ISSN: 2667-4203

ESKİŞEHİR TECHNICAL UNIVERSITY JOURNAL OF SCIENCE AND TECHNOLOGY
C– Life Sciences and Biotechnology

ESKİŞEHİR TEKNİK ÜNİVERSİTESİ BİLİM VE TEKNOLOJİ DERGİSİ
C – Yaşam Bilimleri ve Biyoteknoloji

Volume/Cilt 12 Number/Sayı 1 January / Ocak - 2023

ESKİŞEHİR TECHNICAL UNIVERSITY JOURNAL OF SCIENCE AND TECHNOLOGY
C- Life Sciences and Biotechnology
Eskişehir Teknik Üniversitesi Bilim ve Teknoloji Dergisi C – Yaşam Bilimleri ve Biyoteknoloji

Volume: 12 / Number: 1 / January - 2023

Eskişehir Technical University Journal of Science and Technology C – Life Sciences and Biotechnology (formerly Anadolu University Journal of Science and Technology C – Life Sciences and Biotechnology) is an **peer-reviewed** and **refereed international journal** by Eskişehir Technical University. Since 2010, it has been regularly published and distributed biannually and it has been published biannually and **electronically only since 2016**.

Manuscripts submitted for publication are analyzed in terms of scientific quality, ethics and research methods in terms of its compliance by the Editorial Board representatives of the relevant areas. Then, the abstracts of the appropriate articles are sent to two different referees with a well-known in scientific area. If the referees agree to review the article, full text in the framework of the privacy protocol is sent. In accordance with the decisions of referees, either directly or corrected article is published or rejected. Confidential reports of the referees in the journal archive will be retained for ten years. All post evaluation process is done electronically on the internet. Detailed instructions to authors are available in each issue of the journal.

"Anadolu Üniversitesi Bilim ve Teknoloji Dergisi C- Yaşam Bilimleri ve Biyoteknoloji (Anadolu University Journal of Science and Technology C – Life Sciences and Biotechnology)" published within Anadolu University started to be published within Eskişehir Technical University which was established due to statute law 7141, in 2018. Hence, the name of the journal is changed to "Eskişehir Teknik Üniversitesi Bilim ve Teknoloji Dergisi C- Yaşam Bilimleri ve Biyoteknoloji (Eskişehir Technical University Journal of Science and Technology C – Life Sciences and Biotechnology)".

Indexed by **DOAJ** - Directory of Open Access Journals, **EBSCO** and **ULAKBIM**

ISSN: 2667-4203

ESKİŞEHİR TECHNICAL UNIVERSITY JOURNAL OF SCIENCE AND TECHNOLOGY
C- Life Sciences and Biotechnology
Eskişehir Teknik Üniversitesi Bilim ve Teknoloji Dergisi C – Yaşam Bilimleri ve Biyoteknoloji

Volume: 12 / Number: 1 / January – 2023

Owner / Publisher: Prof. Dr. Tuncay DÖĞEROĞLU for Eskişehir Technical University

CONTACT INFORMATION
Eskişehir Technical Unıversity Journal of Science and Technology
Eskişehir Technical University, Institute of Graduate Programs, 26470 Eskişehir, TURKEY
Phone: +90 222 213 7485
e-mail : btda@eskisehir.edu.tr

ESKİŞEHİR TECHNICAL UNIVERSITY JOURNAL OF SCIENCE AND TECHNOLOGY
C- Life Sciences and Biotechnology
Eskişehir Teknik Üniversitesi Bilim ve Teknoloji Dergisi C – Yaşam Bilimleri ve Biyoteknoloji

Volume: 12 / Number: 1 / January – 2023

ABOUT

Eskişehir Technical University Journal of Science and Technology C- Life Sciences and Biotechnology (formerly Anadolu University Journal of Science and Technology C - Life Sciences and Biotechnology) is an peer-reviewed and refereed international journal by Eskişehir Technical University. Since 2010, it has been regularly published and distributed biannually and it has been published biannually and electronically only since 2016.

The journal issues are published electronically in **JANUARY** and **JULY.**

- **The journal accepts TURKISH and ENGLISH manuscripts.**

AIM AND SCOPE

The journal publishes high quality original research papers, reviews and technical notes in the fields of life sciences: All aspects of biology such as taxonomy, physiology, biochemistry, ecology, environmental biology, biophysics, genetic, toxicology, biodiversity and biotechnology and, agricultural science, health sciences, biomedical sciences and pharmacy.

PEER REVIEW PROCESS

Manuscripts are first reviewed by the editorial board in terms of its its journal's style rules scientific content, ethics and methodological approach. If found appropriate, the manuscript is then send to at least two referees by editor. The decision in line with the referees may be an acceptance, a rejection or an invitation to revise and resubmit. Confidential review reports from the referees will be kept in archive. All submission process manage through the online submission systems.

OPEN ACCESS POLICY

This journal provides immediate open access to its content on the principle that making research freely available to the public supports a greater global exchange of knowledge.

The journal doesn't have Article Processing Charge (APC) or any submission charges.

ETHICAL RULES

You can reach the Ethical Rules in our journal in full detail from the link below:
https://dergipark.org.tr/tr/pub/estubtdc/policy

Ethical Principles and Publication Policy

Policy & Ethics

Assessment and Publication

As a peer-reviewed journal, it is our goal to advance scientific knowledge and understanding. We adhere to the guideline and ethical standards from the Committee on Publication Ethics (COPE) and the recommendations of ICMJE (International Committee of Medical Journal Editors) regarding all aspects of publication ethics and cases of research and publication misconduct to ensure that all publications represent accurate and original work and that our peer review process is structured without bias. We have outlined a set of ethical principles that must be followed by all authors, reviewers, and editors.

All manuscripts submitted to our journals are pre-evaluated in terms of their relevance to the scope of the journal, language, compliance with writing instructions, suitability for science, and originality, by taking into account the current legal requirements regarding copyright infringement and plagiarism. Manuscripts that are evaluated as insufficient or non-compliant with the instructions for authors may be rejected without peer review.

Editors and referees who are expert researchers in their fields assess scientific manuscripts submitted to our journals. A blind peer review policy is applied to the evaluation process. The Editor-in-Chief, if he/she sees necessary, may assign an Editor for the manuscript or may conduct the scientific assessment of the manuscript himself/herself. Editors may also assign referees for the scientific assessment of the manuscript and make their decisions based on reports by the referees. The Editor-in-Chief makes the final decision regarding the publishing of the manuscript.

Articles are accepted for publication by the Editor-in-Chief in accordance with the COPE (Committee on Publication Ethics). Authors can access this information online via the journals' websites (https://publicationethics.org/). Articles are accepted for publication on the understanding that they have not been published and are not going to be considered for publication elsewhere. Authors should certify that neither the manuscript nor its main contents have already been published or submitted for publication in another journal.

The journal adapts the COPE guidelines to satisfy the high-quality standards of ethics for authors, editors, and reviewers:

Duties of Editors-in-Chief and co-Editors

The crucial role of the journal Editor-in-Chief and co-Editors is to monitor and ensure the fairness, timeliness, thoroughness, and civility of the peer-review editorial process. The main responsibilities of Editors-in-Chief are as follows:

• Selecting manuscripts suitable for publication while rejecting unsuitable manuscripts,
• Ensuring a supply of high-quality manuscripts to the journal by identifying important,
• Increasing the journal's impact factor and maintaining the publishing schedule,
• Providing strategic input for the journal's development,

Duties of Editors

The main responsibilities of editors are as follows:

• An editor must evaluate the manuscript objectively for publication, judging each on its quality without considering the nationality, ethnicity, political beliefs, race, religion, gender, seniority, or institutional affiliation of the author(s). Editors should decline any assignment when there is a potential for conflict of interest.
• Editors must ensure the document(s) sent to the reviewers does not contain information of the author(s) and vice versa.
• Editors' decisions should be provided to the author(s) accompanied by the reviewers' comments and recommendations unless they contain offensive or libelous remarks.
• Editors should respect requests (if well reasoned and practicable) from author(s) that an individual should not review the submission.
• Editors and all staff members should guarantee the confidentiality of the submitted manuscript.

• Editors should have no conflict of interest with respect to articles they reject/accept. They must not have a conflict of interest with the author(s), funder(s), or reviewer(s) of the manuscript.
• Editors should strive to meet the needs of readers and authors and to constantly improve the journal.

Duties of Reviewers/Referees

The main responsibilities of reviewers/referees are as follows:

• Reviewers should keep all information regarding papers confidential and treat them as privileged information.
• Reviews should be conducted objectively, with no personal criticism of the author.
• Reviewers assist in the editorial decision process and as such should express their views clearly with supporting arguments.
• Reviewers should complete their reviews within a specified timeframe (maximum thirty-five (35) days). In the event that a reviewer feels it is not possible for him/her to complete the review of the manuscript within a stipulated time, then this information must be communicated to the editor so that the manuscript could be sent to another reviewer.
• Unpublished materials disclosed in a submitted manuscript must not be used in a reviewer's personal research without the written permission of the author. Information contained in an unpublished manuscript will remain confidential and must not be used by the reviewer for personal gain.
• Reviewers should not review manuscripts in which they have conflicts of interest resulting from competitive, collaborative, or other relationships or connections with any of the authors, companies, or institutions connected to the papers.
• Reviewers should identify similar work in published manuscripts that has not been cited by the author. Reviewers should also notify the Editors of significant similarities and/or overlaps between the manuscript and any other published or unpublished material.

Duties of Authors

The main responsibilities of authors are as follows:

• The author(s) should affirm that the material has not been previously published and that they have not transferred elsewhere any rights to the article.
• The author(s) should ensure the originality of the work and that they have properly cited others' work in accordance with the reference format.
• The author(s) should not engage in plagiarism or in self-plagiarism.
• On clinical and experimental humans and animals, which require an ethical committee decision for research in all branches of science;
All kinds of research carried out with qualitative or quantitative approaches that require data collection from the participants by using survey, interview, focus group work, observation, experiment, interview techniques,
Use of humans and animals (including material/data) for experimental or other scientific purposes,
• Clinical studies on humans,
• Studies on animals,
• Retrospective studies in accordance with the law on the protection of personal data, (Ethics committee approval should have been obtained for each individual application, and this approval should be stated and documented in the article.)
Information about the permission (board name, date, and number) should be included in the "Method" section of the article and also on the first/last page.

During manuscript upload, the "Ethics Committee Approval" file should be uploaded to the system in addition to the manuscript file.
In addition, in case reports, it is necessary to include information on the signing of the informed consent/ informed consent form in the manuscript.
• The author(s) should suggest no personal information that might make the identity of the patient recognizable in any form of description, photograph, or pedigree. When photographs of the patient were essential and indispensable as scientific information, the author(s) have received consent in written form and have clearly stated as much.
• The author(s) should provide the editor with the data and details of the work if there are suspicions of data falsification or fabrication. Fraudulent data shall not be tolerated. Any manuscript with suspected fabricated or falsified data will not be accepted. A retraction will be made for any publication which is found to have included fabricated or falsified data.
• The author(s) should clarify everything that may cause a conflict of interests such as work, research expenses, consultant expenses, and intellectual property.
• The author(s) must follow the submission guidelines of the journal.
• The author(s) discover(s) a significant error and/or inaccuracy in the submitted manuscript at any time, then the error and/or inaccuracy must be reported to the editor.
• The author(s) should disclose in their manuscript any financial or other substantive conflicts of interest that might be construed to influence the results or interpretation of their manuscript. All sources of financial support should be disclosed under the heading of "Acknowledgment" or "Contribution".
• The corresponding author should ensure that all appropriate co-authors and no inappropriate co-authors are included in the paper and that all co-authors have seen and approved the final version of the paper and have agreed to its submission for publication. All those who have made significant contributions should be listed as co-authors. Others who have participated in certain substantive aspects of the research should be acknowledged or listed under the heading of "Author Contributions".

Cancellations/Returns
Articles/manuscripts may be returned to the authors in order to increase the authenticity and/or reliability and to prevent ethical breaches, and even if articles have been accepted and/or published, they can be withdrawn from publication if necessary. The Editor-in-Chief of the journal has the right to return or withdraw an article/manuscript in the following situations:

• When the manuscript is not within the scope of the journal,
• When the scientific quality and/or content of the manuscript do not meet the standards of the journal and a referee review is not necessary,
• When there is proof of ruling out the findings obtained by the research, (When the article/manuscript is undergoing an assessment or publication process by another journal, congress, conference, etc.,)
• When the article/manuscript was not prepared in compliance with scientific publication ethics,
• When any other plagiarism is detected in the article/manuscript,
• When the authors do not perform the requested corrections within the requested time (maximum twenty-one (21) days),
• When the author does not submit the requested documents/materials/data etc. within the requested time,
• When the requested documents/materials/data etc. submitted by the author are missing for the second time,
• When the study includes outdated data,

• When the authors make changes that are not approved by the editor after the manuscript was submitted,
• When an author is added/removed, the order of the authors is changed, the corresponding author is changed, or the addresses of the authors are changed without the consent of the Editor-in-Chief,
• When a statement is not submitted indicating that approval of the ethics committee permission was obtained for the following (including retrospective studies):
• When human rights or animal rights are violated,

ETHICAL ISSUES

Plagiarism

The use of someone else's ideas or words without a proper citation is considered plagiarism and will not be tolerated. Even if a citation is given, if quotation marks are not placed around words taken directly from other authors' work, the author is still guilty of plagiarism. Reuse of the author's own previously published words, with or without a citation, is regarded as self-plagiarism.

All manuscripts received are submitted to iThenticate®, which compares the content of the manuscript with a database of web pages and academic publications. Manuscripts are judged to be plagiarized or self-plagiarized, based on the iThenticate® report or any other source of information, will be rejected. Corrective actions are proposed when plagiarism and/or self-plagiarism is detected after publication. Editors should analyze the article and decide whether a corrected article or retraction needs to be published.

Open-access theses are considered as published works and they are included in the similarity checks.

iThenticate® report should have a maximum of 11% from a single source, and a maximum of 25% in total.

Conflicts of Interest

Eskişehir Technical University Journal of Science and Technology A - Applied Sciences and Engineering should be informed of any significant conflict of interest of editors, authors, or reviewers to determine whether any action would be appropriate (e.g. an author's statement of conflict of interest for a published work, or disqualifying a referee).

Financial

The authors and reviewers of the article should inform the journal about the financial information that will bring financial gain or loss to any organization from the publication of the article.

*Research funds; funds, consulting fees for a staff member; If you have an interest, such as patent interests, you may have a conflict of interest that needs to be declared.

Other areas of interest

The editor or reviewer may disclose a conflict of interest that, if known, would be embarrassing (for example, an academic affiliation or rivalry,a close relationship or dislike, or a person who may be affected by the publication of the article).

Conflict of interest statement

Please note that a conflict of interest statement is required for all submitted manuscripts. If there is no conflict of interest, please state "There are no conflicts of interest to declare" in your manuscript under the heading "Conflicts of Interest" as the last section before your Acknowledgments.

AUTHOR GUIDELINES

All manuscripts must be submitted electronically.
You will be guided stepwise through the creation and uploading of the various files. There are no page charges. Papers are accepted for publication on the understanding that they have not been published and are not going to be considered for publication elsewhere. Authors should certify that neither the manuscript nor its main contents have already been published or submitted for publication in another journal. We ask a signed **Copyright Form** to start the evaluation process. After a manuscript has been submitted, it is not possible for authors to be added or removed or for the order of authors to be changed. If authors do so, their submission will be cancelled.

Manuscripts may be rejected without peer review by the editor-in-chief if they do not comply with the instructions to authors or if they are beyond the scope of the journal. After a manuscript has been accepted for publication, i.e. after referee-recommended revisions are complete, the author will not be permitted to make any changes that constitute departures from the manuscript that was accepted by the editor. Before publication, the galley proofs are always sent to the authors for corrections. Mistakes or omissions that occur due to some negligence on our part during final printing will be rectified in an errata section in a later issue.

This does not include those errors left uncorrected by the author in the galley proof. The use of someone else's ideas or words in their original form or slightly changed without a proper citation is considered plagiarism and will not be tolerated. Even if a citation is given, if quotation marks are not placed around words taken directly from another author's work, the author is still guilty of plagiarism. All manuscripts received are submitted to iThenticateR, a plagiarism checking system, which compares the content of the manuscript with a vast database of web pages and academic publications. In the received iThenticateR report; The similarity rate is expected to be below 25%. Articles higher than this rate will be rejected.

Uploading Articles to the Journal

Authors should prepare and upload 2 separate files while uploading articles to the journal. First, the Author names and institution information should be uploaded so that they can be seen, and then (using the additional file options) a separate file should be uploaded with the Author names and institution information completely closed. When uploading their files with closed author names, they will select the "Show to Referee" option, so that the file whose names are closed can be opened to the referees.

Preparation of Manuscript

Style and Format: Manuscripts should be **single column** by giving one-spaced with 2.5-cm margins on all sides of the page, in Times New Roman font (font size 11). Every page of the manuscript, including the title page, references, tables, etc., should be numbered. All copies of the manuscript should also have line numbers starting with 1 on each consecutive page.

Manuscripts must be upload as word document (*.doc, *.docx vb.). **Please avoid uploading *texts in *.pdf format.***

Symbols, Units and Abbreviations: Standard abbreviations and units should be used; SI units are recommended. Abbreviations should be defined at first appearance, and their use in the title and abstract should be avoided. Generic names of chemicals should be used. Genus and species names should be typed in italic or, if this is not available, underlined.

Please refer to equations with capitalisation and unabbreviated (e.g., as given in Equation (1)).

Manuscript Content: Articles should be divided into logically ordered and numbered sections. Principal sections should be numbered consecutively with Arabic numerals (1. Introduction, 2. Formulation of problem, etc.) and subsections should be numbered 1.1., 1.2., etc. Do not number the Acknowledgements or References sections. The text of articles should be, if possible, divided into the following sections: Introduction, Materials and Methods (or Experimental), Results, Discussion, and Conclusion.

Title and contact information

The first page should contain the full title in sentence case (e.g., Hybrid feature selection for text classification), the full names (last names fully capitalised) and affiliations (in English) of all authors (Department, Faculty, University, City, Country, E-mail), and the contact e-mail address for the clearly identified corresponding author.

Abstract

The abstract should provide clear information about the research and the results obtained, and should not exceed 300 words. The abstract should not contain citations and must be written in Times New Roman font with font size 9.

Keywords

Please provide 3 to 5 keywords which can be used for indexing purposes.

Introduction

The motivation or purpose of your research should appear in the "Introduction", where you state the questions you sought to answer, and then provide some of the historical basis for those questions.

Methods

Provide sufficient information to allow someone to repeat your work. A clear description of your experimental design, sampling procedures, and statistical procedures is especially important in papers describing field studies, simulations, or experiments. If you list a product (e.g., animal food, analytical device), supply the name and location of the manufacturer. Give the model number for equipment used.

Results

Results should be stated concisely and without interpretation.

Discussion

Focus on the rigorously supported aspects of your study. Carefully differentiate the results of your study from data obtained from other sources. Interpret your results, relate them to the results of previous research, and discuss the implications of your results or interpretations.

Conclusion
This should state clearly the main conclusions of the research and give a clear explanation of their importance and relevance. Summary illustrations may be included.

Acknowledgments
Acknowledgments of people, grants, funds, etc. should be placed in a separate section before the reference list. The names of funding organizations should be written in full.

Conflict of Interest Statement
The authors are obliged to present the conflict of interest statement at the end of the article after the acknowledgments section.

References
AMA Style should be used in the reference writing of our journal. If necessary, at this point, the reference writings of the articles published in our article can be examined.

Citations in the text should be identified by numbers in square brackets. The list of references at the end of the paper should be given in order of their first appearance in the text or in alphabetical order according to the surname of the fist author. All authors should be included in reference lists unless there are 10 or more, in which case only the first 10 should be given, followed by 'et al.'. Do not use individual sets of square brackets for citation numbers that appear together, e.g., [2, 3, 5–9], not [2], [3], [5]–[9]. Do not include personal communications, unpublished data, websites, or other unpublished materials as references, although such material may be inserted (in parentheses) in the text. In the case of publications in languages other than English, the published English title should be provided if one exists, with an annotation such as "(article in Turkish with an abstract in English)". If the publication was not published with an English title, cite the original title only; do not provide a self-translation. References should be formatted as follows (please note the punctuation and capitalisation):

Journal articles
Journal titles should be abbreviated according to ISI Web of Science abbreviations.

Guyon I. and Elisseeff A. An introduction to variable and feature selection. J. Mach. Learn. Res. 2003; 3: 1157-1182.

Izadpanahi S., Ozcınar C., Anbarjafari G. and Demirel H. Resolution enhancement of video sequences by using discrete wavelet transform and illumination compensation. Turk J. Elec. Eng. & Comp. Sci. 2012; 20: 1268-1276.

Books
Haupt R.L. and Haupt S.E. Practical Genetic Algorithms. 2nd ed. New York, NY, USA: Wiley, 2004.

Kennedy J. and Eberhart R. Swarm Intelligence. San Diego, CA, USA: Academic Press, 2001.

Chapters in boks
Poore JH, Lin L, Eschbach R and Bauer T. Automated statistical testing for embedded systems. In: Zander J, Schieferdecker I, Mosterman PJ, editors. Model-Based Testing for Embedded Systems. Boca Raton, FL, USA: CRC Press, 2012. pp. 111-146.

Conference proceedings
Li R.T.H. and Chung S.H. Digital boundary controller for single-phase grid-connected CSI. In: IEEE 2008 Power Electronics Specialists Conference; 15–19 June 2008; Rhodes, Greece. New York, NY, USA: IEEE. pp. 4562-4568.

Theses
Boynukalın Z. Emotion analysis of Turkish texts by using machine learning methods. MSc, Middle East Technical University, Ankara, Turkey, 2012.

Tables and Figures
All illustrations (photographs, drawings, graphs, etc.), not including tables, must be labelled "Figure." Figures must be submitted in the manuscript.

All tables and figures must have a caption and/or legend and be numbered (e.g., Table 1, Figure 2), unless there is only one table or figure, in which case it should be labelled "Table" or "Figure" with no numbering. Captions must be written in sentence case (e.g., Macroscopic appearance of the samples.). The font used in the figures should be Times New Roman with 9 pt. If symbols such as ×, μ, η, or ν are used, they should be added using the Symbols menu of Word.

All tables and figures must be numbered consecutively as they are referred to in the text. Please refer to tables and figures with capitalisation and unabbreviated (e.g., "As shown in Figure 2…", and not "Fig. 2" or "figure 2").

The resolution of images should not be less than 118 pixels/cm when width is set to 16 cm. Images must be scanned at 1200 dpi resolution and submitted in jpeg or tiff format. Graphs and diagrams must be drawn with a line weight between 0.5 and 1 point. Graphs and diagrams with a line weight of less than 0.5 point or more than 1 point are not accepted. Scanned or photocopied graphs and diagrams are not accepted.

Figures that are charts, diagrams, or drawings must be submitted in a modifiable format, i.e. our graphics personnel should be able to modify them. Therefore, if the program with which the figure is drawn has a "save as" option, it must be saved as *.ai or *.pdf. If the "save as" option does not include these extensions, the figure must be copied and pasted into a blank Microsoft Word document as an editable object. It must not be pasted as an image file (tiff, jpeg, or eps) unless it is a photograph.

Tables and figures, including caption, title, column heads, and footnotes, must not exceed 16 × 20 cm and should be no smaller than 8 cm in width. For all tables, please use Word's "Create Table" feature, with no tabbed text or tables created with spaces and drawn lines. Please do not duplicate information that is already presented in the figures.

ESKİŞEHİR TECHNICAL UNIVERSITY JOURNAL OF SCIENCE AND TECHNOLOGY
C- Life Sciences and Biotechnology

ESKİŞEHİR TEKNİK ÜNİVERSİTESİ BİLİM VE TEKNOLOJİ DERGİSİ
C– Yaşam Bilimleri ve Biyoteknoloji

Volume/ Cilt**: 12 / Number/**Sayı**: 1 / January /** Ocak**- 2023**

CONTENTS / İÇİNDEKİLER

Sayfa / Page

ARAŞTIRMA MAKALESİ / RESEARCH ARTICLE

ESKİŞEHİR TEKNİK ÜNİVERSİTESİ BİLİM VE TEKNOLOJİ DERGİSİ C- YAŞAM BİLİMLERİ VE BİYOTEKNOLOJİ

Eskişehir Technical University Journal of Science and Technology C- Life Sciences and Biotechnology

2023, 12(1), pp. 1-7, DOI:10.18036/estubtdc.982228

RESEARCH ARTICLE

INVESTIGATIONS ON MORPHO-ANATOMICAL AND VOLATILE COMPOUNDS OF CULTIVATED *Fritillaria persica* L. (LILIACEAE)[#]

Merve HAS[1, *], Sevim KÜÇÜK [1], Mine KÜRKÇÜOĞLU [2], Murat KÜRŞAT [3]

[1] Department of Pharmaceutical Botany, Faculty of Pharmacy, Anadolu University, Eskişehir, Turkey
[2] Department of Pharmacognosy, Faculty of Pharmacy, Anadolu University, Eskişehir, Turkey
[3] Department of Biology, Faculty of Arts and Sciences, Bitlis Eren University, Bitlis, Turkey

ABSTRACT

In this study, cultivated *Fritillaria persica* L. (Liliaceae) investigated morpho-anatomical and volatile compounds of bulbs. Purple tepals, 12-28 flowers in inflorescence, yellow-green filaments and dark yellow or purple anthers were notable morphological properties. For the anatomical study, sections were prepared from root, stem and leaf. The cortex of the stem was comprised of parenchyma and sclerenchyma. The collateral vascular bundles were scattered. The leaves were unifacial and amphistomatic. The stomata type was anomocytic. The crushed bulb volatiles of *F. persica* were trapped by the headspace-solid phase microextraction technique and determined by analysis by gas chromatography/mass spectrometry. Eight compounds (83.9%) were identified and the major compounds were found as 1,3-dichlorobenzene (60%), limonene (8.1%) and *p*-cymene (5.1%).

Keywords: *Fritillaria persica* L., Morphological, Anatomical, HS-SPME, GC/MS

1. INTRODUCTION

Fritillaria L., belonging to the Liliaceae family, is a genus with about 150 species in the Northern Hemisphere, excluding some parts of North America [1, 2]. In Turkey, the genus *Fritillaria* includes approximately 45 species [2, 3].

"Fritillus" means chessboard and is especially derived from the checkered colors of *Fritillaria meleagris* L. [4]. *Fritillaria* is referred to as the "Ters Lale" in Turkey because its flowers like tulips and look at the ground, some species are grown as ornamental plants [5]. *Fritillaria persica* L. is one of them, it has different names such as "Kırklale, Ağlayan gelin or Acem şahtuğu" (in Turkish) [3, 5, 6].

Fritillaria species have alkaloids, saponins, terpenoids and many different components [7]. In Chinese medicine, Bulbus Fritillaria (Beimu) is used as an expectorant in respiratory tract diseases [8]. Bulbs of *F. pinardii* Boiss. and *F. crassifolia* subsp. *kurdica* (Boiss. & Noë) Rix, are used for external wound healing in Turkey [9]. In Iran, *F. imperialis* L. bulbs are used for digestive diseases and pain treatments [10]. Moreover, it is known that the strong odor emitted by *F. imperialis* has a repulsive effect on some animals such as mice and mole [11]. Among the *Fritillaria* species distributed in North America, *F. agrestis* Greene is another bad odor species, while *F. striata* Eastw. flowers have been reported to be fragrant [12].

F. persica growing naturally in Anatolia and is in the category of Vulnerable (VU) [13]. The aim of this research is to investigate morpho-anatomical features and volatile compounds of the cultivated *F. persica*. As far as we know, this is the first research made on the anatomy and volatiles of the *F. persica* growing in Turkey.

This article was produced from the master thesis, 2019 and represented as an oral presentation on ANES, Eskişehir
*Corresponding Author: mervehas@anadolu.edu.tr
Received: 10.09.2021 Published: 30.01.2023

2. MATERIALS AND METHODS

2.1. Morphological Method

F. persica bulbs were obtained from Şanlıurfa and grown in Anadolu University Faculty of Pharmacy. The samples (ESSE 15403) are kept in the Herbarium of Anadolu University Faculty of Pharmacy. Morphological characters were examined under with the Wild M5A stereomicroscope (with drawing tube) and the results were compared with Flora of Turkey [14], "Resimli Türkiye Florası" [3].

2.2. Anatomical Method

In the anatomical study, superficial (leaf) and cross (root, stem, leaf) sections of the plant parts were made by hand. Methylene blue was used for staining the stem and leaf anatomical sections. Anatomical sections were investigated with the Olympus BX51T microscope and the photographs were taken by the digital camera.

2.3. HS-SPME (Headspace-solid Phase Microextraction) Procedure, GC/MS (Gas Chromatography/Mass Spectrometry) Analysis and Identification of Compounds

SPME fibre precoated with a 65 μm layer of blue fibre (Polydimethylsiloxane-Divinylbenzene, supplied by Supelco Bellefonte, USA) was used. The crushed bulb volatiles of *F. persica* were trapped with HS-SPME technique and analyzed by GC/MS. The GC/MS analysis was carried out with an Agilent 5975 GC-MSD system [15].

3. RESULT

3.1. Morphological Results

Bulb 5 cm diameter, 6 cm height and spindle shaped. Stem 55-82 cm, thick, dull bluish green, smooth, straight. Leaves 50-60, alternate, dull bluish green, lowest leaves 8-9.5 x 1.5-2.5 cm, ovate-lanceolate, acute-acuminate at apex. Bracts 2.1 × 0.4 cm, present or absent. Inflorescence raceme, flowers 12-28. Perianth narrow campanulate, dark purple, tepal segments 14-17 x 7-10 mm, oblanceolate, oblong-oblanceolate, apex and base obtuse. Nectars 3 mm long, 1.5 mm broad, triangular. Filament 8-11 mm, yellow-green; anthers 2 mm, dark yellow or purple. Stylus 7-7.5 mm, smooth; stigma straight (Figure 1).

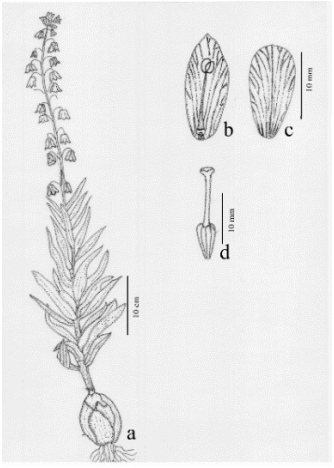

Figure 1. *F. persica*, a: general view, b: inner segment and stamen, c: outer segment, d: pistil.

3.2. Anatomical Results

3.2.1. Root

In the outer region there are small and one row of epidermis cells. Exodermis cells just below the epidermis. The cortex layer usually consists of irregularly shaped parenchyma cells. The endodermis and pericycle cells are evident. In the vascular bundles the metaxylem consists of 6-arms and there are phloem cells between them (Figure 2).

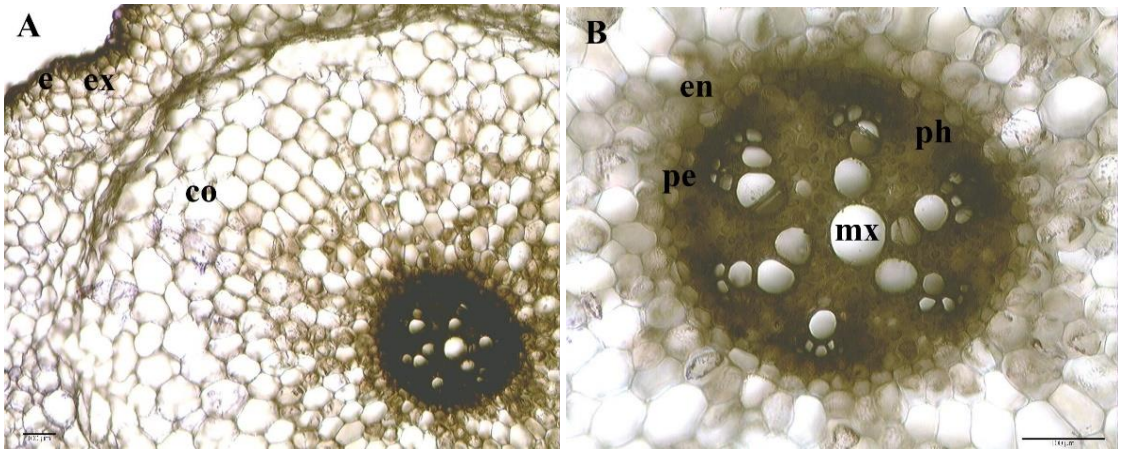

Figure 2. Cross section of *F. persica*; A-B: Root (e: epidermis, ex: exodermis, co: cortex, en: endodermis, pe: pericycle, mx: metaxylem, ph: phloem).

3.2.2. Stem

In the stem section, there is a cuticle followed by a single layer of small, rectangular epidermis cells. Between epidermis and sclerenchyma layers, there are 5-6 rows of round or oval shaped parenchyma cells. They are more oval shape in 1-3 raw under the epidermis. The sclerenchyma layer consists of 5-6 rows of cells. The vascular bundles are numerous and collateral. There are round or oval shaped parenchyma cells in the pith region, but the parenchyma cells in this part are more rounded (Figure 3).

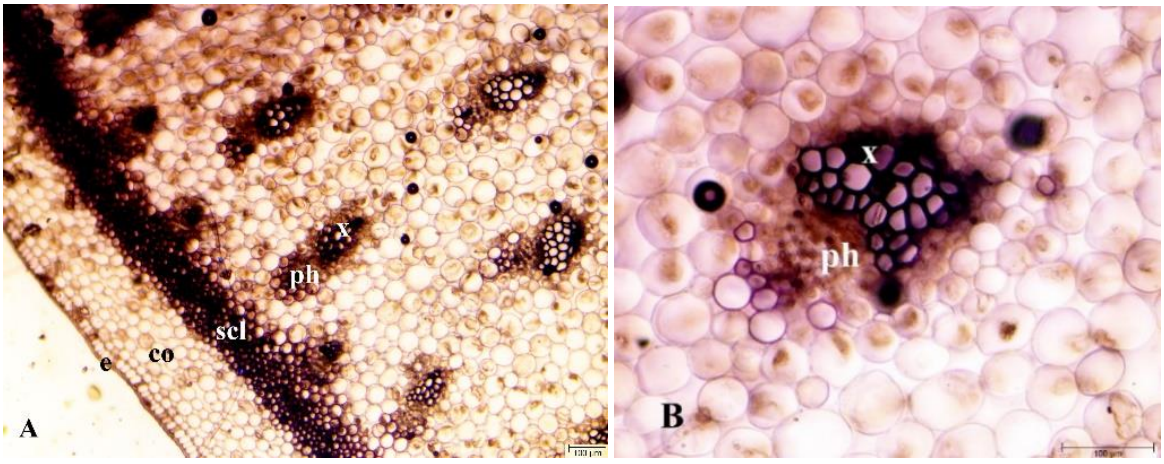

Figure 3. Cross section of *F. persica*; A-B: Stem (e: epidermis, co: cortex, scl: sclerenchyma; ph: phloem, x: xylem).

3.2.3. Leaves

In the leaf section, there is a thick cuticle layer on both surfaces. Epidermis cells are long quadrilateral or asymmetrical and cell walls are undulate. There is not any differentiation as palisade parenchyma and spongy parenchyma (unifacial leaf) and there are large spaces between the parenchyma cells.

In surface sections made from the leaf, there are anomocytic type stomata on both abaxial and adaxial surfaces (amphistomatic). The stomata cells are oval-rounded (Figure 4).

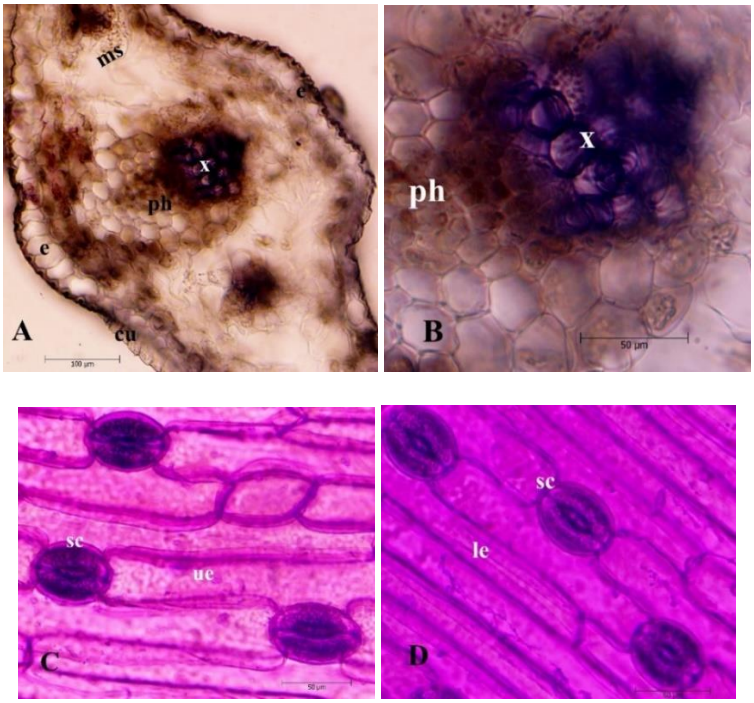

Figure 4. Cross (A-B) and superficial (C-D) section of *F. persica* leaves (cu: cuticula, e: epidermis, ms: mesophyll, x: xylem, ph: phloem, ue: upper epidermis, le: lower epidermis, sc: stomata cell).

3.3. HS-SPME Results

Volatile compounds of *F. persica* were determined by trapping with HS-SPME technique and analyzing by GC/MS. In this study, eight compounds were identified (Table 1).

Table 1. Chemical composition of crushed *F. persica* bulbs.

RRI	Compounds	%	IM
1203	Limonene	8.1	t_R, MS
1272	Styrene	1.9	MS
1280	*p*-Cymene	5.1	t_R, MS
1409	Rosefuran	2.9	t_R, MS
1429	1,3-Dichloro benzene	60.0	MS
1495	2-Ethyl hexanol	3.8	MS
1532	Camphor	1.2	t_R, MS
1896	Benzyl alcohol	0.9	MS

%; calculated from FID data. IM; Identification method. RRI; Relative retention indices calculated against n-alkanes. t_R; identification based on the retention times (t_R) of genuine compounds on the HP Innowax column. MS; identified on the basis of computer matching of the mass spectra with those of the Wiley, Adams and Mass Finder libraries and comparison with literature data.

4. DISCUSSION

In this study, the morphological, anatomical, and volatile compounds of *F. persica* was investigated. The bulbs produced in Şanlıurfa were grown in Eskişehir and studies were carried out with these samples. Tekşen and Aytaç [16] reconsidered the *Fritillaria* of the Mediterranean region in Turkey. According to the work "Resimli Türkiye Florası", *F. persica* and *F. imperialis* the bulbs of are at least 4 cm in diameter, unlike other *Fritillaria* species in Turkey. The differences between these two species are that the flowers of *F. persica* are purple, greenish-purple and the inflorescence is raceme, the flowers of *F. imperialis* are orange to red and the inflorescence is umbella [3]. Morphological properties in this study are similar to comparative studies (Table 2) [3, 14]. But our samples did not contain capsules.

Table 2. Cultivated *F. persica* comparison with the morphological characteristics of the Flora of Turkey [14] and "Resimli Türkiye Florası" [3].

Characteristics	Flora of Turkey (1984)	Resimli Türkiye Florası (2018)	This study
Bulb	3-5 cm diam, 6 cm height	2.5-6 × 2.5-5 cm	5 cm diam, 6 cm height
Stem	20-100 cm	20-100 cm	55-82 cm
Number of leaves	10-25	10-70	50-60
Leaf	15 × 3 cm	4.4-15 × 1.0-3.0 cm (lower) 5.1-10 × 0.5-1.1 cm (middle)	8-9.5 × 1.5-2.5 cm (lowest)
Bracte	-	1.6-6.4 × 0.4-0.5 cm	2.1 × 0.4 cm
Tepal	15-20 × 6-7 mm Purplish, greenish-grey	1-2 × 0.5-0.8 cm (outer) 1-2 × 0.5-0.9 cm (inner) Purple, greenish-purple	14-17 × 7-10 mm Purple
Number of flowers	7-20	3-28	12-28
Nectar	1.5 mm broad	1.5-3 × 1.5-3.5 mm	3 × 1.5 mm
Filament	5-6 mm	5-10 mm, yellow	8-11 mm, yellow-green
Anther	4 mm	1.5-4 mm, brownish or purple	2 mm, dark yellow or purple
Style	6-8 mm	5.5-10 mm	7-7.5 mm

Namazi et al. [17] reported that they had defined a dichotomous key based on anatomical features for *Fritillaria* species in Iran and according to this key, phloem fiber is absent in the anatomy of the stem of *F. persica*. Corneanu and Popescu [18] and Akyol et al. [19], reported that there was no distinction between parenchyma cells in the leaf section of the anatomy of *Fritillaria* species. In some anatomical studies related to the *Fritillaria* species, the stomata type was determined as anomocytic [20, 21] and other studies stated stomata surrounded by four neighboring cells [22, 23] or do not have by-cells [18]. Many of the anatomical features in our study were like other species of *Fritillaria*.

In the *F. persica* volatile components study with the HS-SPME technique, eight compounds corresponding to 83.9% were identified and a high rate of 1,3-dichlorobenzene (60%) was found and other major compounds are limonene (8.1%) and *p*-cymene (5.1%). Compounds consist of monoterpene hydrocarbons (13.2%), oxygenated monoterpenes (4.1%) and others (66.6%). Seyed Saleh et al. (2018), α-bisabolol oxide A, camphor, chamazulene, trans-thujone, α-bisabolone oxide A, δ-3-carene and α-pinene by hydrodistillation, and α-bisabolol oxide A, chamazulene, α-bisabolone oxide A, camphor, trans-thujone, δ-3-carene, α-pinene were reported as major constituents of *F. imperialis* oil obtained by HS-SPME from Iran [24]. Has (2019), volatile compounds of *F. imperialis* bulbs were captured with HS-SPME fiber, analyzed by gas chromatography/mass spectrometry from Turkey. Thirteen compounds represented by 93.6% were identified in the 30 minutes study with the blue fiber, and the main compounds were *p*-cymene (23.6%), 1,8 cineol (22.4%) and limonene (15.2%). Ten compounds represented by 98.2% were identified the at 50°C and 15 minutes study with black fiber, and the main compounds were hexanal (29.8%), styrene (22.5%) and 1-hexanol (21.8%) [21, 25].

As a result, morphological and anatomical properties of cultivated *Fritillaria persica* are parallel within the literature within some size or number variations. Volatile compounds of crushed bulbs of *Fritillaria persica* by HS-SPME technique were reported for the first time in this study. Eight compounds (83.9%) were identified and the main components were found as 1,3-dichlorobenzene (60%), limonene (8.1%) and *p*-cymene (5.1%). Compounds consist of monoterpene hydrocarbons (13.2%), oxygenated monoterpenes (4.1%) and others (66.6%).

ACKNOWLEDGEMENTS

Thank you for the support provided by Scientific Research Projects of Anadolu University (Project No. 1705S178) Commission and for procurement plant bulbs by Menekşe Baykuş.

CONFLICT OF INTEREST

The authors stated that there is no conflicts of interest regarding the publication of this article.

REFERENCES

[1] Rix M, Strange K. *Fritillaria* s*ororum*: Liliaceae. Curtis's Botanical Magazine, 2014; 31(3): 214-222.

[2] Yıldırım H, Tekşen M. *Fritillaria arsusiana* (Lilieae, Liliaceae), a new species from southern Anatolia. Phytotaxa, 2021; 502(2): 133–159.

[3] Tekşen M. *Fritillaria* L. Güner A, Kandemir A, Menemen Y, Yıldırım H, Aslan S, Ekşi G, Güner I, Çimen AÖ. (Eds). Resimli Türkiye Florası, ANG Vakfı Nezahat Gökyiğit Botanik Bahçesi Yayınları. İstanbul, 2018; 2: 880-926. ISBN 978-605-67172-4-6. https://www.turkiyeflorasi.org.tr/view.html?taxon=TR0000002089#Fritillaria (accessed 03.09.2022)

[4] Hibberd S. Familiar Garden flowers. Cassell and company Limited, 1879. https://books.google.com.tr/books?id=FVcDAAAAQAAJ&printsec=frontcover&hl=tr&source=gbs_ge_summary_r&cad=0#v=onepage&q=fritillus&f=false (accessed 26.04.2021).

[5] Baytop T. Türkiye'de bitkiler ile tedavi, geçmişte ve bugünde (2. Baskı). İstanbul: Nobel Tıp Kitabevleri, 1984.

[6] Baytop T. Türkçe bitki adları sözlüğü. Ankara: Türk Dil Kurumu, 2007. pp. 23.

[7] Hao DC, Gu XJ, Xiao PG, Peng Y. Phytochemical and biological research of *Fritillaria* medicinal resources. Chin J Nat Med, 2013; 11(4): 330–344.

[8] Tang W, Eisenbrand G. *Fritillaria* spp. In: Chinese drugs of plant origin. Springer, Berlin, Heidelberg, 1992. pp. 525-526.

[9] Mükemre M, Behçet L, Çakılcıoğlu U. Ethnobotanical study on medicinal plants in villages of Çatak (Van-Turkey). J Ethnopharmacol, 2015; 166: 361–374.

[10] Mosaddegh M, Naghibi F, Moazzeni H, Pirani A, Esmaeili S. Ethnobotanical survey of herbal remedies traditionally used in Kohghiluyeh va Boyer Ahmad province of Iran. J Ethnopharmacol, 2012; 141: 80-95.

[11] Beresford-Kroeger D. A garden for life: The natural approach to designing, planting, and maintaining a north temperate garden. Ann Arbor: University of Michigan Press, 2004. pp. 171. https://books.google.com.tr/books?hl=tr&id=XFJ2OnvqAacC&q=fritillaria#v=snippet&q=fritillaria&f=false (accessed 13.09.2022)

[12] Beetle DE. A monograph of the north American species of *Fritillaria*. California Botanical Society, Madrono, 1944; 7(5): 133-159.

[13] Ekim T, Koyuncu M, Vural M, Duman H, Aytaç Z, Adıgüzel N. Türkiye Bitkileri Kırmızı Kitabı (Eğrelti Ve Tohumlu Bitkiler). Ankara: Türkiye Tabiatını Koruma Derneği, 2000. pp. 178.

[14] Rix EM. *Fritillaria* L. in: Davis PH, Mill RR, Tan K. (Eds). Flora of Turkey and the East Aegean Islands, Vol.8. Edinburgh: Edinburgh University Press, 1984.

[15] Küçük S, Kürkçüoğlu M, Tuyan CS. Headspace volatiles of *Allium subhirsutum* L. growing in Turkey. Ann Phytomed, 2018; 7(2): 180-182.

[16] Tekşen M, Aytaç Z. The revision of the genus *Fritillaria* L. (Liliaceae) in the Mediterranean region (Turkey). Turk J Bot, 2011; 35: 447-478.

[17] Namazi F, Sharifi-Tehrani M, Shabani L. Anatomical study on *Fritillaria* species in Iran. Bangladesh J Bot, 2017; 46(1): 93-101.

[18] Corneanu GC, Popescu GG. Distributional and anatomical studies on *Fritillaria* (Liliaceae) in Romania. Botanischer Garden und Botanisches Museum, Berlin-Dahlem, Willdenowia, 1981; 11: 307-315.

[19] Akyol Y, Yetişen K, Özdemir C. The morphological and anatomical studies on *Fritillaria caucasica* J.F. Adam (Liliaceae). SDU Journal of Science, 2014; 9(1): 12-19.

[20] Wang Q, Zhou SD, Deng XY, Zheng Q, He XJ. Comparative morphology of the leaf epidermis in *Fritillaria* (Liliaceae) from China. Bot J Linn Soc, 2009; 160: 93–109.

[21] Has M. *Fritillaria imperialis* L. ve *Fritillaria persica* L. türleri üzerinde farmasötik botanik ve fitokimyasal araştırmalar. MSc, Anadolu Üniversitesi, Eskişehir, Turkey, 2019.

[22] Alan S. An endemic species in Turkey: Morphological and anatomical investigation on *Fritillaria fleischeriana* Steudel & Hochst. ex Schultes & Schultes fil. (Liliaceae). Ot Sist Bot Dergisi, 2008; 15(2): 115-124.

[23] Pinar SM, Behçet L. Anatomical, morphological and palynological study on Turkish endemic *Fritillaria baskilensis* (Liliaceae). Bocconea, 2012; 24: 305-310.

[24] Seyed Saleh SS, Arabi S, Ashouri F. Comparison of head space solid phase microextraction (HS-SPME) and hydro-distillation (HD) techniques in determination of essential oils in *Fritillaria imperialis*. JMP, 2018; 17(68): 122-135.

[25] Küçük S, Kürkçüoğlu M, Kurşat M, Has M. *Fritillaria imperialis* L. (Liliaceae) türü üzerinde morfolojik, anatomik ve fitokimyasal çalışmalar. 4th International Anatolian Agriculture, Food, Environment and Biology Congress, 2019. pp. 606-608.

ESKİŞEHİR TEKNİK ÜNİVERSİTESİ BİLİM VE TEKNOLOJİ DERGİSİ C- YAŞAM BİLİMLERİ VE BİYOTEKNOLOJİ

Eskişehir Technical University Journal of Science and Technology C- Life Sciences and Biotechnology

2023, 12(1), pp. 8-18, DOI:10.18036/estubtdc.1184733

RESEARCH ARTICLE

COMPARATIVE FLUX BALANCE ANALYSES OF SERINE ALKALINE PROTEASE OVERPRODUCTION IN *Bacillus subtilis* AT GENOME AND SMALL SCALE

Pınar KOCABAŞ *

* Bioengineering Department, Engineering Faculty, Ege University, İzmir, Turkey

ABSTRACT

This work aims to conduct flux balance analysis of serine alkaline protease overproduction in *Bacillus subtilis* using enzyme-constrained genome scale model and to compare the results with fluxes obtained from a smaller, bioreaction-based model. Fluxes of the enzyme constraint genome scale model were calculated using CobraToolbox v3.0 and compared with those of bioreaction-based model for the specific growth rate of zero. The active reaction number first increased and then remained constant with specific growth rate for enzyme constrained genome scale model. The SAP synthesis flux increased with a decrease in specific growth rate for both models. The TCA cycle was active for both models, but with lower fluxes for enzyme-constrained genome scale model. Anaplerotic reactions were active only for bioreaction-based model. Glycolysis pathway fluxes were active for enzyme-constrained genome scale model, meanwhile gluconeogenesis pathway fluxes were active for bioreaction-based model. Oxidative pentose phosphate pathway was inactive for both models and generally higher pentose phosphate pathway fluxes were obtained using bioreaction-based model. The fluxes toward amino acid synthesis pathways and serine alkaline protease synthesis were higher with bioreaction-based model. Since TCA cycle fluxes were lower with enzyme constrained genome scale model, ATP synthesis was lower with enzyme constrained genome scale model compared to bioreaction-based model. For both models, active pathways were the same for TCA cycle, pentose phosphate pathway, amino acid synthesis pathways except glycolysis pathway. The results showed that bioreaction-based model gave more sound results compared to enzyme constrained genome scale model since gluconeogenesis should be active with the carbon source of citrate.

Keywords: Enzyme constrained genome scale metabolic model, Metabolic flux analysis, Bioreaction-based model

Abbreviations

A: Stoichiometric matrix of the metabolic network
ATPS4r: adp[c]+4h[e]+pi[c]⇔ atp[c]+h2o[c]+3h[c]
ala_L[c]: Intracellular L-alanine
arg_L[c]: Intracellular L-arginine
asn_L[c]: Intracellular L-asparagine
asp_L[c]: Intracellular L-aspartate
atp[c]: Intracellular atp
adp[c]: Intracellular adp
BsBRM-2000: Bioreaction-based model [1]
c(t): Metabolite accumulation vector
$\mathbf{c_1(t)}$: Extracellular metabolite accumulation vector
$\mathbf{c_2(t)}$: Intracellular metabolite accumulation vector
$[E_j]$: $Concentration\ of\ enzyme\ j$
ec_iYO844: Enzyme constrained GEM [2]
ec_iYO844-SAP: SAP synthesis reaction [1] and exchange reaction for SAP were added to ec_iYO844 [2] forming ec_iYO844-SAP in this study
EX_SAP(e): SAP exchange reaction
FB: Flux balance
FBA: Flux balance analysis

*Corresponding Author: pinar.kocabas@ege.edu.tr
Received: 30.09.2022 Published: 30.01.2023

gln_L[c]: Intracellular L-glutamine
glu_L[c]: Intracellular L-glutamate
gly[c]: Intracellular glycine
GEM: Genome scale metabolic model
GECKO: Enzymatic Constraints using Kinetic and Omics data
his_L[c]: Intracellular L-histidine
ile_L[c]: Intracellular L-isoleucine
iYO844: *Bacillus subtilis* GEM [3]
leu_L[c]: Intracellular L-leucine
lys_L[c]: Intracellular L-lysine
met_L[c]: Intracellular L-methionine
PPP: Pentose phosphate pathway
PYK: adp[c]+h[c]+pep[c]=>atp[c]+pyr[c]
PGK: 13dpg[c]+adp[c]+8.44309e-07PM_PGK_f ⇔3pg[c]+atp[c]
phe_L[c]: Intracellular L-phenylalanine
pro_L[c]: Intracellular L-proline
pi[c]: Intracellular phosphate
r(t): reaction rate vector
k_{cat}^{j}: Turnover number of enzyme j
SAP: Serine alkaline protease
SAP: SAP synthesis reaction
SAP[e]: Extracellular SAP
ser_L[c]: Intracellular L-serine
thr_L[c]: Intracellular L-threonine
trp_L[c]: Intracellular L-tryptophan
tyr_L[c]: Intracellular L-tyrosine
v_j: $Rate\ of\ reaction\ j$
val_L[c] : Intracellular L-valine

1. INTRODUCTION

Genome scale metabolic models (GEMs) aim to describe the whole metabolism of an organism [4], containing: stochiometric reaction network and gene-protein-reaction-metabolite associations. Functions of GEMs are calculation of intracellular reaction rates in bioprocesses; determination of bottleneck reactions in bioreaction network; increasing yield and selectivity of bioprocesses by changing medium components, bioreactor operation parameters, microorganism or genetic structure; prediction of the results of genetic and environmental changes; determination of the maximum theoretical yield; generation of hypotheses, model-driven discovery; metabolic engineering and strain design [5-6].

Bacillus is an important group of industrial microorganisms acting as microbioreactors in fermentors [5]. *Bacillus subtilis* is the best defined Gram-positive bacteria [2]. *B.subtilis* genome was sequenced and updated totally over the last 20 years by many researchers [7-11], proven to be a model organism for systems biology. Progress in genome sequencing and consequently annotation studies results with reconstructing GEMs with more accurate gene-enzyme-reaction data and therefore with more accurate prediction capacity. There are six basic *B.subtilis* GEMs [12] apart from ec_iYO844 [2]. The first three GEMs are reconstructed with the biochemical and genomic knowledge based on genome sequencing of [7]. Therefore, they are called first generation *B.subtilis* GEMs [3, 13-14]. Second generation *B.subtilis* GEMs [15-16] are based on the genome sequencing of [8]. *i*Bsu1144 [12] is a third generation *B.subtilis* GEM that takes its genome annotation from [9] and ec_iYO844 [2] is a first generation *B.subtilis* GEM since it is based on [3].

Serine alkaline protease (SAP), one of the major industrial enzyme [5] is produced by *Bacillus sp*. at the start of stationary phase of growth [1]. Aims of this work are to conduct flux balance analysis of SAP overproduction in *Bacillus subtilis* using ec_iYO844-SAP that contains 1269 reactions, 1010 metabolites and to compare the results with those of biochemical reaction based model having 147 reactions and 105 metabolites [1] to assess validity of enzyme constrained GEM.

2. MATERIAL AND METHODS

2.1 Flux Balance Analyses

Stream of metabolites in a mathematically represented bioreaction network is computed with flux balance (FB) methodology to analyze metabolic flux distributions. The FB methodology cannot predict metabolite concentrations since it doesn't use kinetic parameters. Using fermentation data as constraints, an allowable solution space is obtained. FBA can determine a single optimum flux distribution on the edge of the allowable solution space by optimizing an objective function [12].

Mass-balance based stoichiometric equations are constructed for each metabolite in the cell, which is considered as a semi-batch microbioreactor. The algebraic sum of all conversion reactions of each metabolite-*i* in the defined reactions plus the transport of metabolite-*i* are equal to the accumulation of metabolite-*i* [6]. The scalar flux balance equations can be shown as a linear vector as follows:

$$A \times r(t) = c(t) \quad (1),$$

Where A is mxn stoichiometric coefficients matrix, m is the number of metabolites and n is the number of reactions [6], r(t) is the flux vector and c(t) is the metabolite accumulation vector. The elements of c(t) are two sub-vectors:

$$c(t)=c_1(t) + c_2(t) \quad (2)$$

where $c_1(t)$ and $c_2(t)$ correspond to extracellular and intracellular metabolite accumulation vectors, respectively. Using pseudo steady state (PSS) approximation for the intracellular metabolites, $c_2(t)$ is set to zero. Intracellular fluxes can be calculated by minimizing or maximizing the objective function Z, specified for a selected component-*i* [17].

$$Z=\sum\alpha_i.r_i \quad (3)$$

where, Z is a linear combination of fluxes (r_i) multiplied by the corresponding stoichiometric coefficient of component-*i* (α_i) for every reaction [12].

In this work, firstly SAP synthesis reaction [1] and exchange reaction for SAP were added to ec_iYO844 [2] forming ec_iYO844-SAP**.** Secondly, the objective function was defined as the exchange reaction of SAP in the cells; whereupon, maximization of the objective function was carried out at citrate's uptake rate at 10 mmole/g_{DW}/h and at various growth rates (μ=0, 0.05, 0.15, 0.25, 0.35, 0.45, 0.55, 0.65, 0.75 h^{-1}) using CobraToolbox v3.0 [18] to determine intracellular reaction rates. Reaction fluxes of metabolites are expressed in mmole/g_{DW}/h; and the flux towards the cell is the specific growth rate, μ(h^{-1}) [12].

2.2 Enzyme Constrained Genome Scale Metabolic Model

The enzymes that catalyze a reaction affect the metabolic flux. Different approaches have been developed to constrain the solution space and improve phenotypic predictions by integrating enzyme concentrations [2]. **GECKO** is the most promising method, using enzymatic data as a new constraint for each metabolic flux, provided that fluxes do not exceed the maximum capacity in a given condition [2, 19].

A set of available enzyme constraints (absolute protein levels and turnover numbers) for the reactions of central carbon metabolism were integrated into the iYO844 GEM of *B. subtilis* [3] following the

principles of the GECKO [2]: An additional constraint that the metabolic flux through the j-th reaction (R_j) does not exceed its maximum capacity (v_{max}), corresponding to the product between the k_{cat} value (converted to h^{-1}) of the enzyme E_j (that catalyzes the j-th reaction) and its abundance [E_j], as shown in Eq 4, was considered to implement the GECKO approach [2].

$$v_j \leq k_{cat}^{j} * [E_j] \qquad \text{for} \quad j=1\ldots17 \tag{4}$$

In this work ec_iYO844-SAP was constructed: Serine alkaline protease (SAP) synthesis reaction [1] (the reaction named as **SAP**) and exchange reaction for SAP (named as **EX_SAP(e)**) were added to ec_iYO844 [2] forming ec_iYO844-SAP containing 1269 reactions and 1010 metabolites. Since the system is underdetermined, optimization was carried out by maximizing the exchange reaction for SAP. SAP synthesis reaction was not chosen as the objective function since it yielded zero SAP production. Citrate uptake rate was considered as 10 mmole/g_{DW}/hr during flux balance analyses, which was conducted in CobraToolbox v3.0 [18]. The reason why citrate was chosen as the carbon source was that the aim of this study is to compare prediction capacities of a genome scale model and biochemical reaction-based model [1], which is named as BsBRM-2000 in this study for intracellular reaction rates/fluxes. Since there is already flux data obtained by theoretical capacity analysis conducted with BsBRM-2000 [1] taking citrate as the sole carbon source with its uptake rate as 10 mmole/g_{DW}/h [1], citrate was chosen to be carbon source.

Added reaction names and reactions are shown below:

SAP: 40 ala_L[c] + 4 arg_L[c] + 18 asn_L[c] + 9 asp_L[c] + 1096 atp[c] + 7 gln_L[c] + 5 glu_L[c] + 35 gly[c] + 5 his_L[c] + 10 ile_L[c] + 16 leu_L[c] + 9 lys_L[c] + 5 met_L[c] + 4 phe_L[c] + 10 pro_L[c] + 32 ser_L[c] + 20 thr_L[c] + 1 trp_L[c] + 13 tyr_L[c] + 31 val_L[c] -> 1096 pi[c] + 1096 adp[c] + SAP[e]

EX_SAP(e): SAP[e] ->

3. RESULTS

Theoretical capacity analysis led to optimized SAP overproduction by using a linear constrained optimization technique for several specific growth rates was conducted and the variation of the fluxes were calculated by fixing the sole carbon source citrate's uptake rate at 10 mmole/g_{DW}/h [1]. The objective function Z was defined as synthesis rate of SAP in the cells, whereupon optimum flux distributions were obtained by maximizing the objective function Z [1]. In this work, it was also aimed to conduct theoretical capacity analysis with ec_iYO844-SAP using the same citrate uptake rate, growth rates and however with different optimization functions (Exchange reaction for SAP, **EX_SAP(e)**) and compare the results with those of literature [1]. In this work, when the optimization function was taken as SAP synthesis reaction, there were no SAP synthesis in all growth rates. Therefore, exchange reaction for SAP was taken as optimization function since it yielded decreasing SAP synthesis with increasing specific growth rates as it was reported [1]. The mathematical model of [1], which is a bioreaction-based model, is named as BsBRM-2000 in this study (Table 1). Prediction capacities of a genome scale model and biochemical reaction-based model for intracellular reaction rates/fluxes were compared for the first time in this study.

Intracellular reaction rates were calculated for ec_iYO844-SAP for different growth rates. Fluxes of common reactions were compared for ec_iYO844-SAP and BsBRM-2000. Active reaction number first increased with growth rate and then remained constant with μ for ec_iYO844-SAP (Table 2). For both models, SAP synthesis rate decreased with growth rate; meanwhile, SAP synthesis flux was higher with BsBRM-2000 (Table 3). The flux ratio of SAP synthesis in two models increased with growth rate (Table 3).

Table 1. Comparison of models.

Gene #	**Reaction #**	**Metabolite #**	**Model**
844	1269	1010	ec_iYO844-SAP
-	147	105	BsBRM-2000

Table 2. Number of active reactions with ec_iYO844-SAP.

μ, h^{-1}	ec_iYO844-SAP	
	Active reaction #	% percent
0	162	12.8
0.05	351	27.7
0.15	351	27.7
0.25	351	27.7
0.35	351	27.7
0.45	351	27.7
0.55	351	27.7
0.65	-	-
0.75	-	-

Table 3. SAP synthesis fluxes.

μ, h^{-1}	Intracellular SAP flux (mmole/g_{DW}/h)		Flux ratio
	ec_iYO844-SAP	BsBRM-2000	
0.0	0.01511	0.0260	1.7
0.05	0.0139	0.0243	1.7
0.15	0.01148	0.0209	1.8
0.25	0.009056	0.0175	1.9
0.35	0.006635	0.0143	2.2
0.45	0.004215	0.0107	2.5
0.55	0.001795	0.0073	4.1
0.65	-	0.0039	-
0.75	-	0.0006	-

TCA cycle fluxes increased with growth rate for ec_iYO844-SAP (Figure 1), while TCA cycle fluxes did not change considerably with respect to growth rate for BsBRM-2000 [1]. Anaplerotic reactions were inactive for ec_iYO844-SAP (Figure 2). Glycolysis pathway fluxes increased with growth rate (Figure 3), oxidative PPP fluxes were inactive for ec_iYO844-SAP (Figure 4).

Fluxes toward serine-group, alanine- group, histidine, aromatic acid- group amino acid pathways decreased at least 1.2- fold with growth rate; meanwhile SAP synthesis flux decreased 8.4- fold with respect to growth rate (Table 4). There were similar decreases with BsBRM-2000 fluxes towards amino acid synthesis pathways (AAP) [1]. ATP synthesis rate increased 2.44- fold with growth rate for ec_iYO844-SAP (Table 5), meanwhile it did not change considerably for BsBRM-2000 [1]. Comparison of two models was carried out only for μ=0 h^{-1} since Çalık et al.[1] reported only central pathway fluxes for μ=0, 0.75 h^{-1} values and ec_iYO844-SAP was infeasible with μ=0.75 h^{-1}. The TCA cycle was active for both models similarly but with lower fluxes of ec_iYO844-SAP (Figure 5). Anaplerotic reactions were active only with BsBRM-2000 (Figure 6).

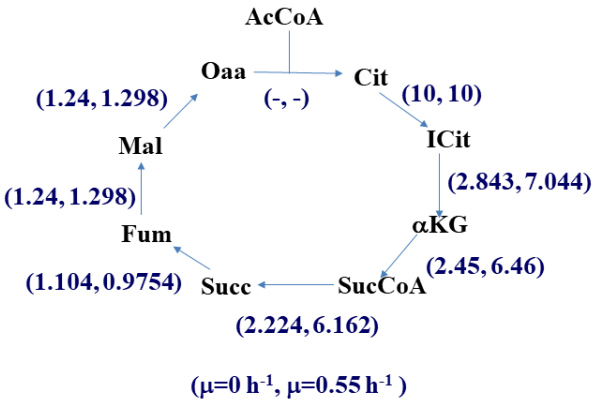

Figure 1. Effects of growth rate on TCA cycle fluxes (mmole/g_{DW}/hr) in SAP production calculated with ec_iYO844-SAP.

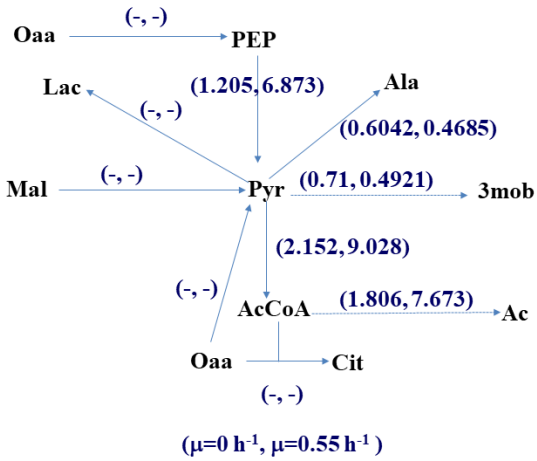

Figure 2. Effects of growth rate on Pyr node fluxes (mmole/g_{DW}/hr) in SAP production calculated with ec_iYO844-SAP.

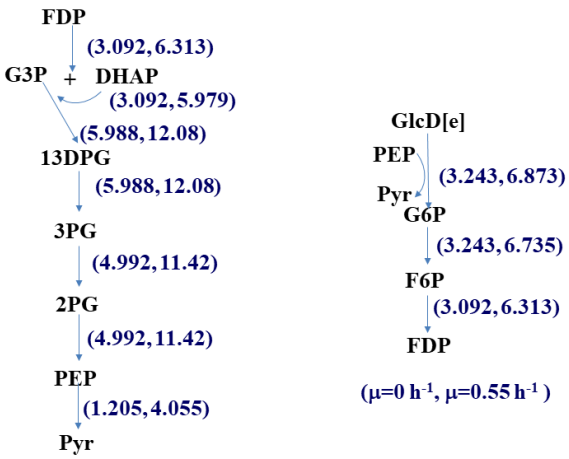

Figure 3. Effects of growth rate on glycolysis pathway fluxes (mmole/g_{DW}/hr) in SAP production calculated with ec_iYO844-SAP.

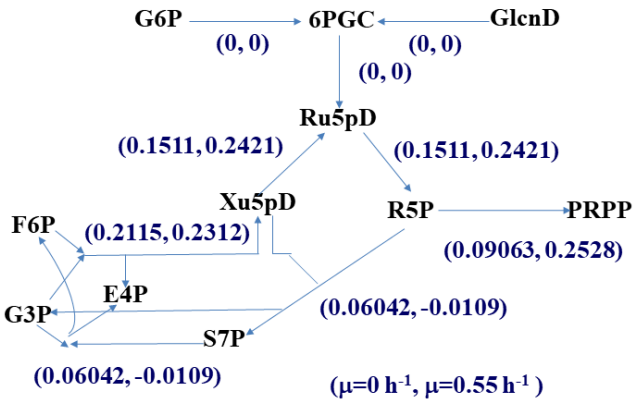

Figure 4. Effects of growth rate on PPP fluxes (mmole/g_{DW}/hr) in SAP production calculated with ec_iYO844-SAP.

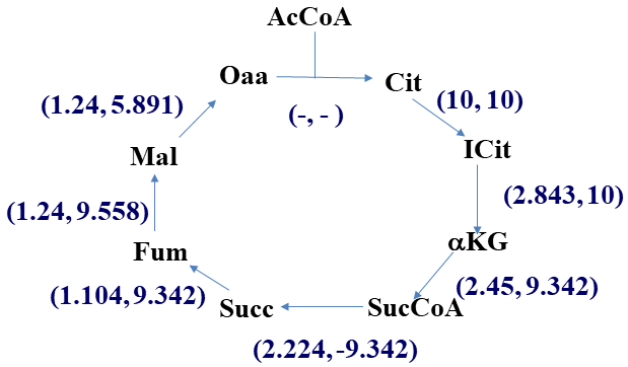

Figure 5. Comparison of TCA cycle fluxes (mmole/g_{DW}/h) in SAP production calculated with ec_iYO844-SAP and BsBRM-2000 for μ=0 h^{-1}, respectively.

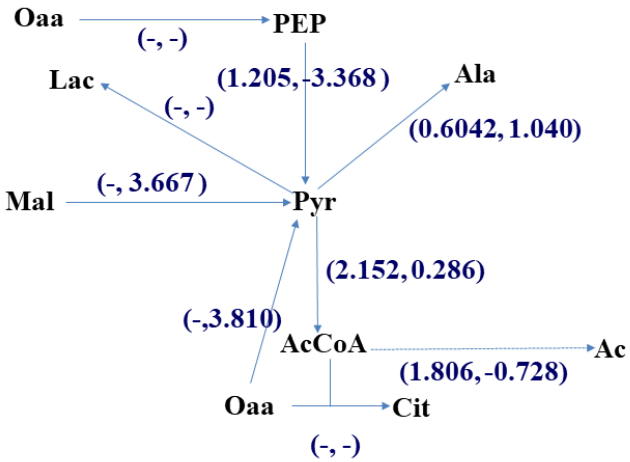

Figure 6. Comparison of Pyr node fluxes (mmole/g_{DW}/h) in SAP production calculated with ec_iYO844-SAP and BsBRM-2000 for μ=0 h^{-1}, respectively.

Table 4. Fluxes towards amino acid synthesis pathways, mmole/g_{DW}/h.

Amino acid synthesis pathways	μ=0 h^{-1}	μ=0.55 h^{-1}	Fold change
Serine group amino acid pathways	0.9959	0.6578	1.5↓
Alanine group amino acid pathways	0.6042	0.4685	1.3↓
Histidine synthesis	0.07553	0.05393	1.4↓
Aspartic acid group amino acid pathways	1.24	1.298	1
Aromatic group amino acid pathways	0.2719	0.2203	1.2↓

Table 5. Effects of growth rate on ATP synthesis fluxes in SAP production calculated with ec_iYO844-SAP.

Reaction	ATP synthesis, mmole/g_{DW}/h	
	μ=0 h^{-1}	μ=0.55 h^{-1}
PYK	1.205	4.055
ATPS4r	20.81	52.24
PGK	5.988	12.08
Total	28.003	68.375

Glycolysis pathway fluxes were active for ec_iYO844-SAP, meanwhile gluconeogenesis pathway fluxes were active for BsBRM-2000 at μ=0 h^{-1} [1]. Oxidative PPP was inactive for both models and generally higher PPP fluxes were obtained with BsBRM-2000 (Figure 7). The fluxes toward amino acid synthesis pathways and SAP synthesis were higher for BsBRM-2000 (Table 6). Since TCA cycle fluxes were lower with ec_iYO844-SAP, ATP synthesis was lower with ec_iYO844-SAP compared to BsBRM-2000 (Table 7).

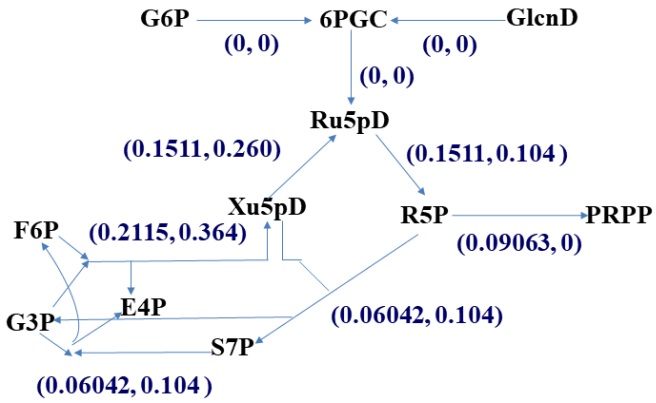

Figure 7. Comparison of PPP fluxes (mmole/g_{DW}/h) in SAP production calculated with ec_iYO844-SAP and BsBRM-2000 for μ=0 h^{-1}, respectively.

Table 6. Comparison of fluxes (mmole/g_{DW}/h) toward amino acid synthesis pathways in SAP production for μ=0 h^{-1}.

Amino acid synthesis pathways	ec_iYO844-SAP	BsBRM-2000	Fold change
Serine-group amino acid pathways	0.9959	1.573	1.6
Alanine-group amino acid pathways	0.6042	1.040	1.7
Histidine synthesis	0.07553	0.130	1.7
Aspartic acid- group amino acid pathways	1.24	2.081	1.7
Aromatic- group amino acid pathways	0.2719	0.273	1.0
Glutamic acid- group amino acid pathways	3.761	7.763	2.1

Table 7. Total ATP synthesis fluxes, mmole/g_{DW}/h: Comparison of ATP synthesis fluxes in SAP production for μ=0 h^{-1}.

ec_iYO844-SAP	BsBRM-2000	Fold change
28.003	52.46	1.9

4. DISCUSSION AND CONCLUSIONS

According to ec_iYO844-SAP and BsBRM-2000 results, active pathways were the same for TCA cycle, PPP and amino acid synthesis pathways except glycolysis pathway. Divergence of fluxes at glycolysis pathway was because enzyme-constraint GEM allows for glucose exchange, otherwise central carbon pathways were inactive with no SAP production at μ=0 h^{-1} with ec_iYO844-SAP. Citrate exerts a negative feedback on glycolysis by inhibiting phosphofructokinase 1 and 6-phosphofructo-2-kinase/fructose-2,6-biphosphatases (PFK2); on the contrary it stimulates pathways consuming ATP such as gluconeogenesis and lipid synthesis [20]. BsBRM-2000 yielded more sound results since an active gluconeogenesis pathway is expected with citrate as sole carbon source.

The number of active reactions increased with respect to growth rate and was maximum as 351. The minimum number of active reactions was 162 at μ=0 h^{-1} as anticipated since stationary phase implies less maintenance energy and less active metabolism inside the cell. This result was in harmony with the literature [21] where active reactions were calculated to be 322, 318, 43 for *Bacillus subtilis* (rBsP) in Period I (0<t<4h), Period II (4<t<12h), Periods III-V (12<t<32 h) calculated using GEM of iYO844 using glucose as carbon source, respectively. The number of active reactions at stationary phase calculated with enzyme constrained iYO844 (ec_iYO844-SAP) is 162, while the number of active reactions at stationary phase calculated with iYO844 [21] is 43 since different carbon sources were utilized in both models.

A bigger model does not necessarily yield better results as it was depicted in this study. The bioreaction - based model produced more sound results probably due to better connectivity. ec_iYO844-SAP could describe the changes in central carbon metabolism with growth rate in a better way. The number of enzyme-constrained reactions is 17 [2] although ec-iYO844-SAP has 1269 reactions and 1010 metabolites. If the number of enzyme-constrained reactions were 1269 for ec-iYO844-SAP, the actual metabolism could have been better depicted with ec-iYO844-SAP. Results will be convergent as more *Bacillus subtilis* reactions are discovered; enzyme information and intracellular regulations are integrated into GEMs.

CONFLICT OF INTEREST

The author confirms to have no competing financial interests or relationships that could influence the work reported in this article.

REFERENCES

[1] Çalık P, Takaç S, Çalık G, Özdamar TH. Serine alkaline protease overproduction capacity of *Bacillus licheniformis.* Enzyme Microb Tech 2000; 26: 45–60

[2] Massaiu I, Pasotti L, Sonnenschein N, Rama E, Cavaletti M, Magni P, Calvio C, Herrgård MJ. Integration of enzymatic data in *Bacillus subtilis* genome-scale metabolic model improves phenotype predictions and enables in silico design of poly-γ-glutamic acid production strains. *Microb Cell Fact* 2019;18:3

[3] Oh YK, Palsson BO, Park SM, Schilling CH, Mahadevan R. Genome-scale reconstruction of metabolic network in *Bacillus subtilis* based on high-throughput phenotyping and gene essentiality data. J Biol Chem 2007; 282: 28791-28799

[4] Wang H, Robinson JL, Kocabaş P, Gustafsson J, Anton M, Cholley PE, Huang S, Gobom J, Svensson T, Uhlen M et al. Genome-scale reconstruction of metabolic networks of model animals represents a platform for translational research. PNAS 2021; 118: 1-9

[5] Çalık P, Özdamar TH. Carbon sources affect metabolic capacities of *Bacillus* species for the production of industrial enzymes: theoretical analyses for serine and neutral proteases and α-amylase. Biochem Eng J 2001; 8: 61-81

[6] Çalık P, Özdamar TH. Bioreaction Network Flux Analysis For Industrial Microorganisms: A Review. Reviews in Chemical Engineering 2002; 18: 553-596

[7] Kunst F, Ogasawara N, Moszer I, Albertini AM, Alloni G, Azevedo V, Bertero MG, Bessieres P, Bolotin A, Borchert S et al. The complete genome sequence of the gram positive bacterium *Bacillus subtilis*. Nature 1997; 390: 249-256

[8] Barbe V, Cruveiller S, Kunst F, Lenoble P, Meurice G, Sekowska A, Vallenet D, Wang T, Moszer I, Medigue C et al. From a consortium sequence to a unified sequence: the *Bacillus subtilis* 168 reference genome a decade later. Microbiol 2009; 155: 1758-1775

[9] Belda E, Sekowska A, Le Fevre F, Morgat A, Mornico D, Ouzounis C, Vallenet D, Medigue C, Danchin A et al. An updated metabolic view of the *Bacillus subtilis* 168 genome. Microbiol 2013; 159: 757-770

[10] Borriss R, Danchin A, Harwood CR, Medigue C, Rocha EPC, Sekowska A, Vallenet D. *Bacillus subtilis*, the model Gram-positive bacterium: 20 years of annotation refinement. Microb Biotechnol 2018; 11: 3-17

[11] Geissler AS, Anthon C, Alkan F, González-Tortuero E, Poulsen LD, Kallehauge TB, Breüner A, Seemann SE, Vinther J, Gorodkin J. BSGatlas: a unified *Bacillus subtilis* genome and transcriptome annotation atlas with enhanced information access. Microb Genom 2021; 7:000524

[12] Kocabaş P, Çalık P, Çalık G, Özdamar TH. Analyses of extracellular protein production in *Bacillus subtilis*-I: Genome-scale metabolic model reconstruction based on updated gene-enzyme-reaction data. Biochemical Engineering Journal 2017; 127: 229-241

[13] Goelzer A, Brikci FB, Martin-Verstraete I, Noirot P, Bessières P, Aymerich S, Fromion V. Reconstruction and analysis of the genetic and metabolic regulatory networks of the central metabolism of *Bacillus subtilis*. BMC Syst Biol 2008; 2:20

[14] Henry CS, Zinner JF, Cohoon MP, Stevens RL. *i*Bsull03: A new genome-scale metabolic model of *Bacillus subtilis* based on SEED annotations. Genome Biol 2009; 10, R69

[15] Tanaka K, Henry CS, Zinner JF, Jolivet E, Cohoon MP, Xia F, Bidnenko V, Ehrlich SD, Stevens RL, Noirot P. Building the repertoire of dispensable chromosome regions in *Bacillus subtilis* entails major refinement of cognate large-scale metabolic model. Nucleic Acids Res 2013; 41: 687-699

[16] Hao T, Han B, Ma H, Fu J, Wang H, Wang Z, Tang B, Chen T, Zhao X. In silico metabolic

engineering of *Bacillus subtilis* for improved production of riboflavin Egl-237,(R,R)-2,3-butanediol and isobutanol. Mol Biosyst 2013; 9: 2034-2044

[17] Çalık P, Çalık G, Takaç S, Özdamar TH. Metabolic flux analysis for serinealkaline protease fermentation by *Bacillus licheniformis* in a defined medium: effects of oxygen transfer rate. Biotechnol Bioeng 1999; 64:151-167

[18] Heirendt L, Arreckx S, Pfau T, Mendoza SN, Richelle A, Heinken A, Haraldsdottir HS, Wachowiak J, Keating SM, Vlasov V et al. Creation and analysis of biochemical constraint-based models: the COBRA Toolbox v3.0. Nat Protoc 2019; 14: 639-702

[19] Sanchez BJ, Zhang C, Nilsson A, Lahtvee PJ, Kerkhoven EJ, Nielsen J. Improving the phenotype predictions of a yeast genome-scale metabolic model by incorporating enzymatic constriants. Mol Syst Biol 2017;13: 935

[20] Iacobazzi V, Infantino V. Citrate--New functions for an old metabolite. Biol Chem 2014; 395: 387-399

[21] Özdamar TH, Şentürk B, Yılmaz ÖD, Kocabaş P, Çalık G, Çalık P. Bioreaction network flux analysis for human protein producing *Bacillus subtilis* based on genome-scale model. Chemical Engineering Science 2010; 65: 574-580.

ESKİŞEHİR TEKNİK ÜNİVERSİTESİ BİLİM VE TEKNOLOJİ DERGİSİ C- YAŞAM BİLİMLERİ VE BİYOTEKNOLOJİ

Eskişehir Technical University Journal of Science and Technology C- Life Sciences and Biotechnology

2023, 12(1), pp. 19-29, DOI:10.18036/estubtdc.1195951

RESEARCH ARTICLE

INVESTIGATION OF THE EFFECTS OF GEOTHERMAL AND MINERAL WATER ON BARLEY (*Hordeum vulgare* L.) AND WHEAT (*Triticum aestivum* L.)

Vasfi KARATEPE [1], Müjgan ELVEREN [2], Etem OSMA [3]

[1] Institute of Natural and Applied Sciences, Erzincan Binali Yıldırım University, Erzincan, Turkey
[2] Medical Services and Techniques, Vocational School of Health Services, Erzincan Binali Yıldırım University, Erzincan, Turkey
[3] Department of Biology, Faculty of Sciences and Arts, Erzincan Binali Yıldırım University, Erzincan, Turkey

ABSTRACT

In the present study, the usability of geothermal and mineral water in agricultural lands was investigated. Geothermal water, mineral water, and tap water with two wheat varieties and one barley variety were used, and six different water groups were formed with geothermal, mineral, and tap water by mixing them at a ratio of 50%. Field soil mixed with 750 g of animal manure was prepared in the growing containers, and then, 6 g of the wheat varieties and 5 g of the barley variety were planted in them. The germinated wheat and barley were irrigated periodically according to their water needs. Barley and wheat were harvested after 15 days. Chlorophyll size, electrolyte leakage, weight, and mineral element concentrations were determined in the harvested crops. The mineral element concentrations of the samples were analyzed in ICP-AES. Additionally, geothermal, mineral and normal water were applied to the soil for three months, and the change between the resulting soil and the starting soil was determined. The collected data were analyzed in the SPSS 22 Statistical Package Program.

Keywords: Barley, Geothermal water, Irrigation, Mineral element, Mineral water, Wheat

1. INTRODUCTION

Water is the most indispensable resource provided by nature. Water exists in nature in many ways, such as oceans, seas, rivers, and groundwater. Due to the increase in world population, intensive agricultural activities and water use in domestic and industrial areas have significantly increased the demand for freshwater [1, 2, 3]. Because of these demands, utilizable freshwater resources are gradually decreasing [4, 5, 6]. Especially, the haphazard use of groundwater for irrigation and its widespread exploitation seem to cause problems in the long run [7, 8, 9]. Insufficient water resources and deteriorating water quality lead to severe concerns for industry, agriculture, and the environment in many parts of the world [10]. The existing total water of the world consists of 97.4% saltwater and 2.6% freshwater. The reclamation and recovery of wastewater, including reuse and recycling, have recently been seen as one of the possible tools that contribute to the better management of water resources [11, 12]. With the race to increase agricultural productivity, irrigation will become more dependent on substandard water resources. Therefore, investigating the effects of irrigation water quality on yield has considerable significance. This approach is extremely critical for maintaining proper food and soil quality as well as providing sufficient crop production for the increasing need [13].

In recent years, the increasing water limitation due to the deterioration of water quality and quantity has started to become a significant problem. In many countries, a large part of urban wastewater is drained from rivers without being treated sufficiently or at all, and the water of these rivers is used for the irrigation of agricultural areas. Rapidly increasing population, rapid urbanization, industrialization, and the extensive use of fertilizers and pesticides in agricultural areas may cause rapid contamination in freshwater resources [14, 15, 16]. Although the distribution of usage areas of water changes, on average, 70% of the total freshwater is used for agriculture, 20% is used for industrial purposes, and 10% is used

*Corresponding Author: eosma@erzincan.edu.tr
Received. 01.10.2022 Published: 30.01.2023

for domestic purposes worldwide. Based on these rates, clean water used for agricultural irrigation creates a large part of the total water used. Depending on the increasing population, it is predicted that by 2050, 70% more food will be produced, and consequently, the amount of water required for agricultural irrigation will increase [17,18]. Thus, it has become an increasingly commonplace practice to use treated wastewater for agricultural irrigation, especially in countries that have water shortages and want to eliminate the cost of wastewater treatment and utilize the rich nutrient resources in wastewater [13, 19].

The use of thermal resources is a very old. The systematic use and development of these resources, which are thought to be used for health and religious purposes in antiquity, coincide with the Roman period. Türkiye is rich in geothermal resources due to the large footprint of active volcanic and tectonic areas. Hence, Türkiye has a significant potential for geothermal tourism. The estimated total number of geothermal resource sites is 1300, and the number of medicinal water sources is over 2000 in Türkiye. Thus, Türkiye is in the top five in the distribution of geothermal resources worldwide [20, 21].

Geothermal water has been used in the tourism sector in many parts of the world, while mineral water is mostly used as drinking water. In this study, the usability of geothermal and mineral water in agricultural areas was investigated.

2. MATERIAL AND METHODS

Geothermal water in Erzincan is among the therapeutic water resources with superior properties such as a temperature of approximately 34° and 660 mg of free carbon dioxide gas per liter. This geothermal water is currently used in geothermal tourism. Mineral water is also located close to the region where geothermal water comes out. It is used for drinking and is always open to the public (Figure 1).

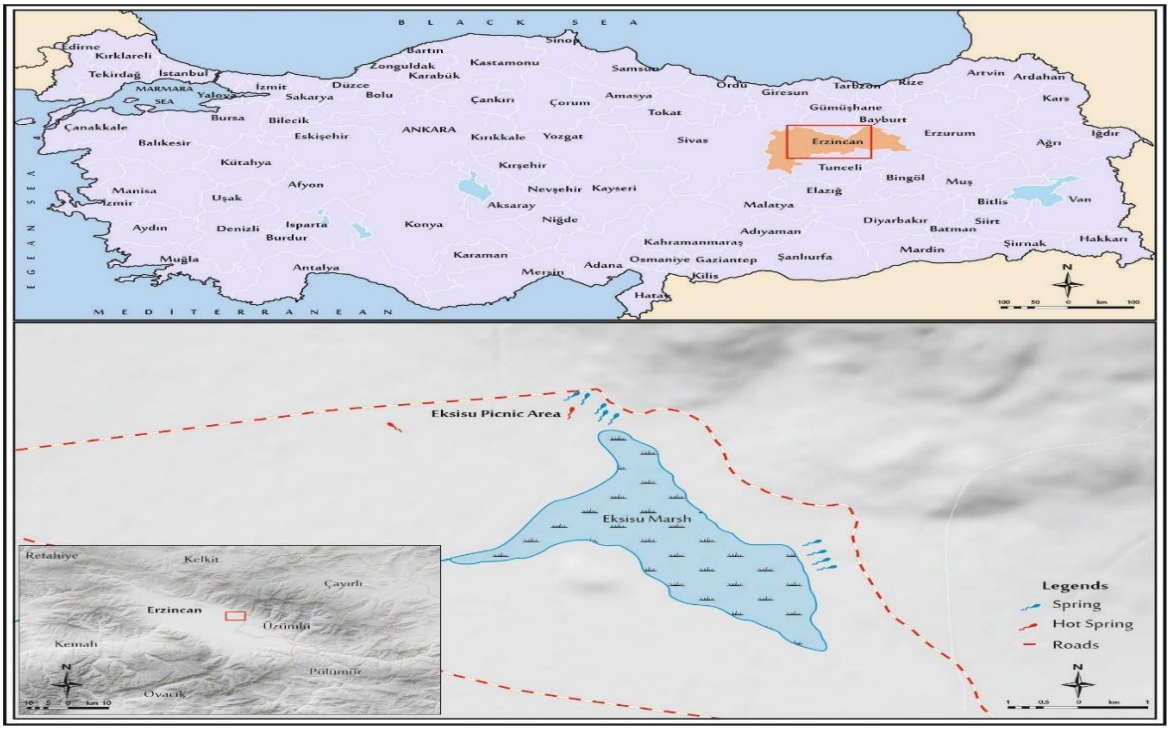

Figure 1. Area of geothermal ve mineral water

The geothermal and mineral water and tap water located within the borders of Erzincan and two wheat (Forblanc, Esperia) and one barley (Akhisar 98) varieties were used in this study. The Forblanc wheat variety is of medium height, and its ears are awn. It has resistance to drought, cold stress, and lying. The

Esperia wheat variety is approximately 80-85 cm in height. Its grain color is red, and its grain structure is hard. It has a winter development nature; its stem is strong, it does not bend easily, and its performance is high in irrigated areas. The Akhisar 98 barley variety is 85-90 cm in height; it is a summer, six-row barley variety. It has medium sensitivity to drought stress and is very productive [22, 23, 24]. In this study, six different water groups were formed using geothermal, mineral, and tap water by mixing them at a ratio of 50%. Additionally, field soil mixed with 750 g of animal manure was prepared in the growing containers, and then, 6 g of the wheat varieties and 5 g of the barley variety were planted in them. The geothermal water was cooled to a suitable temperature before using it for the irrigation of plants. After germination, the plants were irrigated according to the water needs of wheat and barley and the field capacity. Next, barley and wheat were harvested for analyses after an average of 15 days. Chlorophyll size, electrolyte leakage, weight, and mineral element concentrations were determined in the harvested plants. Apart from these analyses, geothermal water, mineral water and tap water were applied to the soil for three months, some properties (e.g., organic matter and salinity) and element concentrations in the resulting soil were determined, and these properties were compared to those of the same soil where no water application was made. Moreover, the water that was used to irrigate the soil was analyzed.

The electrolyte leakage of fresh leaves was determined according to the method reported by [25] 4 mL of distilled water was added to six replicate test tubes containing 0.1 g of fresh leaves and incubated at 4 °C for 24 h. An electrical conductivity meter was used to measure the number of ions present in water.

The Lichtenthaler and Buschmann (2001) method was used to estimate total chlorophyll and carotenoid content in fresh leaves. Fresh leaf tissue (0.5 g) was ground using a mortar and pestle, which contained 5 mL acetone (80%). The absorbance of the corresponding extract was measured at 662 and 645 nm (chlorophyll) and 440 nm (carotenoids). Photosynthetic pigments are expressed as mg/g FW [26].

Samples were dried at 65°C for 24 h, ground, and dried again at 65°C for 12 h. These dried samples were used for the analysis. Needle samples (0.5 g) were prepared using a precision scale and then put into Teflon cells. Eight milliliters of HNO_3 (65%) were added to the ground samples, while 5 mL HNO_3 (65%), 3 ml HCI (37%), and 2 mL HF (48%) were added to the ground soil samples. The samples were put into a microwave oven (Berghof-MWS2). The temperature of the microwave contents was gradually increased to 175°C and held constant for 20 min. The samples and chemicals were filtered with Whatman filters into 50 mL sterile tubes and diluted to 50 mL with ultra-pure water for the ICP-OES analysis. Before the spectroscopic analysis, standards were prepared using a 1,000 ppm multi-element solution. The ICP-OES measurements were made after the calibration using the standards [26].

The data obtained in the study were statistically evaluated. A value of $p \leq 0.05$ was accepted as significant in statistical calculations and comparisons. With SPSS 22 Package Statistical Program, the data were analyzed by ANOVA test at 95% confidence interval and the differences between samples in multiple comparisons were determined by Tukey's B and S-N-K [26].

3. RESULTS AND DISCUSSION

In this study, many parameters were examined, and significant results were obtained (Table 1.). First of all, according to the analysis of the three different types of water, it was determined that the geothermal water had a quite high turbidity at 55.6 NTU, and its conductivity was very high at 7100 µS/cm. On the other hand, the turbidity (0-1 NTU) and conductivity (0-2500 µS/cm) values of the tap and mineral water were within normal ranges. When the pH values of the water were examined, it was seen that the mineral water (5.5) was more acidic, while the other water types were within normal pH ranges (6.5-9.5). Afterwards, due to the application of different irrigation water types (geothermal, mineral, tap) in the soil for three months, the amount of organic matter increased in all three irrigation groups, and differences were observed in other parameters. Changes in the concentrations of elements in the soil were also seen. The concentration of the elements in the soil irrigated with three different types of water

was determined differently than in the non-irrigated soil. Therefore, it was seen that the water used in the study changed the element concentrations of the soil. The data obtained on the differences in element concentrations based on different water treatments are shown in (Table 2).

Table 1. Chemical properties of different waters.

Parametres	Geothermal Water		Mineral Water		Normal Water		Control	
Ph	8,23	Strong Alkaline	7,85	Light Alkaline	8,01	Strong Alkaline	8,03	Strong Alkaline
Ec(Micromhos)	3,22		1,46		1,74		0,91	
Soil Texture	70,00	Clayey-Loamy	68,00	Clayey-Loamy	72,00	Clayey	55,00	Clayey-Loamy
Organic Substance	5,53	High	4,98	High	5,26	High	0,83	Very little
Lime	10,93	Limy	12,10	Limy	10,93	Limy	14,83	Limy
Salt	0,14	Saltless	0,06	Saltless	0,08	Saltless	0,03	Saltless

Table 2. Element concentration in the soil applied to different waters (mg / kg DW).

Element	Al	B	Cu	Fe	Mn	Zn
Geothermal Water	3143,9 ± 57,5^{b}	49,8 ± 0,4^{a}	58,2 ± 0,4^{a}	51258,6 ± 325,1^{b}	613,7 ± 4,3^{a}	65,1 ± 0,6^{a}
Mineral Water	3076,1 ± 41,6^{b}	27,8 ± 0,6^{c}	53,9 ± 0,5^{b}	50732,1 ± 316,2^{b}	609,0 ± 2,5^{a}	64,6 ± 1,5^{a}
Normal Water	3237,7 ± 50,9^{b}	32,8 ± 0,3^{b}	56,8 ± 0,9^{a}	51924,5 ± 479,8^{b}	616,1 ± 5,5^{a}	64,0 ± 0,7^{a}
Control	3550,3 ± 70,7^{a}	27,5 ± 0,1^{c}	55,8 ± 0,5^{b}	53947,7 ± 570,2^{a}	588,8 ± 3,8^{b}	53,5 ± 0,4^{b}

Element	Na	Mg	K	Ca	P
Geothermal Water	1057,4 ± 23,1^{a}	78100,1 ± 652,1^{a}	3233,0 ± 74,9^{a}	43822,4 ± 333,7^{b}	644,1 ± 3,8^{a}
Mineral Water	337,5 ± 12,5^{d}	75350,5 ± 505,9^{b}	2888,6 ± 59,5^{a}	44696,7 ± 296,8^{b}	614,1 ± 6,7^{a}
Normal Water	431,8 ± 10,7^{c}	78401,6 ± 833,4^{a}	3358,6 ± 66,4^{a}	44944,3 ± 457,2^{b}	635,2 ± 7,9^{a}
Control	742,7 ± 20,7^{b}	78233,1 ± 899,8^{a}	2352,4 ± 18,1^{a}	63662,2 ± 959,2^{a}	254,4 ± 6,1^{b}

After applying irrigation water, in the wheat varieties, the concentrations of elements varied in different ranges as: Cu (11.7 ± 1.4-23 ± 0.2 mg/kg DW), Mn (33.6 ± 1.8-45.8 ± 3.9 mg/kg DW) and Zn (23, 5 ± 0.8-39.9 ± 1.3 mg/kg DW), whereas these ranges for the barley variety were as follows: Cu (20.3 ± 0.2-26.5 ± 0.2 mg/kg DW) in barley variety, Mn (31.3 ± 0.6-53.9 ± 2 mg/kg DW) and Zn (33.2 ± 0.7-54.6 ± 0.5 mg/kg DW). It was observed that these values were close to those of the irrigation water. On the other hand the B (27.7 ± 0.2 and 24.0 ± 2.1 mg/kg DW), Fe (199.5 ± 13.1 and 780.1 ± 144.8 mg/kg DW) Na (1027, 6 ± 4.1 and 2049.6 ± 231.6 mg/kg DW) concentrations in the wheat varieties and the Na (9334.5 ± 68.3 mg/kg DW) and B (18.7 ± 0.2 mg/kg DW) concentrations in the barley variety were higher in the samples irrigated with geothermal waters compared to those irrigated with the other water types. According to these results, considerable differences were generally observed between the concentrations of elements due to the application of different types of water (Tables 3-5). Additionally, the chlorophyll levels of the plants differed based on the type of water that was applied. Among the wheat samples, chlorophyll a (5.97-10.56 mg g^{-1} FW), chlorophyll b (2.58-5.18 mg g^{-1} FW), total chlorophyll (8.56-15.61 mg g^{-1} FW) and carotenoid (0.92-2.01 mg g^{-1} FW) levels were higher in the samples where tap water and mineral water were used for irrigation together than those where geothermal water was used, while these values among the barley samples were higher in the samples where tap water was applied.

Table 3. Element concentration in the barley applied to different waters (mg / kg DW) (*p < 0.05, **p < 0.01, ***p < 0.001 significant).

Barley (Akhisar 98)	Al	B	Cu	Fe	Mn	Zn
Geothermal Water	27,1 ± 2,1^{b}	18,7 ± 0,2^{e}	26,5 ± 0,2^{d}	143,6 ± 6,5^{b}	44,7 ± 0,4^{c}	54,6 ± 0,5^{e}
Mineral Water	49,6 ± 5,3^{d}	10,9 ± 0,4^{d}	23,4 ± 0,4^{c}	201,3 ± 15,9^{d}	53,9 ± 2,0^{e}	51,3 ± 1,1^{d}
Normal Water	134,4 ± 0,6^{e}	9,7 ± 0,1^{c}	21,5 ± 0,3^{b}	370,8 ± 9,8^{e}	49,1 ± 0,1^{d}	46,4 ± 0,5^{c}
Geothermal+Normal	40,1 ± 1,7^{c}	10,5 ± 0,1^{d}	21,7 ± 0,3^{b}	175,6 ± 5,2^{c}	38,2 ± 0,3^{b}	40,6 ± 0,4^{b}
Mineral+Normal	7,2 ± 0,2^{a}	7,7 ± 0,1^{b}	20,8 ± 0,2^{ab}	85,8 ± 2,8^{a}	40,6 ± 0,6^{b}	39,9 ± 0,8^{b}
Mineral+Geothermal	7,0 ± 0,4^{a}	7,0 ± 0,0^{a}	20,3 ± 0,2^{a}	83,7 ± 1,9^{a}	31,3 ± 0,6^{a}	33,2 ± 0,7^{a}
Significant	***	***	***	***	***	***

	Na	Mg	K	Ca	P
Geothermal Water	9334,5 ± 68,3^{e}	3876,2 ± 7,1^{de}	73214,1 ± 530,2^{e}	2363,8 ± 16,6^{c}	9148,1 ± 62,5^{c}
Mineral Water	2062,8 ± 62,4^{b}	4068,5 ± 154,0^{e}	57853,9 ± 1155,4^{c}	2420,3 ± 93,6^{c}	11374,8 ± 177,1^{f}
Normal Water	1540,1 ± 14,4^{a}	3781,1 ± 21,0^{d}	53731,1 ± 262,6^{b}	2149,5 ± 25,4^{b}	10441,1 ± 124,7^{e}
Geothermal+Normal	4661,1 ± 72,8^{d}	3213,8 ± 61,5^{c}	61581,8 ± 757,5^{d}	2119,4 ± 43,3^{b}	8134,1 ± 89,4^{b}
Mineral+Normal	1467,4 ± 12,0^{a}	2908,1 ± 80,4^{b}	48344,6 ± 464,2^{a}	1652,5 ± 48,4^{a}	9677,7 ± 140,2^{d}
Mineral+Geothermal	4323,0 ± 48,9^{c}	2512,7 ± 59,9^{a}	54164,1 ± 840,1^{b}	1667,8 ± 35,3^{a}	6256,0 ± 147,9^{a}
Significant	***	***	***	***	***

Table 4. Element concentration in the wheat applied to different waters (mg / kg DW). (*p < 0.05, **p < 0.01,

Wheat (Forblanc)	Al	B	Cu	Fe	Mn	Zn
Geothermal Water	48,9 ± 0,5^{a}	27,7 ± 0,2^{a}	17,6 ± 0,1^{ab}	199,5 ± 13,1^{a}	42,2 ± 0,8^{a}	24,1 ± 0,4^{ab}
Mineral Water	13,8 ± 0,7^{b}	2,8 ± 0,1^{d}	17,5 ± 0,2^{ab}	67,0 ± 3,7^{cd}	36,0 ± 1,2^{c}	23,5 ± 0,8^{b}
Normal Water	13,5 ± 0,7^{b}	3,2 ± 0,2^{d}	18,1 ± 0,1^{a}	90,6 ± 2,5^{b}	38,9 ± 0,7^{b}	25,9 ± 0,4^{a}
Geothermal+Normal	7,6 ± 0,2^{c}	20,1 ± 0,2b	16,5 ± 0,1^{b}	71,2 ± 0,6^{bc}	42,0 ± 0,3^{a}	25,6 ± 0,4^{a}
Mineral+Normal	2,7 ± 0,2^{d}	3,1 ± 0,1^{d}	16,9 ± 0,2^{b}	55,3 ± 1,3^{d}	39,4 ± 0,6^{b}	24,9 ± 0,4^{ab}
Mineral+Geothermal	12,6 ± 1,7^{b}	11,4 ± 0,5^{c}	18,4 ± 0,7^{a}	82,8 ± 3,6^{bc}	40,5 ± 0,6^{ab}	25,4 ± 0,5^{a}
Significant	***	***	***	***	***	**

	Na	Mg	K	Ca	P
Geothermal Water	1027,6 ± 4,1^{a}	2483,4 ± 85,2^{b}	51778,9 ± 536,8^{a}	1483,1 ± 48,0^{a}	6106,5 ± 110,2^{d}
Mineral Water	163,7 ± 6,2^{e}	2163,1 ± 84,8^{d}	45137,0 ± 1373,8^{b}	1275,2 ± 73,4^{b}	8262,1 ± 269,1^{bc}
Normal Water	144,6 ± 2,1^{f}	2334,2 ± 52,2^{cd}	50755,8 ± 909,1^{a}	1537,1 ± 48,0^{a}	8396,5 ± 205,3^{bc}
Geothermal+Normal	782,6 ± 8,3^{b}	2446,7 ± 36,9^{ab}	50263,8 ± 314,0^{a}	1523,9 ± 31,1^{a}	8696,4 ± 101,2^{ab}
Mineral+Normal	185,7 ± 3,1^{d}	2512,4 ± 44,2^{a}	46274,3 ± 775,6^{b}	1557,0 ± 33,9^{a}	9155,3 ± 150,2^{a}
Mineral+Geothermal	720,1 ± 10,3^{c}	2519,0 ± 45,0^{a}	49020,3 ± 781,6^{a}	1510,9 ± 41,7^{a}	7882,9 ± 96,4^{c}
Significant	***	***	***	***	***

***p < 0.001 significant).

Table 5. Element concentration in the wheat applied to different waters (mg / kg DW). (*p < 0.05, **p < 0.01, ***p < 0.001 significant).

Wheat (Esperia)	**Al**			**B**			**Cu**			**Fe**			**Mn**			**Zn**		
Geothermal Water	26,8	±	2,6^{c}	24,0	±	2,1^{a}	18,7	±	0,6^{b}	780,1	±	144,8^{a}	33,6	±	1,8^{b}	28,3	±	2,1^{b}
Mineral Water	146,1	±	2,8^{a}	3,1	±	0,7^{c}	23,4	±	1,5^{a}	461,0	±	14,4^{c}	36,5	±	1,3^{ab}	28,7	±	1,4^{b}
Normal Water	57,3	±	7,0^{b}	7,0	±	0,4^{c}	20,0	±	0,6^{ab}	214,0	±	18,0^{d}	45,8	±	3,9^{a}	39,9	±	1,3^{a}
Geothermal+Normal	58,6	±	5,4^{b}	12,9	±	0,8^{b}	11,7	±	1,4^{c}	600,8	±	3,1^{b}	40,4	±	0,8^{a}	24,0	±	1,9^{c}
Mineral+Normal	28,5	±	6,5^{c}	14,0	±	3,7^{b}	21,3	±	0,5^{ab}	147,6	±	20,7^{d}	43,0	±	2,6^{a}	39,6	±	1,5^{a}
Mineral+Geothermal	13,8	±	0,6^{c}	21,0	±	0,2^{a}	23,0	±	0,2^{a}	85,7	±	2,1^{d}	37,1	±	0,9^{ab}	32,8	±	0,9^{b}
Significant		***			***			***			***			***			**	

	Na			**Mg**			**K**			**Ca**			**P**		
Geothermal Water	2049,6	±	231,6^{a}	4630,0	±	494,5	55030,0	±	1199,5^{d}	2445,4	±	428,0^{ab}	8090,6	±	344,1^{c}
Mineral Water	399,4	±	32,6^{cd}	3438,7	±	228,6	60917,1	±	2564,2^{c}	1955,9	±	84,2^{ab}	10383,5	±	303,6^{b}
Normal Water	327,2	±	11,3^{d}	4463,5	±	274,2	69301,7	±	1819,4^{b}	2591,1	±	165,7^{ab}	14337,2	±	455,3^{a}
Geothermal+Normal	319,6	±	5,5^{d}	4433,9	±	97,7	10041,4	±	1612,3^{e}	1698,3	±	258,5^{c}	6352,2	±	983,1^{d}
Mineral+Normal	647,7	±	161,3^{c}	4298,5	±	207,1	74905,3	±	2728,2^{a}	2849,1	±	395,8^{a}	13746,2	±	297,4^{a}
Mineral+Geothermal	1065,5	±	15,3^{b}	3182,2	±	113,9	76873,8	±	1105,2^{a}	1828,0	±	79,0^{b}	10573,6	±	187,8^{b}
Significant		***			***			***			***			***	

The findings showed that the lowest chlorophyll concentrations in the samples were in the Forblanc wheat variety treated with geothermal water, the Esperia wheat variety treated with mineral water, and the barley variety treated with mineral and geothermal water irrigation. Additionally, there were no significant differences in the chlorophyll and carotenoid analysis results among the irrigation methods applied in the Esperia variety, while there were significant differences in the Forblanc wheat variety and the barley variety. There were differences regarding the weights of the plants after harvesting depending on the water applied, and the weights of the plants irrigated with geothermal water were higher than the weights of others. According to the electrolyte leakage analysis results, there were generally high concentrations in the samples in which mineral water and geothermal water were used together (Figure 2 and 3).

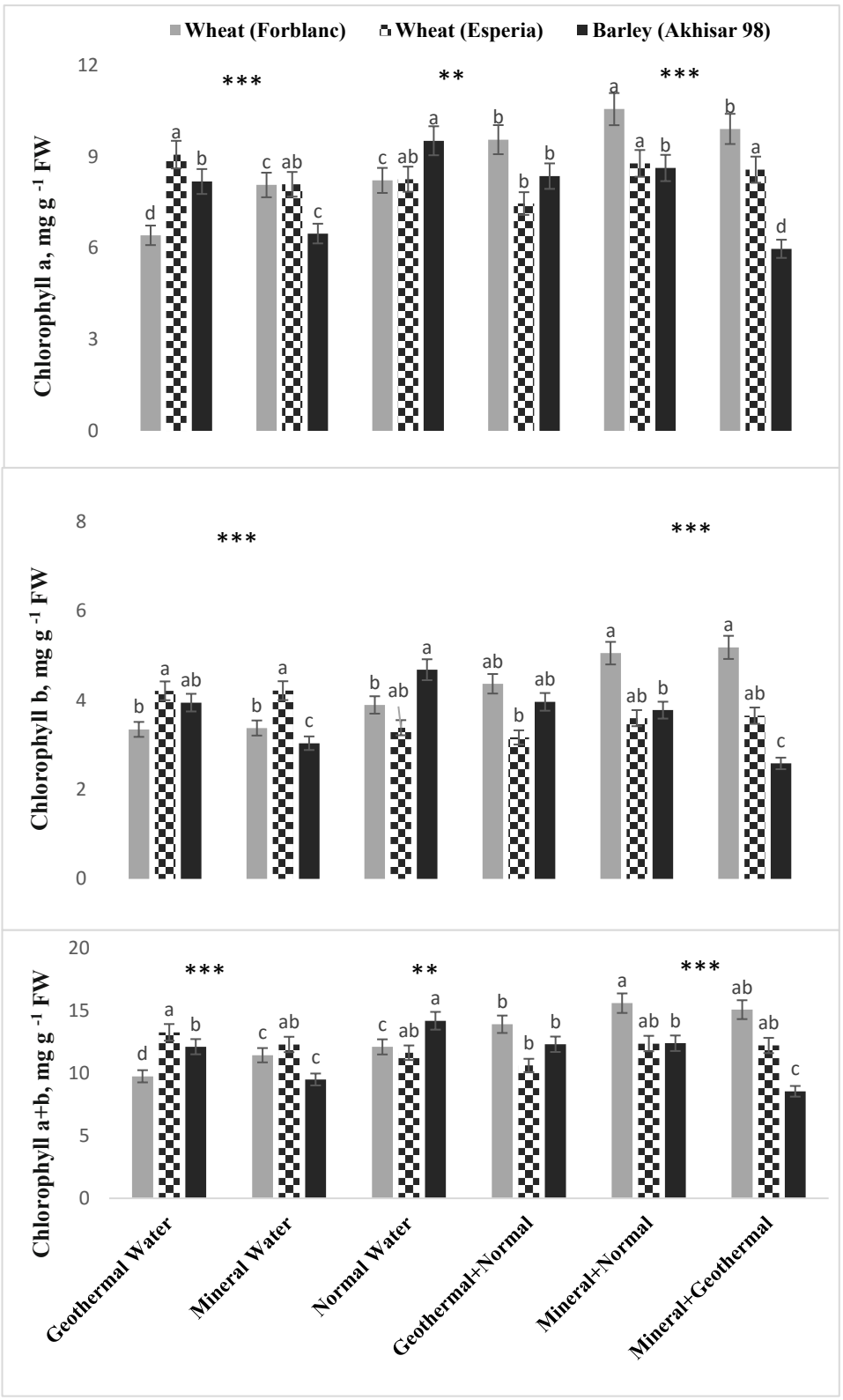

Figure 2. Chlorophyll a, Chlorophyll b, Chlorophyll a+b concentration in wheat and barley using different water

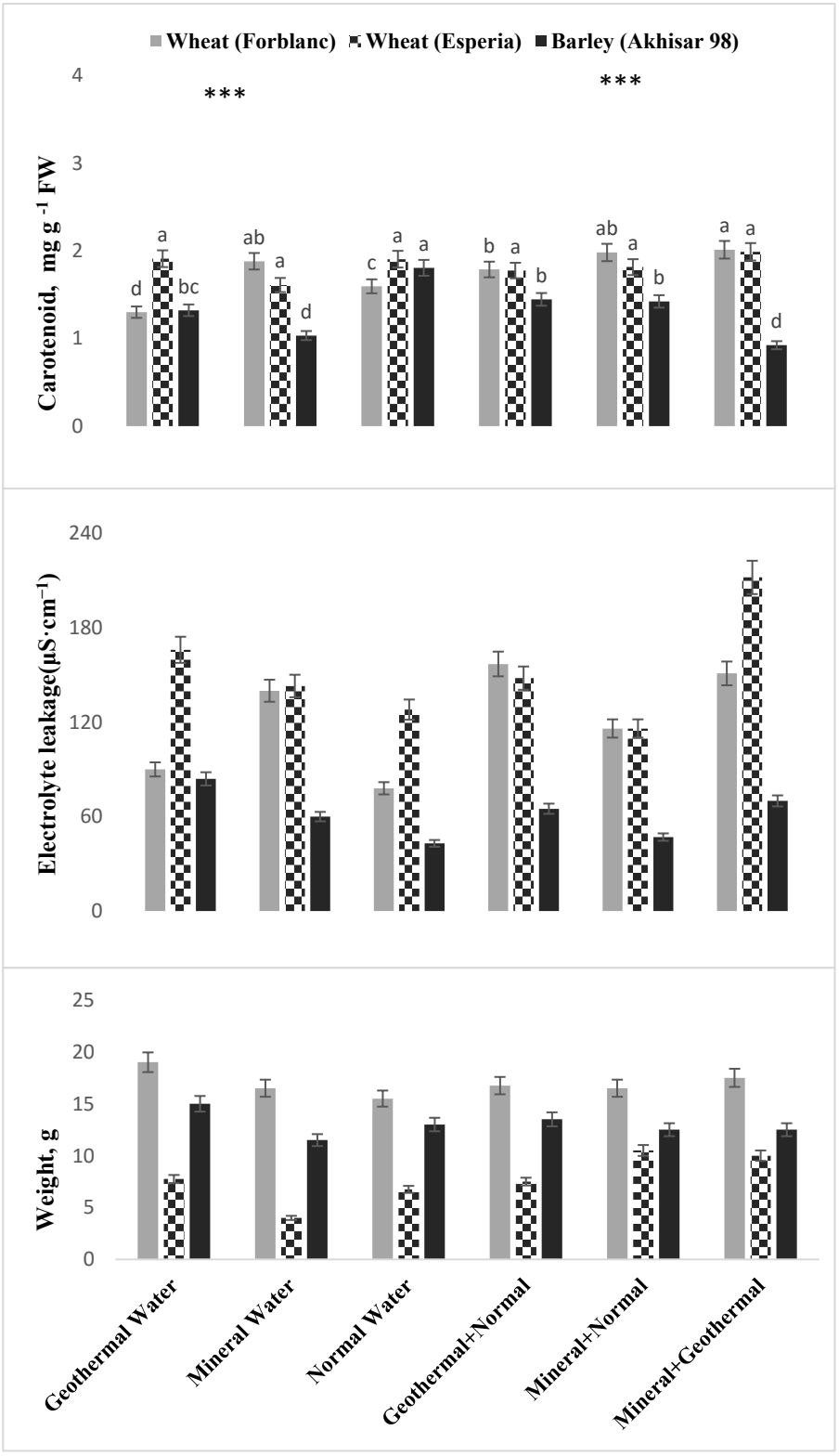

Figure 3. Carotenoid, Electrolyte leakage, Weight concentration in wheat and barley using different water

4. CONCLUSION

In general, geothermal waters are used for energy and therapeutic purposes. On the other hand, mineral waters are mostly used as drinking water. To our knowledge, there is no research on the use of geothermal and mineral waters in plant growing. Studies on groundwater quality for drinking and irrigation purposes have been conducted by many authors [28-32]. The data of this study showed that there were no noticeable differences between the experimental groups that may significantly affect the growth of plants. Türkiye has much thermal water with very different properties about contents, which come to the earth's surface. To investigate the effects of agricultural irrigation, long-term trial studies with different plants and soils should be conducted by determining the contents of these waters. Geothermal and mineral water resources that can be used should be determined in terms of their chemical properties. However, geothermal water can be used as well if it is pre-treated with appropriate methods. More comprehensive studies should be conducted to use such water in the irrigation of plants in case of future water deficiencies regarding this type of water, which is wasted to a substantial extent today. The effects of geothermal and mineral water on the product quality of crops should be examined in depth, and attention should be paid to studies on its use, especially in greenhouse cultivation work with this water.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ACKNOWLEDGEMENT

This study was supported by the Erzincan Binali Yıldırım University BAP (FYL-2019-638).

REFERENCES

[1] Talabi AO, Afolagboye LO, Aladejana, JA, Akinola, OO, Aturamu AO. Assessment of impacts of artisanal and small-scale mining activities on groundwater quality of ijero-ekiti SouthWestern Nigeria. Inter J of Inno Res in Sci Eng and Tec 2015; 4(4): 2194–2206.

[2] Nematollahi MJ, Ebrahimi P, Ebrahimi M. Evaluating hydrogeochemical processes regulating groundwater quality in an unconfined aquifer. Environ Proces 2016; 3(4): 1021–1043.

[3] Shah M, Sircar A, Varsada, R, Vaishnani S. Savaliya U, Faldu M, Vaidy D, Bhattacharya P. Assessment of geothermal water quality for industrial and irrigation purposes in the Unai geothermal field, Gujarat, India. Ground for Sustain Develop 2019a; 8: 59–68.

[4] Amalraj A, Pius A. Assessment of groundwater quality for drinking and agricultural purposes of a few selected areas in Tamil Nadu south India: a GIS-based study. Sustain Water Res Manage 2018; 4(1): 1-21.

[5] Annapoorna H, Janardhana MR. Assessment of groundwater quality for drinking purpose in rural areas surrounding a defunct copper mine. Aquatic Procedi (2015); 4: 685–692.

[6] Shah B, Kansara B, Shankar J, Soni M, Bhimjiyani P, Bhanushali T, Shah M, Sircar A. Reckoning of water quality for irrigation and drinking purposes in the konkan geothermal provinces, Maharashtra, India. Ground for Sustain Develop 2019b; 9: 100247.

[7] Meena RP, Karnam V, Tripathi SC, Jha A, Sharma RK, Singh GP. Irrigation management strategies in wheat for efficient water use in the regions of depleting water resources. Agri Water Manage 2019; 214: 38–46.

[8] Rodell M, Velicogna I, Famiglietti JS. Satellite-based estimates of groundwater depletion in India. Nature 2009; 460: 999.

[9] Tang Y, Zhang F, Engel BA, Liu X, Yue Q, Guo P. Grid-scale agricultural land and water management: A remotesensing- based multiobjective approach. J of Clean Pro 2020; 265: 121792

[10] Zhang F, Yue Q, Engel BA, Guo S, Guo P, Li X. A bi-level multiobjective stochastic approach for supporting environment-friendly agricultural planting strategy formulation. Sci of the Total Environ 2019; 693: 133593.

[11] Asano T. Water from (Waste) Water-The Dependable Water Resource. Stockholm Water Symposium, Stockholm, Sweden 2001.

[12] Polat A. Su Kaynaklarının Sürdürülebilirliği İçin Arıtılan Atıksuların Yeniden Kullanımı. Türk Bilimsel Derlemeler Dergisi, 2013; 6(1): 58-62.

[13] Aydoğdu MH, Mancı AR, Aydoğdu M. The Changes in Agricultural Water Management; Water User Associations, Pricing and Privatization Process. Elect J Soc Sci 2015; 14(52): 146-160.

[14] Aksoy Ö, Erbulucu T, Vatan E. Effects of Waste water from Olive oil and Milk Industry on Growth and Mitosis in Allium cepa Root Apical Meristem. JABS, 2011; 5(3): 75-78,

[15] Çalıseki M, Işık G, Leblebici S. Determination The Effects of The Porsuk River's Water on Seedling Development of *Cucumis sativus* (L.) Seeds. Anadolu University J of Sci. and Tec. C-Life Sci and Biotec 2016; 4(2); 77 – 80.

[16] Yucel E, Yılmaz G. Effects of Different Alkaline Metal Salts (NaCl, KNO_3), Acid Concentrations (H_2SO_4) and Growth Regulator (GA3) on the Germination of *Salvia cyanescens* Boiss. & Bal. Seeds. Gazi University J of Science 2009; 22(3): 123 – 127.

[17] FAO Land & Water. Checked on July 5th, 2019. http://www.fao.org/land-water/home/en/.

[18] Penna D, Geris J, Hopp L, Scandellari F. Water sources for root water uptake: Using stable isotopes of hydrogen and oxygen as a research tool in agricultural and agroforestry systems. Agri Eco and Environ 2020; 291: 106790

[19] Ak M, Top İ. Use of treated municipal wastewater for agricultural irrigation. PAJES 2018; 24(6): 1161-1168.

[20] Akbulut G. The Thermal Spring Tourism in Türkiye and Problems. GAUN-JSS 2010; 9(1): 35-54

[21] Doğanay H, Soylu H. The Importance of Deliçermik Thermal Spring from the Point of View of Tourism. Turkish Geogr Rev 1999; 34: 1-18.

[22] Anonymous http://www.donatlar.com.tr/urunler/tohum/ata-tohum/ekmeklik-bugdayy/forblanc, 2021a

[23] Anonymous https://www.tarimagel.com/urun/esperia-bugday-tohumu/9, 2021b

[24] Anonymous http://www.donatlar.com.tr/akhisar-98-arpa-tohumu, 2021c

[25] Griffith M, Ala P, Yang DSC, Hon WC, Moffatt BA. Antifreeze protein produced endogenously in winter rye leaves. Plant Physiol 1992; 100: 593-596.

[26] Osma E, İlhan V, Yalçın İE. Heavy metals accumulation causes toxicological effects in aquatic Typha domingensis Pers. Braz J of Bot 2014; 37: 461-467.

[27] Elveren M., Osma E. Investigation of Element Accumulation in Vegetables Cooked in Different Pots. BSEU J Sci, 2021; 8(2): 732-738.

[28] Aksever F, Davraz A, Bal Y. Assessment of water quality for drinking and irrigation purposes: a case study of Başköy springs (Aglasun/Burdur/Türkiye). Arabian J Geosci, 2016; 9(20): 748.

[29] Abbasnia A, Yousefi N, Mahvi AH, Nabizadeh R, Radfard M, Yousefi M, Alimohammadi M. Evaluation of groundwater quality using water quality index and its suitability for assessing water for drinking and irrigation purposes: case study of Sistan and Baluchistan province (Iran). Hum Ecol Risk Assess 2019; 25(4): 988–1005.

[30] Akhtar N, Syakir M, Rai S, Saini R, Pant N, Anees M, Qadir A, Khan U. Multivariate investigation of heavy metals in the groundwater for irrigation and drinking in Garautha Tehsil, Jhansi District, India. Anal Lett 2020; 53(5): 774–794.

[31] Hassen I, Hamzaoui-Azaza F, Bouhlila R. Application of multivariate statistical analysis and hydro-chemical and isotopic investigations for evaluation of groundwater quality and its suitability for drinking and agriculture purposes: case of Oum Ali-Thelepte aquifer, central Tunisia. Environ Monit Assess 2016; 188(3): 135

[32] Rufino F, Busico G, Cuoco E, Darrah TH, Tedesco D. Evaluating the suitability of urban groundwater resources for drinking water and irrigation purposes: an integrated approach in the Agro-Aversano area of Southern Italy. Environ Monit Assess 2019; 191(12): 768.

ESKİŞEHİR TEKNİK ÜNİVERSİTESİ BİLİM VE TEKNOLOJİ DERGİSİ C- YAŞAM BİLİMLERİ VE BİYOTEKNOLOJİ

Eskişehir Technical University Journal of Science and Technology C- Life Sciences and Biotechnology

2023, 12(1), pp. 30-41, DOI:10.18036/estubtdc.1199123

RESEARCH ARTICLE

HEALTH RISK ASSESSMENT OF HEAVY METALS IN SEABREAM (*Sparus aurata*) SAMPLED FROM A PUBLIC MARKET IN TÜRKİYE

Burcu YEŞİLBUDAK *

Department of Biology, Faculty of Science and Letters, Çukurova University, Adana, Turkey

ABSTRACT

There is a dearth of data about public health in the regular evaluation of heavy metal levels in seafood obtained from public market. This study aimed to examine the levels of essential (copper, zinc) and nonessential metals (cadmium, lead) in the tissues of cultured seabream (*Sparus aurata*), which was in public market. For this purpose, seabream samples were collected from the public market. And then, copper (Cu), zinc (Zn), cadmium (Cd), and lead (Pb) metal accumulation levels in tissues of the dissected gill, liver, kidney, and muscle were measured by inductively coupled plasma mass spectrometry (ICP-MS). In order to understand whether the fish posed a risk to public health, risk evaluation formulations (estimated daily intake: *EDI*, hazard coefficients: *HQ*, hazard index: *HI*) were calculated for the muscle tissue. The data showed that Zn, Cd and Pb concentrations were maximal in the kidney tissue of *S. aurata* and Cu concentration were in the liver tissue, while the minimum heavy metal levels were in the muscle tissue. As a result, the maximum levels of heavy metals in the edible tissue of seabream were found as 0.11 mg Cu/kg wet weight, 0.72 mg Zn/kg wet weight, 0.12 mg Cd/kg wet weight, and 0.34 mg Pb/kg wet weight. Maximum values of them do not indicate any health risks as they are lower than the allowable tolerable levels specified by the international *EDI* estimation committees. The examined *HQ* and *HI* indicators were observed below 1 in all seasons. However, it is always possible for heavy metal intake to pose potential risks. For this reason, it is essential that the results of the study be interpreted from different perspectives and taken into consideration by other scientists.

Keywords: *Sparus aurata*, Heavy metals, Accumulation, Health risk assessment, Türkiye

1. INTRODUCTION

It is known that fish consumption has many beneficial and protective properties, especially for heart health [1]. Moreover, regular intake of a rich fish oil supplement has been shown to regulate or alter various metabolic processes connected with lipid metabolism, atherosclerosis, thrombosis, and inflammation [2]. Consumption of fish meat is becoming more important every day because it is easier to digest and contains more protein, minerals, vitamins, essential unsaturated fatty acid and especially polyunsaturated fatty acid (omega-3-PUFA) compared to terrestrial organisms [3,4]. However, marine ecosystems are being polluted by heavy metals day by day, and there is an increase in the bioconcentration levels of heavy metals in the bodies of organisms [5,6]. Some heavy metals such as cadmium, lead and mercury accumulate in tissues even at very low concentrations and cause toxic effects, and these show non-essential properties for organisms [7]. On the other hand, copper-zinc in structural and metabolic events which have biological importance in organisms are needed within homeostatic control limits [8,9]. However, copper and zinc can cause public health problems such as neurotoxicity if the tolerable limits for metabolism are exceeded [10]. In case of exceeding the tolerated limits, heavy metals accumulate in the body and cause neurological disorders such as Parkinson's and Alzheimer's, changes in hematological parameters, growth and development disorders, and various metabolic organ function anomalies, which in turn result in a series of fatal health problems [11]. The World Health Organization recommends regular monitoring of the levels of heavy metals such as copper, zinc, cadmium, lead, mercury and aluminum in fish tissues [12]. Seabream (*Sparus aurata*) is a commercially important fish species, and it is recommended to be monitored in the environmental pollution risk analysis [13,14]. According to the Turkish Statistical Institute

*Corresponding Author: yesilbudak@gmail.com
Received:29.10.2022 Published: 30.01.2023

(TUIK), while the average fish consumption per person in Türkiye was 5.49 kg in 2017, it increased by 11.8% to 6.14 kg in 2018 [15]. The rates of toxic substances in food and their effects on human health have been gaining importance in recent years [16]. To this end, the present study was firstly aimed to evaluate the heavy metal accumulation levels in the tissues of *S. aurata* seasonally and to monitor whether an abnormal situation occurred. Secondly, the study was intended to examine the heavy metal levels in the edible muscle tissue of the fish to evaluate its public health risk level through some risk evaluation calculations such as *EDI* (estimated daily intake), *HQ* (hazard coefficients), *HI* (hazard index).

2. MATERIALS AND METHODS

2.1. Sampling Fish

A total of 72 *S. aurata* were obtained from the fish market of Osmaniye province in the winter, spring, summer, fall and winter seasons of 2018 by random sampling method in the research. The total length and total weight of the collected fish samples were measured on a clean bench that could not cause any contamination. The total length measurement of the samples was carried out with ±0.1 mm scale caliper and the total weight measurement was carried out with a balance with ±0.1g precision (Sartorius BP-310S). Height and weight characteristics of the fish according to the seasons were 68.10 ±5.20 g and 13.30 ±1.10 cm for winter, 70.35±4.90 g and 15.25±1.15 cm for spring, 80.55±5.30 g and 20.30±1.30 cm for summer, 80.90±3.90 g and 28.25±0.90 cm for fall. The fish were then stored in a -20 freezer until the metal analysis. All glass and polypropylene materials to be used in the experiment were cleaned according to appropriate protocols to eliminate the presence of various chemical residues [17].

2.2. Sample Preparation of Fish and Heavy Metal Analyses

Fish tissues were dissected, then left to dry in an oven (Memmert UFE 500, Germany) at 150°C for 48 hours. After the tissues came to constant weight, they were weighed by an electronic balance with a precision of ±0.001 g (Sartorius CP-2248). The tissues (about 0.5 g) were transferred to the experiment tubes, and a mixture of 2:1 (v/v) nitric acid and perchloric acid (Merck, %65, S.G.; 1.40 and %60, S.G.; 1.53) was added to them, and the tissues were homogenized on the hot plate (Thermo Scientific, 2200, USA) at 120°C for 3 hours [18]. Following the homogenization, the samples were transferred to polypropylene tubes. The tubes were diluted to 5 ml with ultrapure water so that the samples were ready for metal analysis. The samples were stored in the refrigerator at +4ºC until the time of analysis. Metal concentrations (Cu, Zn, Cd, Pb) in the samples were determined with the ICP-MS Agilent 7500ce model spectrometer (Octopole Reaction Systems, Agilent Technologies, Japan). The instrument calibration of metal analyses was performed according to C-5524 Sigma standards (Sigma Chem. Co. St. Louis, USA). Standard solutions of the metals were diluted with ultrapure water (Millipore Elix Milli-Q, resistivity 18.2 MΩ/cm). The TORT 2 (Lobster hepatopancreas) standard reference material was used to check the reliability of the measurements. The validation parameters of analytic techniques are shown in Table 1.

Table 1. The validation parameters of analytic techniques of Cu, Zn, Cd and Pb.

Element	Recovery (%)	Detection limit (µg/kg)	Quantification limit (µg/kg)	Relative standard deviation (%)	R^2
Cu	95.60	0.60	2.01	3.07	0.99
Zn	97.70	2.45	8.27	3.75	0.99
Cd	91.70	0.45	1.55	2.01	0.99
Pb	95.05	0.35	1.20	2.10	0.99

2.3. Risk Assessment Calculations and Statistical Analysis

The estimation of daily intake rate [$(EDI) = (C_{element} \times D_{fish})Bw^{-1}$] equation was used to estimate the daily intake (*EDI*) of metals (mg/kg body weight/day). The abbreviation $C_{element}$ in the formula refers to the metal concentration in the muscle tissue (mg/kg wet weight (ww)), D_{fish} means the daily fish consumption (g/person/day), and *Bw* (kg) represents the average body weight of the population [19,20]. In Türkiye, the annual fish consumptions of an adult person of 70 kg was found to be 5.5 kg. The hazard quotient [$HQ = EDI/RfD$] relation was used to calculate the hazard coefficient (*HQ*). It is stated in the literature that if the *HQ* value is less than 1, it does not constitute a significant risk potential; in contrast, if the *HQ* value is equal to or higher than 1, then it poses a potential risk for the public health. The abbreviation *RfD* in the formulation refers to the oral reference doses for Cu, Zn, Cd, and Pb, which are specified by the risk assessment information system [21] as 0.3, 0.7, 0.001 and 0.004, mg/kg/day, respectively. The *RfD values* were also utilized as the reference values in the present study. The hazard index [$HI=\Sigma HQs=HQ_{Cu}+HQ_{Zn}+HQ_{Cd}+HQ_{Pb}$] formulation was estimated as the sum of the *HQs* for all the heavy metals in the formula. If the *HI* value of metals is less than 1, then it is stated that the health of local fish consumers is safe, whereas, if the *HI* is equal to or greater than 1, then it is stated that it is harmful for public health [22]. Evaluation of the samples studied in triplicate for every season was performed in the Microsoft Office Excel 2010 program. Mean, standard error, minimum and maximum values were calculated for the data which were grouped according to the seasons. Statistical analysis of the data was carried out using the SPSS 20.0 statistical software (IBM Corp., Armonk, NY, USA). Tukey multiple range test was used to determine whether the metal concentrations varied significantly among tissues based on the season [23].

3. RESULTS AND DISCUSSION

Metal accumulation in tissues and organs of aquatic organisms varies depending on seasonal changes [24], species of the organism [25], morphological structure of the organism such as length and weight [26,27], environmental conditions such as physical and chemical condition of the aquatic ecosystem [28]. The arithmetic mean and standard error values of the heavy metal levels in *S. aurata* tissues (gill, liver, kidney and muscle) were calculated according to the seasons, and the variation in seasonal metal levels were examined by calculating the sum of the squares of the deviations of the data from the arithmetic mean. Accordingly, a summarized graphical representation of the arithmetic mean, standard errors and statistical differences of the metals in the tissues was displayed in Figure 1.

The mean annual Cu, Zn, Cd and Pb concentrations in the gill tissue of *S. aurata* sampled in winter, spring, summer and fall were found to be 0.16, 0.72, 0.12 and 0.25 mg/kg ww, respectively. According to the seasons, Cu accumulation in the gill tissue was the most in the spring season, while the accumulation of Zn was the most in the fall season ($P \leq 0.05$). The highest accumulation of cadmium in the gill tissue was observed in the fall season, but no statistical difference was observed between the accumulation averages of cadmium in the fall and winter seasons and the accumulation rates of lead in the spring and summer seasons ($P \geq 0.05$). The heavy metal accumulation in the gill tissue in the fall and winter season was in the form of Zn>Pb>Cd>Cu, while it was determined as Zn>Cu>Pb>Cd and Zn>Cu>Pb≈Cd in the spring and summer seasons, respectively (Figure 1). The mean annual Cu, Zn, Cd and Pb concentrations in the liver tissue were 0.46, 0.90, 0.15 and 0.23 mg/kg ww, respectively. Cu and Pb accumulation in liver tissue was found to be the highest in the spring, while Zn and Cd accumulations showed the highest accumulation in the summer and fall seasons, respectively ($P \leq 0.05$). While the heavy metal accumulation was in the form of Zn>Cu>Cd>Pb in the fall season, it was determined as Zn>Cu>Pb>Cd in other seasons (Figure 1). The mean annual Cu, Zn, Cd and Pb accumulations in the kidney tissue were 0.29, 1.84, 0.30 and 0.47 mg/kg ww, respectively. The Zn accumulation in the kidney tissue showed the highest accumulation in the summer while the highest accumulations of Cu, Cd and Pb were detected in the fall season compared to the other seasons

($P \leq 0.05$). The heavy metal accumulation sequence in the kidney tissue was observed as Cd>Cu>Zn>Pb in the winter, Zn>Pb>Cd>Cu in the spring, and Zn>Pb>Cu>Cd in the summer and fall (Figure 1).

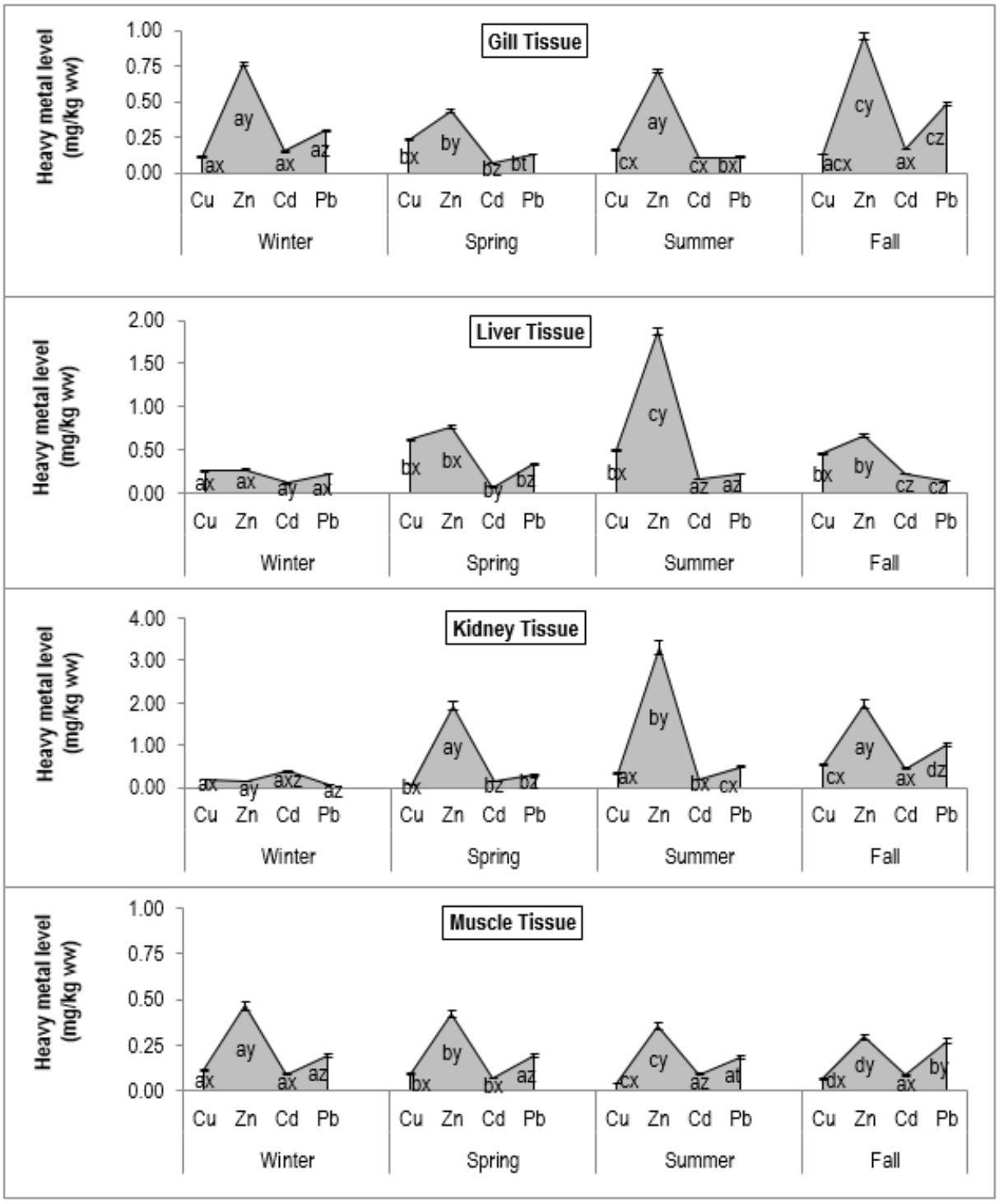

Figure 1. Mean and standard error of heavy metal concentrations in gill, liver, kidney and muscle tissue of *S. aurata* in different seasons. Letters x, y, z and t show differences among Cu, Zn, Cd, Pb metals; a, b, c and d among winter, spring, summer, fall seasons.

The mean annual Cu, Zn, Cd and Pb concentrations in the muscle tissue of *S. aurata* were found to be 0.07, 0.38, 0.08, 0.21 mg/kg ww, respectively. Cu and Zn accumulations in the muscle tissue were determined to be the highest in the winter season, while the highest accumulation of Pb was identified in the fall season ($P \leq 0.05$). Cadmium showed the highest accumulation in the summer season, while statistical difference for the cadmium accumulation was observed only between the spring and summer ($P \leq 0.05$). The heavy metal accumulation of the muscle tissue showed Zn>Pb>Cu>Cd sequence for winter, spring and fall, while it was Zn>Pb>Cd>Cu for summer (Figure 1). Among the heavy metals examined in 10 species including *S. aurata*, zinc was the most detected metal [29], and similarly, the presence of zinc was found at the highest level in our study.

Heavy metals in the contaminated aquatic ecosystem lead to bioaccumulations in various organs and structural units of fish from the food web or in the aquatic environment over time [30]. In a previous study, it was reported that there were significant differences between the metal bioaccumulation levels in fish tissues ($P \leq 0.05$), and that the metal accumulation was higher in the liver and gill organs than in other organs [30,31]. Since metabolic rates of large internal organs such as the liver and kidney are higher than other organs, the heavy metal accumulation in these organs increases in parallel. The negatively charged surface of the gill tissue in fish tends to bind to many positively charged metals [32]. Heavy metals enter aquatic organisms through two metabolic routes: the gills and muscle tissue. Therefore, metal concentrations in these tissues and organs are mostly reflected as an indicator of the ambiance in the aquatic ecosystem [33]. In environmental monitoring studies of heavy metal pollution, various concerns may be stated about the frequency and persistence of being affected by contaminated heavy metals due to the consumption of the muscle tissue of fish. However, if the muscle tissue compared to other soft organs does not have an active structure in the metal accumulation, this situation may be related to low metal binding proteins [34]. This situation is explained in many studies by the fact that the muscle tissue of the fish has less metabolic activity compared to other organs, together with less heavy metal accumulation [31, 35]. The high concentrations of heavy metal determined in the liver tissue of aquatic organisms are due to its high metallothionein production, which has made it an important storage organ as an indicator of chronic metal exposure [31]. In aquatic ecosystems, the entrance of heavy metals to the structure of organisms in the food chain occurs in two ways: the first is directly supplied to the organism through the digestive tract, while the other is by external means such as the muscles and gills [33]. The direct route of uptake of heavy metals from the aquatic ecosystem is more effective because the gills are the main target organ of heavy metal poisoning in fish [36]. The orders of annual metal accumulations according to tissues of the seabream were observed as kidney>liver>gill>muscle for both Zn and Cd, liver>kidney>gill>muscle for Cu, and kidney>gill>liver>muscle for and Pb, in the current study (Figure 1).

In this study, the minimum and maximum levels of metals in the muscle tissue differed between the following ranges, respectively; 0.01-0.11 mg Cu/kg ww, 0.25-0.72 mg Zn/kg ww, 0.05-0.12 mg Cd/kg ww and 0.18-0.34 mg Pb/kg ww (Table 2). Average Cu, Zn, Cd and Pb concentrations in the seabream muscle samples taken from public aquaculture fish in Osmaniye province were found to be far below the maximum levels allowed for fisheries by international organizations (Table 3). Heavy metal samples in the seabream samples obtained from the coastal locations of the Black Sea [29] and the Mediterranean Sea in Türkiye (Table 3) had higher content than the heavy metal levels of the samples in this study. These values in the present study were within the limits set by the Turkish Food Codex Commission Regulation [37]. In a previous study, it was reported that the average values of heavy metals such as total As, Cd, Cu, Pb and Zn in farmed fish were lower than in the wild fish, and the Cd concentration was higher in farmed fish [38]. Table 3 partly shows that the cultured fish in this study differed from wild species.

The *HI* index of heavy metals in wild and farmed seabream sampled from the Algerian coast was determined to be less than one [38]. It has been stated that the consumption of farmed fish *Dicentrarchus labrax* and *S. aurata* analyzed from fish farms in different locations of the

Mediterranean does not pose a serious threat to human health and the fish are in a consumable condition [39]. The international legal limits and indices of trace elements (Ag, Al, As, Ba, Bi, Cd, Cr, Cu, Fe, Li, Mn, Mo, Pb, Sb, Se, Sn, U, V, Zn) in muscle tissues of cultured seabream examined from the Corsican Coasts of the Northwestern Mediterranean were determined to be below the levels that could pose a risk to human health. It was also reported in this study that some elements (As etc.) found in wild *S. aurata* should be consumed in moderation considering their carcinogenic risks [40]. These results in the literature are consistent with the current study with *S. aurata* (Figure 2).

Table 2. Minimum and maximum metal concentrations in muscle tissue of *S. aurata* in different seasons.

Heavy Metals (mg/kg ww)	**Winter** min-max	**Spring** min-max	**Summer** min-max	**Fall** min-max
Cu	0.11-0.12	0.01-0.02	0.03-0.06	0.01-0.02
Zn	0.35-0.58	0.25-0.72	0.26-0.43	0.29-0.31
Cd	0.08-0.10	0.07-0.07	0.05-0.12	0.07-0.11
Pb	0.18-0.20	0.19-0.20	0.16-0.21	0.24-0.34

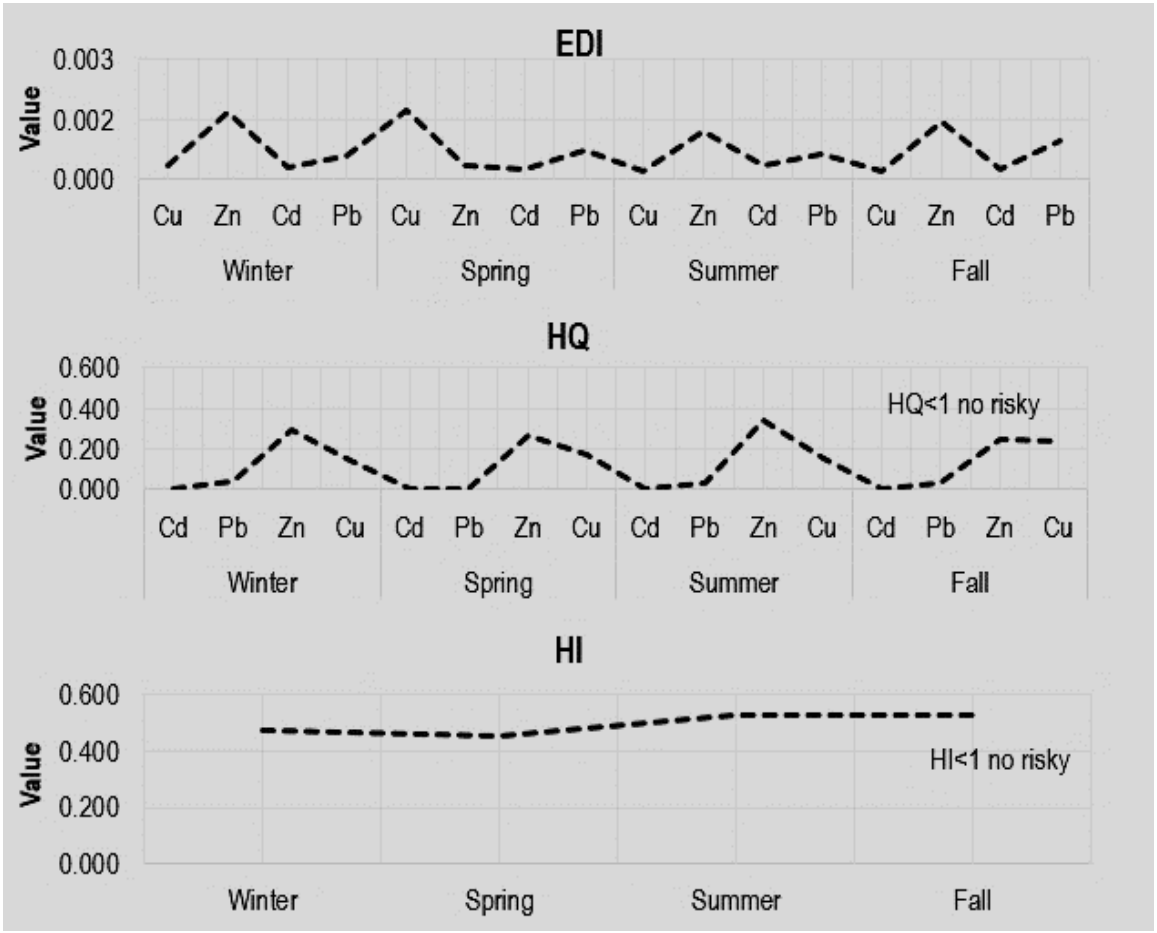

Figure 2. Public health risk values of heavy metals in *S. aurata* from public market in Osmaniye (Türkiye).

Table 3. The maximum limits of heavy metals accepted by international organizations in fishery products and comparison of heavy metal's concentrations in muscle of *S. aurata* with other studies (BDL: Below Detection Limit)

International organizations	**Heavy Metal** (mg/kg ww)				Reference
	Cu	**Zn**	**Cd**	**Pb**	
U.S. Food and Drug Administration	150	100	0.20	1.50	[41]
World Health Organization	30	100	1	2	[42]
Turkish Food Codex	20	50	0.50	0.30	[37]
Other studies					
Tuzla Lagoon (Wild fish)	0.55	-	0.12	1.11	[31]
Beymelek Lagoon (Wild fish)	4.31	7.09	-	-	[43]
Kayseri Province (Market fish)	0.90	33.80	1.24	BDL	[44]
Tirana Province (Wild fish)	-	-	0.35	0.10	[45]
Iskenderun Bay (Wild fish)	6.23	14.35	1.25	3.83	[46]
Elazığ Province (Market fish)	1.19	6.27	0.18	0.44	[47]
Aegean Sea (Cultured fish)	BDL	4.99	BDL	BDL	[48]
Ionian Sea (Cultured fish)	BDL	7.02	BDL	BDL	[48]
Adriatic Sea (Cultured fish)	0.39	4.70	0.01	0.10	[49]
Aegean Sea (Cultured fish)	0.36	2.12	-	-	[35]
Hurmabogazı Lagoon (Wild fish)	1.33	11.1	0.13	0.52	[50]
Çamlık Lagoon (Wild fish)	0.88	4.92	0.64	0.45	[51]
Tuzla Lagoon (Wild fish)	0.39	6.91	0.30	0.32	[51]
Corsica Mediterranean Sea (Wild fish)	0.20	3.55	0.002	0.006	[40]
Corsica Mediterranean Sea (Cultured fish)	0.49	4.34	0.004	0.013	[40]
Croatian (Market fish)	0.94	-	9.4	-	[52]
South Adriatic-Montenegro (Wild fish)	0.17	-	<0.02	<0.1	[53]
Cassidaigne canyon (Wild fish)	-	-	BDL	-	[54]
South Italy (Wild fish)	0.17	0.17	0.04	0.08	[55]
Mediterranean Sea (Wild fish)	0.29	4.13	0.002	0.004	[38]
Mediterranean Sea (Market fish)	0.07	0.38	-	-	[56]
Maximum value detected, (Public market-Cultured fish)	0.11	0.72	0.12	0.34	In this study

4. CONCLUSION

The present study focused on checking the quality of seabream fish in terms of the copper, zinc, cadmium and lead contents. Considering the protein starvation all around the world, seabream is considered a delicious and economically valuable fish. The quality indicators examined in this study showed that seabream is a safe seafood product for consumption given that the calculated data

indicators (*HQ* and *HI*) were below 1 for all seasons (Figure 2). As far as we know, there is still no known scale indicator for the content of heavy metals studied in fish in many countries. Hence, a basic comparison was made by taking into consideration the evaluations in our country. However, when dietary regimens and individual morphological structures are taken into account, what is not a risk for one country may pose a risk for another. Therefore, the acceptable levels of heavy metals should be considered according to the references specified by the Food and Drug Administration (FDA) and the World Health Organization. With this regard, it was concluded that Cu, Zn, Cd and Pb levels in fish species were lower than the maximum levels, but further studies are needed to monitor the course of heavy metals over time.

CONFLICT OF INTEREST

The author stated that there are no conflicts of interest regarding the publication of this article.

REFERENCES

[1] Kris-Etherton PM, Harris WS, Appel LJ. Fish consumption, fish oil, omega-3 fatty acids, and cardiovascular disease. Arterioscler Thromb Vasc Biol 2003; 23(2): 20-30. DOI:10.1161/01.ATV.0000038493.65177.94.

[2] Wang YY, Zhang JX, Tian T, Gao MY, Zhu QR, Xie W, Fu LM, Wang SK, Dai Y. Dietary patterns in association with the risk of elevated blood pressure, lipid profile and fasting plasma glucose among adults in Jiangsu Province of China. Nutr Metab Cardiovasc Dis 2022; 32(1): 69-79. DOI:10.1016/j.numecd.2021.09.004.

[3] Antonijevic B, Jankovic S, Curcic M, Durgo K, Stokic E, Srdic B, Tomic-Naglic D. Risk characterization for mercury, dichlorodiphenyltrichloroethane and polychlorinated biphenyls associated with fish consumption in Serbia. Food Chem Toxicol 2011; 49(10): 2586-2593. DOI:10.1016/j.fct.2011.06.078.

[4] Khalili Tilami S, Sampels S. Nutritional value of fish: lipids, proteins, vitamins, and minerals. Rev Fish Sci Aquacult 2018; 26(2): 243-253. DOI:10.1080/23308249.2017.1399104.

[5] Storelli MM. Potential human health risks from metals (Hg, Cd, and Pb) and polychlorinated biphenyls (PCBs) via seafood consumption: estimation of target hazard quotients (THQs) and toxic equivalents (TEQs). Food Chem Toxicol 2008; 46(8): 2782-2788. DOI:10.1016/j.fct.2008.05.011.

[6] Taş EÇ, Filipuçi I, Çakır DT, Beyaztaş S, Sunlu U, Toğulga M, Özaydın O, Arslan O. Heavy metal concentrations in tissues of edible fish (*Mullus barbatus* L.,1758) from Çandarlı Bay (Turkey). Fresenius Environmental Bulletin 2011; Volume 20 – No 11 : 2834-2839.

[7] Türkmen M, Türkmen A, Tepe Y, Töre Y, Ateş A. Determination of metals in fish species from Aegean and Mediterranean seas. Food Chem 2009; 113(1): 233-237. DOI:10.1016/j.foodchem.2008.06.071

[8] Amundsen PA, Staldvik FJ, Lukin AA, Kashulin NA, Popova OA, Reshetnikov YS. Heavy metal contamination in freshwater fish from the border region between Norway and Russia. Sci Total Environ 1997; 201(3): 211-224. DOI:10.1016/S0048-9697(97)84058-2.

[9] Couture P, Rajotte JW . Morphometric and metabolic indicators of metal stress in wild yellow perch (*Perca flavescens*) from Sudbury, Ontario: a review. J Environ Monit 2003; 5(2): 216-221. DOI:10.1039/B210338A.

[10] DPHHS U. Agency for Toxic Substances and Disease Registry. Toxicological Profile for Zinc. Atlanta, GA: US Department of Public Health and Human Services, 2005.

[11] Isangedighi IA, David GS. Heavy metals contamination in fish: effects on human health. J Aquat Sci Mar Biol 2019; 2(4): 7-12.

[12] Chapman D. Water quality assessments: a guide to the use of biota, sediments and water in environmental monitoring. Boca Raton, Florida, USA: CRC Press, 2021.

[13] Bakiu R, Santovito G, Hoda A, Shehu J, Durmishaj S, Irato P, Piccinni E. Metallothionein (MT): a good biomarker in marine sentinel species like sea bream (*Sparus aurata*). Albanian J Agric Sci 2013; 12(2): 247.

[14] Benhamed S, Guardiola FA, Martínez S, Martínez-Sánchez MJ, Pérez-Sirvent C, Mars M, Esteban MA. Exposure of the gilthead seabream (*Sparus aurata*) to sediments contaminated with heavy metals down-regulates the gene expression of stress biomarkers. Toxicol Rep 2016; 3: 364-372. DOI:10.1016/j.toxrep.2016.02.006

[15] Turkish Statistical Institute. Fisheries Statistics. 2019. https://www.tuik.gov.tr/Home/Index. Accessed July 22, 2022.

[16] Khansari FE, Ghazi-Khansari M, Abdollahi M. Heavy metals content of canned tuna fish. Food Chem 2005; 93(2): 293-296. DOI:10.1016/j.foodchem.2004.09.025

[17] Tuzen M. Toxic and essential trace elemental contents in fish species from the Black Sea, Turkey. Food Chem Toxicol 2009; 47(8): 1785-1790. DOI:10.1016/j.fct.2009.04.029.

[18] Muramoto S. Elimination of copper from Cu-contaminated fish by long-term exposure to EDTA and fresh water. J Environ Sci Health Part A 1983; 18(3): 455-461. DOI:10.1080/10934528309375113.

[19] Griboff J, Wunderlin DA, Monferran MV. Metals, As and Se determination by inductively coupled plasma-mass spectrometry (ICP-MS) in edible fish collected from three eutrophic reservoirs. Their consumption represents a risk for human health? Microchem J 2017; 130: 236-244. DOI:10.1016/j.microc.2016.09.013.

[20] GDFA (General Directorate of Fisheries and Aquaculture). Fisheries Statistics. Republic of Turkey Ministry of Food Agriculture and Livestock, 2018. Accessed May 1, 2022.

[21] The Risk Assessment Information System (RAIS). (2019). University of Tennessee, Available from: https://rais.ornl.gov/index.html.

[22] Li J, Huang ZY, Hu Y, Yang H. Potential risk assessment of heavy metals by consuming shellfish collected from Xiamen, China. Environ Sci Pollut Res 2013; 20 (5): 2937-2947. DOI:10.1007/s11356-012-1207-3.

[23] Sokal RR, Rohlf FJ. Biometry: the principles and practice of statistics in biological research, 3rd edn. New York: WH Freeman and Co, 1995.

[24] Dural M, Göksu MZL, Özak AA. Investigation of heavy metal levels in economically important fish species captured from the Tuzla lagoon. Food Chem 2007; 102(1): 415-421.DOI:10.1016/j.foodchem.2006.03.001.

[25] Türkmen M, Türkmen A, Tepe Y, Ateş A, Gökkuş K. Determination of metal contaminations in sea foods from Marmara, Aegean and Mediterranean seas: twelve fish species. Food Chem 2008; 108(2): 794-800. DOI:10.1016/j.foodchem.2007.11.025.

[26] Damodharan U, Reddy MV. Heavy metal bioaccumulation in edible fish species from an industrially polluted river and human health risk assessment. Fish Aquat Life 2013; 21(1): 19-27. DOI:10.2478/aopf-2013-0003.

[27] Mohammadnabizadeh S, Pourkhabbaz A, Afshari R. Analysis and determination of trace metals (nickel, cadmium, chromium, and lead) in tissues of *Pampus argenteus* and *Platycephalus indicus* in the Hara Reserve, Iran. J Toxicol 2014; Article ID: 576496. DOI:10.1155/2014/576496.

[28] Jezierska B, Witeska M. Metal Toxicity to Fish. Siedlce: Wydawnictwo Akademii Podlaskiej, 2001.

[29] Bat L, Sezgin M, Üstün F, Şahin F. Heavy metal concentrations in ten species of fishes caught in Sinop coastal waters of the Black Sea, Turkey. Turkish J Fish Aquat Sci 2012; 12(5): 371-376. DOI:10.4194/1303-2712-v12_2_24.

[30] Jabeen G, Javed M, Azmat H. Assessment of heavy metals in the fish collected from the river Ravi, Pakistan. Pakistan Vet J 2012; 32(1): 107-111.

[31] Dural M, Göksu MZL, Özak AA. Investigation of heavy metal levels in economically important fish species captured from the Tuzla lagoon. Food Chem, 2007; 102(1): 415-421. DOI:/10.1016/j.foodchem.2006.03.001.

[32] Playle RC. Using multiple metal-gill binding models and the toxic unit concept to help reconcile multiple-metal toxicity results. Aquat Toxicol 2004; 67(4): 359-370. DOI:10.1016/j.aquatox.2004.01.017.

[33] Rajeshkumar S, Liu Y, Zhang X, Ravikumar B, Bai G, Li X. Studies on seasonal pollution of heavy metals in water, sediment, fish and oyster from the Meiliang Bay of Taihu Lake in China. Chemosphere 2018; 191: 626-638. DOI:10.1016/j.chemosphere.2017.10.078.

[34] Squadrone S, Prearo M, Brizio P, Gavienelli S, Pellegrino M, Scanzio T, Guarise S, Benedetto A, Abete MC. Heavy metals distribution in muscle, liver, kidney and gill of European catfish (*Silurus glanis*) from Italian Rivers. Chemosphere 2013; 90(2): 358-365. DOI:10.1016/j.chemosphere.2012.07.028.

[35] Castritsi-Catharios J, Neofitou N, Vorloou AA. Comparison of heavy metal concentrations in fish samples from three fish farms (Eastern Mediterranean) utilizing antifouling paints. Toxicol Environ Chem 2015; 97(1): 116-123. DOI:10.1080/02772248.2014.943226.

[36] Sauliutė G, Svecevičius G. Heavy metal interactions during accumulation via direct route in fish: a review. Zool Ecol 2015; 25(1): 77-86. DOI: 10.1080/21658005.2015.1009734.

[37] Anonymous. Turkish Food Codex Contaminants Regulation Authority Law, 2011. Official Gazette No. 5996, 29.12.2011-28157. Accessed May 2, 2022.

[38] Lounas R, Kasmi H, Chernai S, Amarni N, Ghebriout L, Hamdi B. Heavy metal concentrations in wild and farmed gilthead sea bream from southern Mediterranean Sea-human health risk assessment. Environ Sci Pollut Res 2021; 28(24): 30732-30742. DOI:10.1007/s11356-021-12864-3.

[39] Kalantzi I, Pergantis SA, Black KD, Shimmield TM, Papageorgiou N, Tsapakis M, Karakassis I. Metals in tissues of seabass and seabream reared in sites with oxic and anoxic substrata and risk assessment for consumers. Food Chem 2016; 194: 659-670. DOI:10.1016/j.foodchem.2015.08.072.

[40] Marengo M, Durieux ED, Ternengo S, Lejeune P, Degrange E, Pasqualini V, Gobert S. Comparison of elemental composition in two wild and cultured marine fish and potential risks to human health. Ecotoxicol Environ Saf 2018; 158: 204-212. DOI:10.1016/j.ecoenv.2018.04.034.

[41] Food and Drug Administration. Fish and fisheries products hazards and controls guidance, 3rd edn. US FDA. Washington, DC: Center for Food Safety & Applied Nutrition, 2001.

[42] Mokhtar MB, Aris AZ, Munusamy V, Praveena SM. Assessment level of heavy metals in *Penaeus monodon* and *Oreochromis spp* in selected aquaculture ponds of high densities development area. Eur J Sci Res 2009; 30(3): 348-360.

[43] Uysal K, Emre Y, Köse E. The determination of heavy metal accumulation ratios in muscle, skin and gills of some migratory fish species by inductively coupled plasma-optical emission spectrometry (ICP-OES) in Beymelek Lagoon (Antalya/Turkey). Microchem J 2008; 90(1): 67-70. DOI:10.1016/j.microc.2008.03.005.

[44] Yıldırım Y, Gönülalan Z, Narin I, Soylak M. Evaluation of trace heavy metal levels of some fish species sold at retail in Kayseri, Turkey. Environ Monit Assess 2009; 149(1): 223-228. DOI:10.1007/s10661-008-0196-7.

[45] Ozuni E, Dhaskali L, Abeshi J, Zogaj M, Haziri I, Beqiraj D, Latifi F. Heavy metals in fish for public consumption and consumer protection. Nat Montenegrina 2010; 9(3): 843-851.

[46] Dural M, Genc E, Sangun MK, Güner Ö. Accumulation of some heavy metals in *Hysterothylacium aduncum* (Nematoda) and its host sea bream, *Sparus aurata* (Sparidae) from North-Eastern Mediterranean Sea (Iskenderun Bay). Environ Monit Assess 2011; 174:147-155. DOI:10.1007/s10661-010-1445-0.

[47] İlhak Oİ, Karatepe P, Özçelik M. Heavy metal levels in some fish species sold at retail in Elazığ. Fırat Univ Med J Health Sci 2012; 26(2): 99-103.

[48] Kalantzi I, Black KD, Pergantis SA, Shimmield TM, Papageorgiou N, Sevastou K, Karakassis I. Metals and other elements in tissues of wild fish from fish farms and comparison with farmed species in sites with oxic and anoxic sediments. Food Chem 2013; 141(2): 680-694. DOI:10.1016/j.foodchem.2013.04.049.

[49] Žvab Rožič P, Dolenec T, Baždarić B, Karamarko V, Kniewald G, Dolenec M. Element levels in cultured and wild sea bass (*Dicentrarchus labrax*) and gilthead sea bream (*Sparus aurata*)

from the Adriatic Sea and potential risk assessment. Environ Geochem Health 2014; 36(1): 19-39. DOI:10.1007/s10653-013-9516-0.

[50] Türkmen A, Tepe Y, Türkmen M. Determination of metals in tissues of fish species from Hurmabogazı Lagoon. Indian J Geo-Mar Sci 2016; 45 (2): 277-282.

[51] Tepe Y, Türkmen A, Türkmen M. Comparison of heavy metal accumulation in tissues of economically valuable fish species from two nearby lagoons in Mediterranean coastal area. Indian J Geo-Mar Sci 2017; 46(7): 1333-1338.

[52] Bilandžić N, Sedak M, Čalopek B, Đokić M, Varenina I, Kolanović BS, Luburić ĐB, Varga I, Benić M, Roncarati A. Element contents in commercial fish species from the Croatian market. J Food Compos Anal 2018; 71:77-86. DOI:10.1016/j.jfca.2018.02.014.

[53] Antović I, Šuković D, Andjelić S, Svrkota N. Heavy metals and radionuclides in muscles of fish species in the South Adriatic-Montenegro. In RAP Conference Proceedings 2019; 4: 96-102. DOI:10.37392/RapProc.2019.19

[54] Bouchoucha M, Chekri R, Leufroy A et al. Trace element contamination in fish impacted by bauxite red mud disposal in the Cassidaigne canyon (NW French Mediterranean). Sci Total Environ 2019; 690:1626. DOI:10.1016/j.scitotenv.2019.06.474.

[55] Storelli A, Barone G, Dambrosio A, Garofalo R, Busco A, Storelli MM. Occurrence of trace metals in fish from South Italy: Assessment risk to consumer's health. J Food Compos Anal 2020; 90: 103487. DOI:10.1016/j.jfca.2020.103487.

[56] Łuczyńska J, Pietrzak-Fiećko R, Purkiewicz A, Łuczyński MJ. Assessment of fish quality based on the content of heavy metals. Inter J Environ Res Pub Health 2022; 19(4): 2307. DOI:10.3390/ijerph19042307.

ESKİŞEHİR TEKNİK ÜNİVERSİTESİ BİLİM VE TEKNOLOJİ DERGİSİ C- YAŞAM BİLİMLERİ VE BİYOTEKNOLOJİ

Eskişehir Technical University Journal of Science and Technology C- Life Sciences and Biotechnology

2023, 12(1), pp. 42-52, DOI:10.18036/estubtdc.1211466

RESEARCH ARTICLE

USING *Tilia tomentosa* IN HEAVY METAL POLLUTION MONITORING IN ANKARA PROVINCE, TURKEY

Rüfeyde IŞIK [1], Zeliha LEBLEBİCİ [2,*]

[1] Department of Molecular Biology and Genetics, Institute of Science, Nevsehir Hacı Bektas Veli University, Nevsehir, Turkey
[2] Department of Molecular Biology and Genetics, Faculty of Arts and Sciences, Nevsehir Hacı Bektas Veli University, Nevsehir, Turkey,

ABSTRACT

Heavy metals, the concentration of which in the environment is constantly increasing and which can remain intact in nature for a long time, are a great threat to living things. In addition, due to the fact that it causes bioaccumulation in the structure of living things, the detection of heavy metal concentration is very important. Heavy metals constitute the largest part of air pollution. However, living things in areas where traffic is heavy are exposed to exhaust fumes, and this significantly negatively affects the health of living things. In our study, the areas where heavy traffic are concentrated in Ankara were determined and the possibilities of using linden trees, which were planted abundantly in the middle refuges, as a biomonitor for the change in heavy metal hunters were investigated. Changes of Pb, Fe, Cd, Cr and As elements in soil and plant samples were analyzed in ICP-MS device. SPSS 22 Statistical Package Program was used to evaluate the obtained data. The *Tilia tomentosa* Moench. parts (leaf, flower and branch) are evaluated, it is seen that the heavy metal amounts in the unwashed samples are higher than the washed samples for all heavy metals. The highest metal concentration among the stations was Fe (40681 μgg^{-1}) collected from Gazi station, the lowest concentration was Cd (4.9 μgg^{-1}) collected from the Mogan station on soils.

Keywords: Biomonitor, Heavy Metal, ICP-MS, *Tilia tomentosa* Moench

1. INTRODUCTION

The rapidly increasing world population causes air pollution to increase exponentially, especially due to the use of fossil fuels in large areas [1]. Due to diseases caused by air pollution, millions of people die every year around the world [2]. Heavy metals with an atomic number greater than 20 and with an atomic number of more than 5 g/cm³ in density, which are among the main causes of air pollution, are known as polluting metals. The main sources of heavy metals, which can be found in air, soil and water in different proportions and are released into the atmosphere from different sources, are mostly human-made factors such as urban wastes, chimney and exhaust gases, wastewater, mining and fertilizers [3].

Traffic-based pollution is one of the primary causes of air pollution in our country [4]. Human, plant and animal health are adversely affected by exposure to small amounts of toxic heavy metals such as cadmium (Cd) and lead (Pb) emitted from the exhaust of vehicles [5].

Heavy metals rising to the atmosphere can be mixed with soil and water through precipitation. Thus, it reaches the plants from the soil and the bodies of all living things in contact with the soil. In addition, heavy metals can contaminate surface waters such as streams and rivers, and it leaks from the soil and pollutes underground water resources. In this way, waters rich in heavy metals cause harmful effects for plants, animals and human bodies when used for agricultural areas [7].

As a result of research, the negative effects of heavy metals on human health have been clearly revealed [8]. From research Shaban et al. people who are exposed to high heavy metal concentrations have serious damage to the central nervous system, lungs, kidneys and liver, and this exposure even results in death. Koedrith et al. reported that at low concentrations of heavy metals, discomfort such as nose-throat

*Corresponding Author: zleblebici@nevsehir.edu.tr
Received: 01.11.2022 Published: 30.01.2023

irritation, cough, and shortness of breath were observed; Zeng et al. determined that it causes diseases such as asthma, cough, and respiratory distress in children [9-10-11].

The use of plants as biomonitors is important for monitoring heavy metal pollution. Lichen, moss, flowering plants and epiphytic plants are used regionally and locally [13-14-15-1-8-17]. For this purpose, lichens and mosses are preferred plants because they can accumulate many elements in much greater quantities than their needs [18]. However, since it is not known how long these plants are exposed to heavy metals, especially higher plants have been used in recent years [19-20]. Examples of higher plants are trees, and shrubs. These plants are very popular for this subject due to their ability to accumulate trace elements from soil, water and air [13].

There are differences between deciduous and evergreen species in the calculation of heavy metal accumulation in higher plants. Annual heavy metal accumulation can be calculated in deciduous higher plant species, while long-term heavy metal accumulation can be calculated in evergreen species. In the calculation of heavy metal pollution, samples taken from different organs of the plant are brought to the laboratory environment and analyzed in different devices [21].

Tilia tomentosa (Linden tree) species was used in this study. The reason for using this species is that there are plenty of linden trees in areas with heavy traffic in Ankara, and people want to collect the leaves and flowers of this tree and use it for healing purposes.

For this purpose, the potential for use of the species as a bioindicator will be determined by measuring the heavy metal concentrations in the linden trees in the region and the harms of the results in terms of human health will be evaluated.

3. MATERIAL AND METHOD

3.1. Study Area

In our study, linden samples were collected from six different regions in Ankara: Karapürçek (city center), Samsun Road (main road), Gazi District (main road), Pursaklar (secondary road), Protocol Road (main road), and Mogan (control). Samples are divided three in to branches, leaves, and flowers. Some of the samples were washed, and some were analyzed without washing. In addition, soil samples from which the specimens grew have been collected for analysis (Figure 1).

Figure 1. Study area

3.2. Sample Preparation

The plant samples collected from the study area were brought to the laboratory environment and half of them were washed with double distilled water to save them from surface contamination. All linden samples were dried in an oven at 80 ^{0}C for 24 hours. Samples that got rid of moisture were ground in a mortar and transferred to bags so that they become homogeneous. Soil samples were taken from a 10 cm area after the debris was cleaned from the surface, and from a depth of 15 cm around the tree root using a steel auger. Soil samples were placed in nylon bags to prevent contamination. The samples brought to the laboratory were dried for 1 day, air-dried, and then stored in labelled bags [22].

3.3. Digestion and Metal Determination

The samples were dissolved in a microwave instrument using 10 ml of pure HNO3. After dissolving, the volume of the samples was made up to 10 ml with double distilled water. Element values in the samples were determined using the ICP-MS device. Standard solutions and control samples were used to identify any contamination that could contaminate the samples from the outside. All trials were done in 3 repetitions. Care was taken to ensure that all chemicals used were analytical. (Merck, Darmstadt, Germany) [22].

Soil samples brought to the laboratory were dried in an oven at 80 °C for 24 hours and homogenized using a 2 mm sieve. The samples were dissolved in a microwave instrument using 10 ml of pure HNO_3/HCl mixture. After dissolving, the volume of the samples was made up to 10 ml with double distilled water. Element values in the samples were determined using the ICP-MS device [23].

3.4. Biotransfer Factor (BTF)

Biotransfer factor in plants; It was determined from the samples was calculate to determine the heavy metal transfer capability and yield of the plants depending on the concentration of metals in the soil.

The following formula was used for BTF calculation: TF = Cv/Cs, where Cv is the metal concentration detected in the parts of *Tilia tomentosa* and Cs is the metal concentration in the soil [24].

3.5. Statistical Analysis

For each sample, the mean, standard deviation, minimum and maximum values of the readings with 3 replicates were calculated. The $p \leq 0.05$ value was considered significant in the statistical comparison of the means. In addition, in order to facilitate the evaluation of the data obtained, ANOVA test with a confidence interval of 95% using the SPSS 20 program and Duncan tests for determining the difference in multiple comparisons were applied for the necessary groups and parameters. Means and differences between groups were interpreted by comparing them by means of these tests.

4. RESULTS AND DISCUSSION

The most important heavy metals are Fe, Cu, V, Mn, Zn, Ni, Cr, Mo, Co, Be, Cd, Pb, TI, Sb, Ag, As, Se, Hg, Sn, and Al elements. Among these, elements such as Zn, Mn, Fe, Cu, Ni, and Mo are vital micronutrients for plants and animals, and their high concentrations can cause harmful effects. Others are much more dangerous [25-26].

However, heavy metals are not easily destroyed in nature and tend to bioaccumulation in living things. Factors causing metal accumulation; mineral fertilizers, mining, biocides, wastewater, exhaust and flue gases, and sewage [27]. It can also be emitted into the atmosphere as volatile compounds [28-30]. Heavy metals from industrial sources and carcinogens; They are As, V, Ni, Cd, Zn, Cr and Pb elements [31]. Considering these properties of heavy metals and the dangers they may pose for living things, it is very

important to determine heavy metal concentrations, to find areas that may pose a risk, and to find the risk level in these areas [32].
In our study, the concentrations of Cd, Cr, As, Fe and Pb elements in *Tilia tomentosa* parts (leaf, flower and branch) and soil were investigated (Table 1-3).

Lead is not an absolute requirement for plants, but is very dangerous for health when it exceeds 300 ppm [33]. It significantly affects the plant water regime by negatively affecting cell wall stability and cell turgor. It also affects nutrient uptake as it is retained by the roots of the plant by reducing root growth [34] (Table 1). The highest Pb concentration among the stations was collected from Samsun Road station (43,72 μgg^{-1}) that of linden branch, the lowest concentration was (0,95 μgg^{-1}) collected from the Mogan station that of linden flowers (Table 1).

Although iron toxicity is not very common, in plants, it secretes root secretions that lower the soil pH [35]. It also causes burns on the leaves and stunting on the root and stem whereas negatively affects amino acid binding and protein synthesis in the plant [36-37]. The highest Fe concentration has been found in linden leaves (4637.78 μgg^{-1}) at Pursaklar station whereas the lowest concentration has been found in linden flower (100.9 μgg^{-1}) at Mogan station (Table 1).

Cadmium changes nitrogen and carbohydrate metabolisms in plants in many ways. By inhibiting photosynthesis, it causes the growth of stomata and the consequences of water loss, thus deteriorating the working conditions of chlorophyll [38]. The highest Cd concentration among the stations was collected at Karapürçek station (12,41 μgg^{-1}) on leaves and the lowest concentration was (0,12 μgg^{-1}) collected at Mogan station on flowers (Table 1).
Chromium, which adversely affects the plant body, negatively affects root development in the plant. This reduces the amount of nutrients and water taken from the soil and significantly reduces plant yield and quality [39]. Plants exposed to this stress produce reactive oxygen species to defend themselves, causing many damage to the plant [40]. The highest Cr concentration among the stations was collected from Karapürçek station (151,1 μgg^{-1}) on leaf, the lowest concentration was (37,6 μgg^{-1}) collected from the Mogan station on leafs (Table 2).

Arsenic has a toxic effect, restricting germination in some plants it whereas increasing arsenic concentration causes significant reductions in plant height, grain yield, number of full grains, grain weight, and root biomass [41]. The highest Cr concentration among the stations was collected at Karapürçek station (44,4 μgg^{-1}) on leaf, and the lowest concentration was (0,16 μgg^{-1}) collected at Mogan station on flowers (Table 2).
High concentrations of heavy metals were detected in the unwashed plant parts collected from the stations. This was thought to be due to heavy traffic, in which high level metals are transported. A similar result was achieved by Leblebici et al. (2020) in their studies conducted in Nevsehir, where they determined that there was a higher level of accumulation of Pb and As compared with the other metals that were found [23].

When the linden parts (leaves, flower and branch) are evaluated, it is seen that the heavy metal concentration in the unwashed samples are higher than the washed samples for all heavy metals. The results are similar to the study of Leblebici and Kar (2018) [22]. When the plant parts are evaluated according to the ANOVA test, it is seen that the heavy metal concentrations in flower samples are lower than those in branch and leaves samples (Table 1, 2).

Table 1. Comparison of accumulation of Lead (Pb); Iron (Fe); Cadmium (Cd) in different parts of *Tilia tomentosa;* leaves, flower and branch with washed and unwashed samples (μg g^{-1}).[a]

Heavy Metals	Parts	Stations	Unwashed Samples	Washed Samples
		Karapürçek	30,08±3,12[a]	6,65±0,12[ab]
		Samsun Road	25,66±2,85[ab]	8,91±0,32[ab]
	Leaves	Gazi	25,78±2,63[ab]	15,91±1,14[a]
		Pursaklar	18,21±1,79[b]	10,04±1,06[ab]
		Protokol	13,2±1,54[b]	8,85±0,45[ab]
		Mogan	12,88±0,95[c]	5,38±0,12[b]
		Karapürçek	3,57±0,15[b]	2,24±0,14[b]
		Samsun Road	7,2±0,74[a]	4,47±0,53[a]
Pb	Flower	Gazi	5,62±0,23[ab]	3,09±0,25[ab]
		Pursaklar	3,48±0,11[b]	2,23±0,18[b]
		Protokol	5,43±0,21[ab]	3,99±0,74[ab]
		Mogan	1,82±0,02[c]	0,95±0,08[c]
		Karapürçek	29,56±3,41[ab]	16,55±2,35[ab]
		Samsun Road	43,72±5,15[a]	22,89±5,41[a]
	Branch	Gazi	35,66±4,21[ab]	16,11±2,61[ab]
		Pursaklar	15,8±2,13[b]	8,14±1,42[b]
		Protokol	18,38±2,75[b]	8,87±1,78[b]
		Mogan	11,12±1,02[c]	4,16±0,94[c]
		Karapürçek	3363,16±12,06[b]	1636,93±6,54[ab]
		Samsun Road	3397,03±11,04[b]	1415,52±5,41[b]
	Leaves	Gazi	4291,57±15,84[ab]	2337,55±7,25[a]
		Pursaklar	4637,78±13,42[a]	2092,44±6,25[ab]
		Protokol	2695,74±9,01[b]	1409,64±4,12[b]
		Mogan	424,35±1,54[c]	178,14±1,36[c]
		Karapürçek	312,74±4,10[b]	197,56±2,15[b]
		Samsun Road	813,49±5,03[a]	627,2±5,16[a]
Fe	Flower	Gazi	325,18±3,64[b]	212,67±1,78[b]
		Pursaklar	511,91±5,01[ab]	410,71±2,62[ab]
		Protokol	346,76±2,39[b]	321,14±2,30[b]
		Mogan	193,77±1,03[c]	100,9±1,03[c]
		Karapürçek	1105,42±6,01[a]	1041,3±9,35[a]
		Samsun Road	414,38±3,24[b]	374,6±2,18[b]
	Branch	Gazi	1070,55±4,12[ab]	947,1±8,14[ab]
		Pursaklar	281,45±1,32[b]	187,49±2,74[b]
		Protokol	590,07±2,14[b]	326,07±5,14[b]
		Mogan	137,97±1,02[c]	153,48±1,48[c]
		Karapürçek	12,41±1,02[a]	3,17±1,25[a]
		Samsun Road	5,9±0,62[ab]	1,59±0,89[ab]
	Leaves	Gazi	3,28±0,12[b]	1,92±0,79[b]
		Pursaklar	2,36±0,08[b]	1,05±0,64[b]
		Protokol	1,25±0,04[b]	0,87±0,14[b]
		Mogan	0,69±0,01[c]	0,44±0,09[c]
		Karapürçek	0,96±0,05[b]	0,36±0,07[b]
		Samsun Road	1,01±0,08[ab]	0,67±0,08[a]
Cd	Flower	Gazi	1,36±0,09[a]	0,38±0,04[b]
		Pursaklar	0,74±0,03[b]	0,4±0,05[ab]
		Protokol	0,83±0,04[b]	0,51±0,06[ab]
		Mogan	0,33±0,01[c]	0,12±0,02[c]
		Karapürçek	3,38±0,12[a]	1,92±0,08[a]
		Samsun Road	1,93±0,10[b]	1,66±0,05[ab]
	Branch	Gazi	1,71±0,09[b]	0,77±0,03[b]
		Pursaklar	1,5±0,06[b]	0,78±0,04[b]
		Protokol	1,52±0,07[b]	1,02±0,08[b]
		Mogan	1,21±0,03[c]	0,98±0,02[c]

[a] For a given station, mean concentrations followed by the same letter are not significantly different ($p<0.05$).

Table 2. Comparison of accumulation of Chromium (Cr) and Arsenic (As) in different parts of *Tilia tomentosa;* leaves, flower and branch with washed and unwashed samples (μg g^{-1}).[a]

Heavy Metals	Parts	Stations	Unwashed Samples	Washed Samples
		Karapürçek	151,1±2,03[a]	85,4±3,05[ab]
		Samsun Road	102,2±1,98[b]	71,6±2,75[ab]
	Leaves	Gazi	121,3±2,14[b]	78,5±2,92[ab]
		Pursaklar	98,4±1,42[b]	57,1±1,74[b]
		Protokol	161,4±3,25[ab]	88,6±3,01[a]
		Mogan	45,8±0,95[c]	37,6±0,95[c]
		Karapürçek	124,5±3,14[ab]	114,6±5,41[a]
		Samsun Road	105,6±2,51[b]	102,8±4,12[ab]
Cr	Flower	Gazi	113,4±2,87[b]	107,9±3,25[ab]
		Pursaklar	135,4±3,11[a]	104,7±3,10[ab]
		Protokol	128,3±2,56[ab]	102,7±3,06[ab]
		Mogan	95,2±0,93[c]	76,8±2,01[b]
		Karapürçek	72,8±3,14[a]	68,1±2,36[a]
		Samsun Road	65,7±2,64[ab]	60,3±1,85[ab]
	Branch	Gazi	69,7±2,73[ab]	63,6±1,83[ab]
		Pursaklar	52,8±2,10[ab]	49,1±1,02[b]
		Protokol	63,1±2,16[ab]	61,5±1,87[ab]
		Mogan	60,4±1,23[b]	46,4±0,86[b]
		Karapürçek	44,4±1,12[a]	8,95±0,13[a]
		Samsun Road	24,71±1,01[ab]	6,78±0,11[b]
	Leaves	Gazi	15,13±1,03[ab]	6,47±0,10[b]
		Pursaklar	14,21±0,96[ab]	8,51±0,12[ab]
		Protokol	9,25±0,41[b]	5,8±0,09[b]
		Mogan	9,09±0,12[b]	4,62±0,04[c]
		Karapürçek	0,78±0,05[b]	0,64±0,04[ab]
		Samsun Road	1,98±0,16[ab]	0,81±0,09[ab]
As	Flower	Gazi	2,4±0,41[a]	1,09±0,12[a]
		Pursaklar	1±0,12[ab]	0,75±0,08[ab]
		Protokol	1,6±0,31[ab]	0,52±0,04[b]
		Mogan	0,42±0,04[c]	0,16±0,01[c]
		Karapürçek	12,54±2,01[a]	3,53±0,41[ab]
		Samsun Road	6,99±1,32[ab]	5,29±0,67[a]
	Branch	Gazi	7,13±1,14[ab]	2,56±0,35[b]
		Pursaklar	5,5±1,03[b]	2,59±0,37[b]
		Protokol	5,95±1,06[b]	1,53±0,13[b]
		Mogan	3,07±0,92[c]	0,86±0,10[c]

[a] For a given station, mean concentrations followed by the same letter are not significantly different ($p<0.05$).

*Permissible limits (mg/kg) were adopted from WHO (2000) and The Soil Pollution Control Regulation (2001) on plant and soil [42, 43].The highest metal concentration among the stations was Fe (40681 μgg^{-1}) collected from Gazi station, and the lowest concentration was Cd (4.9 μgg^{-1}) collected from the Mogan station on soils (Table 3). According to the ANOVA test in lead, metal concentration in all stations is different from each other on soil samples ($p<0.05$) (Table 3).

The reason for high levels of heavy metal concentrations in the soil and plant samples can be result of being increased exposure to dissolved chemicals due to heavy traffic and decreased quality of soil [44].

Table 3 Heavy metal concentration (mean ±SD, $\mu g g^{-1}$, n=5 per location) in soils in Ankara Province [a].

Soil	Cd	Cr	As	Pb	Fe
Karapürçek	20.31±2.01[a]	400.61±5.04[ab]	45.68±0.21[b]	168.58±2.14[bc]	16901.2±12.54[b]
Samsun Road	9.54±1.03[b]	653.02±7.12[a]	60.77±0.34[ab]	371.22±2.41[a]	12871.7±8.41[b]
Gazi	9.53±1.42[b]	230.98±3.01[b]	61.59±0.58[ab]	324.86±2.05[ab]	40681.06±24.15[a]
Pursaklar	7.5±2.05[b]	434.56±6.02[ab]	67.08±0.87[a]	125.22±1.52[c]	14845.7±9.74[b]
Protokol	16.46±.15[ab]	226.93±2.98[b]	57.53±0.45[ab]	292.8±2.56[b]	18894.1±10.31[b]
Mogan	4.9±0.12[c]	102.75±1.05[c]	25.16±0.12[c]	103.78±1.03[d]	10627.9±5.14[c]
Permissible Limits*	3	100	20	50	300

For a given metal, mean concentrations followed by the same letter are not significantly different ($p<0.05$).

The correlations of heavy metal, transfer factors and correlations were detected in *Tilia tomentosa* parts and soil samples collected from six different stations.

When the correlation between the concentration of metals in washed and unwashed flower, stem and leaf samples was investigated, the strongest and most positive correlation was determined as Cr, which was found to be the highest in linden flowers and leafs. The correlation was statistically significant ($p<0.01$) (Table 4).

Table 4 Correlation coefficients for heavy metal concentrations between plant parts ($p<0.01$**) ($p<0.05$*).

	Cr	**As**	**Pb**	**Fe**	**Cd**
Leaves	0,883**	0,416	-0,13	0,644*	0,224
Branch	0,609*	0,491	-0,151	0,576*	-0,270
Flower	0,937**	0,520*	-0,171	0,909**	0,371

Permisible limits in plants Pb (0.2 $\mu g\ g^{-1}$), Fe (30 $\mu g\ g^{-1}$), Cd (0.1 $\mu g\ g^{-1}$), Cr (3 $\mu g\ g^{-1}$), As (0.1 $\mu g\ g^{-1}$) [42].

According to the transfer factors from soil to plant parts, the highest transfer factor was determined at 0,656 for As in leaves while the lowest transfer factor was found to be in Pb and Fe (0,019) in flowers (Table 5).

Transport of heavy metals from soil to plant is one of the main components of human exposure to heavy metals through the food chain. This study determined that the BTF values between sampling sites and plant parts were significantly different. The BTF values of all metals were found to be low in terms of transfer factors. This may be because the metal concentration in the soil is higher than the concentrations in plant parts. Similarly, Leblebici et al. reported that there was no linear increase in BTF values of plants as a result of heavy metal concentration in the soil [23] (Table 5).

Table 5. Transfer factors of metals from soil to parts of *Tilia tomentosa* in Ankara province, Turkey

	As	**Cd**	**Pb**	**Cr**	**Fe**
Leaves	0,656	0,615	0,081	0,231	0,113
Branch	0,187	0,166	0,117	0.111	0,027
Flower	0,035	0,066	0,019	0,207	0,019

5. CONCLUSION

In this study, heavy metal accumulation in *Tilia tomentosa* parts (leaves, flower and branch) and soil due to heavy traffic was investigated in the province of Ankara. In addition, according to the criteria published by international organizations, it was determined that the metal accumulation at the stations was at a very critical level. Consequently, measures may need to be taken to prevent heavy metal contamination in soil and reduce heavy metal translocation from soil to food.

As *Tilia tomentosa* is used as a beverage, heavy metal monitoring in soil and plant is important. It is important to reduce metal concentrations in the study area and to investigate their effects on human health. By continuing these studies in areas with heavy traffic, acute and chronic health problems and metal exposure can be prevented.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

FUNDING INFORMATION

This study was supported by the Research Fund of the University of Nevşehir Hacı Bektaş Veli. The Project number is TEZ22F5.

REFERENCES

[1] Turkyilmaz A, Cetin M, Sevik H, Isinkaralar K, Saleh EAA. Variation of Heavy Metal Accumulation in Certain Landscaping Plants Due to Traffic Density. Environ, Develop Sustain, 2018a; 1-14.

[2] Isinkaralar O, Isinkaralar K, Ekizler A, Ilkdoğan C. Changes in the Amounts of CO_2 and Particulate Matter in Kastamonu Province Depending on Weather Conditions and Locations. J Chem Biolog Physic Sci 2017; 7(3): 643- 650.

[3] Karaçağıl D. İstanbul'da Belirlenmiş Sahil Şeritlerinde Toprak Kalitesi ve Ağır Metal Kirliliği. MSc, Başakşehir University, İstanbul, Turkey, 2013.

[4] Baycu G, Tolunay D, Özdem H, Günebakan S, Ecophysilogical and Seasonal Variations in Cd, Pb, Zn and Ni Concentrations in the Leaves of Urban Deciduous Trees in İstanbul. Environ Pollut 2006; 143:545-554.

[5] Çavuşoğlu K. *Cupressus sempervirens* L. ve *Cedrus libani* A.Rich. Yapraklarında Taşıtların Sebep olduğu Kurşun (Pb) Kirliliğinin Araştırılması. BAÜ, Fen Bilimleri Enstitüsü Dergisi 2005; 7(2): 37-56.

[6] Batır M.B. Kurşun (Pb) ve Bakır (Cu) Ağır Metal Stresi Uygulanan Enginar (*Cynara scolymus L.*) Tohumlarının Fidelerinde Oluşan DNA Değişikliklerinin Belirlenmesi. MSc, Eskişehir Osmangazi University, Eşkişehir, Turkey, 2014.

[7] Yeli C, Çakmakcı T, Şahin Ü, Tüfenkçi Ş. Ağır Metallerin Toprak, Bitki, Su ve İnsan Sağlığına Etkileri. Türk Doğa ve Fen Dergisi 2020; 9:103-114.

[8] Ávila- Pérez P, Ortiz-Oliveros H.B, Zarazúa-Ortega G, Tejeda- Vega S, Villava A, Sánchez-Muñoz R. Determining of Risk Areas due to Exposure to Heavy Metals in the Toluca Valley Using Epiphytic Mosses as a Biomonitor. Jo Environ Manage 2019; 241:138-148.

[9] Shaban N, Abdou K, Hassan N.E.H. Impact of Toxic Heavy Metals and Pesticide Residues in Herbal Proucts. Beni-Suef Uni J Bassic Appl Sci 2016; 5(1): 102- 106.

[10] Koedrith P, Kim H, Weon J.I, Se Y.R. Toxicogenomic Approaches for Understanding Molecular Mechanisms of Heavy Metal Mutagenicity and Carcinogenicity. Int J Hyg Environ Healty 2013; 216(5): 587-598.

[11] Zeng X, Xijin X, Boezen M.H, Hou X. Children with health impairment by heavy metals in an e-waste recycling area. Chemosphere 2015; 148: 408- 415.

[12] Cansaran A, Yildirim C, Karavin N. Availability of *Maclura polifera* (Rafin). Schneider as biomonitor for the Heavy Metal Pollution. Bangladesh J Bot 2016; 45(3): 723-726.

[13] Madejón P, Marañón T, Murillo J.M. Biomonitoring of trace elements in the leaves and fruits of wild olive and holm oak trees. Sci Total Environ 2006; 355:187-203.

[14] Yap CK, Fitri M, Mazyhar Y, Tan SG. Effects of Metal Contaminated Soils on the Accumulation of Heavy Metals in Diffirent Parts of *Centella asiatica*: A Laboratory Study. Sains Malays 2010; 39(3): 347-352.

[15] Abril G.A, Wonnaz E.D, Malteas A.C, Invernizzi R, Plá R.R, Pignata ML. Characterization of Atmospheric Emission Sources of Heavy Metals and Trace Elements Through a Localscole Monitoring Network Using *T. capillars*. Ecol Indic 2014; 40: 153-161.

[16] Sevik H, Ozel H.B, Cetin M, Erdem T. Determination of changes in heavy metal accumulation depending on plant species, plant organism and traffic density in some landscape plants. Air Qual Atmos Health 2019; 12(2): 189- 195.

[17] Çobanoğlu O. The Possibilaties of Using Blue Spruce (*Picea pungens* Engelm) as a Bio-Monitor By Measuring the Recent Accumulation of Heavy Metals in Hs Leaves. MSc, Kastamonu University, Kastamonu, 2019.

[18] Mossi MMM. Determination of Heavy Metal Accumulation in the Some of Landscape Plants for Shrub Forms. Ph.D, Kastamonu University, 2018.

[19] Koç İ. Using *Cedrus atlantica*'s annual rings as a biomonitor in observing the change of Ni and Co concentrations in the atmosphere. Environ Sci Pollut Res 2021; 1-7.

[20] Vural H. Trafik Kaynaklı Ağır Metal Kirliliğinin Belirlenmesinde Peyzaj Bitkilerinin Biyomonitor Olarak Kullanılabilirliği. TURKJANS 2021; 8(4): 1174–1186.

[21] Duffus JH. Heavy metals a meaningless term? (UPAC Technical Report). Pure Appl Chem 2002; 74(5): 793-807.

[22] Leblebici Z, Kar M. Heavy metals accumulation in vegetables irrigated with different water sources and their human daily intake in Nevsehir. J Agric Sci Technol 2018; 20(2): 401-412.

[23] Leblebici, Z, Kar M. Başaran L. Assessment of the Heavy Metal Accumulation of Various Green Vegetables Grown in Nevşehir and their Risks Human Health. Environ Monit Assess 2020; 192: 483-490.

[24] Rahmani GNH, Sternberg SPK. Bioremoval of lead from water using *Lemna minor*. Biores Technol 1999; 70(3): 225–230.

[25] Pınar B. Bazı Peyzaj Bitkilerinde Ağır Metal Birikiminin Trafik Yoğunluğuna Bağlı Değişimi. MSc, Kastamonu Üniversity, 2019.

[26] Baycu G, Tolunay D. İstanbul Kentsel Toprakların Cd, Pb, Zn ve Ni İçerikleri. J Fac For Istanbul U 2009; 59(1): 87-105.

[27] Csavina J, Field J, Taylor MP, Gao S, Landázuri A, Betterton E.A, Sáez A.E. A review on the importance of metals and metalloids in atmospheric dust and aerosol from mining operations. Sci Total Environ 2012; 433:58-73.

[28] Csavina J, Taylor MP, Félix 0, Rine K.P, Sáez AE, Betterton EA. Size-resolved dust and aerosol contaminants associted with copper and lead smelting emissions: implications for emission management and human health. Sci Total Environ 2014; 493:750-756.

[29] Chen Y.M, Gao J, Yuan Y.Q, Ma J, Yu S. Relationship between heavymetal contents and clay mineral properties in surface sediments: implications for metal pollution assessment. Cont Shelf Res 2016; 124:125-133.

[30] Shahid M, Khalid S, Abbas G, Shahid N, Nadeem M, Sabir M, Aslam M, Dumat C. Heavy metal stress and crop productivity in: (K.R. Hakeem Ed.) Crop Production and Global Enviranmental Issues SE-1. Springer Intern Publish 2015; 1-25.

[31] Turkyilmaz A, Sevik H, Cetin M. The use of perennial needles as biomonitors for recently accumulated heavy metals. Landsc Ecol Eng 2018; 14(1): 115-120.

[32] Seven T, Can B, Darende BN, Ocak S. Hava ve Toprakta Ağır Metal Kirliliği. Nation J Environ Sci Res 2018; 1(2): 91-103.

[33] Sharma P, Dubey R.S. Lead toxicity in plants. Broz J Plant Physiol 2005; 17(1):35-52.

[34] Kabir AM, Iqbal MZ, Shafiq M. Effects of lead on seedling growth of *Thespesia populnea L.* Plant Soil Environ 2010; 4: 194-199.

[35] Pak O. Kırklareli Sınırları İçerisindeki Otoban Kenarlarında Bulunan Tarım Arazilerinde Bazı Ağır Metal Kirliliğinin Araştırılması. MSc, Namık Kemal University Tekirdağ, 2011.

[36] Kahvecioğlu Ö, Kartal G, Güven A, Timur S. Metallerin Çevre Etkileri-1. UCBAD 2003; 136:4-53.

[37] Kacar B, Katkat V. Bitki Besleme 1. Baskı, 849, Nobel Yayın, İstanbul, 2006.

[38] Gouia H, Garbel MH, Meyer C. Effects of cadmium on activity of nitrate reductase and on other enzymes of the nitrate assimilation pathway in bean. Plant Physiol Biochem 2000; 38: 629-638.

[39] Vajpayee P, Dhawan A, Shanker R. Evaluation of the Alkaline Comet Assay Conducted With the Wetlands Plant *Bacopa monnieri L.* as a Model for Ecogenotoxicity Assessment. Environ Molecul Mutagen 2006; 47: 483- 489.

[40] Kimbrough DE, Cohen Y, Winer AM, Creelman L, Mabuni C. A ciritical assessment of chromium in the environment. Crit Rev Environ Sci 2009; 29(1):1-49.

[41] Abedin MJ, Cotter- Howells J, Meharg AA. Arsenic Accumulation and Metabolism in Rice (*Oryza sativa* L.). Plant Soil 2002; 240:311-319.

[42] FAO/WHO, Codex Alimentarius International Food Standards Codex Stan-179, Codex Alimentarius Commission, 2003.

[43] Turkey Soil Pollution Control Regulation (TSP 24609) From the Ministry of Environment, Official Gazzette, No: 24609, Date: 10.12.2001.

[44] Naser HM, Sultana S, Mahmud NU, Gomes R, Noor S. Heavy metal levels in vegetables with growth stage and plant species variations. Bangladesh J Agric Res 2012; 36(4): 563–574.