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Research Article

Akifer Sistemlerinde Depolanan DNAPL Karışımlarının Yerinde Kimyasal Yıkama Teknolojisi ile Islahının İncelenmesi

Nihat Hakan Akyol[1*], Taylan Acılıoğlu[1]

ÖZET

Trikloroetilen (TCE), tetrakloroetilen (PCE) ve Dikloroetilen (DCE) yeraltı suyu akifer sistemlerinde en çok gözlenen klorlu organik bileşiklerden olup kanserojen etkisinden ötürü insan sağlığı için ciddi tehlike oluşturmaktadır. Bu bileşikler tekil ya da karışım olarak yeraltına genellikle DNAPL olarak girerek çoğunlukla akiferlerin tabanını oluşturan kil ya da ana kaya içerisinde (çanak) yüksek doygunlukta depolanarak uzun süreli çözünmüş faz kirletici kaynağı üretmektedir. Yerinde kimyasal yıkama (ISCF) organik kirleticilerin yeraltından uzaklaştırılması için kullanılan önemli ıslah tekniklerindendir. Çalışmanın amacı; akifer sistemlerin tabanında depolanmış yüksek doygunluktaki DNAPL karışım (TCE, PCE ve DCE) kütlelerinin yıkama ajanları (SDS, Tween 80, MCD ve su) ile ıslah performanslarının etkinliğinin değerlendirilmesidir. Kimyasal yıkama ajanlarıyla (SDS, Tween 80, MCD ve su) heterojen sistemlerde depolanan DNAPL karışım (TCE, PCE, DCE) kaynak zonları büyük oranda ıslah edilmesine rağmen, fiziksel ortam heterojenliği geniş ölçekte aşamalı konsantrasyon davranışı sergilemiştir. Sonuçlara göre, yıkamanın ilk anlarında organik kirletici hidrolik olarak elverişli zonlardan (matriks) gelirken, geç zamanlarda yüksek doygunluktaki hidrolik olarak elverişli olmayan zonlardan (çanak) gelmektedir. Sonuç olarak, bu çalışmadan elde edilecek sonuçlar, ulusal ve uluslararası alandaki önemli bilgi eksikliğine katkıda bulunacak ve arazi uygulamalarının geliştirilmesine yardımcı olacaktır.

Article Info

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Investigation of In situ chemical flushing technology for DNAPL mixtures resided in aquifer systems

ABSTRACT

Contamination of groundwaters by chlorinated solvents such as trichloroethylene (TCE, tetrachloroethylene (PCE) and dichloroethylene (DCE) is awidespread problem worldwide. These compounds causes serious threat for human health due to being human carcinogen. Chlorinated solvents generally enter the subsurface as single and mixture form of dense non aqueous pHase liquids (DNAPLs) and accumulation generally occurs in aquifers where the DNAPL mass is most likely pooled at the bottom of aquifers and become a long-term aqueous pHase source zone for groundwater contamination. Surfactant enhanced aquifer remediation (SEAR) is groundwater remediation techniques used to remove organic pollutants from the subsurface environment. The objective of the project is to test the reagent (SDS, Tween 80, MCD and water) flushing for DNAPL mixtures (TCE, PCE, DCE) trapped in heterogeneous porous media. Results showed that most of DNAPL source zones were remediated but, physical heterogeneity led to extensive sequential concentration behavior. The results also emphasized that in the early stage, some portion of organic liquid is hydraulically accessible (matrix) whereas the later stage of mass removal was controlled by the more poorly-accessible mass (pool) associated with higher-saturation zones.

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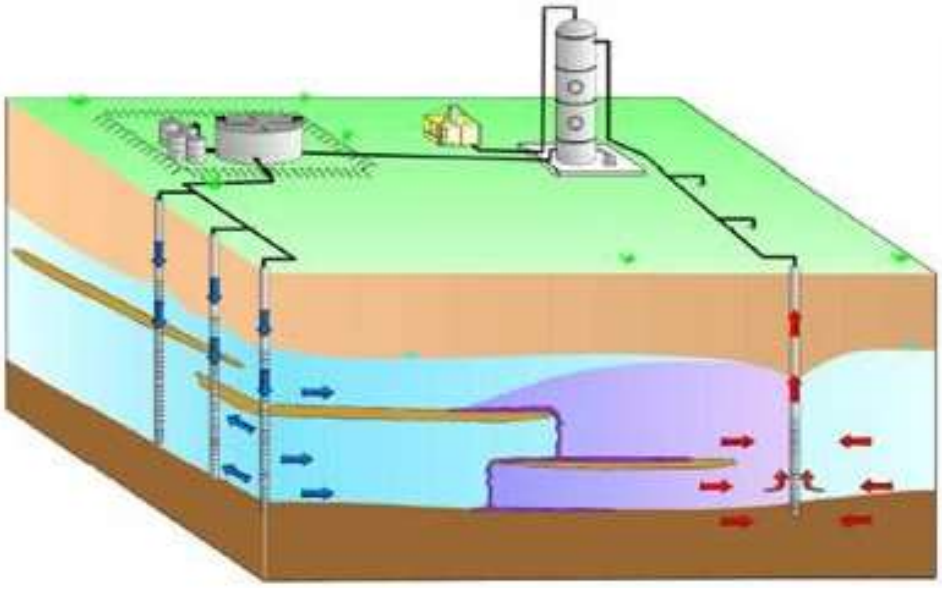
1. GİRİŞ

Klorlu solventler önemli DNAPL (Yoğun Suyla Karışmayan Organik Kirletici) kirletici gruplarından olup metal sanayinde ve havacılıkta yağ çözücü, kuru temizlemede ve elektronik sanayi gibi birçok alanda kimyasal solvent olarak kullanılmaktadır. Klorlu solventler yeraltına çoğunlukla DNAPL (Dense non-aqueous phase liquids) olarak girerler. Dolayısıyla, yeraltında çok az miktarlarda bulunsalar dahi, çözünmüş faz üretme potansiyeline sahip olup, büyük hacimlerde yeraltı su kütlelerini kirletme riski oldukça yüksektir. DNAPL'ların yeraltındaki hareketi DNAPL miktarına, özelliğine, fiziksel ortam özelliklerine ve akım koşullarına bağlıdır [1]. Yeterli miktarda DNAPL mevcudiyetinde, su tablasından aşağıya doğru geçirgenliği düşük bir katmanla karşılaşıncaya kadar göç eder [2]. Doygun zonda DNAPL kütlesinin bir kısmı suda çözünürken, bir kısmı ise matriks içerisinde artık DNAPL olarak depolanabilir Diğer kısmı ise yer altı suyu akifer sistemlerinin tabanını oluşturan geçirimsiz kil ya da ana kaya içerisindeki çatlaklarda veya üzerinde depolanabilmektedir. Bu kütleler özellikle bu noktalarda yüksek doygunlukta DNAPL birikintileri oluşmasına olanak sağlayarak, uzun vadede yer altı su kaynakları için potansiyel risk oluşturmaktadır.

Yerinde kimyasal yıkama teknolojisi (ISCF), akiferlerin kaynak zonlarındaki DNAPL kütlelerini uzaklaştırmada kullanılan önemli ıslah teknolojilerinden biridir (Şekil 1). Bu teknolojide yüzey aktif madde, alkol, kosolvent ya da kompleks şekerler gibi kimyasalların suya ilavesi ile DNAPL'ların çözünürlüğü arttırılarak ve yüzey gerilimi azaltılarak su ile yıkamaya göre daha etkin bir uzaklaştırma sağlanmaktadır [3-9].

DNAPL kaynak zonların yıkama kimyasallarla ıslahı ile ilgili son yıllardaki çalışmalar çoğunlukla tek tip DNAPL kütleler ile yapılmıştır [10-13]. Ancak, klorlu solventlerle kirlenmiş sahaların çoğunda karışım halinde de gözlenmiştir. Çalışmanın amacı; akifer sistemlerin tabanında depolanmış yüksek doygunluktaki DNAPL karışım (TCE, PCE ve DCE) kütlelerinin yıkama ajanları (SDS, Tween 80, MCD ve su) ile ıslah performanslarının etkinliğinin değerlendirilmesidir.



Şekil 1. Yerinde kimyasal yıkama teknolojisinin şematik gösterimi [14].

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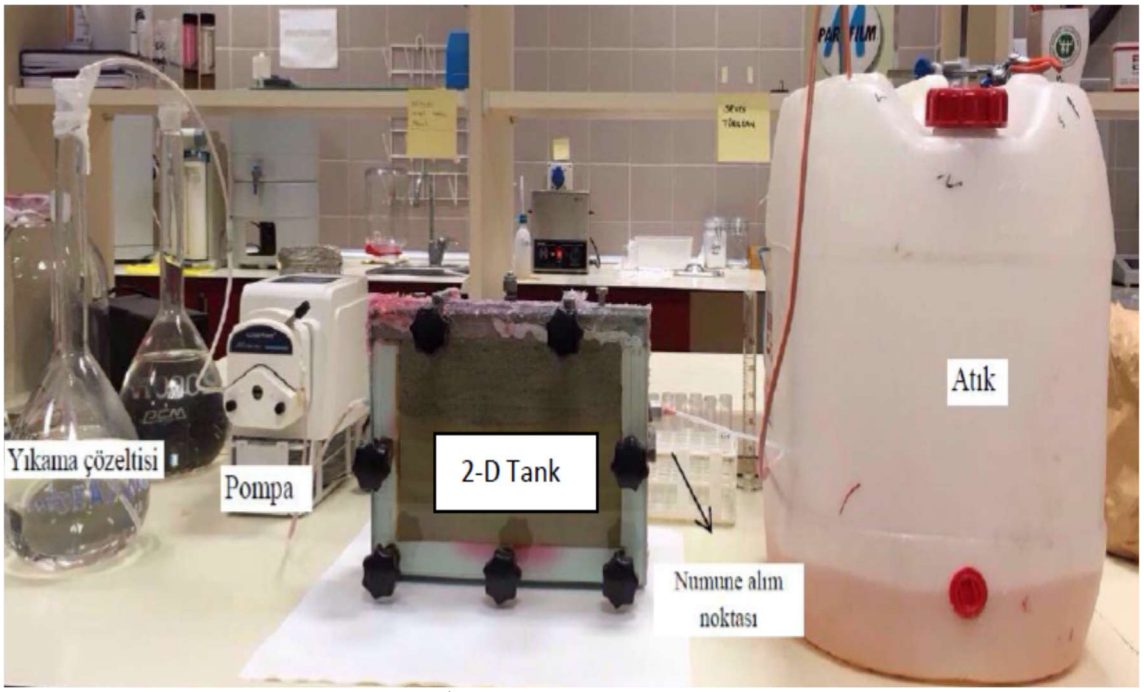
Research Article

2. MATERYAL ve METOD

Deneylerde kullanılan 2-D tank paslanmaz çelikten, ön yüzü ise DNAPL dağılımının gözlemlenebilmesi için cam malzemesinden yapılmıştır (Boy:20 cm, En:30 cm, Kalınlık:5 cm) (Şekil 2). DNAPL enjeksiyonları tankın orta noktasından düşük geçirgenlikteki zona yüksek doygunlukta yapılması amaçlanmıştır. Tankın sol ve sağ yanına solüsyon giriş ve çıkışı sağlayacak enjeksiyon delikleri açılmıştır. Tankın iç kısmına enjekte edilen çözeltinin daha iyi yayılmasını sağlayacak paslanmaz çelik bir filtre (12 µm) yerleştirilmiştir. Tankın girişi paslanmaz çelik boruyla peristaltik pompaya bağlanırken, çıkışında ise bir örnek alma yapısı oluşturulmuştur.

Tank deneyleri ile farklı fiziksel ortam heterojenlikleri yansıtmak amacıyla düşük organik karbon içeriğine sahip (% O.C~0.05) silis kumları kullanılmıştır. Silis kumları Kocaeli Gebze'de faaliyet gösteren Santoz Sanayi Tozları San. Tic. Lim. Şti'den temin edilmiştir. Elde edilen kuvars kumları oluşturulmak istenen farklı fiziksel ortam heterojenliği oluşturması amacıyla elekten geçirilerek arzu edilen boyutlara getirilmiştir. Tank deneyleri kapsamında 2 farklı boyutta (359 µm (40/50 mesh) ve 172µm (70/100 mesh)) silis kumları kullanılmıştır. Bunlar içerisinde, 70/100 mesh geçirimsiz tabandan oluşan çanağı temsil ederken, 40/50 mesh silis kum ise geçirimli ortamı (matriks) temsil etmektedir (Şekil 3).



Şekil 2. İki boyutlu tank deney düzeneği

Heterojen jeolojik sistemlerde depolanan yüksek doygunluktaki DNAPL karışımlarının farklı yıkama ajanlarıyla (Yüzey aktif madde (Tween80 ve SDS), kompleks şeker (MCD) ve su) ile ıslah performanslarının değerlendirilmesi 2 boyutlu tank deneyleriyle amaçlanmıştır. Yıkama deneyleri sırasında zamana bağlı örnekler alınarak TCE, PCE ve DCE analizleri GC FID ile analiz edilmiştir.

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e-ISSN: 2757-9719

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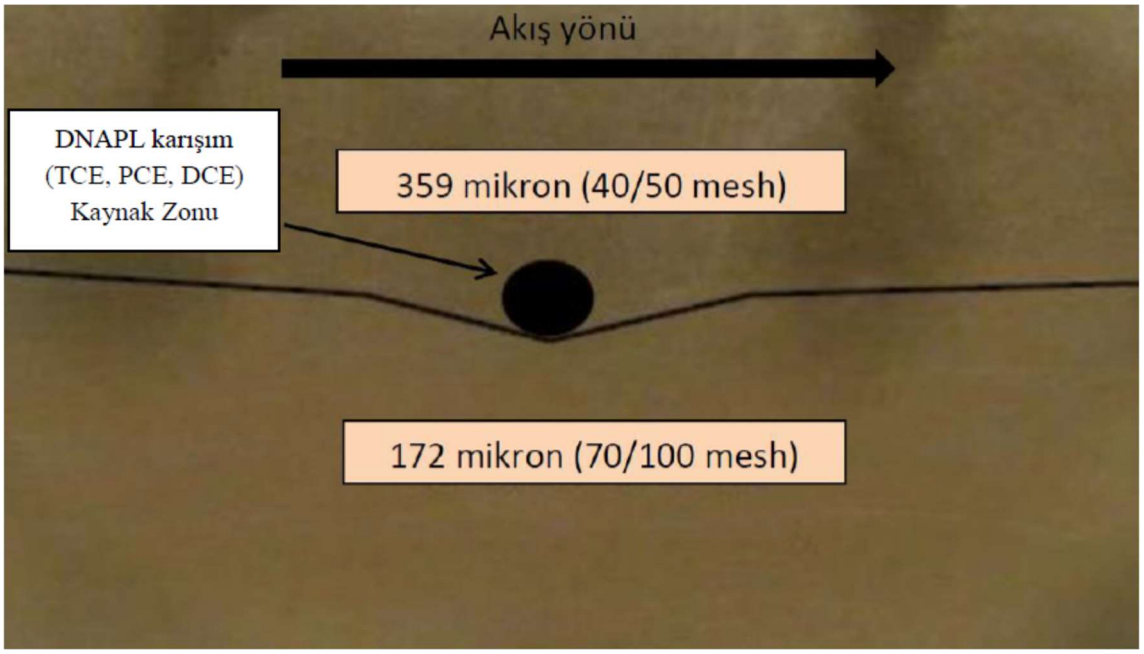

Şekil 3. Silis kumlardan oluşturulan fiziksel heterojenlik ve temsili DNAPL karışım enjeksiyonu

TCE, PCE ve DCE zamana bağlı dağılım grafikleri oluşturularak kütle balans hesaplamaları yapılmıştır. Bu hesaplamalarla kütle (TCE, PCE ve DCE) giderim değerleri elde edilmiştir. TCE, PCE ve DCE giderim yüzdeleri su ile yıkama deneyi hariç %97'nin üzerindedir. Tüm deney koşullarında DNAPL doygunluğu %0.04, gerçek hız 10 cm/saat olarak seçilmiştir.

3. SONUÇLAR ve ÖNERİLER

Klorlu bileşikler tehlikeli atık sahaların çoğunda gözlenebilmektedir. Bu tür bileşikler çoğunlukla akiferlerin tabanında (çanak), DNAPL birikintisi şeklinde depolanabilmektedir. Yeraltında bulunan suda çözünmeyen organik bileşiklerin uzun dönem kirletici kaynağı olduğu ve uygulanan saha ıslahının maliyetini ve ıslah süresini önemli derecede artırdığı bilinmektedir. Çalışma kapsamında; DNAPL karışımlarının (TCE, PCE ve DCE) heterojen özellikteki silis kumlu gözenekli ortamlarda farklı yıkama ajanlarıyla (Tween 80, SDS, MCD ve su) ıslah performansı tank deneyleriyle test edilmiştir.

Silis kumlu heterojenliğin yansıtıldığı birinci deneyde; DNAPL karışım (TCE, PCE, DCE) enjeksiyon sonrası %5 Tween 80 çözeltisi devamlı olarak tanka enjekte edilmiştir. Deney sırasında TCE, PCE ve DCE'nin zamana bağlı değişimi Şekil 4.'de görülmektedir. Tween 80 enjeksiyonun başlaması ile TCE konsantrasyonu hızlı bir şekilde artarak yaklaşık 3,3 saat sonunda en yüksek değer olan 705 mg/L'ye ulaşmıştır. Bu değer daha sonra azalmaya başlamıştır. 9,5. ve 14. saatler aralığında TCE konsantrasyonu 40-45 mg/L civarında duraylı değerlerde seyredip 21. saat sonunda 1 mg/L'nin altına düşmüştür. PCE konsantrasyonu 5,4 saat sonunda en yüksek değer olan 151 mg/L'ye ulaşmıştır. Daha sonra azalmaya başlayan bu değer 9,5. ve 14,7. saatler arasında yaklaşık 20-25 mg/L seviyeler arasında gözlenip 22. saat sonunda 1 mg/L'nin altına düşmüştür. DCE konsantrasyonu 3,3 saat sonunda en yüksek değer olan 1601 mg/L'ye ulaşmıştır. Daha sonra azalmaya başlayan bu değer 6 ve 7. saatler arasında yaklaşık 30 mg/L seviyelerinde duraylı seviyelere erişip 9,8 saat sonunda 1 mg/L'nin altına düşmüştür.

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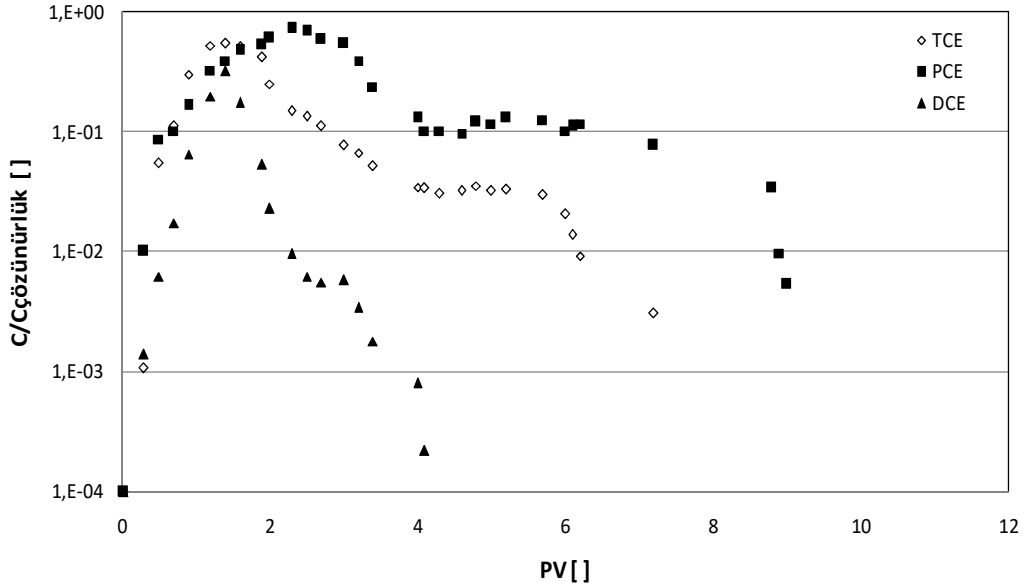
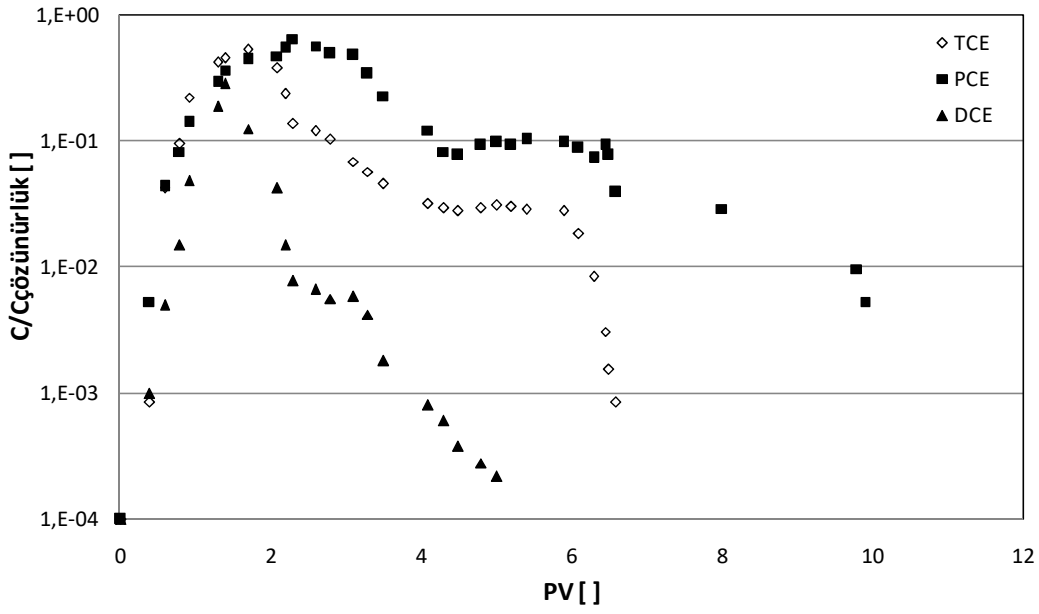


Şekil 4. Yüzey aktif maddeli (Tween80) yıkama ile TCE, PCE ve DCE'nin zamana bağlı değişimi (1PV=2,38saat)

İkinci deneyde tank %5 SDS ile yıkanmıştır (Şekil 5.) SDS enjeksiyonun başlaması ile TCE konsantrasyonu hızlı bir şekilde artarak yaklaşık 4 saat sonunda en yüksek değer olan 684 mg/L'ye ulaşmıştır. Bu değer daha sonra azalmaya başlamıştır. 9,7. ve 14. saatler aralığında TCE konsantrasyonu 35-40 mg/L civarında duraylı değerlerde seyredip 23. saat sonunda 1 mg/L'nin altına düşmüştür. PCE konsantrasyonu 5,5 saat sonunda en yüksek değer olan 133 mg/L'ye ulaşmıştır. Daha sonra azalmaya başlayan bu değer 10. ve 15. saatler arasında yaklaşık 20-25 mg/L seviyeler arasında gözlenip 24. saat sonunda 1 mg/L'nin altına düşmüştür. DCE konsantrasyonu 3,5 saat sonunda en yüksek değer olan 1410 mg/L'ye ulaşmıştır. Daha sonra azalmaya başlayan bu değer 6 ve 7. saatler arasında yaklaşık 30 mg/L seviyelerinde duraylı seviyelere erişip 11 saat sonunda 1 mg/L'nin altına düşmüştür.



Şekil 5. Yüzey aktif maddeli (SDS) yıkama ile TCE, PCE ve DCE'nin zamana bağlı değişimi.

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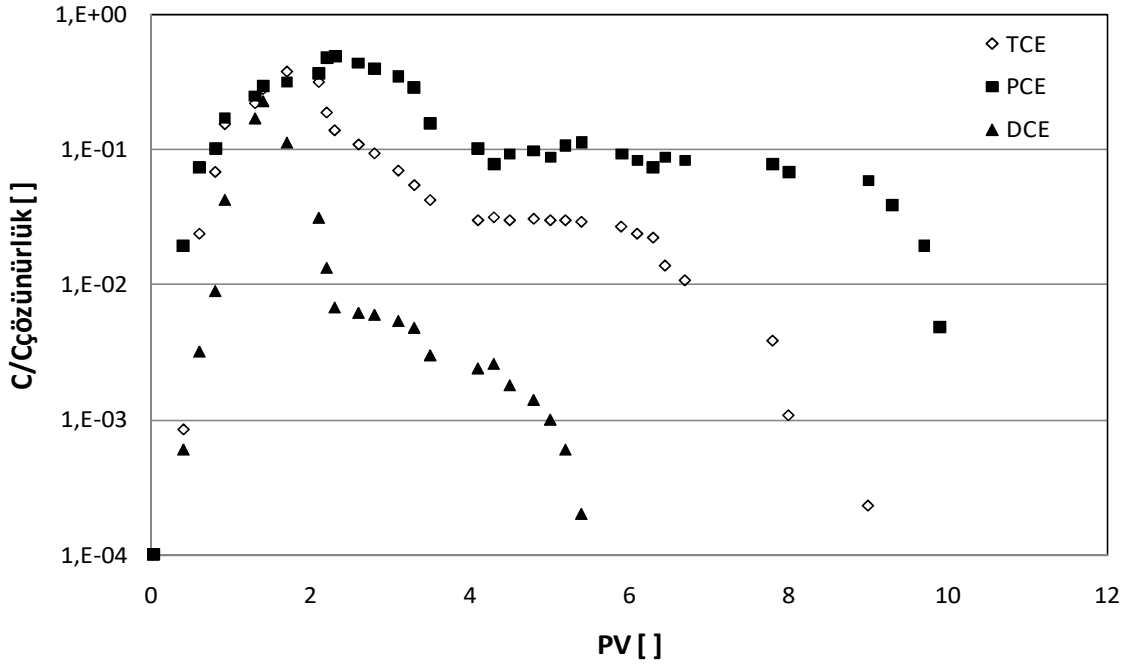
Research Article

(1 PV= 2,38 saat)

Üçüncü deneyde tank % 5 MCD ile yıkanmıştır (Şekil 6). MCD enjeksiyonunun başlaması ile TCE konsantrasyonu hızlı bir şekilde artarak yaklaşık 4 saat sonunda en yüksek değer olan 493 mg/L'ye ulaşmıştır. Bu değer daha sonra azalmaya başlamıştır. 10. ve 15. saatler aralığında TCE konsantrasyonu 30-40 mg/L civarında duraylı değerlerde seyredip 25. saat sonunda 1 mg/L'nin altına düşmüştür. PCE konsantrasyonu 5,5 saat sonunda en yüksek değer olan 101 mg/L'ye ulaşmıştır. Daha sonra azalmaya başlayan bu değer 10. ve 20. saatler arasında yaklaşık 15-20 mg/L seviyeler arasında gözlenip 26. saat sonunda 1 mg/L'nin altına düşmüştür. DCE konsantrasyonu 3,3 saat sonunda en yüksek değer olan 1150 mg/L'ye ulaşmıştır. Daha sonra azalmaya başlayan bu değer 6 ve 8. saatler arasında yaklaşık 30 mg/L seviyelerinde duraylı seviyelere erişip 13 saat sonunda 1 mg/L'nin altına düşmüştür.



Şekil 6. Kompleks şekerli (MCD) yıkama ile TCE, PCE ve DCE'nin zamana bağlı değişimi. (1 PV= 2,38 saat)

Dördüncü deneyde tank su ile yıkanmıştır. Su enjeksiyonunun başlaması ile TCE konsantrasyonu hızlı bir şekilde artarak yaklaşık 4 saat sonunda en yüksek değer olan 210 mg/L'ye ulaşmıştır (Şekil 7). Bu değer daha sonra azalmaya başlamıştır. 10. saat itibariyle TCE konsantrasyonu 30-40 mg/L civarında duraylı değerlerde seyredip 34. saate kadar aynı seviyelerde devam etmiştir. PCE konsantrasyonu 5,5 saat sonunda en yüksek değer olan 58 mg/L'ye ulaşmıştır. Daha sonra azalmaya başlayan bu değer 8. saat itibariyle 15-20 mg/L seviyeler arasında gözlenip 34. saate kadar aynı seviyelerde devam etmiştir. DCE konsantrasyonu 3,3 saat sonunda en yüksek değer olan 421 mg/L'ye ulaşmıştır. Daha sonra azalmaya başlayan bu değer 12. saat itibariyle 10-15 mg/L seviyeler arasında gözlenip 34. saate kadar aynı seviyelerde devam etmiştir.

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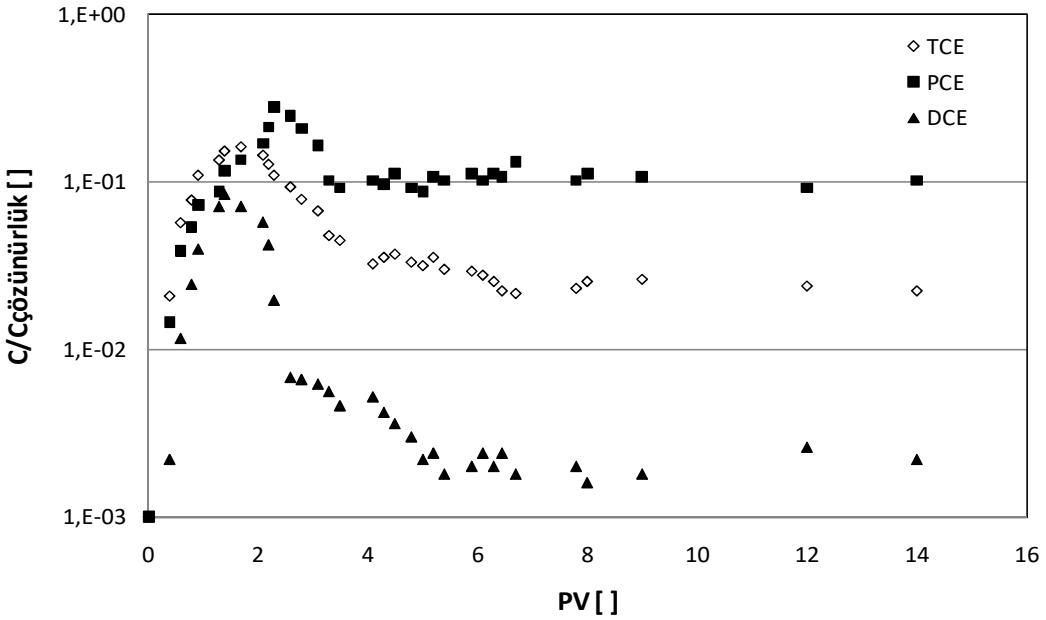

Şekil 7. Suyla yıkama ile TCE, PCE ve DCE'nin zamana bağlı değişimi. (1 PV= 2,38 saat)

Sonuç olarak, kullanılan yıkama ajanları (Tween 80, SDS, MCD) ile DNAPL karışımları ile heterojen jeolojik sistemlerdeki kaynak zonları başarılı bir şekilde ıslah edilmesine rağmen, teknolojinin başarısı sahaya özgü olup saha karakterizasyonu, tutunma, geri-bırakma, çözünme (dissolüsyon) ve oksidasyona etki eden tüm faktörler detaylı bir şekilde laboratuvar ölçeğinde araştırılmalıdır. Karakterize edilen tüm unsurların arazi ölçeğinde uygulamaya geçmeden önce belirlenmesi saha çalışmalarının planlanmasında kullanılması teknolojinin başarısını, süresini ve maliyetini olumlu yönde etkileyeceği düşünülmektedir.

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Çıkar Çatışması/Ortak Çıkar Beyanı

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

Yazarların Katkısı

Yazarlar çalışmaya eşit katkıda bulunmuştur.

Etik Kurul Onayı

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

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KAYNAKLAR

[1] Feenstra S., Cherry J. A. 1988. ‘‘Subsurface contamination by dense non-aqueous phase liquid (DNAPL) chemicals’’, The International Association of Hydrogeologists, Halifax, Nova Scotia.

[2] Mercer J. W., Cohen R. M. (1990). A review of immiscible fluids in the subsurface: Properties, models, characterization, and remediation, Journal of Contaminant Hydrology, 6, 107-163

[3] Boving T. B., Brusseau M. L. (2000). Solubilization and removal of residual trichloroethene from porous media: Comparison of several solubilization agents, Journal of Contaminant Hydrology, (42), 51-67.

[4] Tick G. R., Lourenso F., Wood A. L., Brusseau M. L. (2003). Pilot-scale demonstration of cyclodextrin as a solubility-enhancement agent for the remediation of a tetrachloroethene-contaminated aquifer, Environmental Science and Technology, 37, 5829-5834.

[5] Tick, G.R., Rincon, E.A. (2009). Effect of enhanced solubilization agents on dissolution and mass flux from uniformly distributed immiscible liquid trichloroethene (TCE) in homogeneous porous media. Water, Air, & Soil Pollution, 204, 1-4, 315-332.

[6] Difilippo E. L., Carroll K. C., Brusseau M. L. (2010). Impact of organic-liquid distribution and flow-field heterogeneity on reductions in mass flux, Journal of Contaminant Hydrology, 115, 14–25.

[7] Akyol N. H., Russo Lee A., Brusseau M. L. (2013). Impact of enhanced-flushing reagents and organic liquid distribution on mass removal and mass discharge reduction, Water, Air and Soil Pollution, 224, 1731.

[8] Javanbakht G., Goual L. (2016). Impact of Surfactant Structure on NAPL Mobilization and Solubilization in Porous Media. Industrial and Engineering Chemistry Research, 55, 11736-11746.

[9] Wu B., Li H., Du X., Zhong L., Yang B., Du P., Gu Q., Li F. (2016). Correlation between DNAPL distribution area and dissolvedconcentration in surfactant enhanced aquifer remediation effluent: A two-dimensional flow cell study, Chemosphere, 144, 2142-2149.

[10] Akyol N. H., Yolcubal I. (2013). Oxidation of Nonaqueous PHase Trichloroethylene with Permanganate in Epikarst, Water, Air and Soil Pollution, 224, 1573, 1-19.

[11] Akyol, N.H. 2018. Surfactant-enhanced permanganate oxidation on mass-flux reduction and mass removal relationship for pool-dominated TCE source zones in heterogeneous porous media, Water, Air and Soil Pollution, 229, 285. doi.org/10.1007/s11270.

[12] Akyol, N.H., Turkkan S. (2018). Effect of cyclodextrine-enhanced dissolution on mass removal and mass discharge reduction for TCE source zones in heterogeneous porous media, Water, Air and Soil Pollution, 229.

[13] Karaoglu, A.G., Copty, N.K., Akyol, N.H., Kilavuz, S.A., Babaei, M. 2019. “Experiments and sensitivity coefficients analysis for multiphase flow model calibration of enhanced DNAPL dissolution” Journal of Contaminant Hydrology 225 (2019) 103515.

[14] Naval Facilities Engineering Command (Deniz Tesisleri Mühendislik Komutanlığı)

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Elektromanyetik Radyasyon ve İnsan Sağlığına Etkileri

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ÖZET

Isaac Newton ile başladığı kabul edilen fizik ve ardından gelen diğer bilim dallarındaki gelişmeler insanlık tarihi ile kıyaslandığında çok yeni bir geçmişe sahiptir. Buna rağmen bugün ulaşılan bilimsel ve teknolojik düzey akıl almaz seviyededir. Neredeyse her geçen gün insan hayatını kolaylaştıran, zaman ve enerji kullanımı açısından inanılmaz avantajlar sağlayan yeni icatlar yapılmakta, teknolojik cihazlar kullanıma girmektedir. Yüksek gerilim hatları ve baz istasyonları ile yakın komşuluk şehir hayatının kaçınılmaz bir sonucu olmuştur. Ayrıca ev ve ofislerde kullanılan elektrikli aletler ve hepsinden önemlisi herkesin yanında taşıdığı ve kullanım sıklığı giderek artan cep telefonları artık insan hayatının çok önemli birer parçası haline gelmiştir. Hemen hepsi elektrik enerjisi ile çalışan bu cihazların en önemli dezavantajlarından birisi elektromanyetik alan oluşturmalarıdır. Tıpta tanı ve tedavide kullanılan iyonizan radyasyonun yan etkileri çok iyi bilinirken, noniyonizan bir radyasyon türü olan elektromanyetik dalgaların olası yan etkileri ile ilgili yapılmış çok sayıda çalışma olmasına rağmen halen bir sürü cevaplanmamış soru bulunmaktadır. Bu çalışmada elektromanyetik radyasyon ve insan sağlığına olan olumsuz etkileri konusunda yapılmış çalışmalar derlenmiştir.

Electromagnetic Radiation and its Effects on Human Health

ABSTRACT

Physics, which is considered to have started with Isaac Newton, and the developments in other branches of science that followed have a very short history when compared to humanity's. Still, the scientific and technological advancements reached today is at an unbelievable level. Almost every day, new inventions and technological devices are made that make human life easier and provide incredible advantages in time and energy use. Close neighborhood with high voltage lines and base stations has been an inevitable result of city life. Furthermore, electrical appliances used in homes and offices, and most importantly, mobile phones, which everyone carries with them and whose frequency of use is increasing, have become an important part of human life. One of the most important disadvantages of these devices, which all work with electrical energy, is that they create electromagnetic fields. While the side effects of diagnostic and therapeutic ionizing radiation used in medicine are well known, there are still unanswered questions despite the many studies on the possible side effects of electromagnetic waves, which is a type of non-ionizing radiation. In this paper, studies on electromagnetic radiation and its negative effects on human health have been compiled.

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1. GİRİŞ

Radyasyon, en basit tanımı ile bir kaynaktan dalga ya da parçacık halinde salınan enerjidir [1].

Karşılaştığı atomu etkileyerek çekirdek ya da yörüngeleri ile tepkimeye giren yüksek enerjili radyasyona iyonizan radyasyon adı verilir. İyonizan radyasyon parçacık ya da elektromanyetik dalga formunda olabilir. Alfa, beta ve nötron radyasyonları parçacık tipinde iyonizan radyasyon türüne örnektir. Radyasyonun bu tipinde belirgin bir kütle ve enerjiye sahip, hızla hareket eden parçacıklar söz konusudur. λ (Gama) ve X ışınları ise elektromanyetik dalga formuna sahip, kütlesiz radyasyon çeşitleridir.

Karşılaştığı atomda çekirdek ya da elektron yörüngelerinde değişikliğe neden olamayacak kadar düşük enerji seviyesine sahip radyasyon çeşidine ise noniyonizan radyasyon adı verilir ve bu tip radyasyon elektromanyetik dalga formundadır. Radyo dalgaları, kızıl ötesi dalgalar, mor ötesi ışık ve mikrodalgalar bu radyasyon tipinin en bilinen örnekleridir [2,3].

İyonizan radyasyon, atom düzeyinde oluşturduğu etki ile biyolojik sistemlerde son derece zararlı etkilere sahiptir. Radyasyonun zararına ilk maruz kalan kişi radyoaktivitenin keşfinde rol oynayan bilim insanları olup ilk yan etkilerden biri, Madam Curie tarafından saflaştırılmış radyumu cebinde taşıyan Henri Becquerel'in derisinde oluşan radyasyon yanığıydı [4]. Bu tarihten itibaren iyonizan radyasyonun kanser yapıcı etkisi başta olmak üzere zararları günümüze kadar birçok çalışma ile ortaya konmuştur.

İnsanoğlu 40 μT (mikro Tesla) değerinde bir elektromanyetik alan yayan, Dünya denen dev bir mıknatısta yaşam sürmektedir [5]. Dünya çekirdeği iki kısımdan oluşmakta olup iç çekirdek katı, dış çekirdek ise sıvı haldedir. İç çekirdeğin etrafında hareket eden dış çekirdeğin bu hareketi mıknatıslanma etkisi yaparak manyetik alan oluşturur [6]. Bunun dışında kozmik ışınlar, radon gazı, gama radyasyonu ve radyoizotoplar diğer doğal radyasyon kaynaklarıdır [7]. Özellikle toprakta ve kayalarda bulunan radon gazı, ikamet ettiğimiz ve yaşamımızın büyük kısmını geçirdiğimiz evlerimizin temellerindeki çatlaklardan içeri girerek insanla temas etmekte ve zarar vermektedir [8]. Akciğer kanserine neden olan maddeler arasında sigaradan sonra ikinci büyük neden olarak Radon gazı gösterilmektedir [9].

Doğal radyasyon kaynakları dışında insanoğlunun kendi eli ile ürettiği ve yapay radyasyon olarak adlandırılan radyasyon kaynakları da mevcuttur. En büyük yapay radyasyon kaynakları tıbbi radyasyon ve nükleer radyasyondur [10]. Radyoloji, nükleer tıp ve radyoterapi alanında yaşanan gelişmeler radyasyonun insan sağlığı açısından tanı ve tedavide daha geniş bir alanda kullanılabilmesine imkân sağlamaktadır. Bunların dışında elektrikle çalışan cihazların ürettiği elektromanyetik radyasyon gün geçtikçe artan oranda insan hayatına girmektedir [11]. Son yıllarda, elektromanyetik alanların neden olduğu sağlık riskleri açısından her geçen gün artan sıklıkta bilimsel kanıtlar bulunmaktadır [12]. Bu derlemenin amacı, elektromanyetik dalga formunu tanımak, teknolojik gelişmelerle paralel bir şekilde artan elektromanyetik kirlenmenin sağlık üzerine olan olası etkilerini incelemek ve alınabilecek önlemler hakkında bilgi sahibi olmaktır.

Elektromanyetik Radyasyon

Elektromanyetik radyasyonun keşfi, 1873 yılında James Clerk Maxwell tarafından elektromanyetizma teorisinin tanımı ve matematiksel olarak formüle edilmesi ile gerçekleşmiştir. Maxwell'in bulguları bugün halen elektromanyetik teorinin temelini oluşturmaktadır [13].

Elektromanyetik radyasyon, elektrik ve manyetik alanlardan oluşur [14]. Elektrik ve manyetik alanlar aralarında 90 derecelik bir açı bulunan, birbirine dik düzlemlerde, sinüs dalgası şeklinde hareket ederler. Işık hızında ilerleyen bu dalga hareketi ve düzlemsel değişim esnasında elektrik alan maksimum değere ulaştığında, manyetik alan minimum, elektrik alan minimum değere ulaştığında manyetik alan maksimum değerdedir [15].

Elektrik alanını oluşturan elektrik yükünün varlığıdır ve bu alanın ölçü birimi Volt/metre'dir. Elektrikle çalışan bir cihaz, örneğin saç kurutma makinesi açık olmasa bile fişi takılı ise bir elektrik alan meydana getirir. Kaynaktan uzaklaştıkça elektrik alan şiddeti azalır. Ayrıca yalıtkan nitelikli maddeler elektrik alanı engeller [16].

Elektrik yükleri yer değiştirdiğinde, yani elektrikli cihaz çalışıp bir elektrik akımı oluştuğunda manyetik alan meydana gelir. Manyetik akı yoğunluğu ölçü birimi Tesla (T), manyetik alan ölçü birimi Gauss (G)'tur (1 Tesla=10.000 Gauss). Elektrik akımı ne kadar yüksekse manyetik alan da o kadar büyük olur. Elektrik alandan farklı olarak manyetik alan, yalıtkan olsun ya da olmasın maddeler tarafından engellenemez [16].

Elektromanyetik alan, elektrik ve manyetik alanların birleşiminden oluşur. Elektromanyetik dalgalara ait tanımlayıcı özellikler dalgaların frekans ve dalga uzunluklarıdır. Frekans, 1 saniyedeki dalga sayısıdır ve Hertz (Hz) ile ölçülür. 1 Hz = 1 Sn^{-1}'dir. Yani 1 saniyede gerçekleşen 1 elektromanyetik dalganın frekansı 1 Hz'dir. Dalga uzunluğu (boyu) ise bir dalga ritminin tekrarlayan birimleri arasındaki mesafedir. λ ile gösterilmekte olup birimi uzunluk ölçü birimidir. Dalga boyu küçüldükçe frekans ve enerji artar. Yani düşük frekanslı elektromanyetik dalgalar yüksek dalga boyuna ve düşük enerjiye sahiptir (mikrodalgalar ve radyo dalgaları gibi). Yüksek frekanslı dalgalar ise düşük dalga boyuna ancak yüksek enerjiye sahiptir. Bunlar yüksek enerjili gama ve x-ışınları gibi iyonizan elektromanyetik dalgalardır [16].

Biyomanyetolojiye göre tüm maddelerin doğal bir manyetik alanı vardır [15]. Her madde ve her canlı gibi insan vücudu da, hücrelerinde ve organ sistemlerinde bulunan elektrik devreleri ile kendine has bir elektromanyetik alanlar sistemine sahiptir. Bu manyetik alanların sinyalleri hem kendi aralarında hem de Dünya manyetik alanı ile uyum içerisindedir. Ancak yapay olarak üretilen elektromanyetik alanlar biyolojik elektromanyetik dengeyi bozma riskine sahiptir [6,15,16,17].

Gündelik hayatımızda maruz kaldığımız elektromanyetik radyasyon genel hatlarıyla iki ayrı frekans bandında toplanabilir. Bunlardan birincisi çok düşük frekanslı elektromanyetik alan olarak tanımlanan elektrikle çalışan cihazlardan ve yüksek gerilim hatları-trafolardan yayılan aşırı düşük frekans(ADF) bandıdır. Genel olarak 2 kHz (kilohertz) frekans altındaki alanlar tarafından ADF bandı oluşturulur. Ev içindeki elektrik tesisatının oluşturduğu ADF frekansındaki manyetik alan gücü yaklaşık 0.05 μT olarak ölçülürken yüksek gerilim hatlarının geçtiği bölgelerde yine ev içinde yapılan manyetik alan ölçümleri 100 kat artabilmektedir. Diğer frekans bandı ise cep telefonları, baz istasyonları ve radyo-tv vericilerinden kaynaklanan radyo frekans-mikro dalga(RFMD) bandıdır. Bu dalgalar 100 kHz-300 GHz arasındaki frekans bandındadır [18].

Elektromanyetik radyasyonun canlı organizmada etki mekanizmaları

Elektromanyetik radyasyonun absorbe edildiği madde-doku üzerinde iki yolla etki gösterdiği ortaya konmuştur. Bunlardan biri termal etki, diğeri de nontermal etki olarak adlandırılan ve muhtemelen kimyasal değişikliklere bağlı olan etkidir [19]. Düşük frekanslı (ADF) dalgaların genellikle uzun süre maruziyette ve nontermal etki mekanizması ile etkinlik gösterdiği, yüksek

frekanslı dalgaların ise daha çok termal etkiyle biyolojik açıdan zararlı olabileceği saptanmıştır [20].

Kişisel olarak en fazla ilişkimiz olan elektromanyetik radyasyon kaynağı cep telefonlarıdır. Cep telefonunun mucidi ABD'li John Francis Mitchell ve Martin Cooper olarak bilinir (1973). Türkiye cep telefonu ile ilk kez 1993 yılında tanışmış olup 2G teknolojisi ülkemizde 1994 yılından itibaren kullanılmaya başlanmıştır (1991-1993 yılları arasında 1G teknolojiye sahip araç telefonları kullanılmıştır). İlk cep telefonu görüşmesi ise 23 Şubat 1994 yılında dönemin Cumhurbaşkanı Süleymen Demirel ile dönemin Başbakanı Tansu Çiller arasında yapılmıştır. O günden zamanımıza cep telefonu kullanımı hızla artmıştır.

1 Megahertz (MHz) saniyede bir milyon periyoda denk gelmekte olup analog telefonlar 800-900 MHz, dijital telefonlar 1850-1990 MHz frekanslarla çalışmaktadır [21]. 0-3000 MHz arası frekanslar çok düşük frekanslı elektromanyetik dalgaları oluşturmaktadır [22]. Evlerdeki elektrik tesisat ve güç iletim hatlarından yayılan alanlar 50 Hz civarında olup çok düşük frekanslı elektromanyetik alan sınıfına girmektedir. Yapılan araştırmalar neticesinde çok düşük frekanslı elektromanyetik alanlar ve sağlık üzerindeki etkileri ortaya konmuş, bunun sonucunda Uluslararası Kanser Araştırma Merkezi (IARC) tarafından çok düşük frekanslı manyetik alan hayvan deneylerinde olası kanserojen olarak (2B) sınıflandırılmıştır [23].

Elektromanyetik dalga canlı bir doku ile karşılaştığında doku tarafından emilir. Dokunun bu enerjiyi emme-soğurma hızı ‘’Spesifik Absorbsiyon Hızı’’ ((Spesific Absorption Rate, SAR) olarak tanımlanır. Birimi, doku başına emilen güç miktarı olup ve SI birim sisteminde W/kg (Watt/kilogram) şeklinde ifade edilir [24,25].

Elektromanyetik alana ait termal etkilerin 400 MHz – 3 GHz aralıkta gerçekleştiği ve artan moleküler hareket ve sürtünmeden dolayı oluştuğu iddia edilmiştir. 4 W/kg değerinde bir SAR değerinin 15-20 dakikada insan vücut sıcaklığını 0.2-1 °C artırdığı gösterilmiştir [26,27,28]. Bazı araştırmacılar ise ‘’Nontermal Etki’’ olarak adlandırılan etkilerin artan sıcaklığa karşı hücresel bir tepki olarak yorumlanabileceğini ileri sürmüşlerdir. Hücrelerde mevcut birkaç termoreseptör molekülü olduğu ve ısıya maruz kalma durumunda metabolik hücre stresine karşı hücreyi savunmak için aktive olarak bazı mekanizmalarla gen ekspresyonunu ve ısı şoku proteinlerini aktive ettikleri düşünülmüştür [29].

Uluslararası Noniyonize Radyasyondan Korunma Komitesi (International Commission on Non-Ionizing Radiation Protection, ICNIRP) radyasyonun olası zararlarını minimuma indirmek için bazı limit değerler belirlemiştir. Mesleği gereği elektromanyetik radyasyona maruz kalanlarda 0.4 W/kg, normal halkta 0.08 W/kg değerleri limit olarak belirlenmiştir. Dünya Sağlık Örgütü (The World Health Organization, WHO) cep telefonları için üst limit olarak 0.1 W/kg değerini belirlemiş olup bu değerin üzerinde cep telefonun kullanımından uzak durulmalıdır [30].

Şehirleşmenin, getirdiği avantajların yanında yüksek gerilim telleri, radyo-tv istasyonları ve baz istasyonları gibi elektromanyetik radyasyon kaynaklarına maruziyet açısından dezavantajları da vardır. Yine cep telefonları ve elektrikli aletler bireysel bazda risk oluşturan elektromanyetik alan kaynaklarıdır. Gelişen teknolojiyle birlikte artan elektromanyetik radyasyon maruziyeti nedeniyle elektromanyetik radyasyon alanlarının olası zararlı etkilerini ortaya koymak açısından her geçen gün artan sayıda bilimsel çalışma yapılmakta ve yayımlanmaktadır.

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Elektromanyetik Radyasyonun İnsan Sağlığı Üzerine Etkileri

Balıkçı ve arkadaşlarının yaptığı çalışmada [31] elektromanyetik alana yeterince maruz bırakılan farelerde noradrenalin ve dopamin seviyelerinde artış bulmuşlardır. Yine Koyu ve arkadaşlarının yaptığı benzer bir çalışmada [32] cep telefonlarının yaydığı 900 MHz elektromanyetik alana maruz bırakılan farelerde kortizon seviyesi belirgin miktarda yüksek bulunmuştur. Aynı çalışmada elektromanyetik alan maruziyetinin testosteron seviyesini düşürdüğü de gösterilmiştir. Bu çalışma sonucunda elektromanyetik alanın adrenal korteksi ve testisleri etkilediği sonucuna ulaşılmıştır. Her iki çalışmada da elektromanyetik alanın oluşturduğu termal etkinin primer etyolojik neden olabileceği düşünülmekle beraber elektromanyetik alanın tam olarak tanımlanamayan, stres faktörü olarak yorumlanabilecek nontermal diğer etkileri de etyolojide düşünülmüştür. Neticede elektromanyetik alanların neden olduğu hormonal değişikliklerle motor koordinasyon, duygu durum, öğrenme, hafıza, davranış gibi bazı beyin fonksiyonlarını olumsuz etkileyebileceği düşünülmüştür.

Elektromanyetik alan maruziyetinin sebep olduğu ısı artışının hipotalamik seviyede etkili nonspesifik bir stres faktörüne bağlı olduğu öne sürülmüş, kortizon veya kortikosteron seviyeleri ile ilişkisi ortaya çıkarılmıştır. Vücut sıcaklığındaki artış muhtemel iç düzenleyici mekanizmaları harekete geçirerek bu değişikliklere neden olmaktadır. Bu nedenlerle özellikle cep telefonu gibi kaynaklarda standartlar belirlenirken termal etki göz önünde bulundurulmaktadır [33,34,35].

Mercantepe ve arkadaşları yaptıkları çalışmayla [36] gebelik döneminde maruz kalınan mobil iletişim cihazlarında kullanılan 900MHz frekanslı elektromanyetik alanın, başta nöronlar ve oligodendrositlerde apoptozise neden olarak omurilik üzerinde hasara yol açtığını ortaya koymuşlardır. Manyetik alanların organizma üzerindeki biyolojik etkilerinin araştırıldığı çalışmalarda radyofrekans (RF) dalgalarına maruz bırakılan ratlarda beyin hücrelerinde DNA kırıkları görülmüştür [37]. HL-60 ve HL60R gibi laboratuvarda üretilen hücre soyları üzerinde yapılan çalışmalarda elektromanyetik alanın DNA tamir oranında azalmaya neden olduğu ortaya konmuştur [38]. Hücre proliferasyonu, hücre siklusu ve protein sentezi gibi birçok hayati hücresel fonksiyonun elektromanyetik alan etkisinde bozulabileceği gösterilmiştir [39,40].

900 MHz frekanslı elektromanyetik alanların sıçanların hipokampusunda dopamin seviyelerini azalttığı ve aynı frekansa sahip cep telefonlarının insanlarda öğrenme ve davranış fonksiyonlarını etkilediği gösterilmiştir [40,41]. Balıkçı ve arkadaşlarının yaptığı çalışmada [31] elektromanyetik alanın merkezi sinir sisteminde noradrenalin ve dopamin seviyelerini değiştirerek etkili olabileceği gösterilmiştir. Salford ve arkadaşlarının çalışması [42] sonucunda cep telefonu kullananlarda elektromanyetik alan etkisine bağlı motor koordinasyon, duygu durum ve öğrenme gibi beyin fonksiyonlarının etkilenebileceği gösterilmiştir. Yine Salford ve arkadaşlarının yaptıkları araştırmalarda [42,43] mikrodalga radyasyonun sıçan beyni üzerindeki etkileri incelenmiş ve kan-beyin bariyerinde albümin kaçağına dair bulgular tespit edilmiştir.

Elektromanyetik alan ve çocukluk çağı lösemisi arasındaki ilişkiyi araştıran bir araştırmada [44] 0,4 μT ve üzerindeki bir seviyede elektromanyetik alan maruziyetinde lösemi riskinin 2 kat arttığı gösterilmiştir. Başka bir çalışmada [45] 0,4 μT'den büyük değerlerde elektromanyetik alana gece maruziyetinde 4 yaş altı çocuklarda lösemi riskinin 14.9 kat arttığı iddia edilmiştir. Yine lösemi odağında ancak farklı bir bakış açısı ile yapılmış bir çalışmada [46] lösemi tanısı olan çocuklar incelenmiş ve 0,3 μT ve üzerinde bir değerde gerçekleşen elektromanyetik alan maruziyetinde sağ kalımda azalma tespit edilmiştir. Güney Kore'de bir çalışmada [47], AM

(Amplitude Modulation) radyo yansıtıcılarına 2 km ve daha yakında yaşayan 0-14 yaş grubu çocuklarda lösemi açısından artmış anlamlı risk gösterilmiştir.

Winker ve arkadaşlarının yaptığı çalışmada [48] hücre kültürlerine uygulanan çok düşük frekanslı elektromanyetik alanın kromozom yapısında bozulmalara neden olabileceği gösterilmiştir. Yine benzer hücre kültürlerinde çalışan Wolf ve arkadaşları [49] 24-72 saatlik 0,5-1μT elektromanyetik alan uygulamasının hücre proliferasyon aşamasında ve DNA'da hasar oluşturduğunu göstermişlerdir. Bu tespit, erken gebelik döneminde özellikle kromozomal anomali riski açısından elektromanyetik radyasyon kaynaklarına karşı dikkatli olunması gerektiğini göstermektedir.

Normal popülasyonla karşılaştırıldığında elektrik alan maruziyetinin daha fazla sağlık sorununa neden olduğu kişilerde, elektromanyetik alana karşı bir aşırı duyarlılıktan-hipersensitiviteden söz edilebilir. Bu aşırı duyarlılık özel bir durum olup sıklığının %5 olduğu iddia edilmiştir. En sık karşılaşılan şikayetler ciltte kızarıklık ve yanma gibi dermatolojik bulgular ve yorgunluk, konsantrasyon güçlüğü, baş dönmesi, bulantı, çarpıntı, sindirim sistemi sorunları gibi vejetatif semptomlardır [50].

2008 yılında düşük frekanslı elektromanyetik dalgaların dişi Spague-Dawley fareleri cinsiyet hormonları üzerindeki etkileri incelenmiş olup, gonadotropin düzeylerinde ve over volümlerinde azalma tespit edilmiştir. Bu etkilerin geri dönüşümlü olduğu gösterilmiştir [51]. Agarwal ve arkadaşlarının yaptığı çalışmada [52] cep telefonu kullanım süresi arttıkça, sperm kalitesinin azaldığını belirtmiştir. Yine benzer bir çalışmada [53] elektromanyetik alan maruziyetinde fare spermlerinde anormal kromatin yapısında artış saptanmıştır. Türkiye'de Atatürk Üniversitesi'nde yapılan bir çalışmada [54]; 60 gün boyunca, günde 20 dk arama (23 saat 40 dakika bekleme) durumunda cep telefonu kaynaklı 900 MHz elektromanyetik alana maruz kalan (217 Hz, 2W maksimum pik güç, SAR 0.95 W/kg) domuzlarda sperm sayısında anlamlı düzeyde azalma olduğu ortaya konmuştur.

1997-2004 yılları arasında Amerika, İsveç, Danimarka, Finlandiya, İngiltere, Almanya ve Japonya'da yapılmış olan çalışmaların incelemesinde [55] 10 yıllık cep telefonu kullanımı ile ipsilateral gliom ve akustik nörinom riskinin arttığı gösterilmiştir. Yine İsveç'te yapılan bir başka çalışmada [56] en az 10 yıllık cep telefonu kullanımının akustik nörinom riskini arttırdığı belirtilmiştir. İsviçre'de yapılan bir çalışmada [57], elektromanyetik alana maruz kalan demiryolu işçilerinde beyin tümörü görülme oranının 5.1 kat arttığı gösterilmiştir. Yine Kanada'da yapılan bir çalışmada [58] elektromanyetik alana maruz kalan çalışanlarda glioblastome multiforme görülme oranı 5.3 kat artmış olarak bulunmuştur.

Elektromanyetik alan maruziyeti sonucunda hücresel düzeyde serbest oksijen radikallerinin arttığı ve bu yolla elektromanyetik alanın oksidatif strese neden olduğu gösterilmiştir [59]. Oksidatif stres hücre membranlarında bozulmaya ve ilerleyici hücre hasarı, en sonunda hücre ölümüne yol açabilir [60]. Ayrıca oksijen radikalleri doğrudan DNA hasarına neden olabilir [61]. Yapılan çalışmalarda elektromanyetik alana maruz bırakılan sıçanlarda germ hücrelerinde ve hepatositlerde apoptotik hücre ölümleri görülmüştür [62,63].

Hamileliği sürecinde elektromanyetik alana maruz kalan annelerin bebeklerinde uyku düzensizlikleri izlenmiştir [64]. 2012 yılında, düşük yapan kadınların incelendiği bir çalışmada [65] 14 haftadan önce düşük yapanlarda, 14 haftadan sonra düşük yapanlara kıyasla evlerindeki elektromanyetik alanlar-EMF bantlarında büyük farklılıklar saptanmıştır. Başka bir çalışmada [66]; daha yüksek elektromanyetik alana maruz kalan gebelerde düşük elektromanyetik alan maruziyeti ile kıyaslandığında düşük riski 2.72 kat daha yüksek çıkmıştır.

Mortazavi ve arkadaşlarının yaptığı bir çalışmada [67] telsiz telefon kullanımı ile konsantrasyon bozukluğu arasında anlamlı bir ilişki bulunmuş, yine Mortazavi ekibine ait diğer bir çalışmada [68] ilk ve ortaokul öğrencilerinde cep telefonu kullanımının baş ağrısı, vertigo ve uyku sorunlarına neden olduğu gösterilmiştir. Kucer ve arkadaşları [69], günlük kullanım miktarlarına göre cep telefonu ve bilgisayar kullanıcılarında daha sık oranda baş ağrısı, eklem-kemik ağrısı, işitme kaybı, vertigo ve anksiyete şikayetlerinin görüldüğünü ortaya koymuştur. Ayrıca aynı çalışmada semptomların kadınlarda erkeklere göre daha fazla olduğu söylenmiştir.

2. SONUÇ

İnsan vücudu, hücreler ve organ sistemleri arasında iletişimde elektrik kullanan, elektrik yüklü bir sistemdir. Bir ortamda elektrik akımı var ise o ortamın kendine has elektrik ve manyetik alanları vardır. İçinde yaşadığımız ortamda bulunan gerek doğal gerekse yapay elektromanyetik alanlar biyolojik sistemimizde bulunan elektromanyetik alanlarla etkileşime girerek normal insan fizyolojisini bozma riskine sahiptir. Özellikle bebekler ve çocuklarda sinir-üreme sistemine ait hücrelerde elektromanyetik alan duyarlılığı daha fazladır.

Teknolojik gelişmelerin getirdiği elektromanyetik kirlilik ve artan elektromanyetik alan maruziyeti, beraberinde getirdiği sağlık riskleri sebebiyle son dönemde dikkat çeken bir konudur. Özellikle çocuk yaş grubunun elektromanyetik alanla ilişkisi kontrol edilmeli ve yine özellikle gece elektromanyetik alan maruziyetinden kaçınılmalıdır.

Elektromanyetik alan ve zararlı etkilerinden korunmak için toplumsal ve bireysel bazda uygulanabilecek bazı önlemler insan sağlığını korumak açısından yararlı olacaktır.

Toplumsal-Kurumsal olarak yapılabilecekler;

1. Elektromanyetik alan hakkında bilgilendirme, ölçüm ve değerlendirme konularında hizmet verecek üniversiteler ya da kamu kuruluşlarına ait bağımsız birimler oluşturulmalıdır.
2. Yerleşim yerlerinde, yüksek gerilim hatları, baz istasyonları ve bina içlerinde bulunan elektrik hattı kaynaklı elektromanyetik alanlar ölçülerek gerekli bilgilendirme yapılıp önlemler alınmalıdır.
3. Yeni yerleşim alanları oluştururken elektromanyetik alan oluşumu ve insan maruziyeti açısından en uygun önlemler alınarak gerekli mühendislik ve mimarlık eğitim-çalışmaları yapılmalıdır.
4. Yerleşim alanı olmasa dahi çocukların bulunma ihtimali olan park alanları ve yakınlarından yüksek gerilim hattı geçmemelidir.
5. Elektromanyetik alan etkileri konusunda bilimsel çalışmalara ve kamu spotlarına gereken önem verilmelidir.
6. Baz istasyonları, okul, hastane ve toplu yaşam merkezlerinde sık ölçümler yapılmalıdır.
7. Tüm haberleşme cihazlarının üzerine SAR değerleri yazılmalıdır.

Bireysel olarak yapılabilecekler;

1. Özellikle geceleri elektromanyetik alana olan maruziyeti azaltmak için mümkünse yatak odasında elektrikle çalışan tüm cihazlar uyku öncesinde kapatılmalı, bu mümkün değilse lamba dahil en az 2 metre uzak mesafede bulunmalıdır. Elektromanyetik dalgaların uyku düzenini bozmasına izin verilmemelidir.
2. Cep telefonu alırken SAR değeri düşük olanlar tercih edilmelidir.

3. Telefonla 10 dakikadan fazla görüşme yapılmamalı, konuşurken telefon vücuttan uzakta tutulmalıdır. 0.9 metre uzak tutulan bir telefondan yayılan elektromanyetik dalga 50 kat daha düşüktür [70].
4. Tüm cep telefonu görüşmelerinde kulaklık kullanılmalıdır. Mümkünse tek kulaklık tercih edilmelidir.
5. Cep telefonlarını taşırken kalp hizasında taşımamaya dikkat edilmelidir.
6. Gereksiz hiçbir elektrikli aletin çalışmasına izin verilmemeli veya elektrikle bağlantısı kesilmelidir.
7. Elektrikli masaj yatakları veya elektrikli battaniyeler çalışır durumda uzun süre kullanılmamalıdır.
8. Çocuk beynine elektromanyetik dalgaların girişi daha kolaydır. Bu dalganın etkileri çocuklarda çok daha etkin hissedilir. Cep telefonları çocukların bulunduğu odanın dışında tutulmalıdır.

Başta cep telefonu ve evde kullanılan elektrikli aletler olmak üzere elektromanyetik radyasyon kaynaklarının insan hayatından çıkarılması pek mümkün görünmemekle beraber, özellikle çocuk yaş grubundakiler başta olmak üzere maruziyeti azaltan önlemler ile risklerde azalma sağlanabilir.

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Çıkar Çatışması/Ortak Çıkar Beyanı:

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

Yazarların Katkısı:

İlk yazar %60, ikinci yazar 20%, üçüncü yazar %20 katkıda bulunmuştur.

Etik Kurul Onayı:

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

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KAYNAKLAR

[1] D. Bor, "Radyasyon nedir? (Halkımız için bilgilendirme kılavuzu)," Ankara üniversitesi Mühendislik Fakültesi Fizik Mühendisliği Bölümü, pp. 3, 2015, access address: http://www.radkorder.org/wp-content/uploads/2016/09/RADYASYON-NED%C4%B0R.pdf

[2] Y.E. Togay, "Radyasyon ve Biz," Türkiye Atom Enerjisi Kurumu Yayınları, pp. 2-12, 2002.

[3] M.Z. İnce, "Tanısal Radyolojide Radyasyondan Korunma," Türkiye Atom Enerjisi Kurumu Yayınları, pp. 2-32, 2002.

[4] M. Sekiya ve M. Yamasaki, "Antoine Henri Becquerel (1852–1908): A scientist who endeavored to discover natural radioactivity." Radiol Phys Technol, vol. 8, pp.1–3, 2015.

[5] O. Çerezci, Z. Kartal, K. Pala ve A. Türkkan, "Elektromanyetik Alan ve Sağlık Etkileri," A. Türkkan, Ed., Bursa: F. Özsan Matbaacılık, 2012.

[6] H.H. Sabuncu, "Elektromanyetik Radyasyonlarla veya Elektromanyetik Alanlarda Çalışanların Sağlık Riskleri", Türk Tabipleri Birliği Mesleki Sağlık ve Güvenlik Dergisi, Nurol Matbaacılık, Ankara, 2000.

[7] F.D. Gökharman, S. Aydın ve P.N. Koşar, "Radyasyon güvenliğinde mesleki olarak bilmemiz gerekenler," SDÜ Sağlık Bilimleri Derg., vol. 7, no. 2, pp. 35–40, 2016

[8] T. Hacıosmanoğlu, "Doğal ve yapay radyasyon kaynakları, kişisel doza katkıları," Nucl Med Semin., vol. 3, pp. 166–71, 2017.

[9] H. Halilçolar, D. Tatar, G. Ertuğrul, A. Çakan, M.G. Acıtaş ve B. Kömürcüoğlu, "Epidemiyoloji," In: T. Çavdar, N. Ekim, A. Akkoçlu, C. Öztürk, Eds., "Akciğer kanseri multidisipliner yaklaşım," 1.Baskı. Ankara: Bilimsel Tıp Yayınevi, pp. 17-22, 1999.

[10] Ö. Coşkun, "İyonize Radyasyonun Biyolojik Etkileri," Int J Tech Sci., vol. 1, no. 2, pp. 13–7, 2011.

[11] B.F. Wall, "Ionising radiation exposure of the population of the United States: NCRP report no. 160," Radiat Prot Dosim., vol 136, pp. 136–138, 2009.

[12] L. Hardell ve C.Sage, "Biological effects from electromagnetic field exposure and public exposure standards," Biomedi Pharmacother, vol. 62, pp. 104-109, 2008.

[13] Mevzuat İşleri Daire Başkanlığı, Elektromanyetik alanlara ilişkin genel bilgiler serisi, 2020, Access address: https://www.csgb.gov.tr/media/61322/elektromanyetik-alanlara-iliskin-genel-bilgiler_seri-1.pdf

[14] Bilgi Teknolojileri ve İletişim Kurumu (BTK), 2011, "Elektronik Haberleşme Cihazlarından Kaynaklanan Elektromanyetik Alan Şiddetinin Uluslararası Standartlara Göre Maruziyet Limit Değerlerinin Belirlenmesi, Kontrolü ve Denetimi Hakkında Yönetmelik," Başbakanlık Mevzuatı Geliştirme ve Yayın Genel Müdürlüğü, Resmî Gazete, Vol. 27912, 21 April 2011.

[15] E. Önal, "Elektromanyetik Alanların Canlı Organizmalara Etkilerinin İncelenmesi," İnönü Üniversitesi Elektrik–Elektronik Mühendisliği Anabilim Dalı Fen Bilimleri Enstitüsü Yüksek Lisans Tezi, Malatya, 2005.

[16] TMMOB Elektronik mühendisleri odası, "Elektromanyetik alanın etkileri," access address: https://kitap.emo.org.tr/genel/kitap_goster.php?kodu=105

[17] Okul Sağlığı Güvenliği Kurulu, "Elektromanyetik Kirlilik Nedir? Kirliliği Oluşturan Elektromanyetik Alanları Neler Yaratır?", 2006.

[18] O. Çerezci ve Ş.Ç. Yener, "Çevremizdeki Elektromanyetik Kirlilik ve Sağlığımıza Etkileri," ISEM2016, 3rd International Symposium on Environment and Morality, Alanya, Türkiye, 4-6 November 2016

[19] K.J. Rothman, "Epidemiological evidence on health risks of cellular telephones," Lancet, vol. 356, pp. 25, 2000.

[20] Ş. Özen, "Mikrodalga frekanslı EM radyasyona maruz kalan biyolojik dokularda oluşan ısıl etkinin teorik ve deneysel incelenmesi," Doktora Tezi, Sakarya Üniversitesi Fen Bilimleri Enstitüsü Elektronik ve Haberleşme Y Müh., Adapazarı, Türkiye, 2003.

[21] M.K. Irmak, E. Fadillioglu, M. Guleç, H. Erdogan, M. Yagmurca ve O. Akyol, "Effects of electromagnetic radiation from a cellular telephone on oxidant and antioxidant levels in rabbits," Cell Biochim Funct, vol. 20, pp. 279-283, 2002.

[22] S. Şeker ve O. Çerezci, "Radyasyon Kuşatması," Boğaziçi Üniversitesi Yayınevi, İstanbul, 2000.

[23] International Agency for Research on Cancer (IARC), "IARC monographs on the evaluation of carcinogenic risk to humans," vol. 80, 2002.

[24] D. Elhasoğlu, "Elektromanyetik Kirliliğin Zararlı Etkileri," Yüksek Lisans Tezi, Çukurova Üniversitesi Fen Bilimleri Enstitüsü, Adana, 2006.

[25] R.W. Habash, J.M. Elwood, D. Krewski, W.G. Lotz, J.P. McNamee ve F.S. Prato, "Recent advances in research on radiofrequency fields and health," 2004-2007, J Toxicol Environ Health B Crit Rev, vol. 12, pp. 250-288, 2009.

[26] A. Christ, T. Samaras ve E. Neufeld E, "SAR distribution in human beings when using body–worn RF transmitters," Radiat Prot. Dosimetry, vol. 124, no. 1, pp. 6–14, 2007.

[27] S. Dasdag, M.A. Ketani ve Z. Akdağ, "Whole-body microwave exposure emitted by cellular phones and testicular function of rats," Urol Res., Vol. 27, no. 3, pp. 219-223, 1999.

[28] P. Paredi, S.A. Kharitonov ve T. Hanazawa, "Local vasodilator response to mobile phones Laryngoscope," vol. 111, no. 1, pp. 159-162, 2001.

[29] R. Glaser, "Are thermoreceptors responsible for "non-thermal" effects of RF fields?," Edition Wissenschaft (Bonn, Germany: Forschungsgemeinschaft Funk), no. 21, 2005.

[30] WHO, "THE INTERNATIONAL EMF PROJECT PROGRESS REPORT 1999-2000," erişim adresi: http://www.who.int/peh-emf/publications/reports/en/Progress_report99-00.pdf

[31] K. Balıkçı, "Sıçanlarda in vivo olarak elektromanyetik alana maruz kalmanın beyin dokularındaki katekolaminerjik nörotransmitter düzeylerine etkisi," e-Journal of New World Sciences Academy, vol. 11, no. 3, pp. 1-8, 2016.

[32] A. Koyu, "Cep telefonlarından yayılan 900 MHz elektromanyetik alanın serum kortizol ve testosteron hormonu üzerine etkisi," S.D.Ü. Tıp Fakültesi Dergisi, vol. 12, no. 1, pp. 52-56, 2005.

[33] W.G. Lotz ve S.M. Michaelson, "Temperature and corticosterone releationship in microwaves exposed rats," J Appl Physiol, vol. 44, pp. 438-445, 1978.

[34] W.G. Lotz ve R.P. Podgorski, "Temperature and adrenocortical responses in rhesus monkeys exposed to microwaves," J Appl Physiol, vol. 53, pp. 1565-1571, 1982.

[35] S.T. Lu, N. Lebda, S. Pettit ve S.M. Michaelson, "Microwaveinduced temperature corticosterone and thyrotropin interrelationships," J Appl Physiol, vol. 50, pp. 399-405, 1981.

[36] T. Mercantepe, "Prenatal Dönemde 900Mhz Elektromanyetik Alanın Sıçan Omurilik Dokusu Üzerine Etkilerinin Histopatolojik Olarak İncelenmesi," ACU Sağlık Bil Derg, vol. 9, no. 2, pp. 137-141, 2018.

[37] H. Lai ve N.P. Singh, "Melatonin and a spin-trap compound block radiofrequency electromagnetic radiation-induced DNA strand breaks in rat brain cells," Bioelectromagnetics, vol. 18, pp. 446-454, 1997.

[38] J.G. Robison, A.R. Pendieton, K.O. Monson, B.K. Murray ve K.L. O-Neill, "Decreased DNA repair rates and protection from heat induced apoptosis mediated by electromagnetic field exposure," Bioelectromagnetics, vol. 23, no. 2, pp. 106-112, 2002.

[39] Z.E. Gagnon, C. Newkirk, J.A. Conetta, M.A. Sama ve S. Sisselman, "Teratogenic effect of broad band electromagnetic field on neonatal mice," J Environ Sci Health part A Tox Hazard Subst Environ Eng., vol. 38, no. 11, pp. 2465-2481, 2003.

[40] K. Maaroufi, L. Had-Aissouni, C. Melon, M. Sakly, H. Abdelmelek, B. Poucet ve E. Save, "Spatial Learning, Monoamines and Oxidative Stress in Rats Exposed to 900 MHz Electromagnetic Field in Combination with Iron Overload," Behavioural Brain Research, vol. 258, pp. 80-89, 2014.

[41] C.C. Papageorgiou, E.D. Nanou, V.G. Tsiafakis, E. Kapareliotis, K.A. Kontoangelos, C.N. Capsalis, A.D. Rabavilas ve C.R. Soldatos, "Acute Mobile Phone Effects on Pre-Attentive Operation. Neuroscience Letter," vol. 397, no. 1-2, pp. 99-103, 2006.

[42] L.G. Salford, A.E. Brun, J.L. Eberhardt, L. Malmgren ve B.R.R. Persson, "Nerve Cell Damage in Mammalian Brain after Exposure to Microwaves from GSM Mobile Phones," Environmental Health Perspectives (United States: National Institute of Environmental Health Sciences), vol. 111, no. 7, pp. 881–883, 2003.

[43] L.G. Salford, H. Nittby, A. Brun, G. Grafstrom, L. Malmgren, M. Sommarin, J. Eberhardt, B. Widegren, B.R.R. Persson, "The Mammalian Brain in the Electromagnetic Fields Designed by Man with Special Reference to Blood-Brain Barrier Function, Neuronal Damage and Possible Physical Mechanisms," Progress of Theoretical Physics Supplement (Japan: Physical Society of Japan), vol. 173, pp. 283–309, 2008.

[44] A. Ahlbom, N. Day, M. Feychting, E. Roman, J. Skinner ve J. Dockerty, "A pooled analysis of magnetic fields and childhood leukaemia," Br J Cancer, vol. 83, pp. 692-698, 2000.

[45] J. Schüz, J.P. Grigat, K. Brinkmann ve J. Michaelis, "Residential magnetic fields as a risk factor for childhood acute leukaemia: results from a German population-based case-control study," Int J Cancer, vol. 91, pp. 728-35, 2001.

[46] D.E. Foliart, B.H. Pollock, G. Mezei, R. Iriye, J.M. Silva ve K.L. Ebi, "Magnetic field exposure and long-term survival among children with leukaemia," Br J Cancer, vol. 1, pp. 161-4, 2006.

[47] M. Ha, H. Im, M. Lee, H.J. Kim, B.C. Kim ve Y.M. Gimm, "Radio-frequency radiation exposure from AM radio transmitters and childhood leukemia and brain cancer," Am J Epidemiol, vol. 166, pp. 270-9, 2007.

[48] R. Winker, S. Ivancsits, A. Pilger, F. Adlkofer ve H.W. Rudiger, "Chrosomal damage in human dip loid fibroblasts by intermittent exposure to extremely low-frequency electromagnetic fields," Mutation Research, vol. 585, pp. 43-49, 2005.

[49] F. Wolf, A. Torsello, B. Tedesco, S. Fasanella, A. Boninsegna, M. D'Ascenzo, C. Grassi, G.B. Azzena ve A. Cittadini, "50-Hz extremely low frequency electromagnetic fields enhance cell

proliferation and DNA damage: possible involvement of a redox mechanism," Biochim Biophys Acta, vol. 1743, pp. 120-129, 2005.

[50] WHO, "Fact Sheet 296, Electromagnetic fields and public health," 2005. Access adress: https://www.who.int/peh-emf/publications/facts/EHS_Factsheet_296_English.pdf.

[51] S. Lönn, A. Ahlbom, P. Hall ve M. Feychting, "Mobile phone use and the risk of acoustic neuroma. Epidemiology," vol. 15, pp. 653-659, 2004.

[52] M.A. Al-Akhras, "Influence of 50 Hz magnetic field on sex hormones and body,uterine, and ovarian weights of adult female rats," Electromagn Biol Med., vol. 27, no. 2, pp. 155-163, 2008.

[53] A. Agarwal, F. Deepinder, K.S. Rakesh, G. Ranga ve L. Jianbo, "Effect of cell phone usage on semen analysis in men attending infertility clinic: An observational study," Fertility and Sterility., vol. 89, no. 1, pp. 124-128, 2008.

[54] A.U. Barış, K.K. Dide, G. Fetih, D. Yeter ve U. Ömer, "Effect of Electromagnetic Wave Emitted from Mobile Phone on Some Reproductive Parameters in Adult Male Guinea Pigs," Atatürk Üniversitesi Vet. Bil. Derg., vol. 7, no. 2, pp. 77-84, 2012.

[55] N. Schreier, A. Huss ve M. Röösli, "The prevalence of symptoms attributed to electromagnetic field exposure: a cross-sectional representative survey in Switzerland," Soz Praventiv Med., vol. 51, pp. 202-209, 2006.

[56] L. Hardell, M. Carlberg ve K. Hansson Mild, "Pooled analysis of two casecontrol studies on use of cellular and cordless telephones and the risk for malignant brain tumours diagnosed in 1997-2003," Int Arch Occup Environ Health, vol. 79, pp. 630-639, 2006.

[57] C.E. Minder, D.H. Pfluger, "Leukemia, brain tumors, and exposure to extremely low frequency electromagnetic fields in Swiss railway employees," Am J Epidemiol, vol. 153, pp. 825-835, 2001.

[58] P.J. Villeneuve, D.A. Agnew, K.C. Johnson ve Y. Mao, "Canadian Cancer Registries Epidemiology Research Group. 2002. Brain cancer and occupational exposure to magnetic fields among men: results from a Canadian population-based case-control study," Int J Epidemiol, vol. 31, pp. 210-217, 2002.

[59] E.G. Kıvrak, K.K. Yurt, A.A. Kaplan, I. Alkan ve G. Altun, "Effects of electromagnetic fields exposure on the antioxidant defense system," Journal of Microscopy and Ultrastructure, vol. 5, no. 4, pp. 167–176, 2017.

[60] S.P. LeDoux, N.M. Druzhyna, S.B. Hollensworth, J.F. Harrison ve G.L. Wilson, "Mitochondrial DNA Repair: A Critical Player in the Response of Cells of the CNS to Genotoxic Insults," Neuroscience, vol. 145, no. 4, pp. 1249–1259, 2007.

[61] E. Kaufman ve I.B. Lamster, "The Diagnostik Applications of Saliva— A Review," Sage Journals., vol. 13, no. 2, pp. 197-212, 2002.

[62] Ş. Açıkgöz, "Deneysel Düşük Doz Elektromanyetik Alanın Sıçan Testis ve Semen Dokularına Etkileri ve Kafeik Asit Fenetil Esterin Antioksidan Rolünün Araştırılması," Uzmanlık Tezi, Sakarya Üniversitesi Tıp Fakültesi, Adapazarı, Türkiye, 2019.

[63] E. Şahin, D. Güzel, Ş. Açıkgöz ve N. Tufan, "Effects of Acute and Chronic Exposure to 900 MHz Electromagnetic Field on the Rat Liver Microarchitecture," Proceedings, vol. 2, no. 25, pp. 1585, 2018. Access address: https://www.mdpi.com/2504-3900/2/25/1585

[64] N. Çöl, Ö. Kömürcü-Karuserci ve C. Demirel, "The possible effects of maternal electronic media device usage during pregnancy on children's sleep patterns," Turkish Archives of Pediatrics, vol. 56, no. 3, pp. 254-260, 2021.

[65] M.F. Shamsi, S. Ziaei, M. Firoozabadi ve A. Kazemnejad, "Exposure to extremely low frequency electromagnetic fields during pregnancy and the risk of spontaneous abortion: a case-control study," J Res Health Sci, vol. 13, no. 2, pp. 131-134, 2013.

[66] D-K. Li, H. Chen J.R. Ferber, R. Odouli ve C. Quesenberry, "Exposure to magnetic field non-ionizing radiation and the risk of miscarriage: A prospective cohort study," Scientific reports., vol. 7, no. 1, pp. 1-7, 2017.

[67] S.M. Mortazavi, J. Ahmadi ve M. Shariati, "Prevalence of subjective poor health symptoms associated with exposure to electromagnetic fields among university students," Bioelectromagnetics, vol. 28, pp. 326-330, 2007.

[68] S.M. Mortazavi, M. Atefi ve F. Kholghi, "The pattern of mobile phone use and prevalence of self-reported symptoms in elementary and junior high school students in Shiraz, Iran," Iran J Med Sci., vol. 36, pp. 96-103, 2011.

[69] N. Kucer ve T. Pamukcu, "Self-reported symptoms associated with exposure to electromagnetic fields: a questionnaire study," Electromagn Biol Med., vol. 33, pp. 15-17, 2014.

[70] TUBİTAK-BİLTEN, "Elektromanyetik Dalgalar ve İnsan Sağlığı, Sıkça Sorulan Sorular ve Yanıtları," 2001, Access address: https://www.fmo.org.tr/wp-content/uploads/2010/03/baz_istasyonlari.

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The Relationship Between Oxidative Stress and Selenium

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ABSTRACT

Oxidative stress is characterized by an imbalance between prooxidant molecules in cells and tissues and antioxidant molecules that try to detoxify these reactives molecules. Oxidative stress is affected by both genetic and environmental factors. Selenium (Se) is an essential trace element and inadequacy can lead to various types of diseases, such as cancer and heart disease. Foods are the main source of Se, and it is commonly found in seafood, grains, meat products, milk, vegetables and fruits. Se is an enzyme with antioxidant properties and is included in the structure of seleno enzymes. In this article, the metabolism and antioxidant properties of se are reviewed.

Oksidatif Stres ve Selenyum Arasındaki İlişki

ÖZET

Oksidatif stres, hücrelerdeki ve dokulardaki prooksidan moleküller ile bu reaktif molekülleri detoksifiye etmeye çalışan antioksidan moleküller arasındaki dengesizlik ile karakterize edilir. Oksidatif stres hem genetik hem de çevresel faktörlerden etkilenir. Selenyum esansiyel bir eser elementtir ve yetersizliği kanser ve kalp hastalığı gibi çeşitli hastalıklara yol açabilir. Selenyumun ana kaynağı besinlerdir ve genellikle deniz ürünleri, tahıllar, et ürünleri, süt, sebze ve meyvelerde bulunur. Selenyum antioksidan özelliklere sahip bir enzimdir ve seleno enzimlerinin yapısında yer alır. Bu yazıda selenyumun metabolizma ve antioksidan özellikleri gözden geçirilmiştir.

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1. INTRODUCTION

As an essential trace element, humans absorb selenium (Se) mainly through cereals and meat. According to the World Health Organization (WHO), the value for daily intake of Se value for adults is 50-70 micrograms/day for both men and women [1, 2]. Se plays an important physiological role in thyroid hormone metabolism, immunity, and antioxidant protection. Se deficiency has been reported to be effective in various types of diseases, including cardiovascular disease and cancer [3]. Se is involved in the structure of the enzyme glutathione peroxidase enzyme (GPx). This enzyme is a very important component of the antioxidant defense system of the organism, as it protects lipids, proteins and nucleic acids in the cell structure from oxidative damage [4]. This article deals with the metabolism of Se and its antioxidant role in human health and disease.

Selenium

Se is found in group 16 of the periodic table and is an essential element necessary for human and animal organisms [5]. Depending on dietary habits, the total Se intake comes from meat and fish as well as cereals. Se is widely used in photocells in industry, glass and ceramic photography, rubber industry, steel alloying, Se rectifiers, electronics industry, laser printers and photocopiers [6].

Se compounds are absorbed in the duodenum in humans. Se binds to plasma proteins and lipoproteins (Very low-density lipoprotein (VLDL), low-density lipoprotein (LDL)) in the blood. When Se is taken to meet normal needs, the organs where it is collected in the highest concentration are the liver, kidney, testes, and thyroid. Skeletal muscle accounts for most (40-50%) of the Se pool in the body [7]. The uptake, metabolism and distribution of Se in the body are shown in Figure 1. [8].

In the evaluation of daily Se intake compared with blood Se levels determined in the Turkish population, daily Se intake was calculated to be 43 µg/Lday on average [9]. Se is included in the structure of the enzyme GPx, which is an important member of the antioxidant defense mechanism. Se exerts its biological effects through selenoproteins, which mainly contain the amino acid selenocysteine. Selenocysteine, which is present in the structure of some enzymes, is formed when one of the sulfur atoms in the amino acid cysteine is replaced by a Se atom. The antioxidant properties of selenoproteins help prevent cell damage caused by free radicals. Selenocysteines are anionic at biological pH, thus enabling biological redox reactions by electron exchange [10]. Se is generally found in tissues in two forms: Selenocysteine and selenomethionine. Selenocysteine: it is the biologically active form of Se that forms the active part of enzymes that participate in oxidation and reduction reactions in prokaryotic and eukaryotic life. Selenomethionine cannot be synthesized in the body and is absorbed through the diet. Selenomethionine is believed to be a structure that stores the element Se. When the absorption of this element in the body is blocked, it acts as a source of Se for the organism. Selenocysteine is a Se-containing analog of cysteine, which is defined as the twenty-first amino acid encoded by DNA. Se-containing amino acids, selenomethionine, are derived from plant

sources, while selenocysteine is derived from animal sources. Se is an essential element with antioxidant and anticarcinogenic properties [11, 12]. As a member of the selenoprotein family, Se has structural and enzymatic functions. The best known of its enzymatic functions is its antioxidant and catalytic role in the production of active thyroid hormone. Se acts as an oxidation-reduction center and contributes to the control of intracellular oxidation-reduction. Selenoproteins are protein structures that contain selenocysteine residues. Se deficiency is thought to cause clinical findings that are due to a disturbance in the formation of selenoproteins. At least seven isoenzymes of GPx have been identified [10, 13, 14].

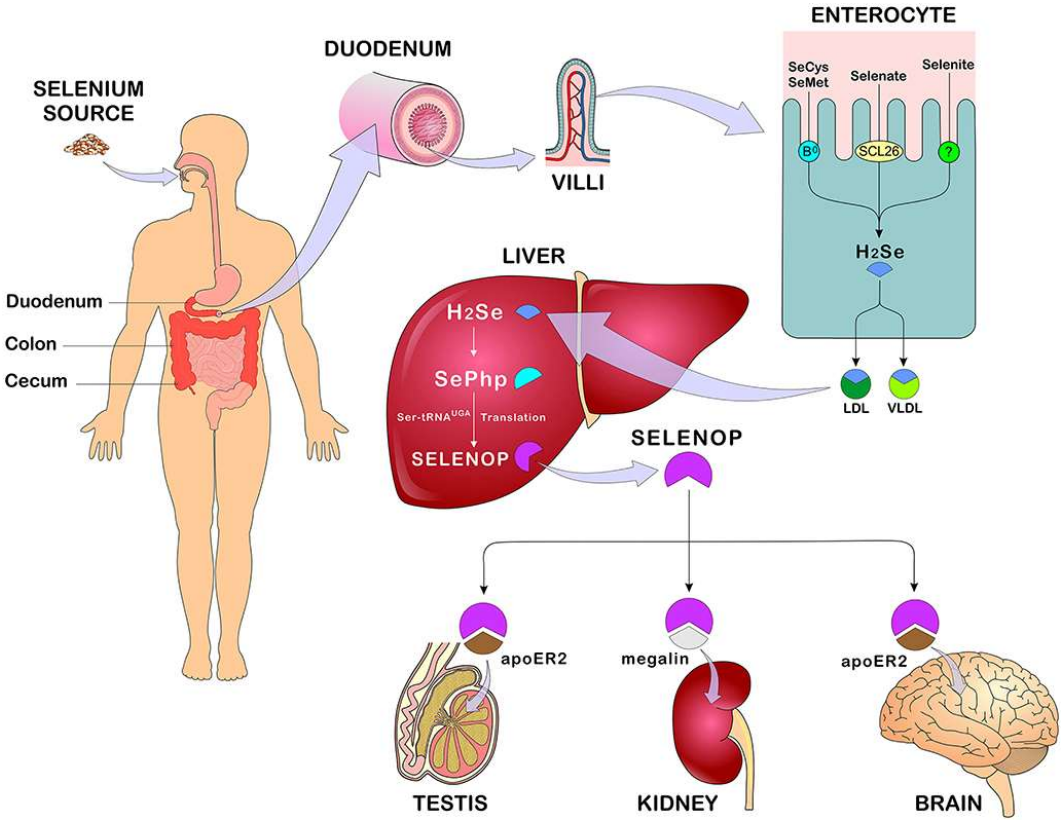

Figure 1. Se absorption, metabolism, and distribution [8]

GPx1 is the most abundant form and is found in almost all mammalian tissues. It catalyzes the reaction of conversion of hydrogen peroxide to water. Thus, it protects the organism from oxidative damage. GPx1 is an antioxidant in the cytoplasm of cells and acts as a se store. When the structure of glutathione peroxidase was studied, it was recognized that it consists of a tetrameric selenoprotein containing a selenocysteine residue. The most important functional part in the structure is the selenocysteine end, and the forms that do not contain selenocysteine are nonfunctional. Although each GPx is a different selenoprotein, they are all antioxidant enzymes that reduce potentially harmful reactive oxygen species such as hydrogen peroxide and oil hydroperoxides by oxidizing glutathione to harmless products such as water and alcohol [15].

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Oxidative stress

Oxidative stress occurs when reactive oxygen species (ROS) is formed in cells that damage or kill cells. Metal ions react with superoxide anions and hydrogen peroxide to produce highly reactive species such as free hydroxyl radicals and metal-oxygen complexes in biological systems that lead to oxidative damage. Oxidative stress occurs when there is an imbalance between the production of ROS and its detoxification (Figure 2.) [16-18].

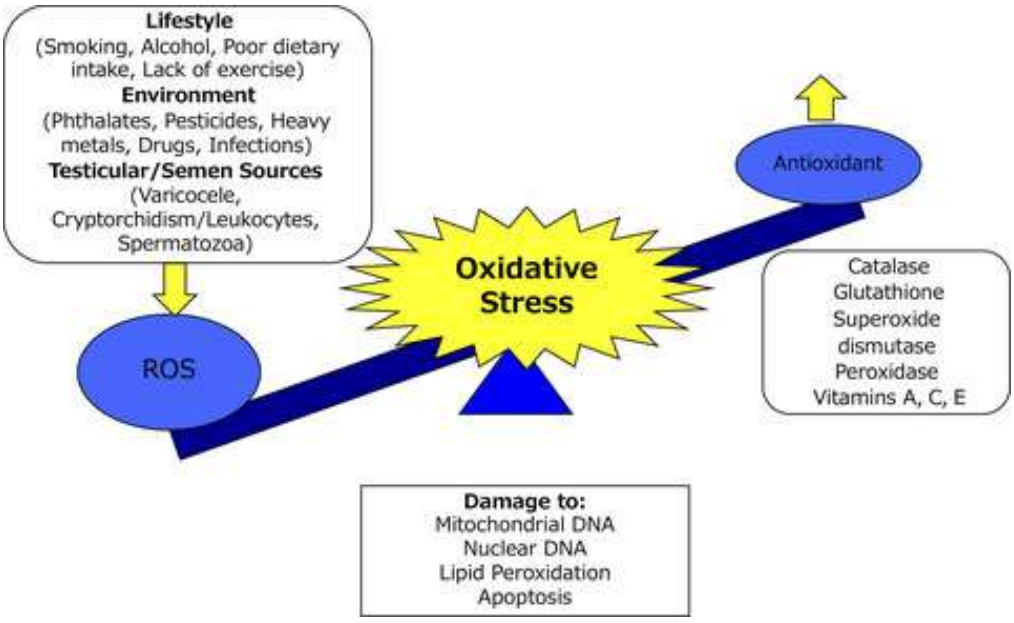

Figure 2. Oxidative stress [18]

All tissues and cells contain systems to detoxify biologically reactive intermediates and prevent or reduce cellular damage from oxidative stress. Biological antioxidants are natural molecules that protect the cell from the uncontrolled formation of free radicals and ROS or prevent them from reacting with biological structures [19]. ROS are superoxide radicals, hydrogen peroxide, and hydroxyl radicals, which are formed in small amounts during normal oxygen metabolism. ROS can initiate radical chain reactions in which various free radicals are formed and cause the formation of various free radicals such as carbon-centred organic radicals, peroxide radicals, alkoxy radicals, and sulfenyl radicals in the cell. ROS and oxygen-free radicals are often unstable and highly reactive. Radicals such as superoxide anion radical, hydroxyl radical, peroxyl radical, nitric oxide, organic peroxide radical are divided into nonradicals such as hydrogen peroxide, lipid hydroperoxide, singlet oxygen, ozone, nitrogen dioxide, and hypochlorous acid. Three major reactive oxygen species, the superoxide radical, hydrogen peroxide, and hydroxyl radical, are normal metabolites continuously produced by mitochondria in growing cells [20, 21].

Free radicals are defined as molecules with one or more unpaired electrons, short-lived, unstable, of low molecular weight and very active. They are formed by the removal of an electron from a nonradical atom or molecule or by the addition of an electron to an atom or

molecule. The radicals formed are not very reactive and are stable. They act as reducing or oxidizing agents in the body because they can donate electrons to or accept electrons from other molecules. Oxidative stress occurs when the balance between free radicals and the antioxidant defense system shifts in favor of the free radicals [22]. Free radicals have different chemical structures such as hydroxyl, superoxide, nitric oxide, and lipid peroxide radicals. The most important free radicals in biological systems are those formed from oxygen. For example, the superoxide radical, which is extremely active and damages many cells, is converted to hydrogen peroxide by superoxide dismutase. Hydrogen peroxide, which is much less effective than superoxide, is rendered harmless by being converted to water and oxygen by enzymes such as catalase and peroxidase in tissues [23]. Free radicals formed in the organism are of endogenous and exogenous origin. Endogenously, mitochondrial electron transport chain (ETZ) in mammals, oxidative reactions in phagocytic and endothelial cells, redox cycles and arachidonic acid metabolism are formed by the action of enzymes such as xanthine oxidase and nicotinamide adenine dinucleotide phosphate (NADPH) oxidase. Exogenous sources include industrial pollutants, drugs, diet, ionizing radiation, ultraviolet light and cigarette smoke. [16, 22, 24].

In the organism, there are protective mechanisms against the harmful effects of free radicals. Some of these mechanisms prevent the formation of free radicals, and others prevent the harmful effects of the formed free radicals. All substances that perform these functions are called antioxidants. Antioxidants are substances that protect cells both directly and indirectly from the adverse effects of xenobiotics, drugs, carcinogens, and toxic radical reactions. Antioxidants act in four different ways. Chain-breaking action: it stops the chemical reactions that generate free radicals, binds the oxygen free radicals to itself, breaks their chains and prevents their function. Suppressive action: it reduces the reaction rate, interacts with the oxygen free radicals and transfers a hydrogen to them, reducing their activity or converting them to an inactive form. Vitamins have this effect. Restorative action; They regenerate the biological molecular damage that occurs in structures such as lipid, protein and DNA. Enzymatic action; they show their action by increasing the synthesis of antioxidant enzymes such as SOD and non-enzymatic antioxidants in the organism.Antioxidants are substances that oxidize at low concentrations and can reduce (with electron transfer) or prevent the oxidation of another substrate, i.e. fight oxidation. Antioxidants can be of endogenous or exogenous origin [19, 22].

Selenium and oxidative stress

Oxidative stress results from the production of endogenous and exogenous ROS and an imbalance between the production of ROS and antioxidant defenses. Oxidative stress plays a role in the development mechanism of many diseases such as cardiovascular disease, acute and chronic kidney disease (CKD), diabetes, hypertension, chronic obstructive pulmonary disease (COPD), neurodegenerative diseases (NDs), biliary disease and cancer. Se is found in the structure of selenoprotein that exhibit antioxidant, anti-inflammatory, and antiviral effects [25, 26].

It has been shown in animal studies that Se supplementation increases plasma GSH levels and decreases malondialdehyde (MDA) levels, and as a biomarker of oxidative stress [26-28]. Some studies reported that Se supplementation could not affect plasma GSH and total antioxidant capacity (TAC) levels in a short time (3 weeks) [29]. Se is one of the essential components of the GPx enzyme, its task is to protect the cell from free radicals. [3, 11, 26]. Se supplementation suppresses the NF-kappa B pathway with selenoproteins. Se uptake may play a role in reducing oxidative stress through free radical inhibition by being involved in the GPx structure. [1, 8, 12].

2. CONCLUSION

Research on Se over the past few years has produced a wealth of evidence showing the important role that Se and its metabolites play in human disease. In particular, our knowledge of the functional roles of the GPx and TrxR groups as essential antioxidant selenoenzymes in protecting cells from oxidative stress has greatly increased. Until specific biomarkers that directly associate Se with disease prevention and treatment are identified, its use as a supplement in health care should be taken with caution. Se increases the levels of antioxidant biomarkers against free radicals, decreases the levels of oxidative markers and thus prevents oxidative stress. Se, oxidative stress and antioxidant status can be studied in oxidative-related diseases.

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REFERENCES

[1] J. Gao, Y. Liu, Y. Huang et al., "Daily selenium intake in a moderate selenium deficiency area of Suzhou, China," Food Chemistry, vol. 126, no. 3, pp. 1088-1093, 2011.

[2] I. Institute of Medicine Subcommittee on, I. Uses of Dietary Reference, and I. Institute of Medicine Standing Committee on the Scientific Evaluation of Dietary Reference, DRI Dietary Reference Intakes: Applications in Dietary Assessment, Washington (DC): National Academies Press (US) Copyright 2000 by the National Academy of Sciences. All rights reserved., 2000.

[3] D. Kang, J. Lee, C. Wu et al., "The role of selenium metabolism and selenoproteins in cartilage homeostasis and arthropathies," Experimental & Molecular Medicine, vol. 52, 2020.

[4] U. Tinggi, "Selenium: its role as antioxidant in human health," Environmental health and preventive medicine, vol. 13, no. 2, pp. 102-108, 2008.

[5] H. Zeng, and G. F. Combs, Jr., "Selenium as an anticancer nutrient: roles in cell proliferation and tumor cell invasion," J Nutr Biochem, vol. 19, no. 1, pp. 1-7, 2008.

[6] D. G. Barceloux, and D. Barceloux, "Selenium," Journal of Toxicology: Clinical Toxicology, vol. 37, no. 2, pp. 145-172, 1999.

[7] Y. Saito, "Selenium Transport Mechanism via Selenoprotein P—Its Physiological Role and Related Diseases," vol. 8, 2021.

[8] R. L. U. Ferreira, K. C. M. Sena-Evangelista, E. P. de Azevedo et al., "Selenium in Human Health and Gut Microflora: Bioavailability of Selenocompounds and Relationship With Diseases," vol. 8, 2021.

[9] B. Giray, and F. Hincal, "Oxidative DNA base damage, antioxidant enzyme activities and selenium status in highly iodine-deficient goitrous children," Free Radic Res, vol. 36, no. 1, pp. 55-62, 2002.

[10] M. P. Rayman, "The importance of selenium to human health," Lancet, vol. 356, no. 9225, pp. 233-41, 2000.

[11] P. Brenneisen, H. Steinbrenner, and H. Sies, "Selenium, oxidative stress, and health aspects," Mol Aspects Med, vol. 26, no. 4-5, pp. 256-67, 2005.

[12] S. R. Stapleton, "Introduction: The selenium conundrum," Cellular and Molecular Life Sciences, vol. 57, no. 13, pp. 1823-1824, 2000.

[13] S. P. Stawicki, M. Lyons, M. Aloupis et al., "Current evidence from phase III clinical trials of selenium supplementation in critically Ill patients: why should we bother?," Mini Rev Med Chem, vol. 7, no. 7, pp. 693-9, 2007.

[14] A. Utomo, X. Jiang, S. Furuta et al., "Identification of a novel putative non-selenocysteine containing phospholipid hydroperoxide glutathione peroxidase (NPGPx) essential for alleviating oxidative stress generated from polyunsaturated fatty acids in breast cancer cells," J Biol Chem, vol. 279, no. 42, pp. 43522-9, 2004.

[15] K. M. Brown, and J. R. Arthur, "Selenium, selenoproteins and human health: a review," Public Health Nutr, vol. 4, no. 2b, pp. 593-9, 2001.

[16] G. J. Burton, and E. Jauniaux, "Oxidative stress," Best practice & research. Clinical obstetrics & gynaecology, vol. 25, no. 3, pp. 287-299, 2011.

[17] G. Pizzino, N. Irrera, M. Cucinotta et al., "Oxidative Stress: Harms and Benefits for Human Health," Oxidative medicine and cellular longevity, vol. 2017, pp. 8416763-8416763, 2017.

[18] T. Takeshima, K. Usui, K. Mori et al., "Oxidative stress and male infertility," Reproductive Medicine and Biology, vol. 20, no. 1, pp. 41-52, 2021.
[19] B. L. Tan, M. E. Norhaizan, W.-P.-P. Liew et al., "Antioxidant and Oxidative Stress: A Mutual Interplay in Age-Related Diseases," Frontiers in pharmacology, vol. 9, pp. 1162-1162, 2018.
[20] C. Migdal, and M. Serres, "[Reactive oxygen species and oxidative stress]," Med Sci (Paris), vol. 27, no. 4, pp. 405-12, 2011.
[21] M. Schieber, and N. S. Chandel, "ROS function in redox signaling and oxidative stress," Current biology : CB, vol. 24, no. 10, pp. R453-R462, 2014.
[22] L. A. Pham-Huy, H. He, and C. Pham-Huy, "Free radicals, antioxidants in disease and health," International journal of biomedical science : IJBS, vol. 4, no. 2, pp. 89-96, 2008.
[23] A. Phaniendra, D. B. Jestadi, and L. Periyasamy, "Free radicals: properties, sources, targets, and their implication in various diseases," Indian journal of clinical biochemistry : IJCB, vol. 30, no. 1, pp. 11-26, 2015.
[24] G. Pizzino, N. Irrera, M. Cucinotta et al., "Oxidative Stress: Harms and Benefits for Human Health," Oxidative Medicine and Cellular Longevity, vol. 2017, pp. 8416763, 2017.
[25] I. Liguori, G. Russo, F. Curcio et al., "Oxidative stress, aging, and diseases," Clinical interventions in aging, vol. 13, pp. 757-772, 2018.
[26] N. Zakeri, M. R. kelishadi, O. Asbaghi et al., "Selenium supplementation and oxidative stress: A review," PharmaNutrition, vol. 17, pp. 100263, 2021.
[27] M. Akil, M. Bicer, E. Menevse et al., "Selenium supplementation prevents lipid peroxidation caused by arduous exercise in rat brain tissue," Bratisl Lek Listy, vol. 112, no. 6, pp. 314-7, 2011.
[28] K. S. Ibrahim, Z. A. Saleh, A. R. Farrag et al., "Protective effects of zinc and selenium against benzene toxicity in rats," Toxicol Ind Health, vol. 27, no. 6, pp. 537-45, 2011.
[29] L. A. Savory, C. J. Kerr, P. Whiting et al., "Selenium supplementation and exercise: effect on oxidant stress in overweight adults," Obesity (Silver Spring), vol. 20, no. 4, pp. 794-801, 2012.

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Research Article

The effects of Potassium Chloride and Calcium Sulphate on Egg Production and Heart Rate in *Daphnia magna* (Crustacea: Cladocera)

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ABSTRACT

Daphnia magna is capable of rapid reproduction in suitable environmental conditions and temperature (15-22 °C). Additionally, reproduction of water fleas under laboratory conditions is quite simple and cheap. Moreover, daphnids are commonly used in laboratory experiments. Therefore, the effects of potassium chloride (KCl) and calcium sulphate ($CaSO_4$) on heart rate and egg production in *D. magna* were assessed in this study. Daphnids of treatment groups were exposed to distinct concentrations of KCl and $CaSO_4$ (37.5, 75, 150 mg/L). Ten individuals were used in each group. All experiments were made in triplicate. Heart rate variability was examined for 96 h and the number of heartbeats was calculated by using a video camera. The number of eggs per individual in each experimental group was recorded for 21 days. An increase in both heart rate and egg production due to exposure to $CaSO_4$ was generally observed, depending on the concentration gradients. However, KCl caused a decrease in the egg production of medium and high-dose groups. Furthermore, although an increase in heart rate was examined for two successive days after exposure to KCl, a decrease in the heart rate of medium and high-dose groups was observed during the last 48 h. When $CaSO_4$ treatment groups compared with the control group, it can be concluded that $CaSO_4$ contributes positively to egg production. When it comes to KCl, it can be concluded that KCl negatively affects egg production in the medium and high dose groups.

Potasyum Klorür ve Kalsiyum Sülfatın *Daphnia magna* (Crustacea: Cladocera) Türünde Yumurta Üretimi ve Kalp Hızı Üzerine Etkileri

ÖZET

Daphnia magna uygun çevre koşullarında ve sıcaklıkta (15-22 °C) hızlı üreme yeteneğine sahiptir. Ayrıca su pirelerinin laboratuvar koşullarında üremesi oldukça basit ve ucuzdur. Dahası su pireleri laboratuvar deneylerinde yaygın olarak kullanılmaktadır. Bu nedenle bu çalışmada potasyum klorür (KCl) ve kalsiyum sülfatın ($CaSO_4$) *D. magna* bireylerinde kalp hızı ve yumurta üretimi üzerine etkileri değerlendirildi. Uygulama gruplarındaki su pireleri KCl ve $CaSO_4$'ün farklı konsantrasyonlarına (37.5, 75, 150 mg/L) maruz bırakıldı. Her grupta on birey kullanıldı. Tüm deneyler üç tekrar halinde yapıldı. 96 saat boyunca kalp hızı değişkenliği incelendi ve video kamera kullanılarak kalp atım sayısı hesaplandı. Her deney grubundaki birey başına düşen yumurta sayısı 21 gün boyunca kaydedildi. $CaSO_4$'e maruz kalma nedeniyle hem kalp hızında hem de yumurta üretiminde konsantrasyon derecelerine bağlı olarak genellikle bir artış gözlendi. Ancak KCl orta ve yüksek doz gruplarında yumurta veriminde azalmaya neden oldu. Ayrıca KCl'ye maruz kaldıktan sonra art arda iki gün boyunca kalp hızında bir artış incelenmiş olmasına rağmen, orta ve yüksek doz gruplarında kalp hızında son 48 saat içinde bir azalma gözlendi. $CaSO_4$ uygulanan gruplar kontrol grubu ile karşılaştırıldığında $CaSO_4$'ün yumurta üretimine olumlu katkı sağladığı sonucuna varılabilir. KCl'ye gelince, KCl'nin orta ve yüksek doz gruplarında yumurta üretimini olumsuz etkilediği sonucuna varılabilir.

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1. INTRODUCTION

Daphnia is a genus of small planktonic crustaceans which is characterized by flattened leaf legs used as the filtering apparatus. Daphnids feed on small, suspended particles which is usually made up of planktonic algae in water. They are the most widely used live food items particularly during the development of some fish species (perch, freshwater bream, catfish, carp, sturgeon etc.) in fish farming [1, 2]. Therefore, water fleas are important members of the food chain in freshwater. *Daphnia magna* is an extensively used model organism in ecotoxicological research because of its common distribution, short life cycle, rapid maturation, and reproduction [3, 4].

As most of the other species in the genus *Daphnia*, *D. magna* reproduces by parthenogenesis. In a typical growth season, sexual reproduction in life cycle of *Daphnia* is also observed. Dormant encapsulated eggs (ephippia) produced by sexual reproduction are usually strongly melanized and contain two large eggs. Ephippium with only one or no egg is not uncommon. Ephippium is triggered by conditions announcing or associated with an unfavorable environment [5, 6]. *Daphnia* is a keystone genus in freshwater and environmental conditions effect offspring sex and whether they reproduce asexually or sexually [7]. Chemical exposure effects not only hatching of dormant eggs, but also *D. magna* hatchling survival and movements [6].

There are two types of circulatory system in animals which are the open circulatory system and the closed circulatory system. Heart is an important component of circulatory system because its muscular contraction provides to move fluid (hemolymph or blood) for distribution of respiratory gases and nutrients. There are two types of animal hearts which are neurogenic and myogenic hearts [8, 9]. Initiation of heartbeat in neurogenic heart is under nervous control. However, initiation of heartbeat in myogenic heart is under muscular control [10, 11].

There is diversity in heart types of Crustacea. This taxon includes species with neurogenic hearts or myogenic hearts. *D. magna* has myogenic heart. Therefore, the heart of *D. magna* and other ancestral crustaceans such as *Triops longicaudatus* bear structural resemblance to the hearts of vertebrate animals. Additionally, *Daphnia* may represent a model system for understanding the myogenic hearts of vertebrates [9]. Moreover, changes in *D. magna* heartbeat have been reported to be an indicator for toxicity evaluation [12, 13]. Therefore, heart rate may be a sensitive physiological indicator that can reflect differential concentration effects of chemical compounds to *D. magna* circulatory system.

Calcium sulphate ($CaSO_4$) is a naturally occurring mineral used in various industrial applications. It has the longest history not only in industrial usage but also in clinical usage. Moreover, to date, many clinical *in vivo* and *in vitro* studies have been conducted. Potassium is one of the three most important minerals which is used to produce the electrical charge to function properly of the body cell. Because the cells produce electrical charges through electrolytes (eg, sodium and potassium). Additionally, KCl helps regulation of heartbeat and blood pressure, contributes nerve and muscle activities, water balance. However, high dose potassium causes a decrease in blood pressure and cardiac arrhythmia [14, 15].

In the present study, the effects of mineral salts constituting water hardness on egg production and heart rate of *D. magna* were analyzed. We evaluated the changes of these features after

exposure to different concentrations of KCl and $CaSO_4$. This species was chosen because of its size and the heart can be easily observed by optical methodologies. Additionally, a 21-day standard reproductive test was carried out to evaluate the effects of KCl and $CaSO_4$ on egg production of *D. magna*.

2. MATERIAL and METHODS

The experiment was carried out with *D. magna* which we have cultured in the laboratory for more than three years. Daphnids were maintained in aerated fresh water at temperature of 20-22 °C with a 14 h light and 10 h dark cycle. Stock solutions were prepared by dissolving potassium chloride (KCl) and calcium sulfate ($CaSO_4$) in fresh water. Three different concentrations of KCl and $CaSO_4$(37.5, 75, 150 mg/L) were diluted from the stock solution. These concentrations were determined based on literature data [16, 17]. The culture medium was renewed twice a week. Daphnids were fed with a mixture of *Spirulina* microalgae and TetraPond fish food once a day. Each beaker contained 60 mL solution and ten daphnids. All experimental samples were made in triplicate. Ten adults (older than 14 d) were used for heart rate analysis. Each daphnid was transferred on a slide with a drop of the culture medium and examined by light microscopy. Heartbeats of each daphnid were recorded in slow motion by a video camera for 10 seconds. Changes in heart rate of *D. magna* were examined for 96 h. In the second part, a 21-day reproductive test was performed with neonates (<24 h). The number of eggs per individual in each experimental group was noted. Data were presented as mean with standard deviation. The differences were compared for statistical significance by one-way ANOVA with post hoc analysis using Tukey test. Statistical evaluations were carried out via PASW statistics (SPSS) 18 software. We set the significance level at $p<0.05$.

3. RESULTS

The effects of KCl and $CaSO_4$ on heart rate and egg production of *D. magna* were analyzed in the current study. Heart contractions were visually counted by slowing down the speed of video playback. The number of eggs per individual in each experimental group (including control, low, medium, and high-dose groups) was also calculated for 21 days. The heart of *D. magna* is located dorsally and anterior from the brood chamber (Figure 1). As a result of the exposure to $CaSO_4$, an increase in heart rate depend on concentration gradients (37.5, 75, 150 mg/L) was generally examined. Heart rate changes were statistically significant in all treatments groups compared to control except for medium-dose group data at 72 and 96 h. Although exposure to different concentration of KCl (37.5, 75, 150 mg/L), caused increasing of heart rate for two consecutive days, a decrease in the heart rate of medium and high-dose groups was observed during the last 48 h. Although a decrease in the heart rate of medium and high-dose groups was observed during the last 48 h, the heart rate of medium group at 96 h was not lower than the control group. When the control group was compared to the treatment groups, it was statistically significant except for the data related to the heart rate of the medium-dose group at 96 h. The mean of heart rate (mean ± s.d.) for each group was presented in Tables 1 and 2.

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Figure 1. Adult *Daphnia magna* of control group. The spherical heart is located on the dorsum of animal and in front of the brood chamber where the eggs are carried. There are two eggs in the brood chamber.

Table 1. The mean and standard deviation (mean ± s.d.) of *Dapnia magna* heart rate in the control group and treatment groups with different concentrations of calcium sulfate ($CaSO_4$) were presented.

Experimental Groups	24 h	48 h	72 h	96 h
Control	392.4±28.49	403.2±12.20	394.2±22.04	399±50.52
37.5 mg/L	401.4±54.26	393±38.06	400.8±72.43	406.2±14.96
75 mg/L	432±32.01	424.2±18.18	418.2±16.08	420±20.07
150 mg/L	441±35.44	437.4±15.74	435±23.43	445.2±43.13

Table 2. The mean and standard deviation (mean ± s.d.) of *Dapnia magna* heart rate in the control group and treatment groups with different concentrations of potassium chloride (KCl) were presented.

Experimental Groups	24 h	48 h	72 h	96 h
Control	370.8±13.36	374.4±21.66	391.2±18.74	387.6±36.51
37.5 mg/L	414.6±9.79	422±15.36	423.6±17.23	424.2±27.42
75 mg/L	444±7.74	444.6±23.12	420.6±28.22	384.6±44.32
150 mg/L	471.6±22.41	480.6±25.53	451.2±17.46	364.2±22.77

Figure 2. Adult *Daphnia magna* in the medium-dose group of KCl bearing melanized eggs known as Ephippium. A closer microscopic photograph of the ephippium with two eggs on the left side.

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The eggs were observed in the brood chamber located dorsally beneath the carapace of *D. magna*. The embryos hatched from the eggs after about 1 day at 20-22 °C but they remained in the brood chamber for further development. After 2-3 days, the young individuals were released by the mother daphnid from the brood chamber. Although the newborn looked more or less like the adult daphnid, the brood chamber was not developed in newborn individuals. The first eggs were examined into the brood chamber of nearly 9-10-day-old female water fleas and daphnids lived for 35-40 days in laboratory conditions. *Daphnia* produces a different type of egg for resting. The resting eggs were enveloped with a chitinous melanized structure called ephippium (Figure 2). Resting eggs were uncommon in our experimental groups and they were rarely observed in only one or two individuals in medium-dose concentration of KCl. After exposure to $CaSO_4$, an increase in egg production depend on concentration gradients was observed. However, exposure to KCl gave rise to a decrease in egg production of the medium-and high-dose groups. Our values were presented by graphs (Figures 3, 4).

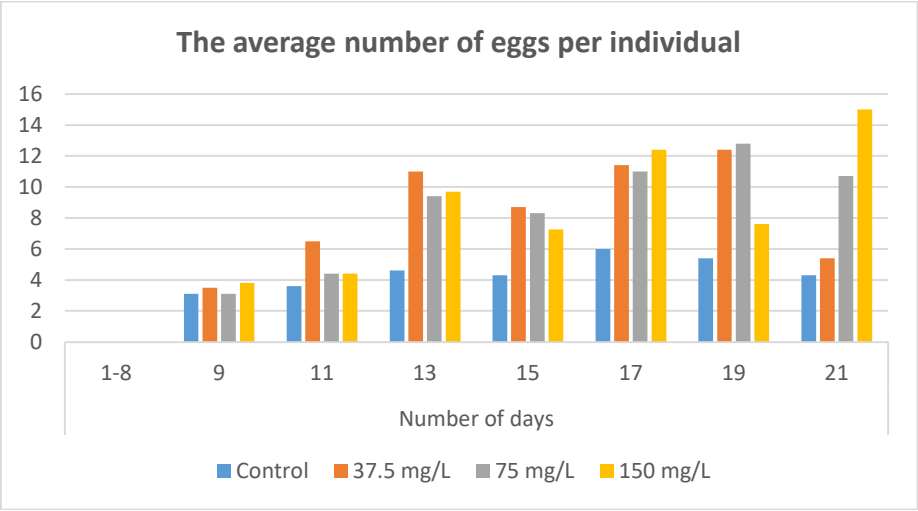

Figure 3. The graph shows the average number of eggs per individual in the control group and the treated groups with different concentrations of calcium sulfate ($CaSO_4$).

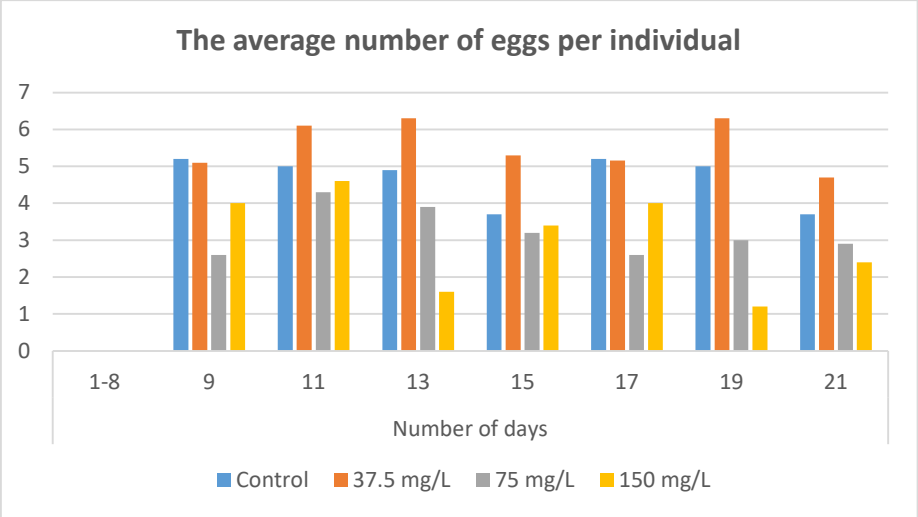

Figure 4. The graph shows the average number of eggs per individual in the control group and the treated groups with different concentrations of potassium chloride (KCl).

4. DISCUSSION

The effects of different concentrations of $CaSO_4$ and KCl on *D. magna*, which is an important part of the food chain in the aquatic ecosystem, were evaluated. $CaSO_4$ is the compound with the longest history not only in industrial usage but also in clinical usage. KCl is a naturally occurring form of potassium salt, typically extracted from land or sea. KCl is found in many foods due to fact that it is considered safe and has high antimicrobial activity compared to sodium chloride. Potassium participates in various functions in the body, such as regulating blood pressure levels, neurotransmission, cardiovascular, skeletal, and muscular system activities [15, 18]. Therefore, the present study is important to evaluate the positive or negative effects of these two compounds on *D. magna* which is an invertebrate model organism, frequently used in laboratory studies and ecotoxicology studies. Moreover, *D. magna* is used in model in the toxicity assessment of pharmaceuticals. Ketoprofen is a nonsteroidal anti-inflammatory and analgesic drug commonly used in human and veterinary medicine. Bownik et al. [19] reported that the results showed ketoprofen induced a time- and concentration-dependent decrease of daphnid heart rate. Additionally, *Daphnia* heart activity was analyzed after exposed to cardioactive drugs: ouabain, metaproterenol, metoprolol, and verapamil. Results indicated that ouabain, metaproterenol and metoprolol caused similar effects on heart of daphnids to those observed on humans due to positive systolic inotropic effects [20].

After exposure to KCl, an increase in heart rate was observed for two consecutive days however a decrease in heart rate was observed in the medium and high-dose groups during the last 48 hours. Cohlmia et al. [21] investigated the effects of KCl and monophosphate glutamate (MSG) on the heart of *D. magna*. According to the results of this study, while there was an increase in heart rate after exposure to MSG, there was a decrease in heart rate after exposure to KCl. Although potassium is the prominent cation in many physiological functions such as regulation of osmotic pressure, conduction of nerve impulse, muscle contraction particularly the cardiac muscle etc. [22], Mattu et al. [23] have reported that high concentrations of potassium give rise to abnormal heart and skeletal muscle function by lowering cell-resting action potential and preventing repolarization, leading to muscle paralysis. Soetan et al. [22] also reported that an increased level in serum potassium caused dilatation of the heart and cardiac arrest.

After exposure to $CaSO_4$, an increase in heart rate of treatment groups (except for second-day data of 37.5 mg/L) was observed compared to the control group, depending on the concentration levels of this compound. Wilson et al. [24] investigated the effects of caffeine and ethanol on the heart rate of *D. magna*. It was noted that while heart rate increased because of caffeine exposure, it decreased because of ethanol exposure. Crustacean species need to periodically shed their old exoskeleton to grow, and the exoskeleton contains $CaCO_3$. Due to continual replacement of exoskeleton at frequent intervals, crustacean species come across massive calcium fluctuations in the internal concentration. To minimize the fluctuation, they have highly advanced calcium regulatory mechanisms occur in epithelial cell layers of crustacean gills, gut, antennal glands, and integument [25].

An increase in egg production was observed depending on the $CaSO_4$ concentration levels. Similarly, Leblanc and Surprenant [16] reported that different concentrations of $CaSO_4$ (200, 290, 460, 910, 2100 mg/L) had a positive impact on the number of offspring produced per

daphnid. Romero et al. [26] who studied with birds, reported that after the animals were fed with a mixture of $CaSO_4$ and zeolite (6.94% of the diet), egg yolk, egg weight and egg quality were increased. When it comes to KCl, it caused a decrease in egg production in the medium- and high-dose groups, although there was an overall increase at the low dose group. Bhattacharya and Kaliwal [27] reported that haemolymph trehalose was significantly increased after supplementing the feed with KCl to the silkworm. Trehalose is the main circulating sugar which is used for oocyte growth [28]. Based on literature data, the increase in egg production of the low-dose group may be related to trehalose level due to KCl. However, a high potassium concentration gives rise to various complex dysfunctions and abnormal cell-membrane-permeability for ion exchanging or molecule transportation [29]. Omana and Wu [30] also reported that a high concentration of KCl was effective on egg proteins due to minimized complex formation between ovomucin and other egg white proteins.

Overall, our results revealed that $CaSO_4$ and KCl induced time- and concentration-dependent changes on heartbeat and egg production of *D. magna*. Due to the fact that the heartbeat mechanism in *Daphnia* is similar in many respects to that of vertebrates, heart rate is a prominent physiological indicator that reflects differential concentration effects of chemical compounds to *D. magna* circulatory system. Given that *D. magna* is a key species in aquatic ecosystems and a significant component of the freshwater food web, our results added information about the effects of $CaSO_4$ and KCl on heartbeat and egg production of this species compared with the studies published to date.

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The Declaration of Conflict of Interest/Common Interest

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

Authors' Contribution

The first author contributed 40%, the second author 30%, the third author 30%.

The Declaration of Ethics Committee Approval

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

The Declaration of Research and Publication Ethics

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

REFERENCES

[1] D. Ebert, "Ecology, epidemiology, and evolution of parasitism in *Daphnia*," National Library of Medicine (US) and National Center for Biotechnology Information (US), Bethesda. ISBN10: 1-932811-06-0, 2005.

[2] S. Savaş and N. L. Çiçek. "Canlı yem organizmalarında L-karnitin," Atatürk Üniversitesi Ziraat Fakültesi Dergisi, vol. 41, no. 1, pp. 71–73, 2010.

[3] S. Arman, E. Akat and S. İ. Üçüncü, "An Investigation on Some Toxic Effects of Carbofuran on *Daphnia magna* Crustacea, Cladocera," Hacettepe Journal of Biology and Chemistry, vol. 44, no. 2, pp. 155–160, 2016.

[4] X. Lv, Y. Yang, Y. Tao, Y. Jiang, B. Chen, X. Zhu, Z. Cai, and B. Li, "A mechanism study on toxicity of graphene oxide to *Daphnia magna*: Direct link between bioaccumulation and oxidative stress," Environmental Pollution, vol. 234, pp. 953–959, 2018.

[5] E. Decaestecker, L. De Meester and J. Mergeay, "Cyclical parthenogenesis in *Daphnia*: sexual versus asexual reproduction," In: Van Dijk P, Martens K, Schön I (eds) Lost Sex. Springer, Berlin, pp. 295–316, 2009.

[6] S. Navis, A. Waterkeyn, T. Voet, L. De Meester and L. Brendonck, "Pesticide exposure impacts not only hatching of dormant eggs, but also hatchling survival and performance in the water flea *Daphnia magna*," Ecotoxicology, vol. 22, no. 5, pp. 803–814, 2013.

[7] A. A. Camp, M. H. Haeba, and G. A. LeBlanc, "Complementary roles of photoperiod and temperature in environmental sex determination in *Daphnia spp*," Journal of Experimental Biology, vol. 222, no. 4, jeb195289, 2019.

[8] S. Burkhard, V. Van Eif, L. Garric, V. M. Christoffels and J. Bakkers, "On the evolution of the cardiac pacemaker," Journal of Cardiovascular Development and Disease, vol. 4, no. 4, pp. 1–18, 2017.

[9] T. J. Pirtle, T. L. Carr, T. Khurana and G. Meeker, "ZD7288 and mibefradil inhibit the myogenic heartbeat in *Daphnia magna* indicating its dependency on HCN and T-type calcium ion channels," Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology, vol. 222, pp. 36–42, 2018.

[10] M. J. Davis and M. A. Hill, "Signaling mechanisms underlying the vascular myogenic response," Physiological Reviews, vol. 79, pp. 387–423, 1999.

[11] I. M. Cooke, "Reliable, responsive pacemaking and pattern generation with minimal cell numbers: the crustacean cardiac ganglion," Biology Bulletin, vol. 202, pp. 108–36, 2002.

[12] J. L. Wilkens, "Evolution of the cardiovascular system in Crustacea," American Zoologist, vol. 39, no. 2, pp. 199–214, 1999.

[13] R. Liang, J. He, Y. Shi, Z. Li, S. Sarvajayakesavalu, Y. Baninla, F. Guo, J. Chen, X. Xu, L. Yu, "Effects of Perfluorooctane sulfonate on immobilization, heartbeat, reproductive

and biochemical performance of *Daphnia magna*," Chemosphere, vol. 168, pp. 1613–1618, 2017.

[14] P. Ducheyne, "Comprehensive Biomaterials," Elsevier: Amsterdam, The Netherlands, 2015.

[15] H. Kahraman and C. C. Karaderi, "The Importance of KCl," Biomedical Journal of Scientific & Technical Research, vol. 19 no. 2, pp. 14170–14171, 2019.

[16] G. A. Leblanc and D. C. Surprenant, "The influence of mineral salts on fecundity of the water flea (*Daphnia magna*) and the implications on toxicity testing of industrial wastewater," Hydrobiologia, vol. 108, no. 1, pp. 25–31, 1984.

[17] A. Bhattacharya and B. B. Kaliwal, "The biochemical effects of potassium chloride on the silkworm, (*Bombyx mori L.*)," Insect Science, vol. 12, no. 2, pp. 95–100, 2005.

[18] W. S. Pietrzak and R. Ronk, "Calcium sulfate bone void filler: a review and a look ahead," The Journal of Craniofacial Surgery, vol. 11, no. 4, pp. 327–333, 2000.

[19] A. Bownik, M. Jasieczek and E. Kosztowny, "Ketoprofen affects swimming behavior and impairs physiological endpoints of *Daphnia magna*," Science of The Total Environment, vol. 725, 138312, 2020.

[20] A. Villegas-Navarro, E. Rosas-L and J. L. Reyes, "The heart of *Daphnia magna*: effects of four cardioactive drugs," Comparative Biochemistry and Physiology Part C: Toxicology and Pharmacology, vol. 136, no. 2 pp. 127–134, 2003.

[21] G. Cohlmia, D. Honarchi, K. Klaiber, E. May and S. Reeves, "Chemical control: observing the excitatory effects of MSG compared to the inhibitory effects of KCl in the heart rate of *Daphnia magna*," Journal of Undergraduate Biology Laboratory Investigations, vol. 2, no. 2, pp. 1–5, 2019.

[22] K. O. Soetan, C. O. Olaiya and O. E. Oyewole, "The importance of mineral elements for humans, domestic animals and plants-A review," African Journal of Food Science, vol. 4, no. 5, 200–222, 2010.

[23] A. Mattu, W. J. Brady and D. A. Robinson, "Electrocardiographic manifestations of hyperkalemia," The American Journal of Emergency Medicine, vol. 18, no. 6, pp. 721–729, 2000.

[24] J. Wilson, J. Collette, A. Miller, A. Hoang and T. Dubose, "The opposite effects of caffeine and ethanol on *Daphnia magna*'s heart rate," Journal of Undergraduate Biology Laboratory Investigations, vol. 2, no. 2, pp. 1–4, 2019.

[25] G. A. Ahearn, P. K. Mandal and A. Mandal "Calcium regulation in crustaceans during the molt cycle: a review and update," Comparative Biochemistry and Physiology Part A: Molecular and Integrative Physiology, vol. 137, no. (2), pp. 247–257, 2004.

[26] C. Romero, E. M. Onyango, W. Powers, R. Angel and T. J. Applegate, "Effect of a partial replacement of limestone by a $CaSO_4$-zeolite mixture combined with a slight protein

reduction on production indices, egg quality, and excreta pH in laying hens,". Journal of Applied Poultry Research, vol. 21, no. 2, pp. 325–334, 2012.

[27] A. Bhattacharya and B. B. Kaliwal, "The biochemical effects of potassium chloride on the silkworm, (*Bombyx mori* L.)," Insect Science, vol. 12, no. 2, pp. 95–100, 2005.

[28] S. N. Thompson, "Trehalose—the insect 'blood' sugar," In: Advances in insect physiology, vol 31. Elsevier, Amsterdam, pp. 205–285, 2003.

[29] T. Yazawa, K. Tanaka and T. Katsuyama, "Neurodynamical control of the heart of healthy and dying crustacean animals," In International Design Engineering Technical Conferences and Computers and Information in Engineering Conference 47438, pp. 1123–1130, 2005.

[30] D. A. Omana and J. Wu, "Effect of different concentrations of calcium chloride and potassium chloride on egg white proteins during isoelectric precipitation of ovomucin," Poultry Science, vol. 88, no. 10, pp. 2224–2234, 2009.

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Research Article

Stem Cell Therapy in Rats with Experimental Intrauterine Adhesions Induced by Trichloroacetic Acid

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ABSTRACT

The effectiveness and role of stem cells obtained from bone marrow in removing the adhesion formed in intrauterine adhesion modeling using chemicals in rats and restoring the implantation of the blastocyst to the endometrium were investigated. The experimental model was created in a single horn using trichloroacetic acid. Three different groups and two subgroups were formed as only culture medium (CM), stem cell and 48-hour medium (Niche). A total of 30 female and 3 male rats were used in the study. Treatment was started immediately after model formation in the acute phase, and 10 days later, the subjects were placed in the same cage with male rats for conception. Then, the pregnancy status and the number of puppies born were evaluated. Histological evaluation was performed with hematoxylin-eosin staining. A statistically significant difference ($p<0.05$) was found in the stem cell applied groups compared to the other groups in both histological and morphological evaluations according to the number of newborns. It has been determined that regional stem cell application in the acute period can remove adhesions in uterine adhesions and make the endometrium more suitable for implantation. As a result, the increase in the number of newborns as a result of the increase in endometrial thickness, number of glands and vascularization, decrease in fibrous areas and regression of adhesive areas in the treatment groups is promising in terms of carrying experimental intrauterine studies to the clinic.

Sıçanlarda Trikloroasetik Asit ile İndüklenen Deneysel İntrauterin Adezyonlarda Kök Hücre Tedavisi

ÖZET

Kemik iliğinden elde edilen kök hücrelerin, sıçanlarda kimyasal kullanılarak oluşturulan intrauterin adezyon modellemesinde rahim içi yapışıklıkların giderilmesinde ve blastosistin endometriuma implantasyonunun yeniden sağlanmasında etkinliği ve rolü araştırıldı. Deney modeli, trikloroasetik asit kullanılarak tek uterin hornda meydana gelen hasara karşı, yalnızca kültür besiyeri (CM), kök hücre ve 48 saatlik Niş kullanılarak farklı grup ve iki alt grup oluşturulmuştur. Çalışmada toplam 30 dişi ve 3 erkek rat kullanıldı. Akut fazda model oluşumundan hemen sonra tedaviye başlandı ve 10 gün sonra denekler gebe kalmaları için erkek sıçanlarla aynı kafese yerleştirildi. Daha sonra gebelik durumu ve doğan yavru sayısı değerlendirildi. Histolojik değerlendirme hematoksilen-eozin boyaması ile yapıldı. Yenidoğan sayısına göre hem histolojik hem de morfolojik değerlendirmelerde kök hücre uygulanan gruplarda diğer gruplara göre istatistiksel olarak anlamlı fark ($p<0.05$) bulundu. Akut dönemde bölgesel kök hücre uygulamasının rahim içi yapışıklıkları ortadan kaldırabileceği ve endometriyumu implantasyona daha uygun hale getirebileceği belirlendi. Sonuç olarak tedavi gruplarında endometrial kalınlık, bez sayısı ve damarlanmanın artması, fibröz alanların azalması ve adeziv alanların gerilemesi sonucu yenidoğan sayısındaki artış deneysel intrauterin çalışmaların kliniğe uyarlanması açısından umut verici olmuştur.

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1. INTRODUCTION

Due to the ability of stem cells to self-renew, differentiate, multiply, and form tissues and organs, they can be used in organ transplants, in the treatment of many diseases or as an alternative to drugs with known negative side effects. Nowadays, stem cells can be obtained from many sources to be used for therapeutic purposes. The most studied sources are bone marrow derived stem cells including hematopoietic, embryonic stem cells and mesenchymal stem cells. Studies in which human embryonic stem cells are used for the treatment of many diseases such as diabetes, Parkinson's, heart failure, neurodegenerative injuries, osteogenesis imperfecta are still in the experimental stage; because embryonic stem cells must be differentiated into target tissue cells before transplantation [1-3]. Intrauterine adhesion (IUA) or Asherman's Syndrome (AS) was described by Fritsch in 1894. Defining the disease picture as "traumatic amenorrhea" for the first time, Dr. It is referred to as AS because it is Joseph G. Asherman [4]. Some authors have proposed to define the term 'Asherman's syndrome' for patients with amenorrhea, those with a completely occluded uterus, or those who have recently had a cesarean delivery (surgical operation). Others use the term 'intrauterine adhesion'; this term is clearer and more descriptive, but patients with surface deficiency of the endometrium without fibrous bridges between the uterine walls are the exception. Women with this condition similarly experience other pregnancy complications such as menstrual bleeding, infertility, recurrent pregnancy loss, uterine growth restriction, problems with implantation, and adhesions [5]. It seems indispensable for the future to develop new treatment modalities that will be more effective and alternative to surgical and hormonal treatment in patients diagnosed with IUA. Because, both in our country and in the world, the number of abortions has increased day by day. At the same time, damage to the uterine tissue may be inevitable during routine gynecological examinations. Stem cell studies in the last decade have developed so rapidly that it has increased the hopes of those seeking cures from cancer, which seems to be incurable, to metabolic diseases, even rheumatic and neurodegenerative diseases. Because these cells are known to positively affect other cells, especially cancer cells, by creating a micro-environment called a niche with the factors they secrete outside of themselves [6]. At this point, we focused on the effects of stem cell therapy as an alternative to surgical methods and medical treatments in endometrial damage.

2. MATERIAL and METHODS

2.1. Experimental Asherman model and stem cell applications

As an implantation failure model, IUA was chemically formed in the single horn of the uterus in female rats with trichloroacetic acid. Under ketamine/xylazine anesthesia, the abdomen was opened with a midline incision and the right and left uterine horns were determined, and then 0.1 ml trichloroacetic acid (IL 33, Istanbul Ilac Sanayi ve Ticaret AS, Umraniye, Istanbul, Turkey) was applied according to the experimental groups one day after the application [7].

Groups:

IUA + CM (G1, n=10); The group given freshly culturer medium (CM),

IUA + Niche (G2=10); The group given 48-hour medium,
IUA + Stem cell + Niche (G3=10); A total of three groups were formed, including the group that was given with CM medium obtained from bone marrow.

Each group was divided into two subgroups and a total of 6 groups were formed. Grouping was formed in such a way that the first subgroup pregnancy was not established, the second subgroup pregnancy was formed and the number of newborns was determined.

2.2. Bone Marrow Mesenchymal Stem Cells (BMSC)

In the study, bone marrow cells taken from the tibia and femur of male rats for bone marrow-derived stromal stem cell decomposition followed by separation in a 25cm2 culture dish with 15% fetal calf serum (FBS) (S0113, Biochrom, Berlin, Germany) added α-MEM (F0915, Biochrom, Berlin, Germany) culture medium was grown in an incubator at 370C and 5% CO2. At the end of the third day, stem cells and non-adherent cells were removed from the medium by changing the medium. Then, the medium of the cells adhering to the culture dish was changed every two days until it became confluent.

2.3. Vaginal smearing

Vaginal smear technique was used between 15.00-16.00 hours from female rats for cycle and pregnancy detection and samples were collected. To perform this procedure, 0.5 ml of saline was drawn into the micro pipette. Saline was sprayed into the vagina with a pipette tip. Without removing the tip of the pipette, the sprayed saline was drawn back into the pipette by creating negative pressure. The extracted liquid was taken on a clean slide and fixed for 5 minutes with methanol. Then, it was stained with rapid hematoxylin and eosin staining method and estrous cycle periods were evaluated cytologically under a light microscope (BX43, Olympus, Japan). In addition, after the proestrus and estrus stages were determined by this method, the subjects of the groups in which pregnancy would be created were placed in cages as three female rats and one male rat and left to mate. Vaginal smears were taken from females released for pregnancy the next day, and the first days of pregnancy were determined in rats whose sperm presence was detected by H&E staining [8].

2.4. Tissue staining

Uterine tissues were harvested by cervical dislocation under general anesthesia after treatment (10 days later). Tissues were fixed for 48 hours with 10% formalin solution for histochemical analysis. Tissues fixed in formalin were washed under running water for 1 night to remove fixative. Afterwards, it was passed through series of ethyl alcohol increasing to 60%, 70, 80 and 100% for 30 minutes each for dehydration. Then, they were kept in xylene-alcohol mixture in a one-to-one ratio for 15 minutes and in two changes of xylene for transparency for 30 minutes, and after applying xylene-paraffin in a one-to-one ratio for 15 minutes in an oven at 60˚C and immersed with two changes of paraffin for 60 minutes, the tissues were transferred to the paraffin blocks. Sections were taken with a microtome and H&E staining was performed. After tissue follow-up, sections of 4-5 µm were taken and stained with hematoxylin and eosin, endometrial thickness was determined using quantitative image processing software (ImageJ-NIH Image). Endometrial thickness measurements were obtained from the lateral edges of each slice; The measurement result was defined as the vertical distances between the luminal surfaces and the serous membranes. In addition, the number of glands was determined by taking

the average of the counts from four random areas by determining the gland abundance points in the stroma**.**

2.5. Statistical analysis

Inflammation, fibrosis, gland count and endometrial thickness were determined by H&E staining and statistical significance was determined in the results obtained from the first groups of the study. In the evaluation, 0= little inflammation and fibrosis, 1= little inflammation and moderate fibrosis, 2= moderate inflammation and fibrosis, 3= much inflammation and fibrosis. The resulting data were compared with the One Way-ANOVA Tukey statistical test, and $p<0.05$ results were considered statistically significant [9].

3. RESULTS

3.1. Vaginal spread findings

Vaginal smear procedure was performed to determine the days of pregnancies we created in our second groups. For this reason, rats in which AS was created and 10 days of medium, niche and stem cell+niche treatments were completed were taken into the same cages as male rats after proestrus and estrus cycles were determined. The next day, a vaginal smear was made again, and whether mating occurred or not was determined according to the presence of sperm detected in the smear. We considered the subjects whose sperm were observed in their vaginal smears to be pregnant. In vaginal smears, it was observed that rat sperm had a hook-shaped head and a long tail. It was observed at the end of the experiment that all of the animals in the vaginal smear preparations we observed sperm were pregnant.

The second subgroups of the groups were categorized according to the sperm findings in the vaginal smear preparations. However, it was determined that the smear was bright in color, the intermediate cells were dense, and the parabasal cells and mucus were rare in the preparations, which belonged to the proestrus group. It was determined that the group consisting of keratinized superficial cells belonged to the oestrus group smears. It was determined that the group with tightly packed multinucleated epithelial cells with cytoplasmic vacuoles and a large number of increased neutrophils belonged to rats in the metestrus cycle (Figure 1).

3.2. Histochemical findings of the groups without pregnancy

In the first groups of the experimental model, in which the IUA model was created as the implantation failure model, both the right and left uterine horns and the groups were evaluated by comparing each other. We applied different treatment agents intraperitoenally for 10 days to rats whose left uterine horns were damaged by acid treatment, and we evaluated the changes in the endometrium after 10 days under a light microscope. They were H&E stained from routine histochemical staining and the morphological and histological changes that occurred in the tissues at the end of the applications were examined (Table 1). In the study, a unilateral IUA model was created in all subject groups [7]. While damage was created by treating the left uterine horn with acetic acid, the right uterine horn was kept as a control and no damage was caused by any chemical or mechanical factor. It was observed that the endometrium degenerated intensively, the synechiae areas increased excessively in the groups to which

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medium and niche were applied (G1-1, G2-1), the number of glands decreased significantly compared to the groups in which only CM and niche were applied (G3-1).

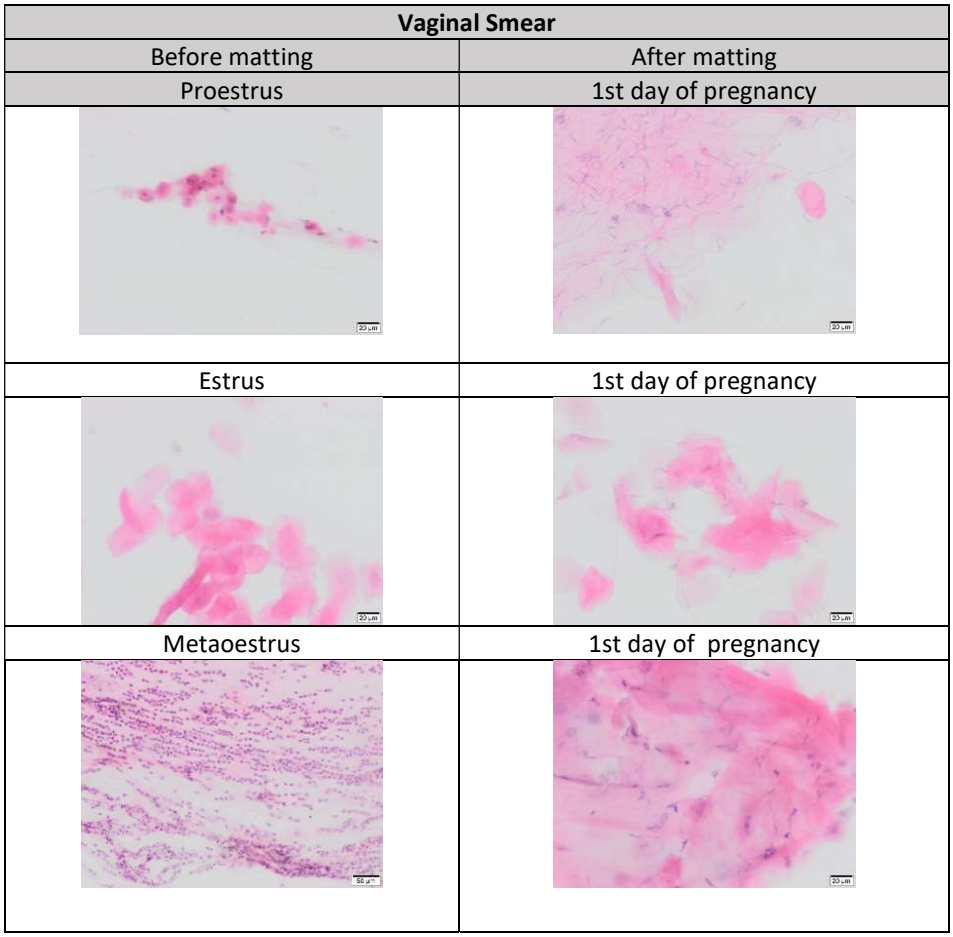

Figure 1. Evaluation results of vaginal smear preparations of IUA subgroups, H&E.

It was observed that the endometrial epithelium of the CM and niche groups was eliminated, and dense fibrous areas were formed in the lamina propria. Again in these groups, both the number of glands decreased and the tubular structure of the gland epithelium was impaired (Figure 2). In addition, the number of newborns in the lower secondary groups was determined and a statistically significant difference was observed ($p>0.05$) (Table 2).

Table 1. Fibrosis, inflammation, endometrial thickness and gland count of the sub-first groups.

	Inflammation	*Fibrosis*	*Endometrial thickness*	*Gland count*
CM	2,4 ± 0,51	2,7 ± 0,48	138, 72 ± 26 µm	5 ± 2
Niche	1,6 ± 0,51	2,3 ± 0,48	154,24 ± 26 µm	5 ± 1
BMSC-Niche	0,5 ± 0,52	0,9 ± 0,73	315,35 ± 32 µm	14 ± 3
Right horn	0,2 ± 0,30	0,7 ± 0,53	333,15 ± 18 µm	16 ± 3

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Table 2. The number of implantation and newborns of the sub-second groups.

	CM G1-2	Niche G2-2	BMSC+Niche G3-2
Newborns	4,66 ± 0,57	5 ± 0,81	8,6± 0,54

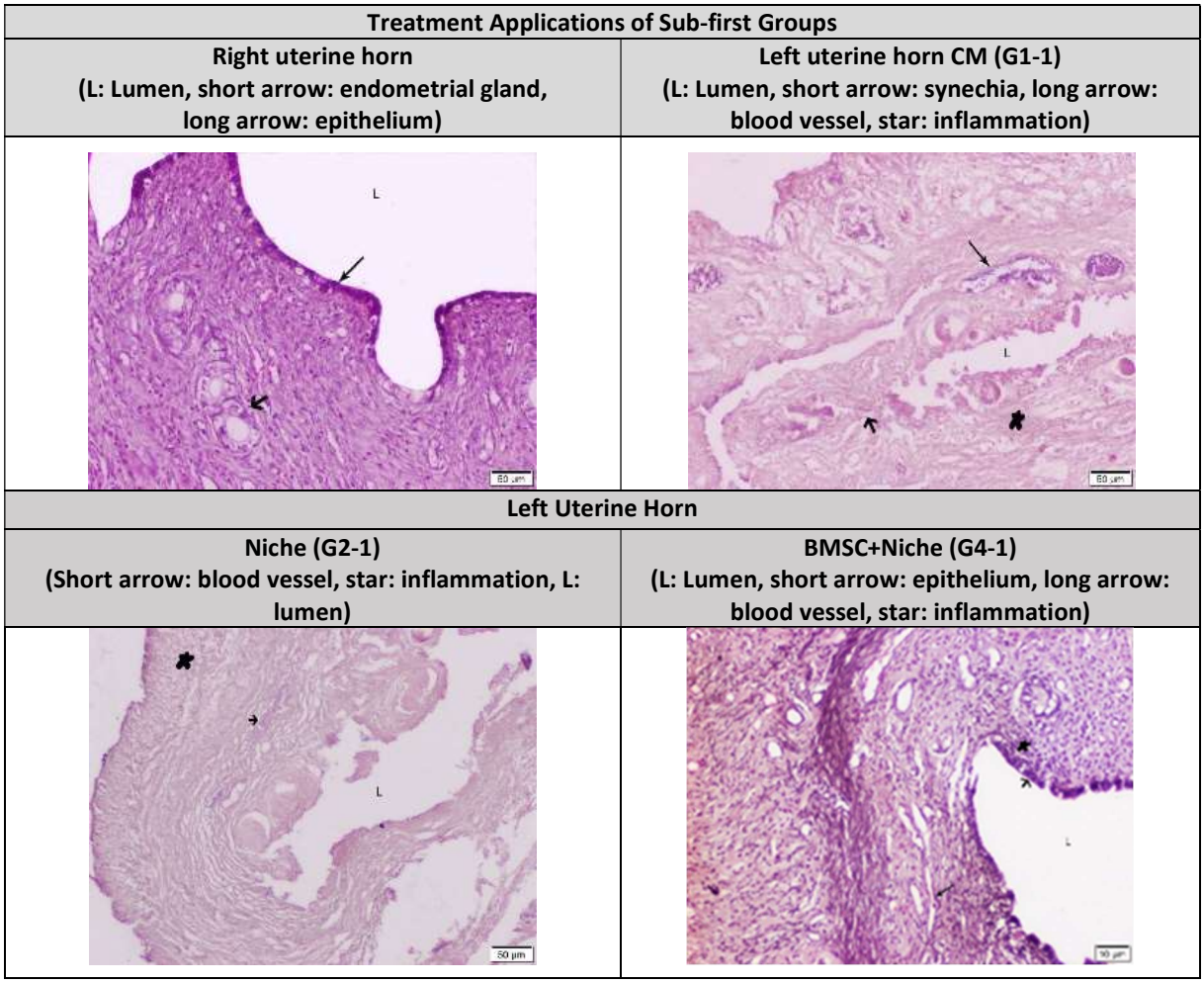

Figure 2. Images of the endometrium after treatment of the sub-first groups, H&E.

4. DISCUSSION

Macroscopic and histological stainings were used to evaluate the changes in the uterus of the CM, Niche, and BMSC + Niche groups treated in the IUA-formed rat uterus according to the number of newborn puppies before pregnancy occurred and in the subjects who became pregnant. In this study, histological images were examined in uterine samples obtained from non-pregnancy and pregnancy groups. The role of CM and its niche in adhesions caused by IUA and its effect on the implantation rate were investigated. Histological findings were obtained showing that the regeneration in the eastern part progressed in the groups treated with BMSC and its niche, new vessel formations increased and glands became more prominent, fibrosis and inflammation that inevitably occur in IUA pathology decreased, and endometrial thickness increased.

There are no randomized controlled trials for the treatment of IUAs. The standard treatment is surgical removal of adhesions by direct observation. With the removal of adhesions, it is aimed to create a cavity with normal anatomy and to provide a functional endometrium. Hysteroscopy is often preferred in current treatment methods. Although the uterine tissue is surgically restored, the recurrence of adhesions and the risk of complications from uterine surgeries have revealed the need for new treatment options. For the treatment of intrauterine formations such as synechia; There were various interventions such as serial flexible hysteroscopies [10], intrauterine adhesion barrier systems [11], fresh amniotic graft, intrauterine insertion of seprafilm [12], hysteroscopic surgery, and hyaluronic acid gels [13].

One of the main problems with intrauterine fillers is the need for concomitant use of antibiotics since a foreign body is present in the uterus. Clinical or subclinical pelvic infections in contact with the vagina may increase secondary infertility.

The point reached recently is that; Estrogen and progesterone supplementation is recommended as the primary option in order for the endometrial tissue to recover and continue its functional activity after surgical procedures. Of course, the effects of this recommendation do not show the same effect in every patient. It has been reported that vaginal administration of micro-sized estradiol further increases the endometrial estradiol concentration, since systemic side effects of oral estrogen supplementation are unavoidable, according to some researchers. However, it is still known that such applications of estrogen cause various contraindications in patients. Such problems cause researchers to work harder for new treatment approaches. Artificial hormone therapy with estrogen is frequently used to promote endometrial proliferation and angiogenesis [14]. Stem cell therapy for endometrial restoration has recently moved from being an edge treatment to becoming a central treatment option. In particular, stem cells obtained from bone marrow have become the most frequently used stem cell source. These cells are isolated directly from the bone marrow by aspiration. Due to their extensive migratory and pluripotent potential, SCs can be easily obtained from both humans and rodents. Stem cells with endometrial regeneration and angiogenesis effects are purified from the bone marrow by immunomagnetic isolation [15]. Ultimately, they prepare the endometrium for implantation. Especially since the endometrium's own stem cells cannot provide endometrial regeneration due to the damage, it has been observed that externally applied transplant stem cells are permanent and increase the success of implantation by functioning. In this study, in which Y-positive cells can be easily demonstrated due to the use of male CM, it has been proven that transplanted cells can remain in the uterus even after three months [16]. Human endometrial tissue is one of the tissues with the strongest dynamism in completing its regeneration perfectly in every menstrual cycle. Although it is generally believed that precursor cells in the functional and basalis layers of the endometrium play a role in this dynamism, there is literature information that BMSCs are also involved in the regenerative ability of the endometrium [17]. In the study, it was tried to remove intrauterine adhesions and to remove these adhesions, which is one of the important infertility problems, by using stem cells, which will contribute to alternative methods and may be the most important treatment tool of the next century. As a matter of fact, both macroscopic findings obtained from the groups; The number of embryos, the number of newborns, the anatomical structure of the endometrium after sacrification and the histopathological findings obtained as a result of microscopic tissue follow-up proved this. Recently, populations of endometrial epithelial and stromal MSCs (Mesenchymal stem cell)

resembling adult MSCs in the basal layer of the human endometrium have been described very rarely [18]. The origin of endometrial stem cells remains unclear. However, BMSCs have been recognized to contain many sources, including menstrual blood-derived mesenchymal stem cells and adipose stem cells [19]. Experimental models have been tried to be designed on the Asherman model using many animals, especially rabbits and rodents, and alternative treatment methods for uterine adhesions have been tried. Especially in rabbits and rodents, mechanical damage, physical damage, laser damage model, curettage model, adhesion developed model with lipopolysaccharide curettage suture and experimental models created using chemicals such as 10% formalin, trichloroacetic acid, polyethylene sponge, phenyl mucilage were designed and fibrotic tissue tried to decrease and increase vascularization [20, 21]. In the light of investigations and presented findings, it was preferred to set up this experimental model using acid [7]. Because it was observed that the synechia formed by the acid creates suitable adhesion conditions for AS.

5. CONCLUSION

Surgical operations only serve to divide adhesions within the cavity, but can do little about endometrial regeneration and adhesion recurrence. So women with Asherman syndrome require more than one approach to provide optimal clinical outcomes. Although stem cells are still in the research phase, new advancing discoveries in this field are leading to new therapeutic strategies every day. We believe that the findings of this study will shed light on stem cell applications in reproductive medicine.

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The Declaration of Conflict of Interest/Common Interest

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

Authors' Contribution

The authors contributed equally to the study.

The Declaration of Ethics Committee Approval

This study was conducted with the approval of the Animal Experiments Local Ethics Committee of Manisa Celal Bayar University, Faculty of Medicine, with the decision number 77637435-050.04.04 dated 05/02/2018.

The Declaration of Research and Publication Ethics

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

REFERENCES

[1] W. Jiang, A. Ma, T. Wang, K. Han, Y. Liu and Y. Zhang. "Homing and differentiation of mesenchymal stem cells delivered intravenously to ischemic myocardium in vivo: a time-series study," Pflugers Archive, vol. 453, pp. 43-52, 2006.

[2] K. Kollar, M.M. Cook, K. Atkinson, and G. Brooke. "Molecular mechanisms involved in mesenchymal stem cell migration to the site of acute myocardial infarction," International Journal of Cell Biology, vol. 904682, 2009.

[3] S.L. Hu, H.S. Luo, J.T. Li, Y.Z. Xia and L. Li. "Functional recovery in acute traumatic spinal cord injury after transplantation of human umbilical cord mesenchymal stem cells," Criteria Care Medicine, vol. 38, pp. 2181-2189, 2010.

[4] J.A. Asherman. "Amenorrhea traumatica (atretica)," An International Journal of Obstetrics and Gynaecology, vol. 55, pp.22-30, 1948.

[5] C.M. March. "Management of Asherman's syndrome," Reproductive BioMedicine Online, vol. 23, pp. 63-76, 2010.

[6] T. Oikawa. "Cancer Stem Cells and Their Cellular Origins in Primary Liver and Biliary Tract Cancers," Hepatology, 2016;doi: 10.1002/hep.28485.

[7] S. Kilic, B. Yuksel, F. Pinarli, A. Albayrak and B. Boztok. "Effect of stem cell application on Asherman syndrome, an experimental rat model," Journal of Assist Reproduction Genetics, vol. 31(8), pp. 975-982, 2014.

[8] A. Köylü, S. Ömeroğlu, S. Ö. Akarca Dizakar, M. Demirel, (2021). "Sıçanlarda Vajinal Yayma Örneklerinin Sitolojik Değerlendirilmesi," Manas Journal of Agriculture Veterinary and Life Sciences, vol. 11 (1), 94-99, 2021.

[9] L. Gan, H. Duan, Q. Xu, Y.Q. Tang and J.J. Li. "Human amniotic mesenchymal stromal cell transplantation improves endometrial regeneration in rodent models of intrauterine adhesions," Cytotherapy, vol. 19, pp. 603-616, 2017.

[10] J.K. Robinson, L.M. Colimon and K.B. Isaacson. "Postoperative adhesiolysis therapy for intrauterine adhesions (Asherman's syndrome)," Fertility and Sterility, vol. 90(2), pp. 409-14, 2008.

[11] J. Abbott, A. Thomson andT. Vancaillie. "SprayGel following surgery for Asherman's syndrome may improve pregnancy outcome," Journal of Obstetrics and Gynaecology, vol. 24(6), pp. 710-11, 2004.

[12] V.S. Tsapanos, L.P. Stathopoulou, V.S. Papathanassopoulou and V.A. Tzingounis. "The role of Seprafilm bioresorbable membrane in the prevention and therapy of endometrial synechiae," Journal of Biomedical Materials Research Part A, vol. 63, pp. 10-14, 2002.

[13] X. Lin, M. Wei, T.C. Li, Q. Huang and D. Huang. "A comparison of intrauterine balloon, intrauterine contraceptive device and hyaluronic acid gel in the prevention of adhesion reformation following hysteroscopic surgery for Asherman syndrome: a cohort study," European Journal of Obstetrics & Gynecology and Reproductive Biology, vol. S0301-2115(13), pp. 00325-4, 2013.

[14] Y. Chen, Y. Chang and S. Yao. "Role of angiogenesis in endometrial repair of patients with severe intrauterine adhesion," International Journal of Clinical and Experimental Pathology, vol. 6, pp. 1343-1350, 2013.

[15] C.B. Nagori, S.Y. Panchal and H. Patel. "Endometrial regeneration using autologous adult stem cells followed by conception by in vitro fertilization in a patient of severe Asherman's syndrome," Journal of Human Reproductive Sciences, vol. 4, 43-8. 2011.

[16] F. Alawadhi, H. Du, H. Cakmak and H.S. Taylor. "Bone marrow- derived stem cell (BMDSC) tranplantation improves fertility in murine model of Asherman's syndrome," PLoS One., vol. 9(5), pp. e96662, 2014.

[17] C.E. Gargett, Masuda H. Adult stem cells in the endometrium. Mol Hum Reprod., vol. 16(11), pp.818-34, 2010.

[18] C.E. Gargett, K.E. Schwab and J.A. Deane. "Endometrial stem/progenitor cells: the first 10 years," Human Reproduction, vol. 22, pp. 137-63, 2016.

[19] J. Tan, P. Li, Q. Wang, Y. Li and D. Zhao. "Autologous menstrual blood-derived stromal cells transplantation for severe Asherman's syndrome," Human Reproduction, vol. 31, pp. 2723-9, 2016.

[20] F. Chen, H. Duan, Y. Zhang and Y.H Wu. "Effect and mechanism of formation of intrauterine adhesion at different dose of estrogen," Zhonghua Fu Chan Ke Za Zhi., vol. 45, 917-20, 2010.

[21] F. Liu, Z.J. Zhu, P. Li and Y.L. He. "Creation of a female rabbit model for intrauterine adhesions using mechanical and infectious injury," Journal of Surgical Research, vol. 183, pp. 183, 2013.

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Developing the Wetland Awareness Scale Through Children's Eyes and Example Application

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ABSTRACT

Wetland losses and pollution in water resources, which are increasing on a global scale, affect biodiversity and sustainable ecosystem structure. The most important trigger of this extinction, which will cause wide-ranging ecological problems, is human activities, and necessary steps must be taken quickly to protect its viability. In the study, it was aimed to develop a measurement tool that measures the awareness level of children about wetlands in a valid and reliable way. In the descriptive study, data were obtained from three different groups. Participants consist of secondary school students between the ages of 9-15 studying in Turkey. Explanatory factor analysis was performed by applying the 26-item candidate scale questions to 245 secondary school students. Then, confirmatory factor analyzes were carried out with the data obtained from the participation of 201 students. SPSS 26.0 and AMOS statistical software were used in the analysis of the data. Evaluations of secondary school students' awareness of wetlands were examined on a group of 446 people. As a result of the validity analyzes made with the data obtained from the application, it was determined that the scale consisted of 14 items with two factors. This structure was confirmed by confirmatory factor analysis. The reliability value of the entire scale was 0.891; 0.704 for the wetland awareness factor in terms of bird species diversity; The importance of wetlands and the awareness of the problems experienced factor was determined to be 0.895. There was no significant difference between gender, age, and having taken wetland lessons before and wetland awareness (p: >0.05). It was determined that there was a significant difference between whether they went to a wetland in the region they lived in before and the wetland awareness of the children (U=15052.5 and p: <0.05). Wetland awareness was found to be higher in students who had gone to wetlands before. It was concluded that the scale developed according to these results in the context of the participants in this study, which was carried out with secondary school students, measured the wetland awareness levels of children in a valid and reliable way.

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Research Article

Çocukların Gözünden Sulak Alan Farkındalığı Ölçeği'nin Geliştirilmesi ve Uygulama Örneği

ÖZET

Küresel ölçekte giderek artan sulak alan kayıpları ve su kaynaklarındaki kirlilik, biyoçeşitliliği ve sürdürülebilir ekosistem yapısını etkilemektedir. Geniş çaplı ekolojik sorunlara neden olacak bu yok oluşun en önemli tetikleyicisi ise insan faaliyetleridir ve sürdürülebilirliğin korunması konusunda gerekli adımların hızla atılması gerekmektedir. Doğa koruma bilincinin oluşturulmasında bütüncül bir yaklaşım gerekmektedir ve atılacak öncelikli adımlardan biri toplumda farkındalık yaratmaktır. Farkındalık, bir canlının çevresinde gelişen olayları bilme, algılama ve duyumsama becerisi olarak tanımlanmaktadır. Yapılan çalışmada çocukların sulak alanlar ile ilgili farkındalık düzeylerini ölçen bir ölçek geliştirilmesi amaçlanmıştır. Betimsel olarak yapılandırılan araştırmada veri toplama aracı olarak geliştirilen aday ölçek maddeleri araştırmacılar tarafından hazırlanmış ve uzman görüşleri doğrultusunda oluşturulmuştur. Katılımcılar, Türkiyede öğrenim gören 9-15 yaş arasındaki ortaokul öğrencilerden oluşmaktadır. 26 maddelik aday ölçek soruları 245 ortaokul öğrencisine uygulanarak açıklayıcı faktör analizi yapılmıştır. Daha sonra 201 öğrencinin katılımından elde edilen verilerle doğrulayıcı faktör analizleri gerçekleştirilmiştir. Verilerin analizinde SPSS 26.0 ve AMOS istatistik yazılımı kullanılmıştır. Ortaokul öğrencilerinin sulak alanlara yönelik farkındalıklarının değerlendirmeleri ise 446 katılımcıdan elde edilen verilerle gerçekleştirilmiştir. Yapılan ilk uygulamadan elde edilen verilerle yapılan geçerlik analizleri sonucunda ölçeğin iki faktörlü 14 maddeden oluşan bir ölçek olduğu tespit edilmiştir. Bu yapı doğrulayıcı faktör analizi ile doğrulanmıştır. Ölçeğin tamamının güvenirlik değeri 0,891; kuş tür çeşitliliği açısından sulak alan farkındalığı faktörü için 0,704; Sulak alanların önemi ve yaşanan sorunlar farkındalığı faktörü için 0,895 olduğu belirlenmiştir. Yapılan örnek uygulamada cinsiyet, yaş ve daha önce sulak alanlarla ilgili ders almaları ile sulak alan farkındalığı arasında anlamlı bir fark bulunmamıştır (p: >0,05). Daha önce yaşadığı bölgedeki bir sulak alana gidip gitmemeleri ile çocukların sulak alan farkındalıkları arasında ise anlamlı bir fark olduğu tespit edilmiştir (U=15052,5 ve p: <0,05). Daha önce sulak alanlara giden öğrencilerde sulak alan farkındalığının daha yüksek olduğu bulunmuştur. Ortaokul öğrencileri ile gerçekleştirililen bu araştırmada yer alan katılımcılar bağlamında bu sonuçlara göre geliştirilen ölçeğin çocukların sulak alan farkındalık düzeylerini geçerli ve güvenilir biçimde ölçtüğü sonucuna ulaşılmıştır.

1. INTRODUCTION

Wetlands are ecosystem environments that have common characteristics and are suitable for a wide range of terrestrial and marine life [1]. Wetlands, which have the richest biodiversity after rainforests, contain 40% of all species and 12% of all animal species in the world [2]. Wetlands, where biodiversity and production are much higher than terrestrial areas, provide important ecosystem services for the sustainability of the ecosystem [3]. Wetlands, one of the world's most productive and valuable ecosystems, provide a wide variety of economic, social, environmental and cultural benefits [4]. These services include maintaining water quality and supply, regulating atmospheric gases, sequestering carbon, protecting coastlines, maintaining authentic local biota, and providing cultural, recreational and educational resources [5].

However, in wetlands, which are one of the ecosystems where human influence is intense, it is stated in many studies that biological diversity and therefore sustainability are damaged [1]; [6-

9]. This shows that people are not sufficiently aware of the ecosystem they live in and the ecosystem services that wetlands offer to people. The destruction of wetlands around the world and the consequent deterioration of ecosystem services will cause significant losses in human well-being and biodiversity, as well as lead to negative economic and social effects in the long run. In this respect, wetlands should be recognized and protected in terms of their hydrological characteristics, biodiversity and physicochemical characteristics. Sustainable protection, management and transfer of these resources to future generations are of great importance [10].

Bird species that have attracted the attention of people throughout history with their colors, songs and flying abilities can be used to monitor wetlands and their healthy structure. Birdwatching is a form of wildlife observation in which bird watching is a fun activity or citizen science [11]. Based on this interest of human beings in bird species; we can use bird watching in raising awareness of nature, creating awareness of ecosystems, monitoring and protecting wetlands. Conservation and restoration of wetlands is essential for the future sustainability of the planet, and provides safety nets for emerging issues such as global climate change, food production for the growing world population, disturbance regulation, clean water and the general well-being of society [3].

One of the primary steps in raising awareness of nature conservation in sensitive ecosystems such as wetlands is to raise awareness in society. Awareness is defined as the skill of knowing, perceiving and sensing the events developing around a living thing. Although the activities related to the protection of our water resources and wetland ecosystems are increasing today, it is not possible to measure the awareness levels and evaluate the effectiveness and diversity of the activities carried out.

The aim of environmental education is to raise citizens who are knowledgeable about the biological environment and its problems, aware of the strategies that can be used to cope with these problems, and actively work for their solution [12]. Traditionally, environmental education has focused on teaching children about “pristine” environments or “wild nature” [13].

Every step to be taken to prevent the extinction and destruction of our wetlands and water resources today is extremely important for children who will face problems that may arise in the future. Increasing the awareness of children, who are the future guardians of wetlands and our water resources and preparing environments where they can contribute to conservation activities will be effective in raising awareness of nature conservation. In the literature review, wetland perceptions of infants and children [14], awareness levels of university students about water resources [15], recognition and protection value of wetlands among primary school students [16] and wetland It has been observed that there are studies on subjects such as education and public perception [17]. However, it has been determined that there is no measurement tool for children's wetland awareness.

The aim of the study was to develop a scale to measure children's awareness of wetlands and to evaluate the wetland awareness of children by making an application example.

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2. MATERIAL AND METHOD

This research is a descriptive research in terms of the evaluation of the technical features of the developed scale and the sample application.

2.1. Research Group

All applications were carried out with secondary school students. In the descriptive study, data were obtained from three different groups. Explanatory factor analyzes (EFA) were conducted with data obtained from a group of 245 secondary school students. Confirmatory factor analyzes (CFA) were conducted with data obtained from a group of 201 secondary school students. Evaluations of secondary school students' awareness of wetlands were examined on a group of 446 people. Participants consist of secondary school students between the ages of 9-15 studying in Turkey.

Participation in the research was on a voluntary basis. Therefore, the sample in the study can be evaluated in the context of purposive sampling [18]. Different opinions in the literature were taken into account while determining the size of the study group in scale development studies. Gorsuch (1974) emphasized that the number of questions should be at least five times the number of participants [19], while Everitt (1975) stated that "the number of participants should be at least ten times the number of items [20]. In line with this information, care was taken to include five times or more participants than the number of items in the measurement tool. Demographic characteristics of the participants in the two groups are given in Table 1.

Table 1. Demographic data of the participants

EFA Group			CFA Group		
Variant		f (%)	Variant		f (%)
Gender	Male	100 (40.8)	Gender	Male	99 (49.3)
	Female	145 (59.2)		Female	102 (50.7)
Age	Average (9-15)	11.64	Age	Average (9-15)	11.98

2.2. Data collection tool

In the light of the information obtained from the literature to determine the candidate scale form elements of the data collection tool developed within the scope of the research 'Wetland Awareness Scale Through Children's Eyes-WASTCE', a question pool was created by the researchers for the candidate measurement tool. The items in the created question pool were presented to expert opinion. Opinions were received from two academicians working in the field of biology, an academician working in the field of measurement and evaluation, and an expert working in a non-governmental organization related to nature conservation. Expert opinions were taken as "appropriate, not appropriate and should be corrected" for the 26 candidate scale items that were corrected. Consistency between expert opinions was examined with the Krippendorff Alpha coefficient [21] and a consistency of .84 was determined. This coefficient indicates a high level of consistency. Detailed information about the analyzes made as a result of the trial application is explained in the "results" section. As a result of the analysis, the final version of the scale was determined as 14 items and 2 factors. As a result, two different total scores are taken from the scale and comments are made on these total scores.

2.3. Implementation of the Data Collection Tool

The candidate scale form was applied to secondary school students with the letter of T.R. Çanakkale Governorship Provincial Directorate of National Education, numbered E-60305806-44-21667935 and dated 04.03.2021. and with the letter of T.R. Fethiye District Directorate of National Education, numbered E-40068571-604.01.01-20426923 and dated 10.02.2021.

Due to the pandemic process, the link created online via google. Forms was sent to the phones of the students' parents and after the parent's approval, it was asked whether the student volunteered to participate. While students who did not have parental consent or did not volunteer to participate did not participate in the study, only the students who volunteered to participate in the study were included in the study.

2.4. Analysis of Data

SPSS 26.0 and AMOS statistical software were used in the analysis of the data. First of all, it was determined that there was no missing data in the data set. Kaiser Meyer Olkin (KMO) Test and Bartlett's Test of Sphericity, which are important criteria of factor analysis, were examined. KMO between 0.801 and 0.900 is considered very good, and above 0.901 is considered excellent. In the sphericity test, the result is expected to be significant ($p<0.05$) [22-24]. The factor structure was revealed by the principal axis factoring (PAF) method. It is a preferred factor extraction method for newly developed scales whose theoretical structure is unknown [25]. “Varimax” axis rotation was performed to clarify the factors. The reference values in Table 2 were taken into account when deciding on the fit indices obtained in confirmatory factor analysis [22, 24, 26-34]. In order to obtain proof of reliability, the Cronbach Alpha reliability coefficient, which shows reliability in terms of internal consistency, was calculated.

Table 2. Confirmatory factor analysis reference values.

Decision	RMSEA	GFI	AGFI	NFI	IFI	CFI	X^2/SD
accept	0,05≤RMSEA≤0,08						2<X^2/SD≤5
excellent	0≤RMSEA≤0,05	0.90 and above	0.90 and above	0.95 and above	0.95 and above	0.95 and above	0≤X^2/SD≤2

With the scale developed within the scope of the research, a sample application was made with the data of 446 participants. Comparison tests were applied according to the variables obtained with questions such as demographic (age, gender) characteristics of the participants and whether they had ever taken a course on wetlands before, whether they had ever been to a wetland before. In the analysis of the data, descriptive statistical analyzes were made using the SPSS program. The Kolmogorov-Smirnov Test was applied to decide which of the parametric or nonparametric tests to use in the comparison tests. In addition, it was decided to use parametric techniques in data sets where the other preconditions, such as homogeneity of variances and whether there was a minimum of 30 samples, were met. It was decided to use nonparametric techniques for data where the prerequisites could not be met [32, 35].

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3. RESULTS

The data obtained from the application with the participation of 245 secondary school students were included in the explanatory factor analysis. Result of the primary analysis, it was determined that the scale has a 10-factor structure with eigenvalues exceeding 1. It is generally accepted that there should be at least three questions in each sub-dimension (factor) in scale development [31]. The item-total correlations and exploratory factor analysis input load values of some items were not at the desired level (0.300 and above) in the literature [35]. However, as a result of the analysis, 2 factors of the scale were found to be very prominent. Eigenvalues are very high in these 2 factors. In the logic of scale development and factor analysis, many variables are explained by reducing them to a small number of factors [32]. From this perspective, it was decided that it would be suitable to conduct factor analysis in a way that would gather under 2 factors. The KMO and Bartlett's Test of Sphericity values for the re-applied factor analysis are given in Table 3.

KMO and Bartlett test results are at the level suggested by the literature, as explained in the data analysis title. As a result of factor analysis, it was determined that 12 items (1, 2, 3, 6, 9, 10, 11, 13, 16, 18, 22 and 23) in the measurement tool could not reach the item-total correlations and factor analysis input load values suggested by the literature. These items were not including to scale. As a result of factor analysis, “Varimax” axis rotation method was used to determine under which factors the items would be grouped. The item total correlations, variance explained by the factors, factor names, input load values and Cronbach Alpha reliability values of the remaining items are given in Table 4.

It is seen that the input load values of the remaining 14 items in the scale vary between 0.216 and 0.703, and the item-total correlations vary between 0.396 and 0.707. According to the literature, these items were determined to be suitable for the scale. The reliability value of the entire scale was 0.891; 0.704 for the wetland awareness (IWAP) factor in terms of bird species diversity; The importance of wetlands and the awareness of the problems experienced (WAITBSD) factor was determined to be 0.895. According to the literature, these values are high reliability levels [32]. The variance explained by the first factor is 38%, and the variance explained by the second factor is 17%. Together, the two dimensions explain 55.112% of the variance in children's awareness of wetlands. This rate is considered low for some sources in the literature [32, 36], sufficient for some sources [35], and between 40% and 70% for some sources in the literature [25]. The scree plot for 14 items and the two-factor structure is shown in Figure 1.

Table 3. KMO and Bartlett values

Kaiser-Meyer-Olkin (KMO)	,910	
Bartlett Test of Sphericity	X^2	1584,293
	SD	91
	p	,000

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Table 4. Factor analysis results

Items	Factors 1	Factors 2	Factor Analysis Item Input Load Value	Item Total Correlations	Factor Name	Variance Explained by the Factor in the Varimax Rotation Result	Factor Cronbach Alpha Confidence Value
S7	,834		,700	,685	Importance of Wetlands and Awareness of Problems (IWAP)	%38,277	0,895
S5	,795		,642	,657			
S4	,783		,635	,674			
S15	,773		,643	,707			
S12	,754		,610	,684			
S14	,657		,565	,688			
S24	,647		,550	,671			
S26	,632		,449	,585			
S25	,618		,426	,566			
S8	,550		,384	,547			
S17	,377		,216	,396			
S21		,838	,703	,354	Wetland Awareness in Terms of Bird Species Diversity (WAITBSD)	%16,835	0,704
S19		,724	,637	,591			
S20		,700	,556	,516			

22 Items Together (All Scale) Cronbach Alpha Reliability Value = 0,891

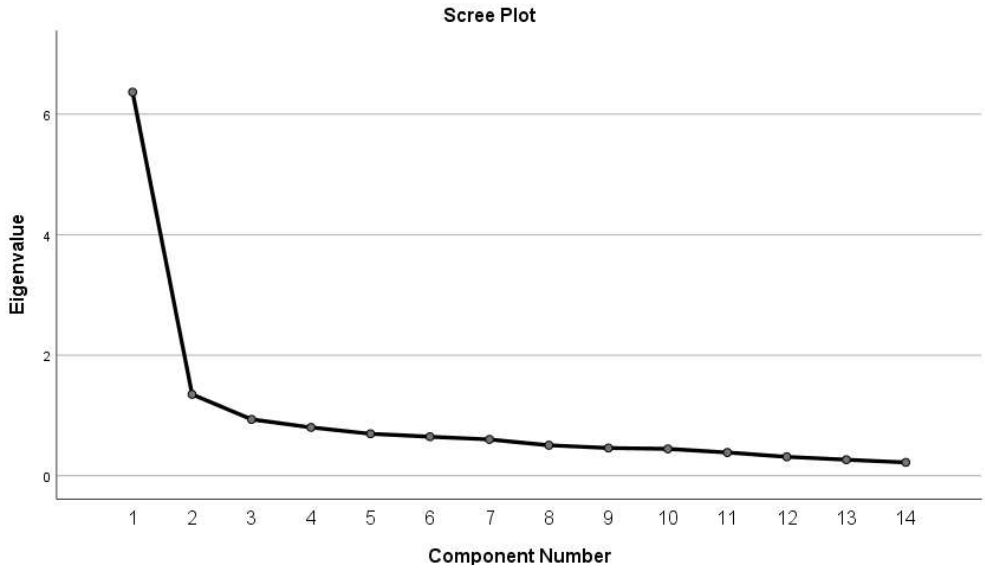

Figure 1. Scree plot of the structure of the Perception of Wetlands in Children's Perception Scale with 2 active 14 items.

After the explanatory factor analysis applied as explained above, a scale structure consisting of 14 items and 2 factors was reached. Another piece of evidence for the validity of this construct

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is the confirmation of the construct. For this reason, a 14-item scale was applied to 201 different students. Confirmatory factor analysis was carried out on the obtained data. Fit indices are shown in Table 5 and the diagram is shown in Figure 2.

Table 5. CFA fit indices

X^2/SD	GFI	AGFI	NFI	IFI	CFI	RMSEA
1,998	0, 909	0,874	0,870	0,930	0,930	0,071

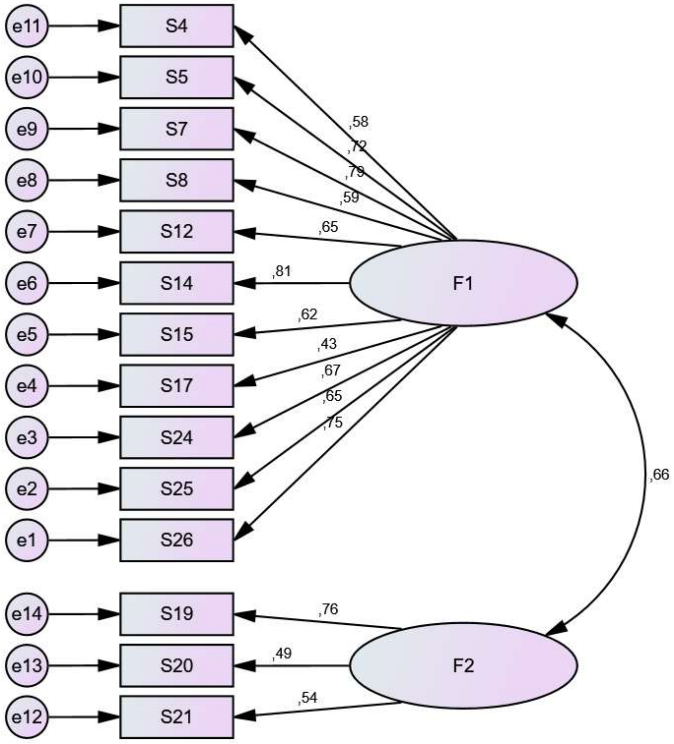

Figure 2. Confirmatory factor analysis diagram of middle school students' wetland awareness scale (standardized values).

The confirmatory factor analysis results are at an acceptable level in the light of the information explained in the data analysis title. It has been concluded that the scale developed according to these results in the context of the participants in this research measures the wetland awareness of secondary school students in a valid and reliable way.

In the sample application made with the children's wetland awareness scale developed within the scope of the research, the awareness of secondary school students towards wetlands was determined. The descriptive statistics of secondary school students' responses to 14 items in the scale are given in Table 6.

Table 6. The final version of the scale items and the distribution of the answers given in the application example

Item No.	Items	N	$\bar{X}$±S. Deviation	I do not agree	Partially Agree	I agree
4	*Today, wetlands are damaged due to human activities.*	446	2.74±0.574	31 %7	52 %11,7	363 %81,4
5	*Wetlands are declining and this is worrying for our future.*	446	2.85±0.455	17 ⁄3,8	35 %7,8	394 %88,3
7	We have to protect wetlands to protect the ecosystem we live in.	446	2.83±0.473	19 ⁄4,3	36 %8,1	391 %87,7
8	*The importance of wetlands is not well known.*	446	2.68±0.570	24 ⁄5,4	93 %20,9	329 %73,8
12	*The fact that there are usually many predators in wetlands negatively affects the animal and plant species there*	446	2.75±0.546	25 ⁄5,6	60 %13,5	361 %80,9
14	*Due to global warming, our wetlands are gradually decreasing.*	446	2.83±0.460	15 ⁄3,4	48 %10,8	383 %85,9
15	*As a result of hunting in wetlands, lead and pellets that settle at the bottom of the water accumulate and affect the wetland ecosystem by causing heavy metal pollution in the future.*	446	2.77±0.509	18 %4	67 %15	361 %80,9
17	*Cutting trees and burning reeds in wetlands cause pollution in wetlands.*	446	2.60±0.666	45 ⁄10,1	90 %20,2	311 %69,7
24	*Our wetlands are rapidly disappearing.*	446	2.80±0.468	13 ⁄2,9	63 %14,1	370 82,9
25	*The number of infectious diseases increases if wetlands are reduced or polluted.*	446	2.72±0.544	21 ⁄4,7	81 %18,2	341 %76,5
26	Wetlands are important in tackling global warming	446	2.79±0.504	19 ⁄4,3	61 %13,9	366 %82,1
19	*If we see many birds in a wetland, this can be considered as an indicator of the healthy structure of the ecosystem in that wetland.*	446	2.62±0.619	33 ⁄7,4	102 %22,9	311 %69,7
20	*The decrease in bird species, diversity and number is evidence of environmental pollution in that area.*	446	2.58±0.685	50 ⁄11,2	89 %20	307 %68,8
21	*If I see or hear a wide variety of birds around me, I think that there is no environmental pollution where I am.*	446	2.38±0.696	55 ⁄12,3	165 %37	226 %50,7
SCALE TOTAL SCORE *(The lowest possible score is 14, and the highest score is 42.)*			**$\bar{X}$±S. Deviation = 37.88±0.239**			

The wetland awareness level of the applied group was calculated as 37.88. Considering that the maximum score that can be obtained from the scale is 45, it has been calculated that the participants have a high level of wetland awareness. The item that the students who participated in the sample of children's wetland awareness scale application least agreed was “If I see or hear a wide variety of birds around me, I think that there is no environmental pollution in my area.” and “The decline in bird species, diversity and numbers is evidence of environmental pollution in that area.” While the items with the highest participation are “Wetlands are decreasing gradually and this is a concern for our future”, “Our wetlands are decreasing gradually due to global warming” and “We have to protect wetlands to protect the ecosystem we live in.” has been. Demographic characteristics of the participants are given in Table 7.

It was tested whether there was a difference in wetland awareness by gender. Since the normal distribution condition could not be met, the nonparametric Mann Whitney U test was applied. No significant difference was found between the wetland awareness scores of girls and boys (U=24047.5 and $p>0.05$).

Table 7. Distribution data of application sample participants by age, gender and other variables

VARIANT	AVERAGE	MINIMUM	MAXIMUM
Age	11,79	9	15
VARIANT	**CATEGORY**	**FREQUENCY**	**PERCENT (%)**
Gender	Girl	247	55,4
	Boy	199	44,6
Have You Taken Any Course About Wetlands Before?	Yes	244	54,7
	No	202	45,3
Have you ever been to a wetland in the area where you live?	Yes	328	73,5
	No	118	26,5

It was tested whether there was a difference between wetland awareness according to age. It was determined that there was no significant relationship in the kruskal-wallis test result, which was performed to test the difference between age and wetland awareness scores ($p>0.05$).

It was tested whether there was a difference between the wetland awareness scores and whether they had taken a course on wetlands before and whether they had been to a wetland before. There was no significant difference between the previous lessons about wetlands and wetland awareness ($p>0.05$). It has been determined that there is a significant difference between the wetland awareness of the secondary school students according to whether they have visited a wetland in the region they lived in before (U=15052.5 and $p<0.05$). Wetland awareness was found to be higher in students who went to wetlands before.

4. DISCUSSION and CONCLUSION

Conservation and restoration of wetlands is essential for sustainability and provides safety nets for emerging issues such as global climate change, food production for a growing world population, clean water, and the general well-being of society [3]. One of the primary steps in raising awareness of nature conservation in sensitive ecosystems such as wetlands is to raise awareness in society. Activities related to the protection of our water resources and wetland ecosystems are increasing today. However, by measuring the awareness levels resulting from these activities, the results of the activities carried out and the effects of their diversity are not sufficiently evaluated.

In addition to facilitating the measurement process, the scales also enable the determination of the quality of the results obtained. Scientific progress is based on measurement, and measurements made with sensitive measurement tools increase this development [37]. In the literature review, it was seen that there is no measurement tool that can be used to measure wetland awareness for children and youth. The aim of the study was to develop a scale to measure children's awareness of wetlands and to evaluate the wetland awareness of children by making an application example.

As a result of the KMO, Bartlett test and factor analysis performed with the data set obtained in the study conducted for this purpose, 12 items (1, 2, 3, 6, 9, 10, 11, 13, 16, 18, 22 and 23) among the candidate scale items were excluded from the scale because the item-total

correlations and factor analysis could not reach the input load values suggested by the literature. These items are as follows; *I have enough information about wetlands, I have enough information about the creatures living in wetlands, Wetlands are the habitat of many living species, The destruction of wetlands around us will not have a negative effect on us, Lessons and practices that explain the importance of wetlands and water should be given more place in schools, The use of pesticides in agricultural activities around wetlands causes pollution in wetlands, If the agricultural lands around wetlands use water uncontrollably, this will adversely affect wetlands, It is very difficult and much more costly to clean up the pollution caused by the disposal of factory and household waste, Wetland The reeds around the areas need to be cleaned by burning, A lot of mosquitoes and insects breed in the wetlands, so the swamp and reeds parts of the wetlands need to be dried, If I see or hear a wide variety of birds around me, that's where I am. I think there is no environmental pollution and We have no connection with our clean drinking and utility water and wetlands in nature.*

After the explanatory factor analysis, a scale structure consisting of 14 items and 2 factors was reached. To verify this structure, a 14-item scale was applied to 201 different students. Confirmatory factor analysis was performed on the data obtained. It has been concluded that the scale developed according to these results in the context of the participants in this research measures the wetland awareness of secondary school students in a valid and reliable way.

Fisman (2005), in his study examining the effects of the urban environmental education program on children's awareness of their local biophysical environment, showed that the program had a significant positive effect on students' knowledge of local environmental awareness and environmental concepts [13]. Anderson and Moss (1993) in their research on wetland perceptions of infants, children and adults; They stated that conditioning negatively affects adults' perception of wetland. In this respect, they stated that it is very important to plan well-planned written and visual media, promotional posters and training programs in order to increase awareness about wetlands and contribute to conservation activities. In addition, within the scope of the research, they determined that one of the important factors that increase awareness for children is technical trips to wetlands [14]. In the study, it was determined that there is a significant difference between the awareness of the wetland according to whether the secondary school students went to a wetland in the region where they lived before (U=15052.5 and $p<0.05$). Wetland awareness was found to be higher in students who had gone to wetlands before. Parallel to this finding, different studies show that constant contact with a particular place improves children's environmental knowledge and anxiety in the best way [38-40].

In his study, the survey study he conducted using the photographs of the biodiversity elements in the environment where the secondary school students live, found that the education of parents, income level of the family, and watching a television program about nature were not statistically effective on the awareness of the students about biodiversity [41]. In the study, it was found that there was no significant difference between wetland awareness by gender, wetland awareness by age, and whether they had taken a course on wetlands before and wetland awareness ($p>0.05$).

Wetland losses experienced today, decrease in biodiversity and pollution in water resources show that we need to reach positive behavioural changes more quickly in order to protect the sustainability of these resources. For this reason, it is very important to increase children's

awareness of wetlands. Based on the results of the scale application, it is recommended that technical trips be made to the wetlands in their regions for secondary school students on dates such as world wetlands day or bird watching day in order to increase students' awareness of wetlands. Bird watching can be used as an important tool to increase the awareness of children in technical trips to be planned. Bird watching is a form of wildlife observing where bird watching is a fun activity or citizen science [11]. This recreational activity, starting from this interest of human beings in bird species; It can be used in raising children's awareness of wetlands, monitoring and protection of wetlands.

It was concluded that the scale developed according to these results in the context of the participants in this study, which was carried out with secondary school students, measured the wetland awareness levels of children in a valid and reliable way. The reliability value of the entire scale was 0.891; 0.704 for the wetland awareness (KTÇSAF) factor in terms of bird species diversity; The importance of wetlands and the awareness of the problems experienced (SAÖYSF) factor was determined to be 0.895. As such, the scale can be used to measure the awareness of similar groups towards research.

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The Declaration of Conflict of Interest/ Common Interest

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

Authors' Contribution

The first author contributed 35%, the second author 20%, the third author 15%, the fourth author 15% and the fifth author %15. İU, NS, BEU, KT and AÇU designed the study, designed the data collection tool, collected the data and created the models. İU performed the data analysis. İU, NS and AU drafted the paper. All authors approved the final version to be published.

The Declaration of Ethics Committee Approval

This study does not require ethics committee permission or any special permission. The scale form was applied to secondary school students with the letter of T.R. Çanakkale Governorship Provincial Directorate of National Education, numbered E-60305806-44-21667935 and dated 04.03.2021. and with the letter of T.R. Fethiye District Directorate of National Education, numbered E-40068571-604.01.01-20426923 and dated 10.02.2021.

The Declaration of Research and Publication Ethics

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

REFERENCES

[1] S. S. Çağırankaya, and B. T. Meriç, "Türkiye'nin Önemli Sulak Alanları: Ramsar Alanlarımız" Orman ve Su İşleri Bakanlığı-Doğa Koruma ve Milli Parklar Genel Müdürlüğü, Hassas Alanlar Dairesi Başkanlığı, Ankara. 2013.

[2] WWF, "Ramsar Sites Assessment Report in Turkey Ed. (Sea Chilli Worshipers)" WWF. 2008.

[3] B. R. Clarkson, A. E. Ausseil, and P. Gerbeaux, "*Wetland ecosystem services. In Dymond JR ed. Ecosystem services in New Zealand – conditions and trends*" Manaaki Whenua Press, Lincoln, New Zealand. 2013.

[4] R. Costanza, R. d'Arge, R. de Groot, S. Farber, M. Grasso, B. Hannon, K. Limburg, S. Naeem, R. V. O'Neill, J. Paruelo, R. G. Raskin, P. Sutton, M. van den Belt "The value of the world's ecosystem services and natural capital" Nature 387: 253–260. 1997.

[5] N. B. Dise, "Peatland response to global change" Science 326: 810–811, 2009.

[6] M.U. Altundağ, and M. Canlı, "Effects of Destruction of Wetlands and Example of Lake Amik" Nature's Voice Magazine, 2(4), pp. 49-66, 2019.

[7] Ç. H. Şekercioğlu, "Ecosystem functions and services" Pp. 45-72 in Sodhi, N.S. and Ehrlich, P.R. (eds.). Conservation Biology for All. Oxford University Press. Oxford. 2010.

[8] D. Y. Karakoç, "Wetlands in Turkey" Ankara University 5th Environment Days International Symposium Abstract Booklet. 2017.

[9] İ. Uysal, and İ. Uysal, "Ornithofauna of Suvla Salt Lake (Çanakkale/Turkey) and the Diversity of Water Birds in Monthly Periods" Environmental Toxicology and Ecology, 1(1), 14-26. 2021.

[10] S. Gül, "Unwritten laws in Kızılırmak delta: The role of traditional ecological knowledge in the protection of a wetland" International Journal of Geography and Geography Education (IGGE), 42, 303-327. 2020.

[11] P. Dunne, "*Pete Dunne on Bird Watching"* Boston: Houghton Mifflin. ISBN 0-395-90686-5, 2003.

[12] W. B. Stapp, et al. "The Concept of Environmental Education" (PDF). The Journal of Environmental Education. 1 (1): 30–31. 1969.

[13] L. Fisman, "The Effects of Local Learning on Environmental Awareness in Children: An Empirical Investigation" The Journal of Environmental Education, 36:3, 39-50, DOI: 10.3200/JOEE.36.3.39-50. 2005.

[14] S. Anderson, and B. Moss, "How wetland habitats are perceived by children: consequences for children's education and wetland conservation" International Journal of Science Education. Volume 15 (5), pp. 473-485, 1993.

[15] Ç. Kılıçarslan, E. M. True, R. Tunalıoğlu, "A Research On Awareness Of The Unıversıty Students On Water Resources And Wetlands: Ege Unıversıty, Turkey Sample" 2 nd International Conference- Water resources and wetlands. 11-13 September, 2014 Tulcea (Romania).

[16] M. A. Weston, C. L. Tzaros, M. J. Antos, "Awareness of wetlands and their conservation value among students at a primary school in Victoria, Australia" Ecologıcal Management & Restoratıon Vol 7 No 3 December 2006. p:223. 2006.

[17] I. Ibrahim, N. Aminudin, M. A.Young, A. I. Yahya, "Education for Wetlands: Public Perception in Malaysia" Procedia - Social and Behavioral Sciences 42 (2012) 159 – 165. 2010.

[18] L. B. Christensen, R. B. Johnson, and L. A. Turner, "*Research methods, design, and analysis*" The USA, Boston: Pearson Education, Inc. 2014.

[19] R. L. Gorsuch, "Factor analysis" The USA: Saunders, 1974.

[20] B. S. Everitt, "Multivariate analysis: the need for data, and other problems" Br J Psychiatry; 126: 237-40. 1975.

[21] K. Krippendorff, "*Content analysis an introduction to its methodology"* The USA: Sage Publications. 2004.

[22] Ö. Çokluk, G. Şekercioğlu, and Ş. Büyüköztürk, "*Multivariate statistics for the social sciences*" Ankara: Pegem Academy. 2010.

[23] A. Field, "*Discovering statistics using IBM SPSS Statistics*" The USA: Sage. 2018.

[24] B. G. Tabachnick, and L. S. Fidell, "*Using multivariate statistics"* The USA: Pearson Education. 2013.

[25] R. M. Warner, "*Applied statistics, from bivariate through Multivariate Tecniques*" The USA: SAGE Publications, Inc. 2008.

[26] J.C. Anderson, and D.W. Gerbing, "The effect of sampling error on convergence, improper solutions, and goodness of fit indices for maximum likelihood confirmatory factor analysis" Psychometrika, 49(2), pp.155-173, 1984.

[27] P. M. Bentler, "Comparative fit indexes in structural models" Psychological Bulletin, 107(2), 238-246, 1990.

[28] D. Hooper, J. Coughlan, and M. R. Mullen, "Structural equation modelling: Guidelines for determining model fit" The Electronic Journal of Business Research Methods, 6(1), 53-60. 2008.

[29] L. T. Hu, and P. M. Bentler, "Cut off criteria for fit indexes in covariance structure analysis: Conventional criteria versus new alternatives." Structural Equation Modeling: A Multidisciplinary Journal, 6(1), 1-55. 1999.

[30] T. J. B. Kline, "*Psychological testing, a practical approach to design and evaluation*" The USA: Sage. 2005.

[31] H. W. Marsh, J. R., Balta and R. P. McDonald, "Goodness of fit indices in confirmatory factor analysis: The effect of sample size" Psychological Bulletin, 103(3), 391-410. 1988.

[32] K. Özdamar, "Statistical data analysis with package programs" 1st volume. Eskisehir: April. 2013.

[33] Ö. F. Şimşek, "*Yapısal eşitlik modellemesine giriş: Temel ilkeler ve LISREL uygulamaları*" İstanbul: Ekinoks. 2007.

[34] A. L. Vieira, "*Interactive LISREL in practice, getting started with a SIMPLIS Approach"* London: Springer. https://doi.org/10.1007/978-3-642-18044-6. 2011.

[35] S. Büyüköztürk, "*Manual of data analysis for social sciences*" Ankara: Pegem, 2013.

[36] J. F. Hair, W. C. Black, B. J. Babin, and R. E. Anderson, "*Multivariate data analysis"* The UK, Harlow: Pearson Education Limited. 2014.

[37] E. Tavşancıl, "Measuring Attitudes and Data Analysis with SPSS" Ankara: Nobel publishing house. 2002.

[38] R. Hart, "Children's participation: The theory and practice of involving young citizens in community development and environmental care" London: Earthscan. 1997.

[39] D. Sobel, "Ecophobia" Great Barrington, MA: The Orian Society. 1996.

[40] J. J. Vaske, and K. C. Kobrin, "Place attachment and environmentally responsible behavior" The Journal of Environmental Education, Vol. 32 No. 4, pp. 16-21. 2001.

[41] U. G. Şahin, "Investigation of Secondary School Students' Awareness of Biodiversity According to Various Variables" Post Graduate Facility, Antalya University, pp.75. 2018.

Appendix 1. Wetland Awareness Scale Through Children's Eyes (English)

Item No.	Wetland Awareness Scale Through Children's Eyes	I do not Agree	Partially Agree	I Agree
1	*Today, wetlands are damaged due to human activities.*			
2	*Wetlands are declining and this is worrying for our future.*			
3	We have to protect wetlands to protect the ecosystem we live in.			
4	*The importance of wetlands is not well known.*			
5	*The fact that there are usually many predators in wetlands negatively affects the animal and plant species there*			
6	*Due to global warming, our wetlands are gradually decreasing.*			
7	*As a result of hunting in wetlands, lead and pellets that settle at the bottom of the water accumulate and affect the wetland ecosystem by causing heavy metal pollution in the future.*			
8	*Cutting trees and burning reeds in wetlands cause pollution in wetlands.*			
9	*Our wetlands are rapidly disappearing.*			
10	*The number of infectious diseases increases if wetlands are reduced or polluted.*			
11	Wetlands are important in tackling global warming			
12	*If we see many birds in a wetland, this can be considered as an indicator of the healthy structure of the ecosystem in that wetland.*			
13	*The decrease in bird species, diversity and number is evidence of environmental pollution in that area.*			
14	*If I see or hear a wide variety of birds around me, I think that there is no environmental pollution where I am.*			

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Appendix 2. Wetland Awareness Scale Through Children's Eyes (Turkish)

Sıra	Çocukların Gözünden Sulak Alan Farkındalığı Ölçeği	Katılmıyorum	Kısmen Katılıyorum	Katılıyorum
1	*Günümüzde sulak alanlar insan faaliyetleri nedeniyle zarar görmektedir.*			
2	*Sulak alanlar giderek azalmaktadır ve bu durum geleceğimiz için endişe vericidir.*			
3	*Yaşadığımız ekosistemi korumak için sulak alanları korumak zorundayız.*			
4	*Sulak alanların önemi yeterince bilinmemektedir*			
5	*Sulak alanda çok sayıda avcı olması oradaki hayvan ve bitki türlerini olumsuz etkiler*			
6	*Küresel ısınma nedeniyle sulak alanlarımız giderek azalmaktadır*			
7	*Sulak alanlarda yapılan avcılık sonucu su dibinde çöken kurşun ve saçma parçaları birikerek gelecekte ağır metal kirliliğine yol açarak sulak alan ekosistemini etkilemektedir*			
8	*Ağaçların kesilmesi, sulak alandaki sazlıkların yakılması sulak alanlarda doğal yapının bozulmasına neden olur.*			
9	*Sulak alanlarımız hızla yok olmaktadır*			
10	*Sulak alanlarımız azalarısa ya da kirlenirse bulaşıcı hastalıkların sayısı artar*			
11	*Sulak alanlar küresel ısınmayla mücadelede önemlidir.*			
12	*Bir sulak alanda çok sayıda kuş görüyorsak, o sulak alandaki ekosistem sağlıklıdır.*			
13	*Sulak alanlarda yaşayan canlıların sayısının azalması çevre kirliliğine işarettir*			
14	*Etrafımda çok çeşitli kuşları görüyor ya da duyuyorsam bulunduğum yerde çevre kirliliği olmadığını düşünürüm.*			

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Effect of Amitriptyline on Serum Biochemistry of Gilthead Sea Bream (*Sparus aurata*)

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ABSTRACT

Amitriptyline is a tricyclic antidepressant that is frequently used in the treatment of many neuropathic and inflammatory diseases as well as depression. As a result of contamination to the aquatic environment, the effects of amitriptyline antidepressant, which has long lasting properties, on the serum biochemistry of gilthead sea bream fish was investigated in this study. For this purpose, gilthead sea bream were exposed to amitriptyline concentrations of 0.2 µg/L, 10 µg/L, 100 µg/L and 1000 µg/L for 14 days. Glucose, albumin, globulin, total protein, triglyceride, cholesterol, aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase and lactate dehydrogenase biochemical parameters were determined from the blood serum samples of the fish taken on the 7th and 14th days of the study. Significant changes occurred on the 14th day in the values of triglyceride, cholesterol, aspartate aminotransferase, alkaline phosphatase and lactate dehydrogenase, which did not show a significant difference on the 7th day. These results reveal that different amitriptyline concentrations cause negative changes in the fish biochemistry.

Amitriptilinin Çipura (*Sparus aurata*) Serum Biyokimyası Üzerindeki Etkisi

ÖZET

Amitriptilin, depresyonun yanında, birçok nöropatik ve inflamatuar hastalığın tedavisinde sıklıkla kullanılan trisiklik antidepresandır. Bu çalışmada, sucul ortama karışması sonucunda uzun süre kalıcılık gösterme özelliğine sahip olan amitriptilin antidepresanının çipura (*Sparus aurata*) balıklarının serum biyokimyasına olan etkisi araştırılmıştır. Bu amaçla çipura balıkları 14 gün boyunca 0,2 µg/L, 10 µg/L, 100 µg/L ve 1000 µg/L'lik amitriptilin konsantrasyonlarına maruz bırakılmıştır. Çalışmanın 7. ve 14. gününde alınan balıkların kan serum örneklerinden glukoz, albumin, globulin, total protein, trigliserid, kolesterol, aspartat aminotransferaz, alanin aminotransferaz, alkalen fosfataz ve laktat dehidrogenaz biyokimyasal parametreleri belirlenmiştir. 7. günde anlamlı bir farklılık göstermeyen tüm serum biyokimya parametrelerinden, trigliserid, kolesterol, aspartat aminotransferaz, alkalen fosfataz ve laktat dehidrogenaz değerlerinde 14. günde anlamlı değişimler meydana gelmiştir. Bu sonuçlar farklı amitriptilin konsantrasyonlarının balık biyokimyasında olumsuz yönde değişimlere neden olduğunu ortaya koymaktadır.

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1. INTRODUCTION

Amitriptyline is a tricyclic antidepressant that has been widely used in the world for many years due to its high efficacy and low cost [1-3]. Acting as a serotonin and noradrenaline reuptake inhibitor, amitriptyline is widely prescribed to treat depression, severe neuropathic and inflammatory diseases in both humans and animals [2-5]. It is known that amitriptyline ranked 56th (5.91 tonnes) among the 100 most consumed pharmaceutical compounds by mass in the UK in 2000 [6].

Studies of amitriptyline on aquatic organisms are very scarce [2]. However, it has been reported that amitriptyline is present in low detectable concentrations in aquatic environment [2,4]. Amitriptyline can be detected up to 72 ng/L in surface waters and up to 223 ng/L in wastewater treatment plant wastes [7-10]. Amitriptyline at concentrations as low as 10 ng/L can impair the immune system of aquatic organisms and affect their reproduction, growth and development [2,10-12]. Along with these findings, it is stated that amitriptyline has the potential to bioaccumulate and is observed up to 1.8 ng/g in aquatic organisms [9-10,13-14]. It has been reported that amitriptyline is one of the five substances that may cause problems on aquatic organisms in the future, based on accumulation and biodegradation in sewage treatment plants [6]. In addition, the classification of chronic aquatic toxicity of amitriptyline hydrochloride is indicated as very toxic effect, long-term persistence in the aquatic environment [15].

It has been reported that gilthead sea bream exposed to amitriptyline at doses of 0.2 µg/L and 10 µg/L for 7 days had residues in the brain and gill tissues, and 33 amitriptyline metabolites were encountered in gall, liver and plasma [10]. In another study, it was determined that sea bream exposed to 0.2 µg/L amitriptyline for 7 days had changes in the brain and liver metabolome [14].

In a study on investigating the effect of amitriptyline at 1, 10, 100 ng/L, 1, 10, 100 µg/L and 1 mg/L concentrations on zebrafish (*Danio rerio*) embryos, it was determined that both incubation time and body length in embryos were significantly reduced due to increasing concentrations of amitriptyline [2]. They also found that amitriptyline caused changes in physiological and biochemical parameters in zebrafish embryos [2]. In the mentioned study, it was stated that the negative effects of amitriptyline on fish embryos could be measured at concentrations as low as ng/L levels [2].

When the results of these studies are evaluated together, amitriptyline, which is used extensively in veterinary and medicine, can cause various problems on aquatic organisms with the increasing residue problem as a result of contamination to wastewater, however, it is seen that the studies in this field are quite limited. The aim of this study is to determine the biochemical effects of 0.2 µg/L, 10 µg/L, 100 µg/L and 1000 µg/L concentrations of amitriptyline antidepressant, which is reported to have long-term persistence in sea water and in the aquatic environment, on gilthead sea bream fish.

2. MATERIAL and METHODS

The fish, obtained from Çanakkale İda Gıda Tarımsal Üretim İç ve Dış Pazarlama A.Ş., were brought to Çanakkale Onsekiz Mart University Faculty of Marine Sciences and Technology Live Source Laboratory, where the study was carried out, for acclimatisation. At the end of the 2-week acclimatisation period, 150 *Sparus aurata* with an average weight of 20 ± 5 g (average mean ± S.D.) were used for the study. After an adaptation period, the fish were transferred into 15 tanks (180 L) as 10 for each group in triplicate. The natural sea water used for the experiment was transferred to the aquariums after the necessary filtration processes. The temperature, salinity and pH of the sea water was determined as 22±0.5°C, ‰26 and 8.04, respectively. Photoperiod was set to 14 hr:10 hr (light:dark). Required ethics committee approval was approved by Çanakkale Onsekiz Mart University Animal Experiments Local Ethics Committee with the decision numbered 2021/08-07.

2.1. Chemical Used in the Study and Its Application

Amitriptyline antidepressant was used in this study. For this purpose, a commercial preparation containing 28.30 mg of amitriptyline hydrochloride equivalent to 25.0 mg of amitriptyline was obtained. Dose range was determined and implemented in experimental aquariums as 0 µg/L(control), 0.2 µg/L, 10 µg/L, 100 µg/L and 1000 µg/L concentrations based on previous studies [2,10]. Water inlet and outlet were stopped in the aquariums for 14 days during the experiment.

On the 7^{th} and 14^{th} blood sampling days, fish were anesthetized with 20 mg/mL clove oil, which is a natural product and widely used [16]. Then, in order not to mix the blood with the mucous membrane, after thoroughly cleaning the posterior part of the anal fin with alcohol, approximately 500 µL of blood was collected by entering the caudal vein with an insulin injector without harming the fish. Blood samples were placed in serum tubes and centrifuged at 5000 g for 10 minutes. Obtained serum samples were stored at -80 °C until the time of biochemical analysis.

2.2. Biochemical Analysis

Biochemical analyzes of blood serum were performed spectrophotometrically using a commercial kit (Bioanalytic) used in fish experiments [17]. Glucose (GLU), albumin (ALB), globulin (GLO), total protein (TP), triglyceride (TG), cholesterol (CHO), Aspartate transaminase (AST), alanine aminotransaminase (ALT), alkaline phosphatase (ALP) and lactate dehydrogenase (LDH) biochemical parameters were determined.

2.3. Statistical Analysis

SPSS v.17 package program (SPSS Inc., Chicago, IL, USA) was used to statistical analyze of all data obtained from this study. Tukey test was performed at 95% confidence level to determine the differences between the groups.

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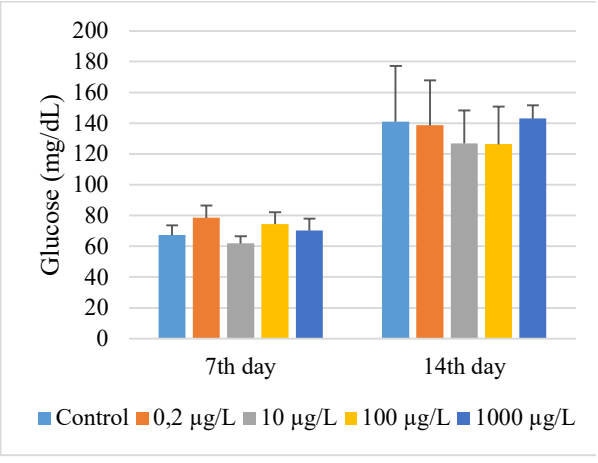
3. RESULTS and DISCUSSION

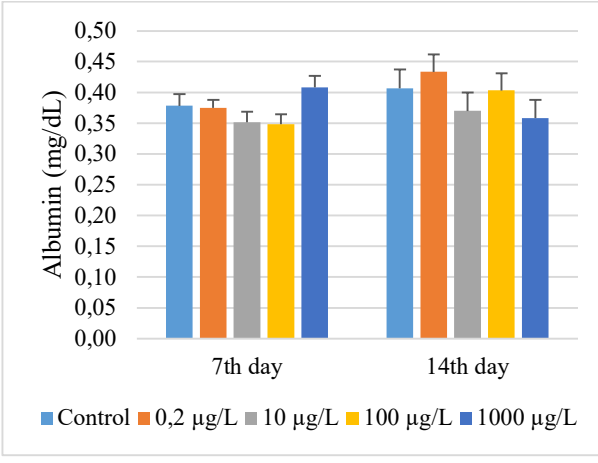
It is known that blood parameters of fish are affected by various factors as health status, season, age, water quality variables, sex, genetic characteristics, nutrition, transportation, other environmental factors, sampling and laboratory analysis methods [18-20]. While amitriptyline can be found at low concentrations in aquatic environments, it has been reported in various studies that it causes damage to aquatic organisms even at these concentrations [2,7-12]. The aim of this study is to reveal the changes in the biochemical parameters of gilthead sea bream, caused by amitriptyline antidepressant.

At the end of the study, the changes in biochemical parameters of GLU, ALB, GLO, TP, TG, CHO, AST, ALT, ALP and LDH from biochemical analyzes are given in Figure 1, respectively.

In this study, it was determined that GLU levels did not differ significantly on the 7th and 14th days compared to the control (P>0.05) (Fig. 1a). The amount of GLU in the blood is the most important parameter that determines the stress status of fish [21-22]. It is known that increased GLU affects stress hormones, such as adrenaline, in muscle and liver [22-23]. It has been reported that persistent hyperglycemia often causes slow growth in fish [22,24].



(a)



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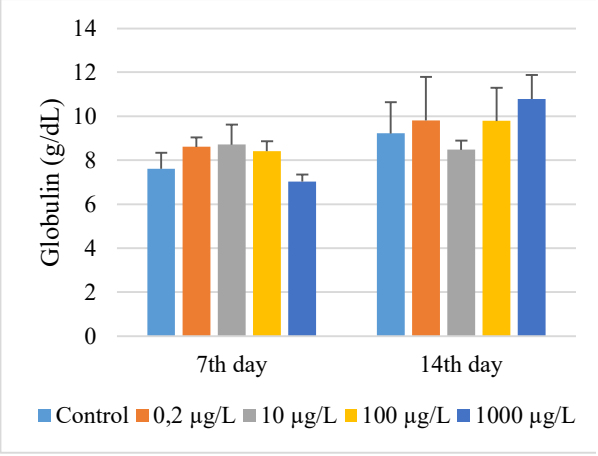

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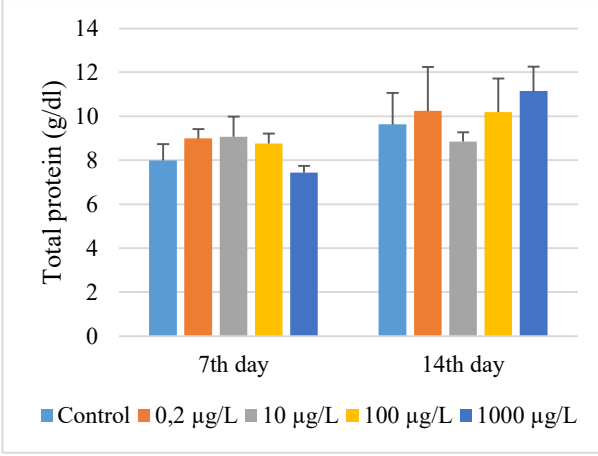

(d)

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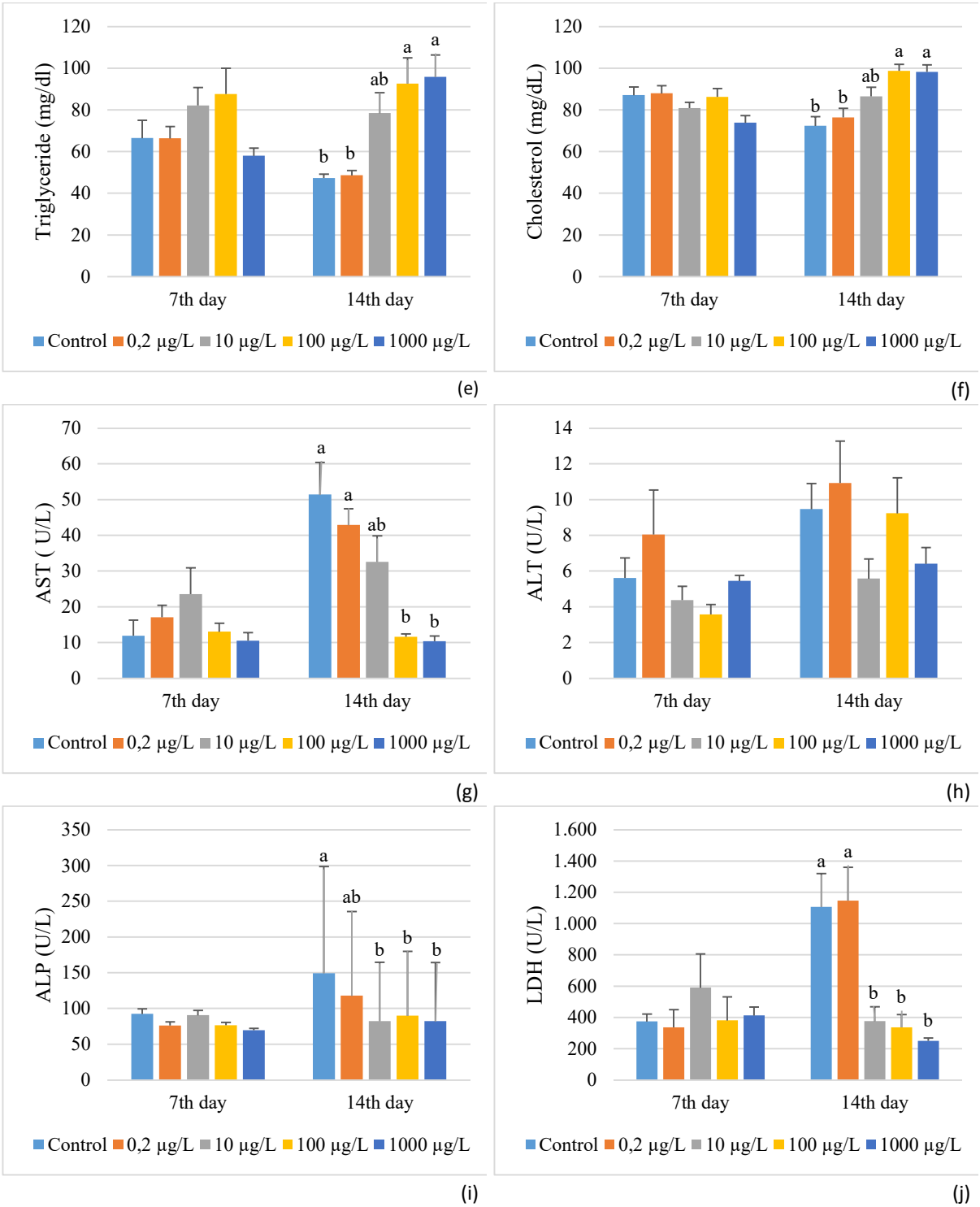

Figure 1. The serum parameters of gilthead sea bream exposed to amitriptyline on days 7th and 14th, a) GLU, b) ALB, c) GLO, d) TP, e) TG, f) CHO, g) AST, h) ALT, i) ALP and j) LDH (P<0,05)

According to our findings, there was no significant change in the GLO levels of gilthead sea bream exposed to amitriptyline compared to the control (Fig. 1c) (P>0.05). GLO, a protein that is insoluble in water but soluble in dilute salt water, is a simple protein that precipitates in a medium semi-saturated with ammonium sulfate or saturated with sodium and magnesium

sulfate [28]. In some immune system diseases, an increase in the amount of GLO occurs [28-29].

In the present study, no significant change was found between the groups in TP values on the 7th and 14th days (P>0.05) (Fig. 1d). TP in the blood is considered as an element of the non-specific immune system and provides information about the state of the immune system [22,27,30]. Serum protein is suppressed as a result of various stress sources and prolonged fasting conditions [21-22,26]. Therefore, the increase in TP will enable the fish to be more resistant to stress conditions.

Considering the TG values on day 14th of our study, it was determined that the doses of 100 µg/L and 1000 µg/L showed a significant increase compared to the control group (P<0.05) (Fig. 1e). With its high caloric value and low water content, TGs (triglycerides) are fatty acid esters of glycerol, where energy is actively stored in the fat storage [28,31]. There is a positive correlation between plasma TGs and CHO concentrations, which vary with age, gender, and diet [28,32]. TG values decrease in long-term fasting conditions [28,29].

In this study, there was no change in CHO values on the 7th day. A difference was detected in the 100 µg/L and 1000 µg/L dose groups compared to control and 0.2 µg/L doses on the 14th day and this difference was in the form of an increase (P<0.05) (Fig. 1f). CHO, a component of plasma membranes in all eukaryotic organisms, is essential for cell survival and growth in higher organisms. However, the buildup of CHO ester plaques can cause atherosclerosis, so excess CHO can be lethal. CHO is also a precursor to steroid hormones such as testosterone, progesterone, estradiol, and cortisol [33-34]. In a study on human CHO and fatty acid biosynthesis of antidepressant drugs, it was determined that antipsychotic drugs activate sterol regulatory element binding protein (SREBP) transcription factors in human and rat glial cells, resulting in upregulation of many downstream genes involved in CHO and fatty acid biosynthesis [35].

ALT, AST, ALP and LDH enzymes found in blood serum have been accepted as stress indicators and are widely used in the diagnosis of fish diseases and in the detection of tissue damage caused by environmental pollution [36]. As a result of this study, no change was observed in the AST, ALT, ALP and LDH values between all groups on the 7th day, while a decrease was observed in the AST, ALP and LDH values on the 14th day of the 100 µg/L and 1000 µg/L dose groups compared to the control group (P<0.05) (Fig. 1g, Fig. 1h, Fig. 1i, Fig.1j). These results showed that sea bream exposed to high concentrations of amitriptyline for 14 days became stressed. In a study of hematological and biochemical changes induced by the antidepressant amitriptyline in male rats, it has been reported that significant increases in ALP, AST, lipid profiles (CHO, TGs, LDL and HDL) in amitriptyline-treated rats compared to the control group [37].

Unlike our results (P>0.05), which found that amitriptyline caused significant changes in AST, ALP, LDH and cholesterol values in gilthead sea bream, in a study on examining the changes in biochemical parameters of trout (*Oncorhynchus mykiss*) as a result of contamination of sertraline antidepressant with water from food sources, no significant difference was found between the control and experimental groups for these parameters [38]. Similar to our results,

it was also stated that there was no dose-related change in GLU, ALB and TP values, the difference between the control and experimental groups was insignificant [38]. In a study on investigating the effect of amitriptyline on the liver of male rats, it was determined that high dose of amitriptyline increased affect the liver activity by increasing all liver enzymes due to the cytotoxic effects of amitriptyline [37,39].

1. CONCLUSIONS and FUTURE OUTLOOK

As a result, according to the findings obtained in this study, it was determined that there were significant changes in some serum biochemical parameters of fish exposed to amitriptyline antidepressant for 14 days. We think that there is a need to investigate the physiological, hematological and genotoxic effects of amitriptyline in fish in future studies.

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The Declaration of Conflict of Interest/Common Interest

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The Declaration of Ethics Committee Approval

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The Declaration of Research and Publication Ethics

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

REFERENCES

[1] C. Barbui, M. Hotopf, "Amitriptyline v. the rest: still the leading antidepressant after 40 years of randomised controlled trials" Br J Psychiatry, vol. 178, pp. 129-144, Feb 2001, doi: 10.1192/bjp.178.2.129. PMID: 11157426.

[2] M. Yang, W. Qiu, J. Chen, J. Zhan, C. Pan, X. Lei, M. Wu, "Growth inhibition and coordinated physiological regulation of zebrafish (*Danio rerio*) embryos upon sublethal exposure to antidepressant amitriptyline". Aquat Toxicol., vol. 151, pp 68-76, Jun 2014

[3] K.A. Demin, T.O Kolesnikova., S.L. Khatsko, D.A. Meshalkina, E.V. Efimova, Y.Y. Morzherin, A.V. Kalueff, "Acute effects of amitriptyline on adult zebrafish: Potential relevance to antidepressant drug screening and modeling human toxidromes" Neurotoxicol Teratol., vol. 62, pp. 27-33, Jul 2017, doi: 10.1016/j.ntt.2017.04.002. Epub 2017 Apr 22. PMID: 28438663.

[4] V. Calisto, V.I. Esteves, "Psychiatric pharmaceuticals in the environment". Chemosphere, vol: 77 (10), pp. 1257–1274, 2009.

[5] M.R. Bautista-Ferrufino, M.D. Cordero, J.A. Sánchez-Alcázar, M. Illanes, A. Fernández-Rodríguez, P. Navas, M. de Miguel, "Amitriptyline induces coenzyme Qdeficiency and oxidative damage in mouse lung and liver" Toxicol. Lett., vol. 204 (1), pp. 32–37, 2011.

[6] I. M. Sebastine, R. J. Wakeman. "Consumption and environmental hazards of pharmaceutical substances in the UK." Process Safety and Environmental Protection vol. 81.4, pp. 229-235, 2003.

[7] A. Lajeunesse, C. Gagnon, S. Sauvé, "Determination of Basic Antidepressants and Their N-Desmethyl Metabolites in Raw Sewage and Wastewater Using Solid-Phase Extraction and Liquid Chromatography−Tandem Mass Spectrometry". Anal. Chem., vol. 80 (14), pp. 5325–5333, 2008, doı: 10.1021/ac800162q

[8] D. R. Baker, B. Kasprzyk-Hordern, "Multi-residue analysis of drugs of abuse in wastewater and surface water by solid-phase extraction and liquid chromatography– positive electrospray ionisation tandem mass spectrometry". J. Chromatogr., vol. 1218 (12), pp. 1620–1631, 2011, doı: 10.1016/j.chroma.2011.01.060

[9] H. Ziarrusta, L. Mijangos, A. Prieto, N. Etxebarria, O. Zuloaga, M. Olivares, "Determination of tricyclic antidepressants in biota tissue and environmental waters by liquid chromatography-tandem mass spectrometry". Anal. Bioanal. Chem., vol., 408 (4), pp. 1205–1216, 2016. doı: 10.1007/s00216-015-9224-y.

[10] H. Ziarrusta, L. Mijangos, U. Izagirre, M.M. Plassmann, J.P. Benskin, E. Anakabe, M. Olivares, O. Zuloaga, "Bioconcentration and biotransformation of amitriptyline in gilthead bream". Environmental science & technology, vol. 51(4), pp. 2464-2471, 2017.

[11] P. P. Fong, A. T. Ford, "The biological effects of antidepressants on the molluscs and crustaceans: A review". Aquat. Toxicol., vol. 151, pp. 4−13, 2014.

[12] L. Minguez, E. Farcy, C. Ballandonne, A. Lepailleur, A. Serpentini, J-M. Lebel, R. Bureau, M-P. Halm-Lemeille, "Acute toxicity of 8 antidepressants: What are their modes of action?" Chemosphere, vol. 108, pp. 314−319, 2014.

[13] S.L. Klosterhaus, R. Grace, M.C. Hamilton, and D. Yee, "Method validation and reconnaissance of pharmaceuticals, personal care products, and alkylphenols in surface waters, sediments, and mussels in an urban estuary" Environment international, vol. 54, pp.92-99, 2013.

[14] H. Ziarrusta, A. Ribbenstedt, L. Mijangos, S. Picart-Armada, A. Perera-Lluna, A. Prieto, U. Izagirre, JP Benskin, M. Olivares, O. Zuloaga, N. Etxebarria, "Amitriptyline at an Environmentally Relevant Concentration Alters the Profile of Metabolites Beyond Monoamines in Gilthead Bream". Environ Toxicol Chem., vol. 38(5): pp. 965-977, May 2019. doi: 10.1002/etc.4381. Epub 2019 Apr 2. PMID: 30702171.

[15] https://assets.lgcstandards.com/sys-master%2Fpdfs%2Fh17%2Fhb6%2F10373775786014%2FSDS_BP+016_ST-WB-MSDS-3547340-1-1-1.PDF (Erişim Tarihi: 01.03.2022)

[16] M. Iversen, B. Finstad, R. S. McKinley, R.A. Eliassen, "The efficacy of metomidate, clove oil, Aqui-S™ and Benzoak® as anaesthetics in Atlantic salmon (*Salmo salar* L.) smolts, and their potential stress-reducing capacity". Aquaculture, vol. 221: pp. 549-566, 2003.

[17] S. Yılmaz, S. Ergün, "Effects of garlic and ginger oils on hematological and biochemical variables of sea bass *Dicentrarchus labrax"*. Journal of Aquatic Animal Health, vol. 24: pp. 219-224, 2012.

[18] C.E. Bond,. "Circulation, respiration, and the gas bladder". In C. E. Bond (ed.) Biology of Fishes. W. B Saunders Company Press, London, UK, 1979, pp. 347-374.

[19] G. Rey Vázquez, G.A. Guerrero, "Characterization of blood cells and hematological parameters in *Cichlasoma dimerus* (Teleostei, Perciformes)". Tissue and Cell, vol. 39, pp. 151-160, 2007.

[20] M. Kanyılmaz, N. Tekellioğlu, "Effects of dietary zeolite levels on some blood parameters of gilthead seabream (*Sparus aurata*) juveniles". Journal of Aquaculture Engineering and Fisheries Research, vol. 2(3), pp. 119-127, 2016.

[21] D.G. McDonald, C.L. Milligan, "Chemical Properties of the Blood". Academic Press, Inc., California. pp. 56-113, 1992.

[22] O. Demir, "Tilapia Oreochromis mossambicus (Peters, 1852) Balıklarının Yemlerinde Balık Yağı Yerine Yerfıstığı Yağı Kullanılmasının Büyüme Performansı, Yem Değerlendirme, Vücut Kompozisyonu Ve Serum Biyokimyasına Etkisi", Muğla Sıtkı Koçman Üniversitesi Fen Bilimleri Enstitüsü Su Ürünleri Mühendisliği Anabilim Dalı, 2019, Muğla.

[23] J.D. Morgan, G.K. Iwama, " Measurements of Stressed States in the Field". Cambridge University Press, Cambridge. pp. 247-270, 1997.

[24] T.W. Moon, "Glucose Intolerance in Teleost Fish: Fact or Fiction?". Comp Biochem Phys B, vol. 129: pp. 243-249, 2001

[25] G.G. Gunter, L. L Sulya, B. E. Box, "Some Evolutionary Patterns in Fishes' Blood". Biological Bulletin. pp. 121. 1961, 10.2307/1539434.

[26] G.H. Satchell, "Physiology and Form of Fish Circulation" Cambridge University Press, Cambridge. 1991, pp. 235.

[27] G.F.Wiegertjes, R.J.M. Stet, H.K. Parmentier, W.B. Van Muiswinkel, "Immunogenetics of disease resistance in Changes in biochemical, histological and specific immune parameters 151 fish; a comparable approach". Dev. Comp. Immunol. 20, pp. 365–381, 1996.

[28] E. Ş. Çelik, S. Bilgin, "Bazı balık türleri için kan protein ve lipidlerinin standardizasyonu". Erciyes Üniversitesi Fen Bilimleri Enstitüsü Fen Bilimleri Dergisi, vol. *23*(1), pp. 215-229, 2007.

[29] S. Mayer, "A review of the scientific justifications for Maintaining cetaceans in captivity, (edit. By Frances Clarke), A report for the whale and dolphin conservation society (WDCS)", 35 p., 1998

[30] B. Magnadottir, "Innate Immunity of Fish (overview)". Fish Shellfish Immun, vol. 20(2): pp. 137–151, 2006.

[31] M.F. Laker, "Klinik Biyokimya" (Ulukaya, E., Tokullugil A., Gür, E., Dirican, M., Tuncel, P., Ulukaya, E., vd. çev.), s. 343, Uludağ Üniv. Tıp Fak. Biyokimya ABD., Nobel Tıp Kitapevleri Ltd.Şti., İstanbul, 1996.

[32] H. Karagül, A. Altıntaş, U.R. Fidancı, T. Sel, "Klinik Biyokimya", s. 430, Cilt 1, Medisan Yayın Evi, Yayın No: 45, Ankara, 2000.

[33] L. Stryer, "Biochernistry", Freeman and Company, San Francisco, pp 470-476. 1975.

[34] M. Atamanalp, M. Yilmaz, H.İ. Haliloğlu, "Comparing Serum Cholesterol Types and Levels of Three Trout Species (*Salvelinus alpinus, Salmo trutta fario and Oncorhynchus mykiss*)", Journal of Applied Animal Research, vol. 23:2, pp. 223-226, 2003. doi: 10.1080/09712119.2003.9706425

[35] M. B. Raeder, J. Fernø, M.Glambek, C.Stansberg, & V. M. Steen, "Antidepressant drugs activate SREBP and up-regulate cholesterol and fatty acid biosynthesis in human glial cells." Neuroscience letters, 395(3), 185-190, 2006.

[36] Ö. Fırat, H.Y. Cogun, T.A. Yüzereroğlu, G. Gök, Ö. Fırat, F. Kargin, Y. Kötemen, "A comparative study on the effects of a pesticide (cypermethrin) and two metals (copper, lead) to serum biochemistry of Nile tilapia, *Oreochromis niloticus*". Fish Physiology and Biochemistry, vol. *37*(3), pp. 657-666, 2011

[37] A. El Atrash, E. Tousson, A. Gad, & S.Allam, "Hematological and biochemical changes caused by antidepressants amitriptyline induced cardiac toxicity in male rats". Asian Journal of Cardiology Research, 23, 1-6, 2019.

[38] J. Vaclavik, P. Sehonova, N. Hodkovicova, L. Vecerkova, J. Blahova, A. Frangi, P. Marsalek, J. Mares, F. Tichy, Z. Svobodova, C. Faggio, "The effect of foodborne sertraline on rainbow trout (*Oncorhynchus mykiss*)". Science of the Total Environment, vol. 708, 135082, 2020

[39] M. Afify, M.D.E. Abd Elmaksoud, T. Mosa, M. Elshaer, N. Kotb, "Differential effects of amitriptyline treatment on testicular and liver functions". New York Science Journal,3(3): pp. 10-182010