG, and the results are seen in Table 3.

## miRNA-Target Enrichment Analysis

The miRNAs which have the potential to regulate the expression of more than one gene associated with dopaminergic system among the investigated mRNAs were shown in Table 4. Common miRNAs for SLC18A2 and DRD2: hsa-miR-141-3p and hsa-miR-200a-3p; for SLC18A2 and DRD1: hsa-miR-142-5p and hsa-miR-5590-3p; for DRD2 and SLC18A2: hsa-miR-9-5p; for COMT, SLC6A3, and DRD1: hsa-miR-30a-5p, hsa-miR-30b-5p, hsa-miR-30c-5p, hsa-miR-30d-5p, and hsa-miR-30e-5p; for COMT and MAOA: hsa-miR-5000-5p; for ALDH2 and TH: hsa-miR-1-3p; for ALDH2, DBH, DRD5, and MAOB: hsa-miR-335-5p; for ALDH2 and COMT: hsa-

**Table 1.** Expression values of the genes associated with dopamine neurotransmission in PCPG and normal tissue

| Gene ID | Pheochromocytoma and Paraganglioma (PCPG) | Normal tissue |
|---|---|---|
| TH* | 1,830.03 | 24.87 |
| DDC* | 599.55 | 3.34 |
| DBH* | 2,853.17 | 20.21 |
| PNMT* | 113 | 8.91 |
| MAOA* | 6.97 | 22.01 |
| MAOB* | 8.26 | 39.2 |
| COMT* | 112.21 | 256.67 |
| ALDH2* | 69 | 346.45 |
| DRD1* | 0.05 | 0.24 |
| DRD2* | 86.66 | 0.98 |
| DRD3 | - | - |
| DRD4 | 0.32 | 0.19 |
| DRD5 | 0.02 | 0.01 |
| SLC6A3 | 0.02 | 0.01 |
| SLC18A1* | 145.98 | 0.37 |
| SLC18A2* | 133.17 | 1.03 |

*significant differential expression pattern between PCPG and normal tissue

miR-16-5p; for PNMT and DRD3: hsa-miR-26b-5p.

Pheochromocytoma and Paraganglioma (PCPG) are rare and potentially lethal neuroendocrine tumors. Despite most of the tumors are benign, approximately 10-15% are classified as malignant and develop metastases in nonchromaffin tissues such as bone, liver, and lymph nodes [17]. It is

**Table 2.** The median, log2, and percentage values of the over-expressed genes in PCPG

| Gene ID | Median (Tumor) | Median (Normal) | Log2 (Fold change) | Percentage |
|---------|----------------|-----------------|--------------------|------------|
| TH | 4539.601 | 616.945 | 2.877 | 1.00e+0 |
| DDC | 1548.065 | 385.690 | 2.002 | 1.00e+0 |
| DBH | 6086.722 | 303.910 | 4.319 | 1.00e+0 |
| PNMT | 2178.772 | 465.310 | 2.225 | 9.44e-1 |
| SLC18A1 | 759.997 | 20.487 | 5.146 | 1.00e+0 |
| SLC18A2 | 534.371 | 89.529 | 2.564 | 1.00e+0 |
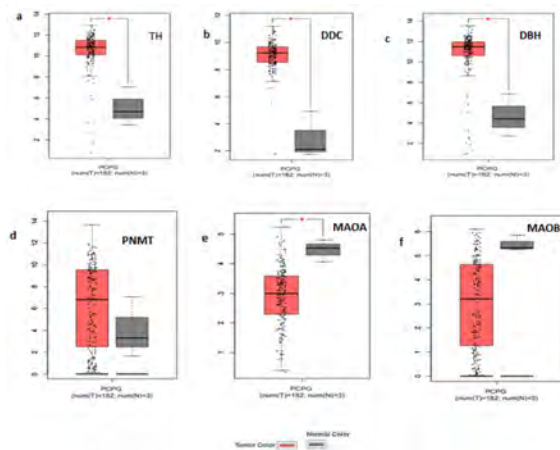


**Figure 1.** Differential expressions of TH, DDC, DBH, PNMT, MAOA, and MAOB genes in PCPG
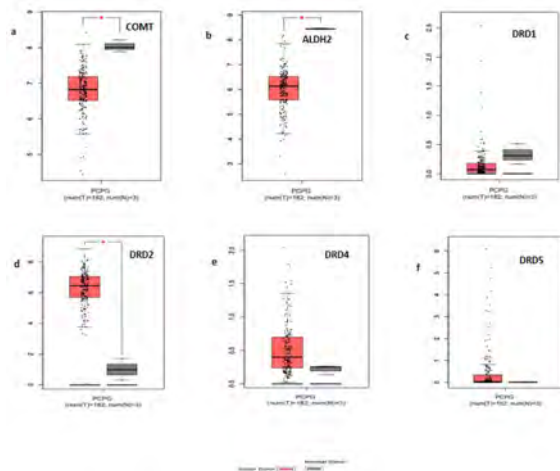


**Figure 2.** Differential expressions of COMT, ALDH2, DRD1, DRD2, DRD4, and DRD5 genes in PCPG



**Figure 3.** Differential expressions of SLC6A3, SLC18A1, and SLC18A2 genes in PCPG

significant to diagnose PCPG in an accurate and early way to treat patients and also affected members in family cases [18]. PCPG usually secrete high amounts of catecholamines. Tumors that predominantly or exclusively produce DA are rare and often malignant and metastatic [17, 19].

Dopamine, biogenic monoamine, is a member of catecholamine neurotransmitters and generated in both central nervous system and periphery. The dopaminergic system is implicated in several biological functions such as motivation, cognition, motor activity, maternal behavior, reward system, and reproductive behavior. Dopamine is synthesized directly from tyrosine or indirectly from L-phenylalanine. Tyrosine hydroxylase is the rate limiting enzyme in DA synthesis that converts tyrosine to L-DOPA and L-DOPA may be converted into DA by DOPA decarboxylase. Subsequently, DA is sequestered into the synaptic vesicles through vesicular monoamine transporter 2 (VMAT2) in dopaminergic neurons, preventing oxidation. DA may be further converted into NE or EPI by modifications from DBH and PNMT in adrenergic and noradrenergic cells [20]. In a non-acidic microenvironment, DA is metabolized by MAO and COMT enzymes [21]. Dopamine exerts its functions via binding to G protein-coupled receptors (DRD1, DRD2, DRD3, DRD4, DRD5) [22].

The dopaminergic phenotype of PCPG is composed of a rare subtype of PCPG that predominantly or exclusively secrete DA (no significant NE and EPI levels) [23]. In contrast to the symptoms that are seen in epinephrine and norepinephrine-secreting tumor in high amounts, the tumors that predominantly or exclusively produce DA are normotensive and asymptomatic [23]. DA secreting PCPG are generally larger tumors than epinephrine and norepinephrine-secreting tumors due to delayed diagnosis (incidentally identified). There are several significant differences, such as the risk of recurrence, malignancy, and peri-operative cardiovascular collapse between DA secreting PCPG and NE and EPI secreting PCPG in terms of management [24]. Moreover, DA secreting PCPG have high malignancy risk compared to NE and EPI secreting PCPG [25].

In the current study, we have analyzed the genes as-
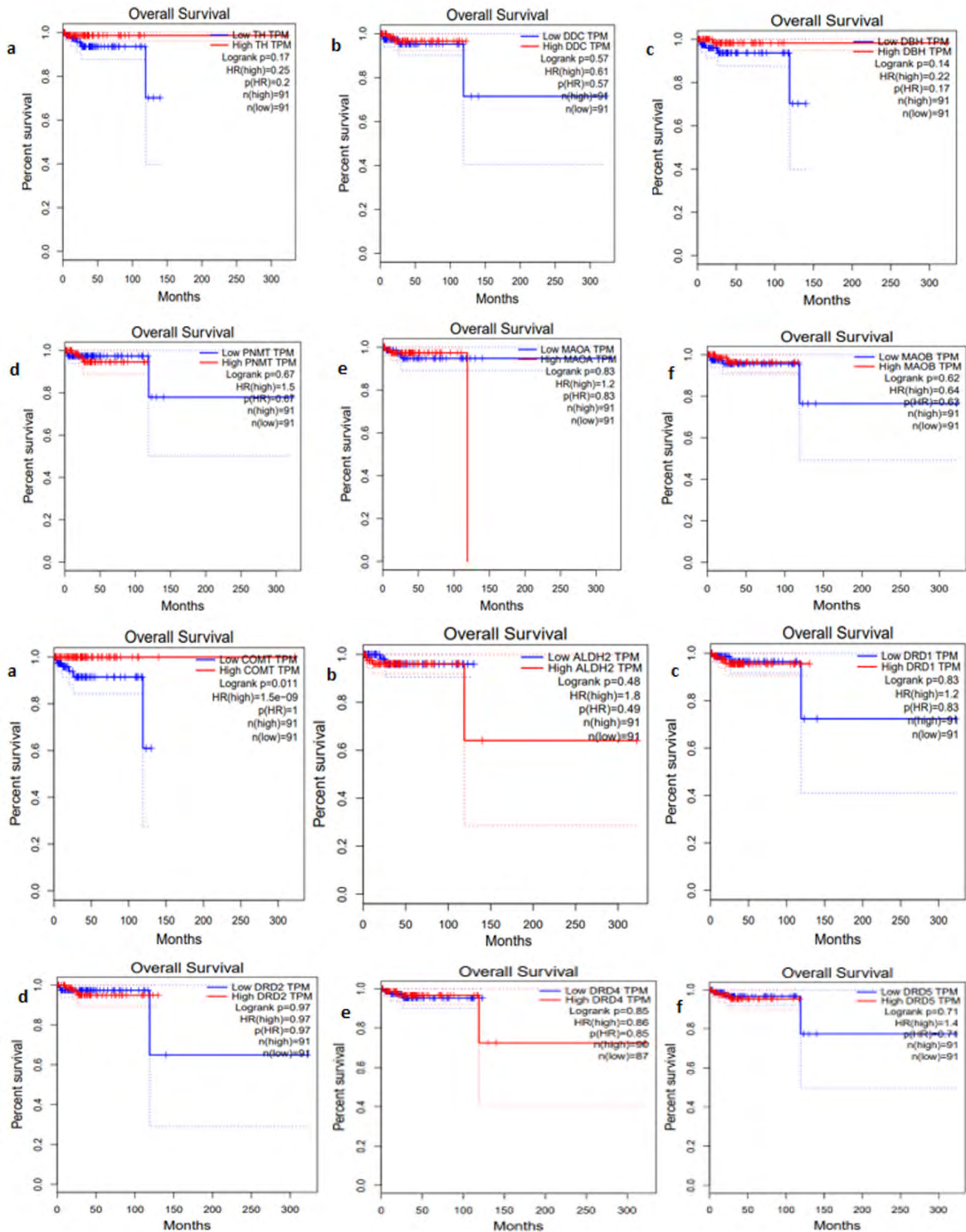
**Figure 4.** Associations of the genes that are associated with dopamine neurotransmission with overall survival in PCPG

sociated with dopamine neurotransmission and specifically focused on the genes that encode the main enzymes involved in DA synthesis and metabolism. High DA levels have been demonstrated in metastatic paragangliomas in consequence of reduced expression level of DBH [26]. In a study conducted with 21 PCPG patients, lower MAOA and

COMT expression levels were detected in tumor tissues [27]. It has been reported that the amounts of mRNA encoded by TH, DDC, and DBH genes were higher in pheochromocytomas compared to the normal adrenal medulla, whereas the PNMT mRNA levels were higher in the normal tissue [28]. According to the survival analysis of the current study,
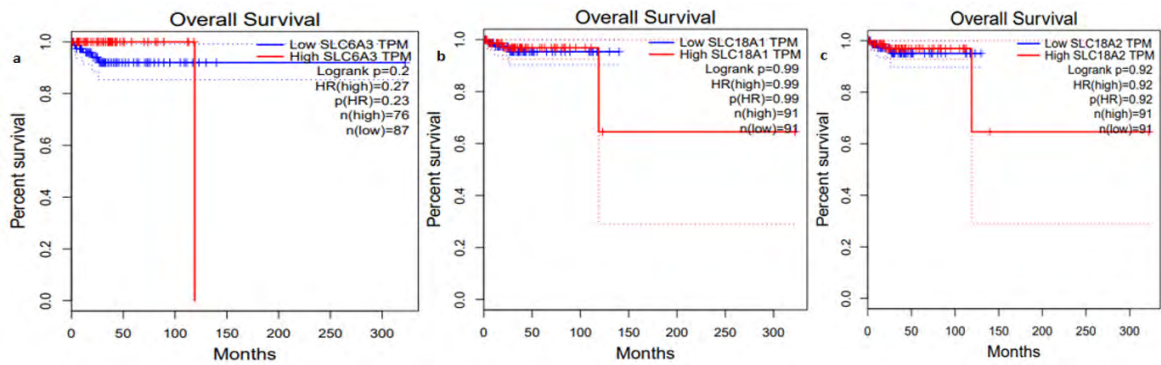
Figure 4. Continued
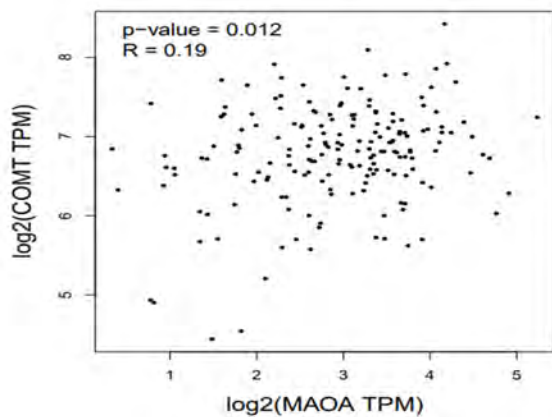


Figure 5. Spearman correlation analysis of MAOA and COMT genes with PCPG
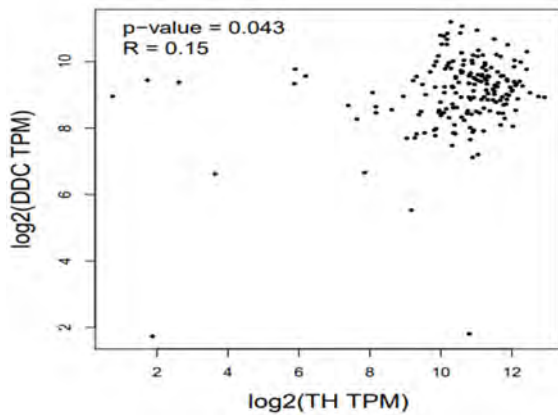


Figure 6. Spearman correlation analysis of TH and DDC genes with PCPG
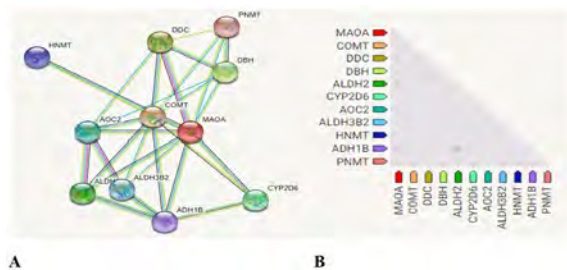


Figure 7. A. The predicted partners of MAOA B. Coexpression pattern of the genes that correlates with the gene encodes MAOA

the COMT gene was significantly less expressed in PCPG
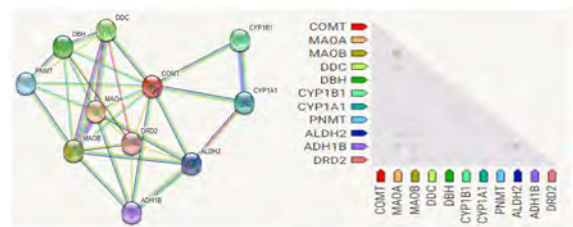


Figure 8. A. The predicted partners of COMT B. Coexpression pattern of the genes that correlates with the gene encodes COMT
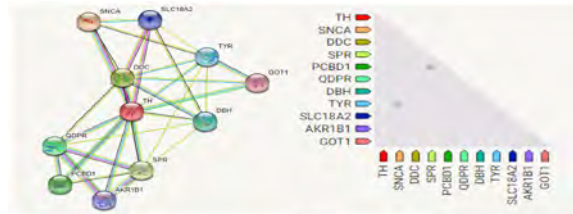


Figure 9. A. The predicted partners of TH B. Coexpression pattern of the genes that correlates with the gene encodes TH
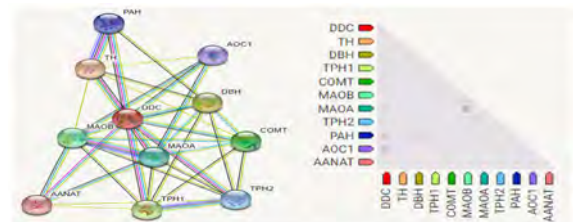


Figure 10. A. The predicted partners of DDC B. Coexpression pattern of the genes that correlates with the gene encodes DDC

compared to the normal tissue. Therefore, COMT may serve as a molecular biomarker for PCPG. DNA methylation and miRNAs directly can modulate gene expression. Based on the findings of the present study, the COMT gene has 4 CpG islands that are associated with DNA methylation. COMT protein significantly interacts with MAOA protein. hsa-miR-5000-5p regulates the expression of both COMT and MAOA genes (p=0.00215, FDR=0.127). In this regard, our findings suppose that COMT may potentially be implicated in tumor suppressive mechanism in PCPG. In patients with dopamine secreting PCPG, post-surgical observation

**Table 3.** The position and features of CpG islands of TH, DDC, MAOA, COMT genes

| Gene | Island no | Island size | Island start | Island end | GC% percent | O/E ratio |
|------|-----------|-------------|--------------|------------|-------------|-----------|
| TH | Island 1 | 120 | 103 | 222 | 50.0 | 0.6 |
| | Island 2 | 198 | 292 | 489 | 50.0 | 0.6 |
| | Island 3 | 604 | 583 | 1186 | 50.0 | 0.6 |
| | Island 4 | 193 | 1273 | 1465 | 50.0 | 0.6 |
| DDC | Island 1 | 257 | 132 | 388 | 50.0 | 0.6 |
| MAOA | None | | | | | |
| COMT | Island 1 | 142 | 16 | 157 | 50.0 | 0.6 |
| | Island 2 | 180 | 367 | 546 | 50.0 | 0.6 |
| | Island 3 | 99 | 605 | 703 | 50.0 | 0.6 |
| | Island 4 | 189 | 773 | 961 | 50.0 | 0.6 |

**Table 4.** miRNA-target enrichment analysis result for the genes associated with dopamine neurotransmission

| miRNA family | p-value | FDR | Odd ratio | Number of interactions | Target Gene 1 | Target Gene 2 | Target Gene 3 | Target Gene 4 |
|--------------|---------|-----|-----------|------------------------|---------------|---------------|---------------|---------------|
| hsa-miR-141-3p/hsa-miR-200a-3p | 0.175 | 0.774 | 0.382 | 2 | SLC18A2 | DRD2 | | |
| hsa-miR-142-5p/hsa-miR-5590-3p | 0.189 | 0.774 | 0.401 | 2 | SLC18A2 | DRD1 | | |
| hsa-miR-9-5p | 0.330 | 0.774 | 0.586 | 2 | DRD2 | SLC18A2 | | |
| hsa-miR-30a-5p/hsa-miR-30b-5p/hsa-miR-30c-5p/ hsa-miR-30d-5p/hsa-miR-30e-5p | 0.139 | 0.774 | 0.443 | 3 | COMT | SLC6A3 | DRD1 | |
| hsa-miR-5000-5p | 0.00215 | 0.127 | 0.0349 | 2 | COMT | MAOA | | |
| hsa-miR-1-3p | 0.233 | 0.280 | 0.459 | 2 | ALDH2 | TH | | |
| hsa-miR-335-5p | 0.258 | 0.293 | 0.654 | 4 | ALDH2 | DBH | DRD5 | MAOB |
| hsa-miR-16-5p | 0.469 | 0.494 | 0.775 | 2 | ALDH2 | COMT | | |
| hsa-miR-26b-5p | 0.573 | 0.593 | 0.933 | 2 | PNMT | DRD3 | | |

and genetic assessment has a notable effect on the prognosis of the disease. The expression values of COMT and MAOA genes, related CpG islands, and hsa-miR-5000-5p may have the potential to be used in the genetic evaluation of the pathogenesis and prognosis of PCPG. Further research is required to further enlighten the relationship between dopamine neurotransmission and the carcinogenesis of PCPG.

## CONCLUSION

The present study reported that the COMT gene is significantly associated with PCPG. The findings of *in silico* analysis demonstrated that COMT might potentially be implicated in tumor suppressive mechanism. Delayed diagnosis and poorer prognosis are still a problem, and some of the tumors are recognized at autopsy. Therefore, expression analysis may be considered as a component of the clinical evaluation of patients. It requires further *in vitro* and *in vivo* dopamine neurotransmission studies in order to understand the molecular mechanism of the dopaminergic system in the pathogenesis of PCPG and its metastases features.

## CONFLICT OF INTEREST

Authors approve that to the best of their knowledge, there is not any conflict of interest or common interest with an institution/organization or a person that may affect the review process of the paper.

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# Mineralogy and Geochemistry of Sediments from Lake Tuz

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## ABSTRACT

Lake Tuz is a closed basin in the center of Anatolia (Turkey) with shallow hypersaline water. In this study, mineralogical and geochemical features of the lake sediments sampled by core drillings were investigated. Halite, polyhalite, calcite, magnesite, dolomite, huntite, quartz, and albite minerals were found in bulk sample and montmorillonite and vermiculite minerals were determined in the clay fraction XRD analyses. In geostatistical evaluations, elements are grouped into four clusters which are named Clastic, Hydrothermal, Evaporite-carbonate and Evaporite-sulfate. Trace elements included in the clastic cluster were used to constrain provenance and tectonic setting. The Light Rare Earth Element (LREE)-enriched REE pattern suggests a cratonic provenance for the lake sediments, except for the low negative Eu anomaly. Trace element ratios of La/Sc, La/Co, Th/Sc, Th/Co, Zr/Sc, Zr/Co, Ba/Sc, and Ba/Co, which are critical for provenance, show a provenance of "felsic-intermediate magmatic" composition. According to the La-Th-Sc diagram, the tectonic setting of the source area was found as "Continental Island Arc".

**Keywords:**
Central Anatolia; Lake Tuz; Provenance; Sediment geochemistry; Tectonic setting

## INTRODUCTION

Lake Tuz is located in the center of Anatolia (Fig. 1), and is the second largest lake of Turkey with a surface area of 1665 km². The lake includes hypersaline water, is fed by groundwater and rainwater in the autumn-winter season and without outflow [1]. The most important streams reaching the lake are Uluırmak, İnsuyu and Peçeneközü. The lake consists of two different parts; a shallow (main lake, hereafter Lake Tuz) with a large area and a deep one with a smaller area [2]. The depth of the lake water does not exceed one meter. Towards the end of the summer, approximately half of the lake floor is exposed following the evaporation of the saline water [3-6]. Also, both table salt and industrial salt are produced in the salt pans, which are separated from the lake with barriers [7].

According to Irion and Müller [8], playa sediments are largely composed of mud (silty clay) varying in colour from white through grey to black, and contains huntite, dolomite, magnesite, polyhalite, and gypsum. Uygun and Şen [9] showed that the geochemistry of the water does not show a standard composition due to the large seasonal variations. Çamur and Mutlu [10] stated that the sediments are mainly composed of gypsum, do-

lomite, huntite, magnesite, and polyhalite minerals. Kılıç and Kılıç [11] reported that the lake water is of Na-K-Mg-Cl-SO$_4$ type and the muddy sediments up to 20 cm deep under the salt crust contain gypsum, magnesite, thenardite, polyhalite, aragonite, and montmorillonite.

In this study, the mineralogy and geochemistry of the lake sediments, which are sampled by core drillings not exceeding one meter in depth, were investigated. It is aimed to evaluate the sediment composition by geostatistical analyses, and to infer their provenance and tectonic settings by geochemical data.

### Geological Background

The current (Holocene) Lake Tuz [13] is located within the Tuzgölü basin [14-19]. The east-northeast of the lake is bordered by the Tuzgölü Fault Zone (TFZ) [20-22]. To the east of this fault zone is the Kırşehir Massif [23-30]. It is stated that the detrital material supply to the lake is mostly originates from Kırşehir Massif due to the difference in elevation [18]. The south and west of the lake are surrounded by plains, where the Quaternary aged old lake terraces take place [31].
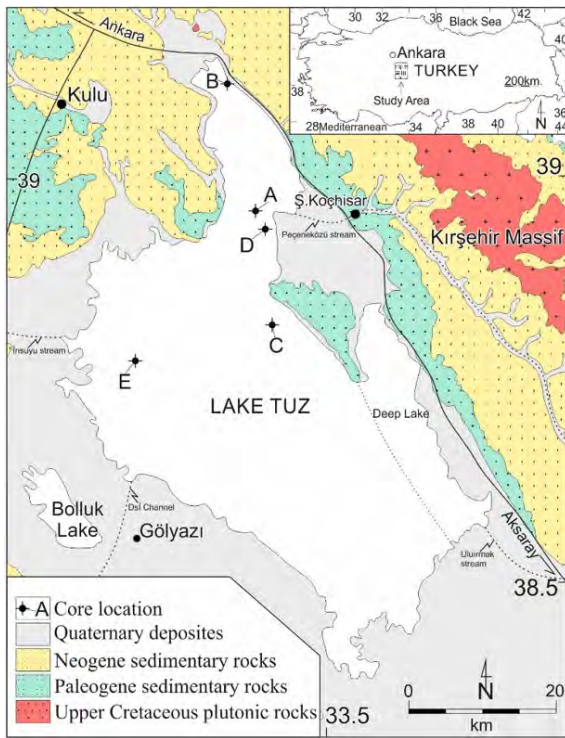
**Figure 1.** The geological map of the close vicinity of the Lake Tuz (modified from [12]) and the drilling locations (A-E).



**Figure 2.** Sediment cores and depth of samples.

## MATERIAL AND METHODS

Five cores with depths varying between 48 and 78 cm were taken from the lake floor with a hand-held drilling machine (Fig. 2). Samples were arranged from different depths of these cores. XRD analyses of the bulk samples were performed in MTA (Turkey) laboratories. Powdered samples were analysed with Bruker D-8 Advance brand machine that have a 2.2 kW cupper X-Ray anode. XRD analyses of the clay fraction were made by standard, ethylene glycol, 300 ºC heat-treated and 550 ºC heat-treated samples. SEM-EDX analyses were performed in Selçuk-İLTEK (Turkey) laboratories using the ZEISS EVO-LS10 brand SEM device with EDX addition and using $LaB_6$ fi-
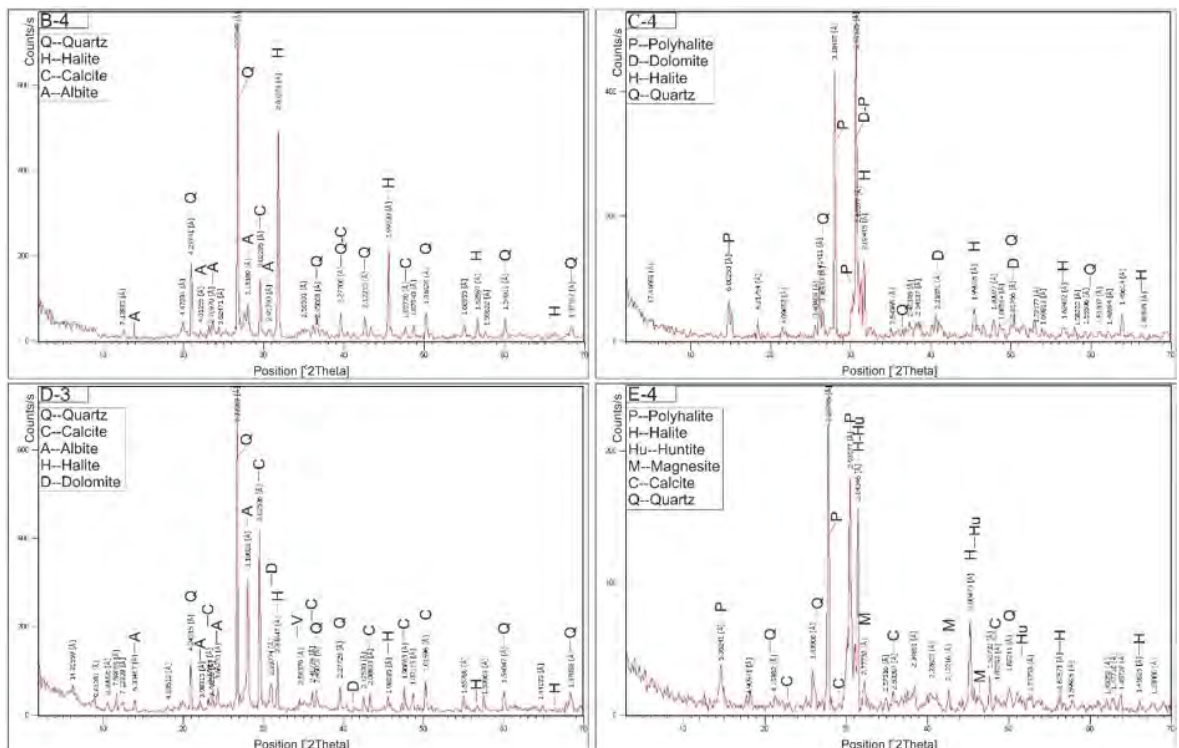


**Figure 3.** XRD analyses of the bulk samples from depths B-4, C-4, D-3 and E-4.

lament as an electron source. Geochemical analyses were carried out in ACME (Canada) laboratories by Lithium Borate Fusion method. A 0.2 g weighed powder sample was poured into a graphite crucible and mixed with 1.5 g of $LiBO_2/Li_2B_4O_7$ flux. The mixture was melted and then dissolved in 100 ml of 5% $HNO_3$. Solution samples were analysed by ICP-ES and ICP-MS. The LOI (Loss on Ignition) was calculated by the weight difference for a 1 g. sample after ignition at 950 °C for 90 minutes. Total carbon (TOT/C) and total sulfur (TOT/S) contents were measured by Leco. Chondrite [32] and Post Archean Australian Shale (PAAS) contents [33] were used for normalization. Chondrite normalized Ce and Eu anomalies were calculated by $(Ce/Ce^*)_{cn}=[Ce_{cn}/[(La_{cn}).(Pr_{cn})]^{0.5}])$ and $(Eu/Eu^*)_{cn}=[Eu_{cn}/[(Sm_{cn}).(Gd_{cn})]^{0.5}]$ formulas, respectively (cn: chondrite normalized).

## RESULTS AND DISCUSSION

### Mineralogy

The lake sediments sampled with cores are white-grey-brown in colour. In XRD analyses of samples representing different depths, mineral compositions consisting of halite, polyhalite, calcite, magnesite, dolomite, huntite, quartz, and albite were detected (Fig. 3). In the clay fraction XRD analyses montmorillonite and vermiculite minerals were detected (Fig. 4). The presence of quartz and polyhalite crystals were observed in SEM-EDX analyses (Fig. 5).
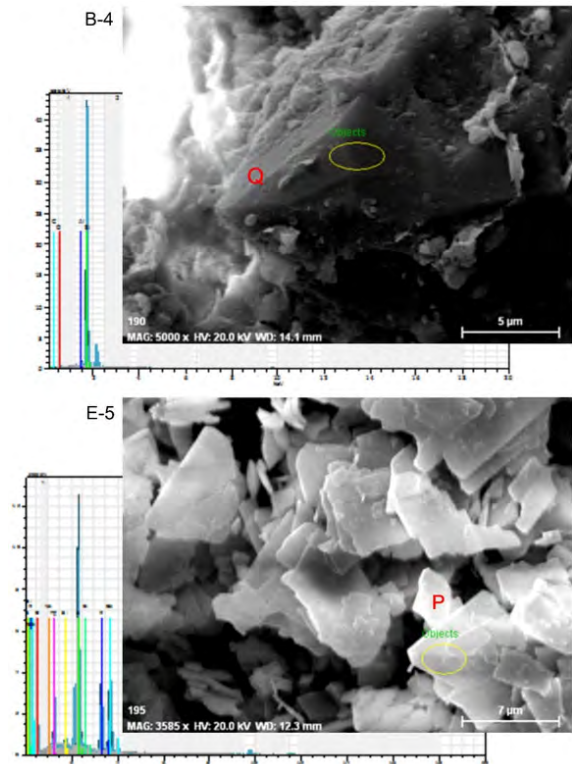


**Figure 5.** SEM images and EDX analyses (yellow circle) of euhedral quartz (Q) in sample B-4 and euhedral polyhalite (P) crystals in sample E-5.
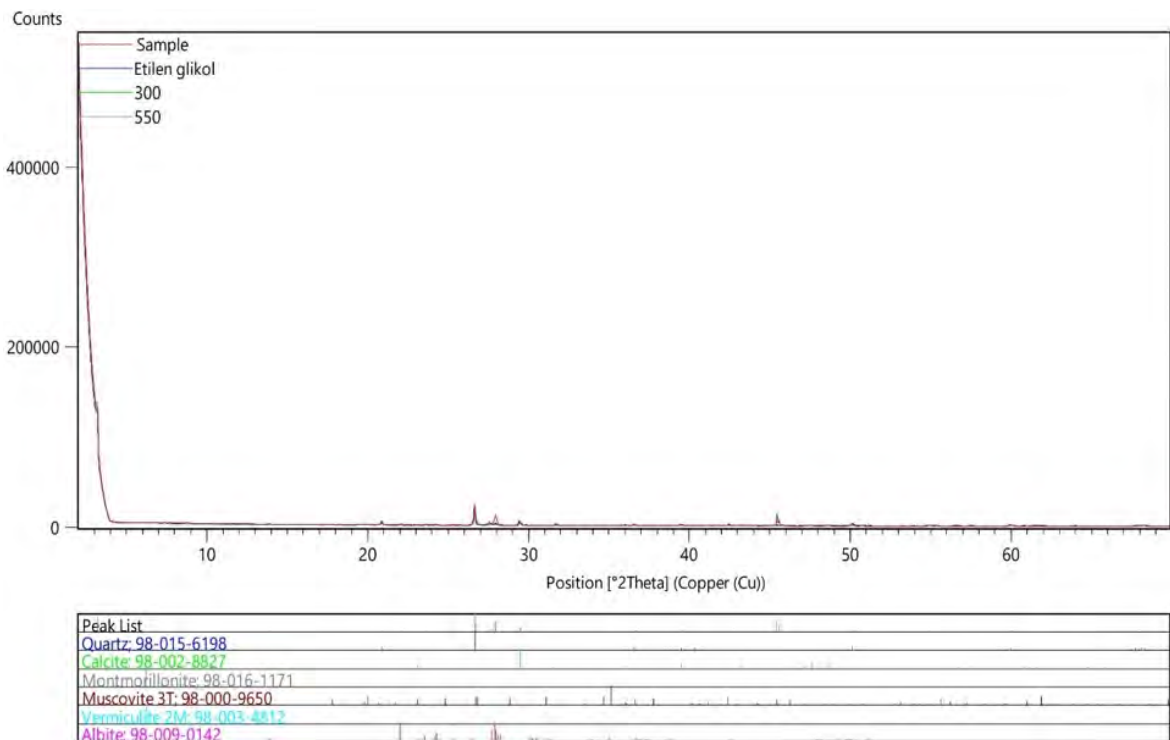


**Figure 4.** XRD analyses of the clay fraction.

## Whole-rock Geochemistry

The major and trace element concentrations of 20 samples taken from different depths of five cores are shown in Table 1.

Major oxide concentrations were highly variable in samples (Table 1). Mineralogical compositions suggest that this variability is due to the mixture of evaporites and clastics at different ratios. In Fig. 6, the major oxides were divided by their $Al_2O_3$ concentrations, and compared with PAAS [34]. Such normalization may reveal major oxide enrichments originating from the evaporitic phase. Considering the sample average in the graph, $Fe_2O_3/Al_2O_3$, $TiO_2/Al_2O_3$, $P_2O_5/Al_2O_3$, and $MnO/Al_2O_3$ overlapped with that of PAAS. However, there is a significant enrichment in $MgO/Al_2O_3$, $CaO/Al_2O_3$, $Na_2O/Al_2O_3$, and $K_2O/Al_2O_3$ ratios of the samples, probably due to the evaporite minerals, as determined in XRD analyses. The slight enrichment in $SiO_2/Al_2O_3$ ratio is due to the abundance of quartz. In addition, the enrichment of $Cr_2O_3/Al_2O_3$ may indicate a contribution from an ophiolitic source [35].

The PAAS normalized plot of trace element concentrations divided by $Al_2O_3$ is shown in Fig. 7. In the graph, $Sc/Al_2O_3$, $Ba/Al_2O_3$, $Co/Al_2O_3$, $Cs/Al_2O_3$, $Ga/Al_2O_3$, $Hf/Al_2O_3$, $Nb/Al_2O_3$, $Rb/Al_2O_3$, $Th/Al_2O_3$, $V/Al_2O_3$, $Zr/Al_2O_3$, $Cu/Al_2O_3$, and $Y/Al_2O_3$ overlapped with PAAS. However, $Sr/Al_2O_3$, $U/Al_2O_3$, $Mo/Al_2O_3$, $Pb/Al_2O_3$, and $Ni/Al_2O_3$ were enriched. Sr enrichment may have occurred due to the evaporitic phase. The enrichment of Pb and Zn may be due to hydrothermal solutions or Pb-Zn mineralizations at the source. U and Mo may be enriched due to the redox conditions [36]. Ni may be enriched due to the ophiolitic source.

In the similarity dendrogram prepared according to the Pearson Correlation Coefficients, the variables showing strong similarity with each other form 4 clusters (Fig. 8). The first cluster consists of $SiO_2$, $Al_2O_3$, $Fe_2O_3$, $TiO_2$, $P_2O_5$, MnO, $Cr_2O_3$, Sc, Ba, Co, Ga, Hf, Nb, Rb, Th, V, Zr, Cu, Ni, and ΣREE variables; the second cluster Cs, Pb, Zn, and As variables; the third cluster consists of MgO, $Na_2O$, TOT/C, U, Mo, and Ce/Ce* variables; and the fourth cluster consists of CaO, $K_2O$, LOI, TOT/S, and Sr variables. The first cluster is named "Clastic" because of the associations with $SiO_2$, $Al_2O_3$, $TiO_2$, and ΣREE. The second cluster originates from possible Pb-Zn mineralization or hydrothermal solutions in the source area and was named "Hydrothermal". The third cluster was named "Evaporite-carbonate" because of its association with TOT/C. It is known that U and Mo in this cluster are enriched under reducing conditions. The Ce anomaly association shows that the elements in this group are affected from redox conditions. Finally, the fourth clus-
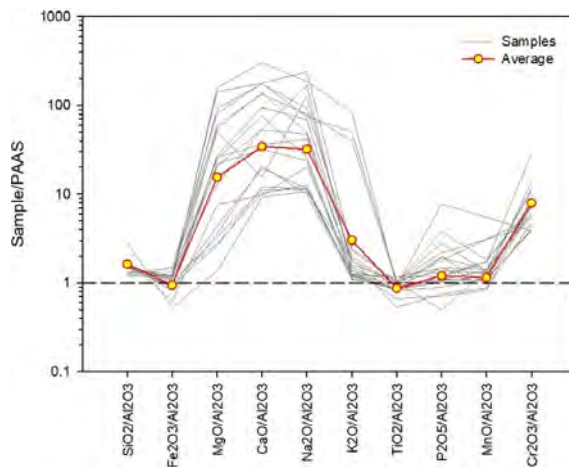


**Figure 6.** Element/$Al_2O_3$ comparison of major oxides with PAAS [34].
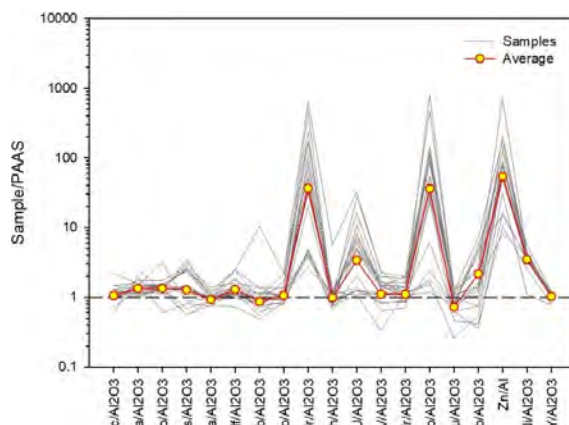


**Figure 7.** Element/$Al_2O_3$ comparison of trace elements with PAAS [34].
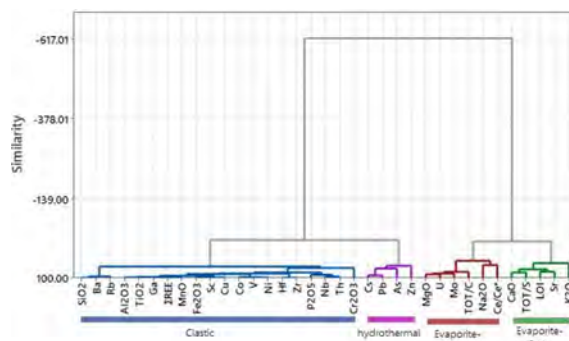


**Figure 8.** Dendrogram of variables

ter represents sulfates due to their association with TOT/S and was named "Evaporite-sulfate". It is understood that the LOI in this group is of gypsum origin and secondary carbonate origin due to the connection of the fourth cluster with the third cluster.

## Provenance

There is a significant enrichment in the concentrations of MgO, CaO, $Na_2O$ and $K_2O$ which are associated with evaporites (Fig. 6). Also, they are not associated with clas-

**Table 1a.** Major (%) and trace element (ppm) concentrations.

| | A1 | A2 | A3 | A4 | B1 | B2 | B3 | B4 | C1 | C2 | C3 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| $SiO_2$ | 24.03 | 12.09 | 19.71 | 48.63 | 73.98 | 50.31 | 48.06 | 46.26 | 4.54 | 6.31 | 9.13 |
| $Al_2O_3$ | 4.89 | 2.41 | 3.90 | 8.30 | 7.87 | 9.23 | 9.33 | 9.58 | 0.94 | 1.27 | 1.79 |
| $Fe_2O_3$ | 1.75 | 0.84 | 1.36 | 2.00 | 1.60 | 3.37 | 3.71 | 4.36 | 0.43 | 0.53 | 0.61 |
| $MgO$ | 12.52 | 7.63 | 11.87 | 7.35 | 1.25 | 2.82 | 3.56 | 3.09 | 15.59 | 13.54 | 11.50 |
| $CaO$ | 10.69 | 15.66 | 9.69 | 5.55 | 5.05 | 7.68 | 7.23 | 6.74 | 11.52 | 15.28 | 16.95 |
| $Na_2O$ | 7.37 | 10.51 | 10.37 | 10.76 | 5.33 | 6.65 | 6.63 | 7.60 | 14.27 | 7.43 | 5.63 |
| $K_2O$ | 1.07 | 0.85 | 1.16 | 2.28 | 2.91 | 2.24 | 2.15 | 2.24 | 0.43 | 0.61 | 0.69 |
| $TiO_2$ | 0.22 | 0.12 | 0.17 | 0.29 | 0.22 | 0.40 | 0.42 | 0.45 | 0.05 | 0.07 | 0.09 |
| $P_2O_5$ | 0.08 | 0.03 | 0.04 | 0.05 | 0.05 | 0.07 | 0.08 | 0.09 | 0.03 | 0.03 | 0.03 |
| $MnO$ | 0.04 | 0.02 | 0.04 | 0.04 | 0.04 | 0.06 | 0.06 | 0.06 | 0.01 | 0.01 | 0.01 |
| $Cr_2O_3$ | 0.026 | 0.022 | 0.024 | 0.052 | 0.032 | 0.030 | 0.031 | 0.033 | 0.006 | 0.006 | 0.012 |
| LOI | 24.30 | 27.00 | 21.80 | 14.40 | 1.40 | 16.70 | 18.10 | 4.10 | 27.10 | 27.70 | 25.80 |
| Sum | 87.03 | 77.16 | 80.08 | 99.69 | 99.68 | 99.54 | 99.32 | 84.66 | 74.91 | 72.81 | 72.21 |
| TOT/C | 3.15 | 1.79 | 2.74 | 1.29 | 1.01 | 2.03 | 1.98 | 1.72 | 4.38 | 3.47 | 2.89 |
| TOT/S | 4.41 | 11.52 | 6.65 | 4.04 | 0.12 | 0.23 | 0.17 | 0.18 | 4.53 | 7.55 | 8.51 |
| Sc | 4 | 2 | 3 | 5 | 4 | 9 | 10 | 10 | 1 | 1 | 2 |
| Ba | 259 | 114 | 184 | 371 | 537 | 377 | 342 | 326 | 56 | 66 | 69 |
| Co | 8 | 5 | 7 | 12 | 6 | 13 | 14 | 16 | 2 | 2 | 3 |
| Cs | 5 | 2 | 4 | 4 | 5 | 10 | 11 | 11 | 2 | 3 | 2 |
| Ga | 5 | 3 | 4 | 7 | 6 | 9 | 9 | 10 | 1 | 2 | 2 |
| Hf | 2 | 1 | 1 | 2 | 3 | 3 | 2 | 3 | 0 | 1 | 1 |
| Nb | 4 | 2 | 4 | 4 | 4 | 7 | 8 | 9 | 1 | 1 | 2 |
| Rb | 34 | 20 | 34 | 60 | 92 | 89 | 89 | 92 | 11 | 15 | 17 |
| Sr | 2242 | 1957 | 1646 | 406 | 327 | 410 | 362 | 265 | 4339 | 2244 | 1014 |
| Th | 3 | 2 | 4 | 4 | 5 | 7 | 8 | 9 | 1 | 1 | 1 |
| U | 4 | 2 | 3 | 3 | 1 | 2 | 2 | 2 | 4 | 3 | 3 |
| V | 56 | 32 | 40 | 42 | 21 | 60 | 63 | 88 | 18 | 17 | 19 |
| Zr | 55 | 30 | 38 | 65 | 99 | 91 | 88 | 113 | 12 | 17 | 28 |
| Mo | 10 | 7 | 5 | 12 | 1 | 1 | 1 | 1 | 40 | 8 | 8 |
| Cu | 8 | 6 | 9 | 10 | 5 | 19 | 19 | 20 | 3 | 4 | 4 |
| Pb | 4 | 2 | 4 | 4 | 5 | 12 | 45 | 50 | 4 | 2 | 3 |
| Zn | 1199 | 839 | 1103 | 386 | 1110 | 2109 | 3643 | 645 | 541 | 1186 | 724 |
| Ni | 58 | 29 | 42 | 62 | 25 | 92 | 93 | 102 | 11 | 16 | 19 |
| As | 39 | 13 | 11 | 20 | 17 | 15 | 25 | 38 | 22 | 15 | 15 |
| Y | 7 | 4 | 6 | 10 | 9 | 14 | 14 | 15 | 2 | 2 | 3 |
| La | 10.20 | 5.70 | 9.80 | 11.40 | 12.10 | 16.90 | 17.70 | 18.10 | 2.60 | 2.90 | 4.40 |
| Ce | 19.10 | 10.70 | 17.20 | 20.80 | 22.70 | 33.60 | 34.30 | 35.20 | 4.80 | 6.00 | 8.80 |
| Pr | 2.09 | 1.16 | 1.87 | 2.39 | 2.53 | 3.65 | 3.76 | 3.91 | 0.48 | 0.61 | 0.98 |
| Nd | 7.80 | 4.70 | 7.80 | 8.60 | 8.90 | 14.50 | 15.30 | 14.00 | 1.60 | 2.40 | 4.00 |
| Sm | 1.41 | 0.76 | 1.17 | 1.66 | 1.70 | 2.61 | 2.69 | 2.82 | 0.27 | 0.41 | 0.68 |
| Eu | 0.38 | 0.21 | 0.31 | 0.50 | 0.45 | 0.63 | 0.65 | 0.70 | 0.07 | 0.11 | 0.16 |
| Gd | 1.23 | 0.63 | 1.10 | 1.65 | 1.46 | 2.41 | 2.52 | 2.52 | 0.26 | 0.43 | 0.58 |
| Tb | 0.20 | 0.12 | 0.18 | 0.27 | 0.24 | 0.40 | 0.43 | 0.44 | 0.04 | 0.07 | 0.08 |
| Dy | 1.19 | 0.72 | 0.89 | 1.45 | 1.34 | 2.32 | 2.38 | 2.44 | 0.29 | 0.39 | 0.56 |
| Ho | 0.22 | 0.14 | 0.19 | 0.33 | 0.31 | 0.46 | 0.49 | 0.54 | 0.05 | 0.08 | 0.09 |
| Er | 0.65 | 0.33 | 0.54 | 0.92 | 0.87 | 1.39 | 1.42 | 1.55 | 0.15 | 0.22 | 0.26 |
| Tm | 0.10 | 0.07 | 0.08 | 0.15 | 0.15 | 0.23 | 0.23 | 0.23 | 0.02 | 0.04 | 0.04 |
| Yb | 0.65 | 0.41 | 0.56 | 0.89 | 0.93 | 1.27 | 1.42 | 1.53 | 0.16 | 0.21 | 0.28 |
| Lu | 0.09 | 0.06 | 0.09 | 0.14 | 0.14 | 0.21 | 0.22 | 0.25 | 0.02 | 0.03 | 0.04 |
| ΣREE | 45.31 | 25.71 | 41.78 | 51.15 | 53.82 | 80.58 | 83.51 | 84.23 | 10.81 | 13.90 | 20.95 |
| ΣLREE/ΣHREE | 9.38 | 9.28 | 10.42 | 7.73 | 8.81 | 8.20 | 8.10 | 7.79 | 9.85 | 8.38 | 9.77 |
| $(La/Yb)_{cn}$ | 10.86 | 9.63 | 12.12 | 8.87 | 9.01 | 9.21 | 8.63 | 8.19 | 11.25 | 9.56 | 10.88 |
| $(Ce/Ce^*)_{cn}$ | 0.99 | 1.00 | 0.96 | 0.96 | 0.98 | 1.03 | 1.01 | 1.00 | 1.03 | 1.08 | 1.02 |
| $(Eu/Eu^*)_{cn}$ | 0.88 | 0.92 | 0.83 | 0.92 | 0.87 | 0.76 | 0.76 | 0.80 | 0.80 | 0.80 | 0.77 |

**Table 1b.** Major (%) and trace element (ppm) concentrations (continued). (-: below detection limits, nv: no value)

| | C4 | D1 | D2 | D3 | E1 | E2 | E3 | E4 | E5 | Average | PAAS |
|---|---|---|---|---|---|---|---|---|---|---|---|
| $SiO_2$ | 5.86 | 20.87 | 46.55 | 43.03 | 9.56 | 13.37 | 11.62 | 3.14 | 4.59 | 25.08 | 62.80 |
| $Al_2O_3$ | 1.32 | 4.02 | 8.88 | 8.65 | 2.39 | 3.22 | 2.63 | 0.61 | 1.08 | 4.62 | 18.90 |
| $Fe_2O_3$ | 0.67 | 1.70 | 2.81 | 2.96 | 1.12 | 1.44 | 1.25 | 0.26 | 0.61 | 1.67 | 7.23 |
| MgO | 9.19 | 6.13 | 3.72 | 4.38 | 5.73 | 9.30 | 15.42 | 11.26 | 10.26 | 8.31 | 2.20 |
| CaO | 12.17 | 14.70 | 12.73 | 11.66 | 8.81 | 17.07 | 2.81 | 12.72 | 13.08 | 10.89 | 1.30 |
| $Na_2O$ | 6.64 | 12.04 | 5.62 | 6.39 | 25.68 | 5.29 | 21.52 | 7.21 | 5.10 | 9.40 | 1.20 |
| $K_2O$ | 10.36 | 1.00 | 1.91 | 2.06 | 0.64 | 0.78 | 0.82 | 10.02 | 10.64 | 2.74 | 3.70 |
| $TiO_2$ | 0.07 | 0.25 | 0.41 | 0.45 | 0.13 | 0.20 | 0.15 | 0.03 | 0.06 | 0.21 | 1.00 |
| $P_2O_5$ | 0.02 | 0.04 | 0.08 | 0.08 | 0.01 | 0.04 | 0.03 | 0.04 | 0.02 | 0.05 | 0.16 |
| MnO | - | 0.04 | 0.06 | 0.07 | 0.02 | 0.02 | 0.02 | - | - | 0.03 | 0.11 |
| $Cr_2O_3$ | 0.006 | 0.097 | 0.103 | 0.084 | 0.012 | 0.024 | 0.016 | 0.002 | 0.004 | 0.03 | 0.02 |
| LOI | 27.30 | 9.20 | 16.60 | 19.90 | 30.40 | 26.30 | 29.50 | 38.00 | 26.80 | 21.62 | nv |
| Sum | 73.59 | 70.06 | 99.51 | 99.73 | 84.50 | 77.01 | 85.74 | 83.32 | 72.27 | 84.64 | 104.59 |
| TOT/C | 0.80 | 2.15 | 2.00 | 2.31 | 1.37 | 2.04 | 3.58 | 1.65 | 1.13 | 2.17 | nv |
| TOT/S | 12.47 | 4.52 | 1.42 | 0.64 | 2.67 | 8.58 | 0.65 | 12.10 | 13.10 | 5.20 | nv |
| Sc | 1 | 4 | 7 | 8 | 3 | 4 | 3 | - | 2 | 4 | 16 |
| Ba | 64 | 211 | 414 | 363 | 102 | 179 | 95 | 54 | 58 | 212 | 650 |
| Co | 2 | 15 | 15 | 14 | 4 | 6 | 4 | 1 | 2 | 8 | 23 |
| Cs | 3 | 3 | 4 | 5 | 5 | 7 | 5 | 2 | 3 | 5 | 15 |
| Ga | 1 | 4 | 9 | 8 | 3 | 4 | 3 | 1 | 1 | 5 | 20 |
| Hf | 0 | 3 | 3 | 3 | 1 | 2 | 1 | 0 | 0 | 2 | 5 |
| Nb | 1 | 4 | 6 | 6 | 3 | 4 | 3 | 6 | 1 | 4 | 19 |
| Rb | 19 | 31 | 60 | 65 | 23 | 27 | 24 | 12 | 20 | 42 | 160 |
| Sr | 2427 | 2370 | 1921 | 445 | 2039 | 3737 | 882 | 4030 | 2640 | 1785 | 200 |
| Th | 1 | 4 | 6 | 6 | 2 | 3 | 2 | 3 | 1 | 4 | 15 |
| U | 2 | 3 | 2 | 2 | 2 | 4 | 3 | 3 | 1 | 3 | 3 |
| V | 15 | 64 | 75 | 79 | 28 | 36 | 31 | 11 | 14 | 40 | 150 |
| Zr | 16 | 84 | 113 | 110 | 31 | 73 | 42 | 14 | 20 | 57 | 210 |
| Mo | 7 | 7 | 3 | 1 | 7 | 19 | 20 | 15 | 5 | 9 | 1 |
| Cu | 4 | 9 | 15 | 15 | 7 | 7 | 9 | 2 | 3 | 9 | 50 |
| Pb | 2 | 7 | 3 | 15 | 7 | 19 | 20 | 2 | 3 | 11 | 20 |
| Zn | 521 | 1346 | 664 | 317 | 541 | 801 | 2065 | 1941 | 640 | 1116 | 85 |
| Ni | 17 | 65 | 89 | 99 | 27 | 30 | 31 | 8 | 14 | 47 | 55 |
| As | 10 | 19 | 5 | 4 | 19 | 29 | 34 | 11 | 8 | 18 | nv |
| Y | 2 | 7 | 13 | 13 | 4 | 6 | 4 | 1 | 2 | 7 | 27 |
| La | 2.90 | 9.70 | 16.30 | 16.00 | 5.60 | 9.50 | 7.10 | 1.90 | 2.80 | 9.18 | 38.00 |
| Ce | 5.40 | 18.60 | 30.30 | 30.60 | 11.40 | 18.60 | 13.60 | 3.90 | 5.00 | 17.53 | 80.00 |
| Pr | 0.60 | 2.14 | 3.37 | 3.36 | 1.22 | 2.02 | 1.43 | 0.40 | 0.53 | 1.93 | 8.83 |
| Nd | 2.30 | 7.40 | 11.70 | 12.50 | 4.70 | 7.90 | 5.70 | 1.30 | 2.00 | 7.26 | 32.00 |
| Sm | 0.44 | 1.51 | 2.47 | 2.47 | 0.86 | 1.23 | 0.98 | 0.26 | 0.37 | 1.34 | 5.60 |
| Eu | 0.10 | 0.36 | 0.65 | 0.67 | 0.20 | 0.30 | 0.25 | 0.07 | 0.10 | 0.34 | 1.10 |
| Gd | 0.39 | 1.42 | 2.25 | 2.10 | 0.78 | 1.16 | 0.85 | 0.29 | 0.36 | 1.22 | 4.70 |
| Tb | 0.06 | 0.21 | 0.39 | 0.38 | 0.12 | 0.17 | 0.13 | 0.04 | 0.06 | 0.20 | 0.77 |
| Dy | 0.42 | 1.17 | 2.10 | 2.07 | 0.63 | 0.98 | 0.71 | 0.19 | 0.31 | 1.13 | 4.40 |
| Ho | 0.06 | 0.22 | 0.44 | 0.44 | 0.12 | 0.20 | 0.12 | 0.04 | 0.05 | 0.23 | 1.00 |
| Er | 0.22 | 0.76 | 1.33 | 1.27 | 0.32 | 0.59 | 0.41 | 0.09 | 0.16 | 0.67 | 2.90 |
| Tm | 0.03 | 0.11 | 0.21 | 0.20 | 0.05 | 0.08 | 0.06 | 0.01 | 0.02 | 0.11 | 0.41 |
| Yb | 0.20 | 0.78 | 1.43 | 1.33 | 0.42 | 0.64 | 0.43 | 0.14 | 0.19 | 0.69 | 2.80 |
| Lu | 0.03 | 0.12 | 0.20 | 0.20 | 0.06 | 0.08 | 0.06 | 0.01 | 0.02 | 0.10 | 0.40 |
| ΣREE | 13.15 | 44.50 | 73.14 | 73.59 | 26.48 | 43.45 | 31.83 | 8.64 | 11.97 | 41.93 | 182.91 |
| ΣLREE/ΣHREE | 8.26 | 8.22 | 7.68 | 8.13 | 9.51 | 10.06 | 10.40 | 9.58 | 9.15 | 8.55 | 9.46 |
| $(La/Yb)_{cn}$ | 10.04 | 8.61 | 7.89 | 8.33 | 9.23 | 10.28 | 11.43 | 9.40 | 10.20 | 9.17 | 9.40 |
| $(Ce/Ce^*)_{cn}$ | 0.98 | 0.98 | 0.98 | 1.00 | 1.05 | 1.02 | 1.02 | 1.07 | 0.98 | 1.00 | 1.05 |
| $(Eu/Eu^*)_{cn}$ | 0.73 | 0.75 | 0.84 | 0.89 | 0.74 | 0.76 | 0.83 | 0.77 | 0.83 | 0.82 | 0.65 |

tic cluster in close similarity (Fig. 8) Since this situation directly controls the chemical concentrations of major oxides in the clastic phase, diagrams using major oxides for provenance determination becomes useless. Therefore, trace elements whose concentrations are associated with clastic cluster were used in this study (Fig.8).

Rare earth elements (REEs) exhibit coherent, insoluble and mostly immobile geochemical behaviours in their trivalent states [33, 37]. They are transported as suspension loads or by holding to fine-grained detritus during weathering, erosion, recycling and sedimentation [38]. REEs are quantitatively transferred to the clastic sedimentary records and is subsequently unaffected by secondary processes such as diagenesis and metamorphism. [39].

Basic rocks have lower Light REE (LREE$_{La-Sm}$) contents compared to Heavy REE (HREE$_{Gd-Lu}$) and do not contain Eu anomaly. However silicic rocks have higher LREE/HREE ratios and large negative Eu anomalies [40]. Due to their redox properties, Eu (+2/+3) and Ce (+3/+4) may behave differently from other trivalent REEs. The existence of the negative Eu anomaly is generally attributed to the retention of Eu by plagioclase, which is stable up to 40 km depth and under highly reducing ambient conditions. On the other hand, Ce tends to oxidize easily under surface conditions. Therefore, Ce anomaly occurs depending on whether the environment is oxidative or reductive [41].

REE concentrations of the samples are significantly lower than PAAS (Fig. 9). But the values of average LREE/HREE, (La/Yb)$_{cn}$, (La/Sm)$_{cn}$, and (Gd/Lu)$_{cn}$ ratios are getting closer to PAAS (Table 1). The Ce anomaly is not obvious. The effect of negative Eu anomaly is lower than that of PAAS. The specific Eu excess is probably due to plagioclase contribution from the source. The average REE pattern, which shows similarity to PAAS, except for the low negative Eu anomaly, suggests a cratonic provenance.

Elements potentially helpful for provenance identification are found in different concentrations in silicic and basic rocks. REEs, Zr, Ba and Th concentrations are higher in silicic sources, while Sc, Ni and Co concentrations are higher in basic sources [42]. These elements are also immobile and are not fractionated during sedimentary processes. Because of these features concentrations of these elements in the source rock is preserved in sediments [43].

Comparing the ratios of these preservative trace elements to each other with the average igneous rock compositions is useful to reveal the composition of the source [44]. The average values of La/Sc, La/Co, Th/Sc, Th/Co, Zr/Sc, Zr/Co, Ba/Sc, and Ba/Co ratios obtained were compared with the compositional variations (granite, felsic volcanic,
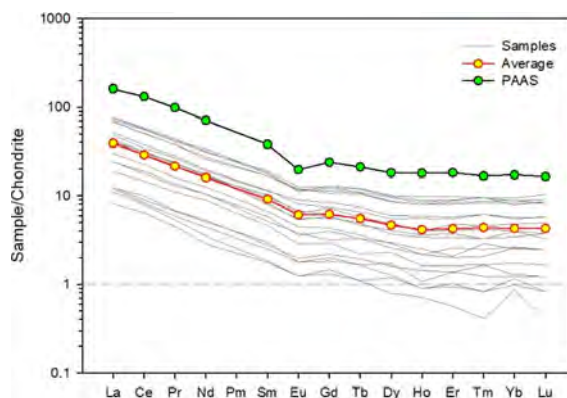


**Figure 9.** Comparison of REE with PAAS.

andesite and basalt) of Condie [45] (Table 2). This comparison suggests a provenance in "silicic-intermediate magmatic" composition for the lake sediments.

Considering the topographic elevation difference and the drainage network reaching the lake, the magmatic and metamorphic complex of the Kırşehir Massif in the east-northeast of the lake is the most likely the source for the sediments [27, 46-50].

**Table 2.** Critical element ratios for provenance.

| Rocks | *Granite | *Felsic Volcanic | *Andesite | *Basalt | Tuz Lake |
|-------|----------|------------------|-----------|---------|----------|
| La/Sc | 8.00 | 2.15 | 1.11 | 0.33 | 2.36 |
| La/Co | 13.33 | 4.67 | 0.91 | 0.31 | 1.37 |
| Th/Sc | 3.60 | 0.78 | 0.22 | 0.07 | 0.83 |
| Th/Co | 6.00 | 1.70 | 0.18 | 0.07 | 0.59 |
| Zr/Sc | 50.00 | 16.54 | 8.89 | 3.97 | 14.25 |
| Zr/Co | 83.33 | 35.83 | 7.27 | 3.74 | 8.45 |
| Ba/Sc | 160.00 | 65.38 | 36.11 | 12.42 | 53.55 |
| Ba/Co | 266.67 | 141.67 | 29.55 | 11.71 | 31.50 |
| | | | | | *Data from [45] |

**Tectonic Setting**

Trace element diagrams of Bhatia and Crook [51] are frequently used to determine the tectonic setting of the source area. These diagrams were found reliable by La-Maskin et al. [52]. In this study, the La-Th-Sc diagram was used (Fig. 10). In the diagram, the tectonic settings are divided into four sections. These are A-Oceanic Island Arc, B-Continental Island Arc, C-Active continental margin, and D-Passive continental margin. In the diagram, the sample average falls on the "Continental Island Arc" region. This setting represents an arc on the continental margin along the subduction zone.

Görür et al. [18] suggested the existence of an Inner Taurus Ocean between the Menderes Taurus Block and the Kırşehir Block in the tectonic evolution model of the Tuzgölü Basin. According to this tectonic model, a continental

arc developed on the Kırşehir Block by the subduction of the Inner Taurus Ocean. The "Continental Island Arc" tectonic setting of the diagram shows that the tectonic setting of the source region coincides with the tectonic setting of Görür et al. [18].
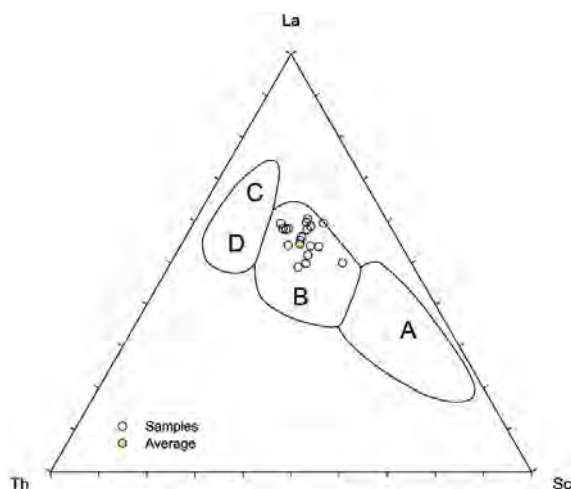


**Figure 10.** La-Th-Sc tectonic setting diagram [51]. A-Oceanic Island Arc, B-Continental Island Arc, C-Active continental margin, and D-Passive continental margin.

Furthermore, Yapıcı et al. [53], compared the Central Anatolian granitoids geochemically and stated that they were classified as well-developed calc-alkaline. These granitoids in the source area also support the tectonic setting model associated with the subduction.

## CONCLUSION

Tuz Lake deposits sampled with cores with a depth not exceeding one meter, were determined to have halite, polyhalite, calcite, magnesite, dolomite, huntite, quartz, and albite minerals by the standard XRD analyses. In the clay fraction XRD analysis, montmorillonite and vermiculite minerals were detected. The geostatistical analyses show four groups namely Clastic, Hydrothermal, Evaporite-carbonate and Evaporite-sulfate. LREE enriched REE pattern suggests "cratonic" provenance except for the low negative Eu anomaly. The low Eu anomaly indicates that a plagioclase-rich source contributed to the sediments. Critical element ratios for provenance such as La/Sc, La/Co, Th/Sc, Th/Co, Zr/Sc, Zr/Co, Ba/Sc, and Ba/Co suggest provenance in "acidic-intermediate magmatic" composition. The most likely candidate for provenance in this composition is the Kırşehir Massif, considering the topography and catchment area. In the La-Th-Sc diagram, the tectonic setting of the source region was found as the Continental Island Arc.

## CONFLICT OF INTEREST

Authors approve that to the best of their knowledge, there is not any conflict of interest or common interest with an institution/organization or a person that may affect the review process of the paper.

## AUTHOR CONTRIBUTION

First author: Conceptualization, methodology, software, investigation, writing-review and editing. Second author: Conceptualization, methodology, supervision, project administration.

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# Expression Analysis of Some Stress-Related Genes Induced by Cadmium on Tomato (*Solanum Lycopersicum* L.) Plants

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## ABSTRACT

Environmental pollution occurs in nature as air, soil and water pollution and as a result it affects whole ecosystem including human beings. Although industrialization and technological developments have made life easier than before, in recent years, they have triggered environmental pollution. Cadmium, which is a toxic pollutant for all living things, is one of the most important element in heavy metal pollutants. In this study, it was aimed to determine gene expression changes in tomato plant under Cd stress. Molecular response of tomato plants to Cd stress was examined by transcript accumulation analysis of two stress-related genes: (i) MT-2 (metallothionine-2) gene encodes metal binding protein and (ii) The GR-1 (glutathione reductase-1) gene encodes the glutathione reductase enzyme and is a marker of the ROS scavenging mechanism. Expression differences in MT-2 and GR-1 genes in tomato seedlings exposed to cadmium stress at different concentrations ranging from 20 to 1280 mg L-1 for 24 hours were determined performing quantitative real-time PCR. The results obtained from this study were showed that MT-2 and GR-1 genes play an important role in the mechanism of protection against heavy metal of Cd stress. In addition, the physiological properties of tomato have been associated with cadmium accumulation.

**Keywords:**
Cadmium stress; Tomato; Metallothionein; Glutathion reductase; qRT-PCR

## INTRODUCTION

Tomato is an important fruit crop grown mainly as an annual and economically valuable plant for growers in the Mediterranean basin (*Solanum lycopersicum* L. formerly Lycopersicon esculentum Mill. belongs to the Solanaceae family). It is an important source of vitamins, minerals, fiber and a dietary antioxidants [1]. It is also among the anti-carcinogenic foods due to the carotenoids it contains. Consumed as fresh and dried fruit, tomatoes are also processed in industry as tomato paste. Turkey, Egypt, Italy, Spain, Greece and Morocco are among the world's largest tomato producers and exporters [2].

With the increasing population, unplanned urbanization and developing technology, heavy metal pollution has become an important environmental problem worldwide. In addition to these, as a result of industrial activities, mining, using pesticide in agriculture, metallurgy, combustion of fossil fuels, faulty waste disposal, metal-enriched some materials, automotive emissions and domestic wastes and many other factors [3-5]. Heavy metal pollution, one of the most important environmental pollutants, is reported to accumulate in soil and water at high concentration, causing genotoxicity and damage to many functional biomolecules in living things. Heavy metals, which accumulate intensely in soil and water ecosystems, can be included in the food chain, especially by means of plant-based nutrients [6,7]. Thus, the heavy metals included in the food chain, may deteriorate the structure of many biomolecules such as proteins, enzymes and especially nucleic acids [8-11].

Very few metals such as Zinc, Copper, Nickel, Manganese and Iron are required nutrients in low concentrations for plant life and normal growth. This situation is similar for humans and animals, too [12,13]. These metals act as co-factors for many enzymes in most metabolic pathways. Especially in the structural and catalytic functions of proteins. On the other hand, the presence of the same metals in high concentrations in tissues causes toxic effects [10,14]. Thus, it adversely affects many biological molecules. For example, reactive

oxygen species (ROS) such as hydrogen peroxide and singlet oxygen formed due to heavy metal toxicity, cause conformational changes in enzymes involved in important metabolic formation pathways such as protein and nucleic acid. This causes oxidative damage, impairment of cellular homeostasis and stress, in plants as well as in many living things [15-17]. Cadmium (Cd) heavy metal in particular is a highly toxic pollutant for all living things, especially plants. Cadmium, which is not an essential element especially for plants, is generally found in low amounts in the soil and adversely affects plant growth and development. It is not an essential nutrient for plants, but it quickly enters the cells. This metal has some serious effects on plants such as growth inhibition, decrease in enzyme activities, photosynthesis and nutrient intake. Cadmium accumulated in plant tissues can cause serious damage to biological molecules such as proteins, enzymes and nucleic acids [6,18-20]. For example; ROS formed via cadmium stress may cause changes such as incompatibilities in DNA bases and instability of the double helix structure [16,21]. This causes changes in the expression of some genes that play a role in dealing with stress caused by heavy metal pollution. This change in gene expression allows plants to cope with stress [3,7,22-24].

In the present work, gene expression changes due to Cd stress in tomato plant were analyzed by examining two different stress-related genes. (i) MT-2 (metallothionine-2) gene encodes metal binding protein and (ii) The GR-1 (glutathione reductase-1) gene encodes the glutathione reductase enzyme and is a marker of the ROS scavenging mechanism [9,25-28].

Metallothionein is a protein that combines with metals to form complex structures as chelators. Many studies have shown that metallothioneins are highly expressed in metallophyte tolerant plant varieties. Thus, the plants can protect themselves against metal stress [26-29]. These metallothionein proteins are able to bind heavy metals by the thiol groups in the cysteine residue. Also, metallothionein proteins are involved in scavenging of ROS. Glutathione reductase is an enzyme that reduces oxidized glutathione. Glutathione reductase, an enzyme especially active in the ascorbate-glutathione (ASH-GSH) pathway, plays a role in defense against ROS by maintaining the low status of GSH [30]. By determining the changes in gene expression levels of metallothionein and glutathione reductase in plants, the tolerance of plants to various types of stress can be determined [29-33].

The results suggest that the early molecular response of hydroponically cultivated tomato plants might develop different strategies to cope with Cd toxicity by manipulating the expression level of stress-related genes. Therefore, in this study, changes in metallothionein and glutathione

reductase genes in tomato seedlings exposed to cadmium heavy metal stress at different concentrations for a certain period of time were demonstrated. The quantitative real-time PCR technique was performed to determine the change in gene expression levels. Finally, the data obtained from the study; showed that the change in the expression levels of these genes in the tomato plant could serve as an additional Cd-tolerance mechanism to deal with the toxic effect of cadmium.

## MATERIALS AND METHODS

### Growth of Plant Samples and Cadmium Stress Treatments

Before planting tomato seeds, their surfaces were sterilized with 70 % alcohol and 30 % sodium hypochlorite solution. The seeds were then washed three or four times with distilled water. For the germination and growth of tomato seeds, viols prepared using sterile perlite were arranged. Tomato seeds were germinated in sterile perlite by irrigating with 0.2 L modified 1/10 Hoagland solution and grown hydroponically. Tomato seeds planted in three biological replicates were grown in a controlled environmental growth chamber at 23-26 $^{o}$C with 250 mmol m-2 s-1 photosynthetic photon flux and 50-60 % relative humidity. After 21 days of growing period, tomato seedlings were exposed to cadmium solution at different concentrations of 20, 40, 80, 160, 320, 640, 1280 mg L-1 in the growth chamber for 24 hours. At the end of the 24 hour period, the roots and shoots of tomato seedlings were harvested and stored at -80 $^{o}$C until RNA isolation.

Root and shoot fragments (~200 mg) taken from samples which exposed to cadmium stress were powdered using liquid nitrogen. Subsequently, RNA was isolated from these samples. For RNA extracting, Trizol RNA extraction protocol was followed by RNeasy mini kit (Qiagen, Cat no: 74104) to cleanup. Quantity and quality measurement of isolated genomic RNAs were determined by Nanodrop (NanoDrop ND-1000 Spectrophotometer, Thermo Scientific). And then it was confirmed by 1.5 % agarose (containing 0.05 μl ml-1 EtBr) gel electrophoresis.

### First Strand cDNA Synthesis Assay

For the first strand cDNA synthesis assay, a two-step procedure was performed for real-time reverse transcriptase-polymerase chain reaction (RT-PCR). The reverse transcription reaction was performed using a high quality cDNA synthesis kit (Roche). According to the protocol; 2 μg isolated RNA, 2.5 μM Anchoredoligo (dT)18, 1X Transcriptor High Fidelity Reverse Transcriptase Reaction Buffer, 20 U RNase Inhibitor, 1 mM deoxynucleotide

mix and 10 U Transcriptor high fidelity reverse transcriptase were used. Quantity and quality measurements of cDNA were determined by Nanodrop (NanoDrop ND-1000 Spectrophotometer, Thermo Scientific).

## The Quantitative Real-time PCR Analysis of GR-1 and MT-2 Genes

Following cDNA synthesis, Real-Time PCR applications were performed using SYBR Green I Master dye via Light Cycler Nano (Roche) device. Sequences of the target genes identified in the study were searched/determined from the NCBI database and primers specific to these regions were designed using the Primer-3 program [36]. The designed primers were commercially synthesized. In addition, the Actin (ACT) gene was selected as the housekeeping gene to be used in the normalization process. During the Real-Time PCR reaction, Melting curve analysis was performed to determine the efficiency of PCR and to observe if there was any dimer formation, following the quantification (quantification = determination of expression) performed using SYBR Green I dye. Before starting the actual experimental work, the optimization of the reaction conditions was ensured. As a result of the experiments, the most suitable primer and cDNA concentrations were determined. The sequences and melting temperatures (Tm) of the primers used throughout the reactions are given in Table-1 and the homology analysis information of the qRT-PCR amplified transcripts in the NCBI database are given in Table 2.

**Table 1.** Sequences and melting temperatures of primers used in qRT-PCR

| Genes/Primers name | Sequence (5'-3') | Tm (°C) |
|---|---|---|
| MT-2F | GCTGTGGATCTAGCTGCAAGTGCG | 58-60 |
| MT-2R | AAGGGTTGCACTTGCAGTCAGATC | |
| GR-1F | CGTGCTGTGATACTTGGTGG | 58-60 |
| GR-1R | TCGTGCAAGGATGCATAGTG | |
| ACT-F | GGGATGGAGAAGTTTGGTGGTGG | 58-60 |
| ACT-R | CTTCGACCAAGGGATGGTGTAGC | |

Real-Time PCR reactions were performed in triplicate (as technical iterations) using the optimal conditions obtained as follows were initial denaturation 10 minutes at 95 °C, (40 cycles) 95 °C for 10 seconds, 58-60 °C for 20 seconds, 72 °C for 20 seconds, and increasing incrementally from 55 °C to 95 °C temperature 0.5 °C min-1.

## Normalization and Statistical Analysis of Real-Time PCR Results

Gene expression results determined as Ct (Cycle Tres-

**Table 2.** Homology analysis information of qRT-PCR amplified transcripts in NCBI database.

| Genes | Length | Homology | Accession no. |
|---|---|---|---|
| MT-2 | 170 bp | *Solanum lycopersicum* type 2 metallothionein mRNA, partial cds | EU884310 |
| GR-1 | 87 bp | *Solanum lycopersicum* glutathione reductase mRNA, partial cds | FJ265823 |
| ACT | 398 bp | *Solanum lycopersicum* ACT mRNA for actin, partial cds | AB199316.1 |

hold) value, ACT (actin) and control conditions used in the study were normalized by considering housekeeping gene (Livak and Schmittgen 2001). The obtained data were normalized according to the 2-ΔΔCt method of Livak and Schmittgen [37].

ANOVA, Tukey and Dunnett multiple comparison tests were performed to reveal differences between groups. The homogeneity of variances was determined with the statistical program (IBM SPSS Statistic-21) and Levene's test. The post-hoc Tukey HSD and Dunnett test were applied to the homogeneously distributed variables (also Dunnett's test to confirm the results) and the Dunnett T3 test was applied to the non-homogeneously distributed variables. $P < 0.05$ was considered to be statistically significant.

## RESULTS AND DISCUSSION

It is one of the most important effects of heavy metal toxicity known to inhibit root and shoot development. The toxicity of heavy metals due to increased concentrations negatively affects the development of roots and shoots and seed germination in plants. Similarly, regression in root development of tomato plants used in the study was detected. As expected, these results are similar to previous studies [8,9,16,30].

Metallothionein binding proteins (MTs) protect plants from metal stress and toxicity. Numerous studies have shown that MTs are highly expressed in many plants exposed to heavy metal toxicity [7,8,27]. Cysteine residue has the ability to bind heavy metals both physiologically (such as zinc, copper, and selenium) and xenobiotically (such as cadmium, mercury, silver, and arsenic) via thiol groups. In addition, it has been reported that MTs have important roles in both metal chaperoning and scavenging of ROS [28]. Many studies have shown that arsenic, cadmium and copper stresses induce metallothionein expression and accumulation [26,27,29].

Glutathione reductase (GR) is an important enzyme in the ASH-GSH (Ascorbate-Glutathione) pathway in enzymatic antioxidant system in plants, as in many living things. It plays a critical role in the defense system against

ROS, which occurs as a result of stress factors, by maintaining the GSH level and acts as a substrate for glutathione-S-transferases. In many recent studies, it was stated that GR-1 expression increased against cadmium stress in various plants [7,9,31-35].

Considering the mRNA expression profiles of genes (MT-2 and GR-1) and actin (ACT) used as a housekeeping gene and control conditions of root and shoot samples of tomato plants with different concentrations of cadmium (Cd) stress applied by Real-time PCR (Light CyclerNano, Roche). Normalized according to the 2-ΔΔCt method of Livak and Schmittgen [37]. In addition, the quantification and melting curve analysis of the transcripts are given in Figure 1. Normalized gene expression data were averaged and according to the results obtained, the changes in the concentration-dependent expression level of MT-2 and GR-1 genes occurring in different tissues of each tomato samples were shown on the graphs. For accuracy of results, MT-2, GR-1 and ACT transcript levels of all samples were measured in triplicate for different concentration of cadmium stress.

In this current study, different amounts of GR-1 accumulation were observed depending on varying concentration ranges in roots and shoots samples taken from tomato seedlings exposed to cadmium heavy metal stress. When the GR-1 expression data in both root and shoot parts were evaluated; The changes in the expression levels of the GR-1 gene, depending on the concentration, in the root samples of tomato seedlings subjected to cadmium stress are given in the Figure 2. Compared to the control group, the GR-1 gene expression level was approximately 5.9 and 7.8-fold, with the highest concentrations of 160 and 320 mg L-1, and the lowest at 1280 mg L-1 concentration, approximately 1.5-fold. GR-1 gene expression change in shoots; it was detected at the highest level with 80 mg L-1 and 320 mg L-1 concentrations, 8.8 and 11.9 fold, respectively. Similarly, fold change was observed as 7.0 fold at 160 mg L-1 concentration. On the other hand, the lowest expression data is; it was detected at the concentration of 1280 mg L-1 with approximately 0.9 fold (Figure 2).

Additionally, the GR-1 expression data in root and shoot samples were examined; an abruptly decrease was observed after the cadmium concentration of 160 mg L-1 in the root and 320 mg L-1 in the shoot, especially. This result decreased almost to the control group expression level with the increase of the cadmium concentration. This also shows that; it is the ineffectiveness of the resistance mechanism against cadmium stress in both roots and shoots after the specified concentrations. It was determined that the GR pathway was inhibited due to possible cellular damage and it could not provide protection against cadmium stress by using the stress recovery pathway [7,31-34].
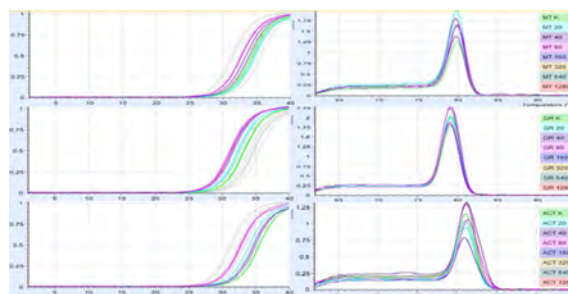


**Figure 1.** The quantification and melting curve analysis of the transcripts. The graphs on the left side shows transcript accumulation; right side shows the melting curve analysis of MT-2, GR-1 and Actin genes.
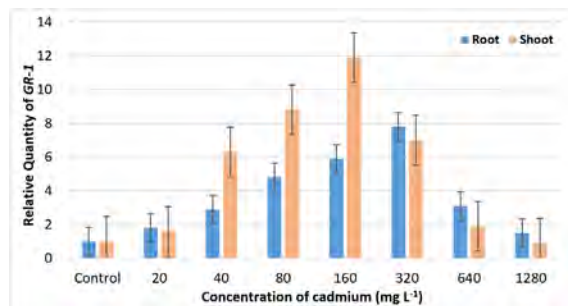


**Figure 2.** The changes in the expression levels of the Glutathion reductase-1 (GR-1) gene, depending on the concentration, in the root and shoot samples of tomato seedlings subjected to cadmium stress

On the other hand, metallothionein-2 (MT-2) gene expression data were examined, MT-2 results similar to GR-1 data were obtained. When the expression data of root and shoot samples of tomato seedlings under cadmium stress were evaluated; MT-2 gene expression level was found to be 3.4 and 4.4 fold in root samples at 80 and 160 mg L-1 concentrations, respectively. After the concentration of 160 mg L-1, there was a remarkable decrease in the expression of the MT-2 gene like GR-1. Similarly, 4.2 and 4.7 fold changes were detected in shoots at 80 and 160 mg L-1 concentrations, respectively (Figure 3). It is cleared from the results obtained that the change in the expression of the metallothionein binding protein gene MT-2 can induce cadmium toxicity tolerance in tomato plant [7,29,32,33].

In addition support these results, the change in GR-1 and MT-2 expressions data in both root and shoot samples was found to be statistically significant (P < 0.05). Detailed
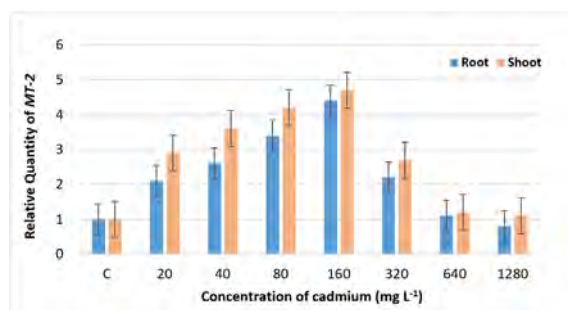


**Figure 3.** The changes in the expression levels of the metallothionein-2 (MT-2) gene, depending on the concentration, in the root and shoot samples of tomato seedlings subjected to cadmium stress

**Table 3.** Sequences and melting temperatures of primers used in qRT-PCR

| | Genes | Concentrations | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | 20 mg L$^{-1}$ | 40 mg L$^{-1}$ | 80 mg L$^{-1}$ | 160 mg L$^{-1}$ | 320 mg L$^{-1}$ | 640 mg L$^{-1}$ | 1280 mg L$^{-1}$ |
| root | GR-1 | * | * | *** | ** | *** | * | * |
| | MT-2 | * | Ns | * | *** | * | *** | * |
| shoot | GR-1 | * | * | ** | ** | * | * | * |
| | MT-2 | * | * | ** | * | * | Ns | * |

* p<0,05, ** p<0,01, *** p<0,001, Ns: non significant

information on the statistical significance levels of GR-1 and MT-2 expressions data in root and shoot samples of tomato seedlings exposed to cadmium stress at different concentrations compared to the control group has been given in the Table 3.

The results obtained with the GR-1 and MT-2 genes and their activities in this current study support different studies in the literature [7-9,26,27,33]. Stress related genes such as GR-1 and MT-2 are used in different applications (especially phytoremediation) against various stress factors. It has been reported that the evaluation of the expression data of these genes is effective in determining the level of damage to the living thing by the pollution in question and determining the molecular biological limits of the defense mechanism [7,26,28-35]. According to Tombuloglu et al. (2012) the expression data of GR-1 and MT-2 genes, which are stress-related genes due to boron stress, were examined in tomoto seedlings. While gene expression increased due to increasing concentration, a decrease was observed after certain concentrations [27]. Also, in our previous study (2019), tomato seedlings were exposed to varying concentrations of zinc heavy metal stress and the expression levels of the same genes were investigated. Although similar results were obtained, its toxic effect was revealed at higher concentrations (eg: 320 mg L-1 - 640 mg L-1) than cadmium toxicity, since zinc is a microelement. After these concentrations, sudden decreases were observed in the same way. The results of the present study also support these [7]. Finally, Wang et al., (2018) cloning and characterization of the glutathione reductase gene and Rono et al. (2021) showed that metallothionein-like gene groups were identified for cadmium detoxification and potential phytoremediation. Thus, it has been shown that there are different and new stress-related genes or clones as GR-1 and MT-2 [31,33].

## CONCLUSIONS

In the present study, it was shown that the activation of MT-2 and GR-1 like protein transcripts under cadmium stress. These genes expression increased at first and then, the expression curve was showed a descending profile due to inhibition of stress mechanisms which regulates the cellular homeostasis under high cadmium level. Due to the increased concentration of cadmium heavy metal, the change of these genes, which are known to be stress-related in plants, such as tomato, has shown that these genes play an important role in the mechanism of protection against heavy metal stress. Also, this study results have indicated that tomato has physiological traits associated with accumulation of cadmium. The early molecular response of hydroponically cultivated tomato plants might develop different strategies to cope with Cd toxicity by manipulating the expression level of stress-related genes. Furthermore, these results showed that the resistance mechanism could not cope with cadmium stress and toxicity due to possible cellular damage using the GR pathway. Finally, the data obtained from the study; showed that the change in the expression levels of these genes in the tomato plant could serve as an additional Cd-tolerance mechanism to deal with the toxic effect of cadmium.

## CONFLICTS OF INTEREST

Author had no any financial or personal relationships with other individuals or organizations that might inappropriately influence this work during the submission process.

## STATEMENT OF ETHICS

There is no need for an ethics committee decision for the studies in the article.

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# Foam-mat Drying of Carrot Juice and Thin Layer Modeling of Drying

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## ABSTRACT

Drying of fruit and vegetables is critical step of processing which can be very destructive for nutrients and especially for bioactive compounds. However, novel drying methods like foam-mat drying helps to decrease the drying period and exposure to drying air therefore protect the bioactives against thermal degradation as well as improving final powder quality. The foam-mat drying of carrot juice and modeling of experimental drying data with the theoretical models has not yet been studied in the literature. In this study, the effects of foam-mat drying at 50, 60 and 70°C on the drying behavior of carrot juice with the addition of 15% egg albumen (EA) and 15% egg albumen+ 10% whey protein isolate (WPI) as foaming agents and thin-layer modeling of the foams at different thicknesses were evaluated. Compared to the control sample (only carrot juice), the drying time of the foamed carrot juice was reduced by 25% to 60% depending on the foam thickness and drying temperature. These results were consistent with the effective diffusion coefficients ($D_{eff}$), since the control sample had comparably low $D_{eff}$ value than the 15% EA and 15% EA+10% WPI foams. Among the fitted mathematical models, Midilli *et al.* had better prediction capacity with the highest adjusted correlation coefficients, in addition to the lowest sum of squared error and root mean square error values for every formulation, foam thicknesses and drying temperatures compared to other theoretical models.

Keywords:
Carrot juice; Foam-mat drying; Modeling; Thin-layer

## INTRODUCTION

Fresh fruits and vegetables are highly perishable because of their high moisture content and should be consumed without any deterioration if only stored properly or food preservation methods such as; freezing, canning, chemical treatments, or drying are employed for increasing their shelf life [1].

Drying is one of the oldest food preservation methods used because it increases the shelf-life of foodstuffs by reducing the water activity, therefore the dried products can be stored for later use. Besides, microbial activity that is causing the spoilage of the food is prevented, and at the same time, most of enzymes that is evoking chemical changes in the food cannot perform their functions due to moisture removal. Thus, dried foods can be stored for a longer period [2].

Drying methods using hot air with natural or forced convection are mostly preferred for drying foods. However, since the chosen method is effective on the quality characteristics of the final product, drying methods such as; contact drying [3], convective drying [4], radiation drying [5], freeze-drying [6], osmotic drying [7] are used for drying of agricultural products like; vegetables, fruits, and cereals. Alternative drying methods are constantly being developed, since the quality of the final product is important. Foam-mat drying is a novel technique developed to increase the moisture transfer during the drying of liquid and semi-liquid foods. The foam-mat drying process, which is carried out by the addition of foaming agents and stabilizers, has come to the forefront due to its advantages such as shortening the drying time with hot air, and better preservation of the dried food quality, and many studies have been carried out on foam drying [8]. The drying of agricultural products using foam drying methods has been studied by many researchers. In these studies, vegetable and fruits such as; instant yam (Dioscorea rotundata) [9], banana [10], tomato pulp [11], blackcurrant pulp [12], papaya nectar [13], mango [14], muskmelon [15], yacon juice [16]

and crab apple juice [8] were dried by this method. Although there are a few studies about carrot powder production with foam-mat drying by incorporation of some other foaming agents like Tween 80, methylcellulose and egg white, these studies mostly focused on chemical composition of the powders or powder yield [17, 18]. Moreover, the foam-mat drying of carrot juice including different animal-based protein sources as foaming agents and the mathematical modeling of drying has not been studied yet. Therefore, the objective of this study was to determine the drying behavior of carrot juice by foam-mat drying method and mathematical modeling of the experimental drying data by exploring the presence of egg albumen (EA) and egg albumen + whey protein isolate (WPI) in the formulation together with the foam thickness at different drying temperatures.

## MATERIALS AND METHODS

Fresh carrots and whole eggs were purchased from a local supermarket in Corum, Turkey. Whey protein isolate with 96% protein was supplied from local distributor of Hipro Iso whey (Bionet Tic. A.S., Istanbul).

Fresh carrot juice was extracted according to the previous study of Cakmak and Ozyurt [19]. The extracted juice were filled into the glass bottles and heat-treated at 95°C for 5 min [20] in a water bath (Wise Bath, WB22, Daihan Scientific, South Korea), and cooled to 4°C.

### Production of Carrot Juice Foams

The most stable foam structure was obtained from the 15% EA+ 10% WPI foam formulation according to the previous study of the authors which was mixed at the highest speed with a hand-blender (Arzum Pasto AR-183, Turkey) for 8 min whipping time. In addition to this formulation, 15% EA including foams were prepared similarly to the given foaming conditions.

### Thin Layer Drying of Carrot Juice Foams

15% EA and 15% EA+ 10% WPI foams together with control (carrot juice without foaming) were spread evenly on petri dishes (OD: 90 mm) at two different thicknesses, in order to equilibrate the mass on each petri dishes. For control, the samples were placed with the thickness of 2.5 and 3.2 mm, whereas 15% EA and 15% EA+ 10% WPI including foams the thickness was arranged as 5 and 6 mm. The samples were dried at 50, 60 and 70°C in a preheated built-in oven (Model no: NV60K7140BB, Samsung, Turkey) with upper-lower heating function at 0.9 m/s steady air velocity until constant weight was observed. Drying experiments performed at least five parallels and the mass of petri dishes were recorded with an analytical ba-

**Table 1.** Thin layer models fitted to experimental drying datas.

| Model | Model eq. | Reference |
|---|---|---|
| Lewis | $MR = e^{(-kt)}$ | [8], [21] |
| Page | $MR = e^{(-kt^n)}$ | [8], [21] |
| Henderson & Pabis | $MR = ae^{(-kt)}$ | [8], [21] |
| Logarithmic | $MR = ae^{(-kt)} + c$ | [8], [21] |
| Two-term | $MR = ae^{(-k_0 t)} + be^{(-k_1 t)}$ | [8], [21] |
| Midilli et al. | $MR = ae^{(-kt^n)} + bt$ | [8], [21] |
| Modified Midilli et al. | $MR = e^{(-kt^n)} + bt$ | [8], [21] |

lance (Precisa Gravimetrics, XB220A, Switzerland) every 10 min for first half hour, and every 30 min until the constant weight was observed. The drying curves of the samples were obtained from the plot of drying rate (kg water/ hm$^2$) versus free moisture content (kg water/ kg dry solid) with respect to the removed free water during aforementioned time intervals at constant surface area exposed during drying.

### Mathematical Modeling of Foam-mat Drying

Fick's second law of diffusion was employed for evaluation of the moisture transfer from the control and carrot juice foam samples. The diffusion equation for an infinite slab at falling rate drying period is given in Eq.1;

$$MR = \frac{M - M_e}{M_0 - M_e}$$
$$= \frac{8}{\pi^2} \sum_{n=0}^{\infty} \frac{1}{(2n+1)^2} \exp\left[ -(2n+1)^2 \frac{\pi^2}{4} \frac{D_{eff} t}{L^2} \right] \quad (1)$$

here MR shows the dimensionless moisture ratio, $M_0$ is initial moisture and $M_e$ is the equilibrium moisture content. $M$ represents the moisture at any time $t$, $L$ is the thickness of the slab in m, and $D_{eff}$ represents effective diffusion coefficient (m$^2$/s).

The experimental drying data of the control and carrot juice foams were fitted to the thin layer models given in Table 1 by using Matlab R2016A (MathWorks Inc., USA). The goodness of model fit was evaluated with respect to the Adj-R$^2$ (adjusted correlation coefficient), SSE (sum of squared error) and RMSE (root mean square error) values.

## RESULTS AND DISCUSSION

The drying rate curves of the samples are shown Fig. 1a, 1b and 1c for drying at 50, 60 and 70°C together with the lowest foam thicknesses (for control: 2.5 mm, for 15% EA and 15% EA + 10% WPI: 5 mm), respectively. It is seen that the control sample and 15% EA foam had both constant and falling rate period at all drying temperatures. In addition, it was observed that the 15% EA + 10% WPI
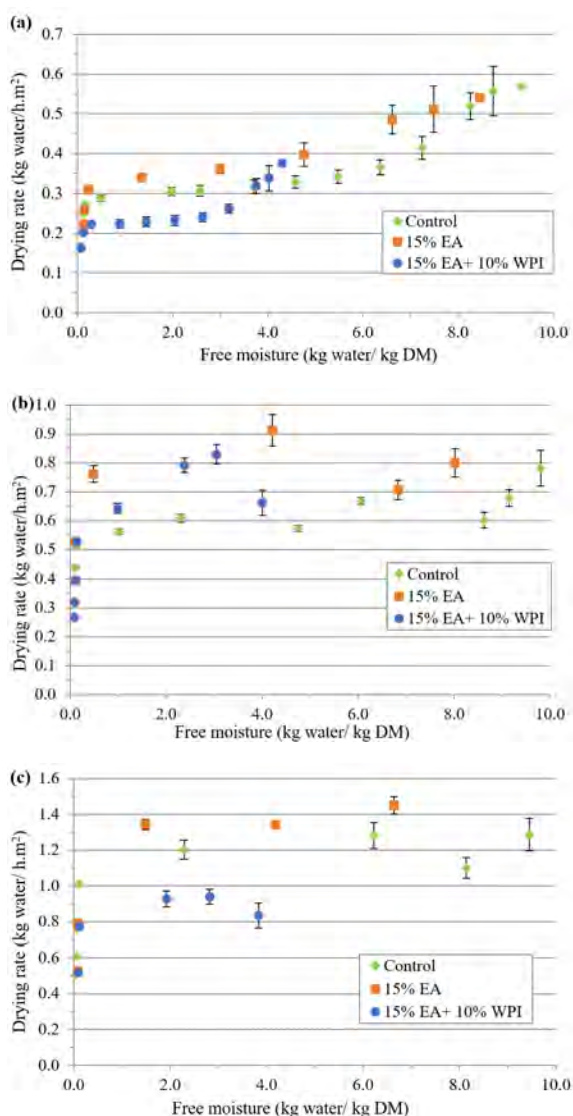
**Figure 1.** Drying rate curves of carrot juice (control), 15% EA and 15%EA+ 10% WPI foams at (a) 50°C, (b) 60°C and (c) 70°C, respectively.

sample had both constant and falling rate period only at 50°C, and only falling rate period at 60 and 70°C. Besides, the increase in drying temperature increased the drying rate by accelerating the moisture transfer at the elevated temperatures. The initial moisture content of control sample was reduced from 9.965 kg water/ kg DM to 0.125 kg water/ kg DM at 50°C, to 0.111 kg water/ kg DM at 60°C and to 0.047 kg water/ kg DM at 70°C for 2.5 mm thickness. Besides, the initial moisture content of 15% EA foam was reduced from 9.542 kg water/ kg DM to 0.151 kg water/ kg DM, 0.105 kg water/ kg DM, 0.078 kg water/ kg DM for drying at 50, 60 and 70°C, respectively.

Drying period of the samples reaching the constant weight was found dependent on the drying temperature. For 50°C, the drying period was observed between 360-480 min for control sample, whereas it was between 210-240 min for

15% EA foam and between 240-270 min for 15% EA+ 10% WPI at both thicknesses. Similarly, the drying period at 70°C lasted 150 min for the control sample; while the drying period of 15% EA sample was 90 min and 60 min for the 15% EA + 10% WPI sample at both thicknesses. As can be seen from these observations, the foam-mat drying method shortened the drying time by 25-60% depending on the drying temperature and the foam thickness (amount).

The effective diffusion coefficients are influenced by the drying temperature, although the foam viscosity may hinder the moisture transfer [22]. The $D_{eff}$ of the control sample was found between $9.403\times10^{-9}$- $9.803\times10^{-8}$ m²/s, for 15% EA it was between $1.421$-$6.262\times10^{-7}$ m²/s and for 15% EA+ 10% WPI foam it was between $5.499\times10^{-8}$- $5.990\times10^{-7}$ m²/s, respectively. In accordance with the drying period values, foam-mat drying improved the moisture diffusion compared to control sample due to increased water-air interface area due to foaming [8, 22, 23].

The results of regression analysis employed for finding the best thin layer model representing the foam-mat drying of carrot juice foams are given in Table 2, 3 and 4. The Adj-R² values of the tested mathematical models were found between 0.93-0.99 and very successful in terms of representing the experimental drying data of carrot juice and foams at any drying temperature and foam thickness. But the most successful model was determined as Midilli *et* al. with the highest Adj-R² together with the lowest SSE and RMSE values. The model constants of Midilli *et* al. model are also shown in Table 5.

Foam-mat drying offers several advantages like increasing the moisture transfer rate by increasing the air-water interface due to volume expansion via foaming. Thus, this method decreases the energy consumption, improves reconstitution capacities of produced powders thus product quality, as well as protecting the bioactive compounds against thermal degradation compared to the conventional drying methods by encapsulation like mechanism of the proteins [8], [24], [25], [26].

Similar to the present study, the foam-mat drying reduced the drying period of apple juice [24], mango puree [23], crab apple juice [8] and date puree [27].

The effective diffusion coefficients can be affected from the foam formulation and the drying temperature [23], and increasing the drying temperature increases the $D_{eff}$ values because of faster moisture transfer from the material [26]. Chaux-Gutiérrez et al. [23] stated in their study that the $D_{eff}$ values of foam-mat drying of mango pulp was found between $2.15$-$6.12\times10^{-10}$ m²/s, whereas the $D_{eff}$ values of lime juice foams $8.980\times10^{-9}$ and $1.138\times10^{-8}$ m²/s [22]. These values are in accordance with the $D_{eff}$ values of carrot juice foams.

**Table 2.** Statistical results of tested models for drying at 50°C.

| Sample- thickness | Model | Adj-R² | SSE | RMSE |
|---|---|---|---|---|
| Control-2.5 mm | Lewis | 0.9657 | 0.05653 | 0.06595 |
| | Page | 0.9794 | 0.03130 | 0.05107 |
| | Henderson & Pabis | 0.9642 | 0.05438 | 0.06732 |
| | Logarithmic | 0.9642 | 0.05438 | 0.06732 |
| | Two-term | 0.9570 | 0.05442 | 0.07377 |
| | Midilli et al. | 0.9846 | 0.02147 | 0.04418 |
| | Modified Midilli et al. | 0.9794 | 0.03130 | 0.05107 |
| Control-3.2 mm | Lewis | 0.9427 | 0.09119 | 0.08071 |
| | Page | 0.9692 | 0.04551 | 0.05916 |
| | Henderson & Pabis | 0.9421 | 0.08560 | 0.08115 |
| | Logarithmic | 0.9421 | 0.08560 | 0.08115 |
| | Two-term | 0.9316 | 0.08561 | 0.08822 |
| | Midilli et al. | 0.9819 | 0.02672 | 0.04534 |
| | Modified Midilli et al. | 0.9735 | 0.03917 | 0.05489 |
| 15% EA-5 mm | Lewis | 0.9781 | 0.03021 | 0.05793 |
| | Page | 0.9888 | 0.01378 | 0.04150 |
| | Henderson & Pabis | 0.9780 | 0.02699 | 0.05808 |
| | Logarithmic | 0.9780 | 0.02699 | 0.05810 |
| | Two-term | 0.9706 | 0.02699 | 0.06707 |
| | Midilli et al. | 0.9888 | 0.01204 | 0.04147 |
| | Modified Midilli et al. | 0.9888 | 0.01378 | 0.04150 |
| 15% EA - 6 mm | Lewis | 0.9781 | 0.03021 | 0.05790 |
| | Page | 0.9872 | 0.01564 | 0.04421 |
| | Henderson & Pabis | 0.9709 | 0.03570 | 0.06680 |
| | Logarithmic | 0.9780 | 0.02699 | 0.05808 |
| | Two-term | 0.9706 | 0.02699 | 0.06707 |
| | Midilli et al. | 0.9888 | 0.01204 | 0.04147 |
| | Modified Midilli et al. | 0.9888 | 0.01378 | 0.04150 |
| 15% EA + 10% WPI-5 mm | Lewis | 0.9734 | 0.03667 | 0.06383 |
| | Page | 0.9888 | 0.01378 | 0.04150 |
| | Henderson & Pabis | 0.9717 | 0.03467 | 0.06583 |
| | Logarithmic | 0.9709 | 0.03570 | 0.06680 |
| | Two-term | 0.9706 | 0.02700 | 0.06709 |
| | Midilli et al. | 0.9888 | 0.01204 | 0.04147 |
| | Modified Midilli et al. | 0.9888 | 0.01378 | 0.04150 |
| 15% EA + 10% WPI-6 mm | Lewis | 0.9737 | 0.03629 | 0.06350 |
| | Page | 0.9861 | 0.01704 | 0.04615 |
| | Henderson & Pabis | 0.9717 | 0.03467 | 0.06583 |
| | Logarithmic | 0.9734 | 0.03264 | 0.06387 |
| | Two-term | 0.9706 | 0.02699 | 0.06707 |
| | Midilli et al. | 0.9888 | 0.01204 | 0.04147 |
| | Modified Midilli et al. | 0.9888 | 0.01378 | 0.04150 |

**Table 3.** Statistical results of tested models for drying at 60°C.

| Sample- thickness | Model | Adj-R² | SSE | RMSE |
|---|---|---|---|---|
| Control-2.5 mm | Lewis | 0.9583 | 0.05841 | 0.08056 |
| | Page | 0.9877 | 0.01534 | 0.04379 |
| | Henderson & Pabis | 0.9611 | 0.04850 | 0.07786 |
| | Logarithmic | 0.9611 | 0.04850 | 0.07786 |
| | Two-term | 0.9481 | 0.04850 | 0.08991 |
| | Midilli et al. | 0.9892 | 0.01183 | 0.04111 |
| | Modified Midilli et al. | 0.9877 | 0.01534 | 0.04379 |
| Control-3.2 mm | Lewis | 0.9476 | 0.08417 | 0.09174 |
| | Page | 0.9873 | 0.01834 | 0.04514 |
| | Henderson & Pabis | 0.9519 | 0.06954 | 0.08790 |
| | Logarithmic | 0.9519 | 0.06954 | 0.08790 |
| | Two-term | 0.9381 | 0.06955 | 0.09968 |
| | Midilli et al. | 0.9900 | 0.01279 | 0.03998 |
| | Modified Midilli et al. | 0.9873 | 0.01834 | 0.04514 |
| 15% EA-5 mm | Lewis | 0.9516 | 0.05243 | 0.09347 |
| | Page | 0.9940 | 0.00498 | 0.03304 |
| | Henderson & Pabis | 0.9506 | 0.04465 | 0.09450 |
| | Logarithmic | 0.9438 | 0.05079 | 0.10080 |
| | Two-term | 0.9176 | 0.04465 | 0.12200 |
| | Midilli et al. | 0.9945 | 0.00437 | 0.03154 |
| | Modified Midilli et al. | 0.9945 | 0.00498 | 0.03154 |
| 15% EA - 6 mm | Lewis | 0.9671 | 0.04077 | 0.07630 |
| | Page | 0.9981 | 0.00203 | 0.01840 |
| | Henderson & Pabis | 0.9616 | 0.04075 | 0.08241 |
| | Logarithmic | 0.9786 | 0.02272 | 0.06154 |
| | Two-term | 0.9711 | 0.03065 | 0.07150 |
| | Midilli et al. | 0.9983 | 0.00176 | 0.01717 |
| | Modified Midilli et al. | 0.9983 | 0.00177 | 0.01877 |
| 15% EA + 10% WPI-5 mm | Lewis | 0.9806 | 0.02246 | 0.05664 |
| | Page | 0.9974 | 0.00260 | 0.02195 |
| | Henderson & Pabis | 0.9879 | 0.01200 | 0.04472 |
| | Logarithmic | 0.9860 | 0.01387 | 0.04809 |
| | Two-term | 0.9663 | 0.02231 | 0.07468 |
| | Midilli et al. | 0.9974 | 0.00241 | 0.02082 |
| | Modified Midilli et al. | 0.9970 | 0.00296 | 0.02219 |
| 15% EA + 10% WPI-6 mm | Lewis | 0.9706 | 0.04013 | 0.07082 |
| | Page | 0.9973 | 0.00320 | 0.02153 |
| | Henderson & Pabis | 0.9726 | 0.03270 | 0.06835 |
| | Logarithmic | 0.9783 | 0.02589 | 0.06082 |
| | Two-term | 0.9697 | 0.02590 | 0.07197 |
| | Midilli et al. | 0.9975 | 0.00256 | 0.02071 |
| | Modified Midilli et al. | 0.9975 | 0.00300 | 0.02081 |

**Table 4.** Statistical results of tested models for drying at 70°C.

| Sample- thickness | Model | Adj-R² | SSE | RMSE |
|---|---|---|---|---|
| Control-2.5 mm | Lewis | 0.9660 | 0.04750 | 0.07705 |
| | Page | 0.9966 | 0.00421 | 0.02451 |
| | Henderson & Pabis | 0.9688 | 0.03815 | 0.07383 |
| | Logarithmic | 0.9688 | 0.03815 | 0.07383 |
| | Two-term | 0.9636 | 0.03815 | 0.07974 |
| | Midilli et al. | 0.9966 | 0.00365 | 0.02447 |
| | Modified Midilli et al. | 0.9966 | 0.00421 | 0.02451 |
| Control-3.2 mm | Lewis | 0.9556 | 0.06283 | 0.08862 |
| | Page | 0.9951 | 0.00609 | 0.02950 |
| | Henderson & Pabis | 0.9624 | 0.04648 | 0.08149 |
| | Logarithmic | 0.9624 | 0.04648 | 0.08149 |
| | Two-term | 0.9562 | 0.04648 | 0.08802 |
| | Midilli et al. | 0.9953 | 0.00503 | 0.02897 |
| | Modified Midilli et al. | 0.9951 | 0.00605 | 0.02940 |
| 15% EA-5 mm | Lewis | 0.9720 | 0.02313 | 0.06802 |
| | Page | 0.9945 | 0.00365 | 0.03019 |
| | Henderson & Pabis | 0.9731 | 0.01782 | 0.06675 |
| | Logarithmic | 0.9731 | 0.01782 | 0.06675 |
| | Two-term | 0.9462 | 0.01782 | 0.09440 |
| | Midilli et al. | 0.9952 | 0.002371 | 0.02811 |
| | Modified Midilli et al. | 0.9950 | 0.003306 | 0.02875 |
| 15% EA - 6 mm | Lewis | 0.9509 | 0.04271 | 0.09243 |
| | Page | 0.9905 | 0.00662 | 0.04067 |
| | Henderson & Pabis | 0.9385 | 0.04286 | 0.10350 |
| | Logarithmic | 0.9351 | 0.04523 | 0.10630 |
| | Two-term | 0.9457 | 0.03785 | 0.09727 |
| | Midilli et al. | 0.9915 | 0.00440 | 0.03840 |
| | Modified Midilli et al. | 0.9905 | 0.00662 | 0.04070 |
| 15% EA + 10% WPI-5 mm | Lewis | 0.9491 | 0.04307 | 0.09357 |
| | Page | 0.9945 | 0.00377 | 0.03070 |
| | Henderson & Pabis | 0.9539 | 0.03171 | 0.08904 |
| | Logarithmic | 0.9438 | 0.03866 | 0.09831 |
| | Two-term | 0.9412 | 0.04044 | 0.10050 |
| | Midilli et al. | 0.9952 | 0.00330 | 0.02870 |
| | Modified Midilli et al. | 0.9947 | 0.00360 | 0.03010 |
| 15% EA + 10% WPI-6 mm | Lewis | 0.9636 | 0.03122 | 0.07901 |
| | Page | 0.9963 | 0.00190 | 0.02506 |
| | Henderson & Pabis | 0.9539 | 0.03168 | 0.08900 |
| | Logarithmic | 0.9615 | 0.02644 | 0.08130 |
| | Two-term | 0.9505 | 0.03396 | 0.09214 |
| | Midilli et al. | 0.9966 | 0.00188 | 0.02400 |
| | Modified Midilli et al. | 0.9961 | 0.00270 | 0.02590 |

**Table 5.** Model constants of the best fitting theoretical model.

| Temperature (°C) | Sample | Midilli et al. model constant |
|---|---|---|
| 50 | Control-2.5 mm | a=0.932, b=2.338*10$^{-14}$, k=0.0003, n=1.556 |
| | Control-3.2 mm | a=0.943, b=3.185*10$^{-12}$, k=0.0001, n=1.659 |
| 60 | Control-2.5 mm | a=0.953, b=2.223*10$^{-14}$, k=0.0005, n=1.659 |
| | Control-3.2 mm | a=0.947, b=4.233*10$^{-10}$, k=0.0002, n=1.859 |
| 70 | Control-2.5 mm | a=0.978, b=1.943*10$^{-12}$, k=0.0021, n=1.626 |
| | Control-3.2 mm | a=0.973, b=2.332*10$^{-14}$, k=0.0009, n=1.682 |
| 50 | 15% EA-5 mm | a=0.962, b=2.244*10$^{-14}$, k=0.0029, n=1.363 |
| | 15% EA-6 mm | a=0.962, b=2.309*10$^{-14}$, k=0.0028, n=1.363 |
| 60 | 15% EA-5 mm | a=0.977, b=1.189*10$^{-9}$, k=0.0014, n=1.874 |
| | 15% EA-6 mm | a=1.002, b=2.256*10$^{-14}$, k=0.0036, n=1.536 |
| 70 | 15% EA-5 mm | a=1.000, b=2.417*10$^{-14}$, k=0.0012, n=1.502 |
| | 15% EA-6 mm | a=0.999, b=4.348*10$^{-14}$, k=0.0006, n=2.203 |
| 50 | 15% EA + 10% WPI-5 mm | a=0.962, b=1.559*10$^{-11}$, k=0.0028, n=1.363 |
| | 15% EA + 10% WPI-6 mm | a=0.962, b=2.222*10$^{-14}$, k=0.0029, n=1.363 |
| 60 | 15% EA + 10% WPI-5 mm | a=0.999, b=2.245*10$^{-14}$, k=0.0069, n=1.354 |
| | 15% EA + 10% WPI-6 mm | a=0.994, b=2.221*10$^{-14}$, k=0.0036, n=1.444 |
| 70 | 15% EA + 10% WPI-5 mm | a=0.986, b=2.220*10$^{-14}$, k=0.0034, n=1.657 |
| | 15% EA + 10% WPI-6 mm | a=0.997, b=2.221*10$^{-14}$, k=0.0048, n=1.596 |

Since carrot juice is a valuable source of carotenoids, the encapsulation of these bioactive compounds with wall materials including the proteins or stabilizers by foam-mat drying like this present study will promote longer stability of carotenoids [28]. Therefore, the efforts related with finding better drying conditions in terms of selecting different dryers such as non-thermal or hybrid dryers together with modifying the drying temperature and air velocity will help to provide an insight for further foam-mat drying of similar juices.

## CONCLUSION

It has been determined that the foam-mat drying process shortens the drying time of carrot juice by 25-60% depending on the drying temperature and the foam thickness. These results in accordance with the effective diffusion coefficients, since the drying of foamed juices had higher $D_{eff}$ values compared to the control sample.

Consequently, the compatibility of experimental drying data of carrot juice with the tested theoretical models was evaluated, and the adjusted correlation coefficients of the tested theoretical models varied between 0.93-0.99, which showed that the fitted models had a high ability to represent the drying behavior of the carrot juice, 15% EA and 15% EA+ 10% WPI foams. However, among these models, regardless of the foam composition, drying temperature, or foam thicknesses, the best results were found with the Midilli *et* al. model. Future studies may focus on prediction of the drying data of different fruit juice foams by the same theoretical model.

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## CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

## AUTHOR CONTRIBUTION

Hülya Çakmak: Funding acquisition, Formal analysis, Investigation, Conceptualization, Methodology, Writing - original draft, Writing - review & editing. V. Hazal Özyurt: Formal analysis, Conceptualization, Investigation, Methodology, Writing - original draft, Writing - review & editing. Both authors read and approved the final manuscript.

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