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 11 Number/Sayı 2 July / Temmuz - 2022

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: 11 / Number: 2 / July - 2022

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: 11 / Number: 2 / July – 2022

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

CONTACT INFORMATION
Eskişehir Technical University Journal of Science and Technology
Eskişehir Technical University, Institute of Graduate Programs, 26555 Eskişehir, TURKEY
Phone: +90-222-321 35 50 /**ext.:** 1767
Fax: +90-222 335 41 22
e-mail : btdc@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: 11 / Number: 2 / July – 2022

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 accepts TURKISH and ENGLISH manuscripts.**
- **The journal is indexed by EBSCO, DOAJ and ULAKBIM.**

AIM AND SCOPE

The journal publishes research papers, reviews and technical notes in the fields of theoretical sciences such as Physics, Biology, Mathematics, Statistics, Chemistry and Chemical Engineering, Environmental Sciences and Engineering, Civil Engineering, Electrical and Electronical Engineering, Computer Science and Informatics, Materials Science and Engineering, Mechanical Engineering, Mining Engineering, Industrial Engineering, Aeronautics and Astronautics, Health Sciences, Pharmaceutical Sciences, and so on.

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

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. Manuscripts judged to be plagiarised or self-plagiarised, based on the iThenticateR report or any other source of information, will not be considered for publication.

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.***

Manuscripts should be written in Turkish or English.

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

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**: 11 / Number/**Sayı**: 2 / July /** Temmuz **- 2022**

CONTENTS / İÇİNDEKİLER

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

2022, 11(2), pp. 39-49, DOI:10.18036/estubtdc.1045591

RESEARCH ARTICLE

SOME MORPHOLOGICAL TRAITS AND HEAVY METAL ACCUMULATION IN MUSCLE TISSUE OF *Ruditapes decussatus* (Linnaeus, 1758)

Burcu YEŞİLBUDAK *

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

ABSTRACT

This study aimed to investigate some morphological characteristics and heavy metal accumulation in muscle tissue of *Ruditapes decussatus* (Linnaeus, 1758) in the spring and autumn seasons of 2018 in the Yumurtalık Coastline of İskenderun Bay located in the northeastern Mediterranean Sea. To this end, shell length (SL), total weight (TW), shell height (SH), shell inflation (SI), shell weight (SW), roundness index (RI), cup index (CI) and total length-weight relationship (LWR) of Bivalvia and heavy metal accumulation in the muscle tissue of Bivalvia were measured for two seasons. Minimum and maximum values of SL, TW, SH, SI, SW, RI and CI were determined as 21.50-39.00 mm, 0.80-22.15 g, 10.50-28.70 mm, 9.11-20.90 mm, 3.08-4.66 g, 1.03-2.14 and 0.58-0.67 respectively. The relationship between total length and total weight of *R. decussatus* was calculated to be $W=0.0052*SL^{2.54}$ for spring and $W=0.0031*SL^{2.88}$ for autumn. The growth type of Bivalvia specimens was determined as isometric growth (2.971). Heavy metal accumulation in the muscle tissue in spring and autumn seasons was found as statistically significant only for zinc and copper in different seasons ($P<0.05$). Biometric data of Bivalvia speciemens and muscle tissue heavy metal level are given and discussed in comparison with the results obtained from other studies. Considering the height-weight relationship, growth type and tissue heavy metal accumulation, it can be said that this area is ecologically suitable for *R. decussatus*.

Keywords: Bivalvia, *Ruditapes decussatus*, Morphological traits, Heavy metal

1. INTRODUCTION

Biometric measurements based on the morphology of the studied Mollusca species are very important in terms of giving information about the population. The shells of Mollusca, which have an important place among the living groups where morphological studies are carried out, can show a variable structure by being affected by environmental and climatic changes [1, 2]. The bivalves are in some ways the most highly modified of all the mollusks [3] and shell part of mollusks has variable structure in the chaotic area [1]. It has been reported in various studies that growth changes in bivalves are used to obtain information about the conditions that cause global and local environmental changes [4, 5]. *R. decussatus* is a member of the family Veneridae. This family (common name: venus clams) is a very large family of marine bivalve mollusks, and hence, makes up a significant proportion of bivalves. A great proportion of Veneridae family is exploited as food sources in other countries such as Portugal, Egypt, and Spain [6, 7, 8, 9]. It has been suggested to grow members of this family in wastewater from aquaculture [9]. Additionally, it has been reported that some species belonging to Veneridae family prefer suspension, endogenous, and coralligen beds as their habitat and can go down to 700 m [10]. Veneridae family lives in areas protected from possible strong waves such as sheltered bays and sea lakes because it is a rare and sensitive bivalve species; also, it belongs to the group of endogenous suspensivores, burying itself at a certain depth in loose sediments [10, 11]. Distribution of *R. decussatus* has been reported along the Atlantic coast, from Norway to Congo and on the West and South coasts of the British Islands, and in the North Sea only in Norway and Denmark and the Atlantic Ocean. In addition, it can be found through the Mediterranean Sea as well as in the Red Sea where this species migrated through the Suez Canal. *R. decussatus* also exists from the south to western Morocco and Senegal, West Africa [12, 13]. Since mussels and oysters feed by filtering the water, they can

*Corresponding Author: yesilbudak@gmail.com
Received: 25.12.2021 Published: 26.07.2022

absorb the metals in the water. They can accumulate heavy metals in the processes of exchanging and binding dissolved ions in water with their absorption in water to get nutrients. Therefore, Mollusca has a high potential for monitoring heavy metals uptake from the water along with the sediments [14]. Heavy metals are toxicants that disrupt the ecological balance and are carried to living beings through the food chain or waterway in the aquatic environment and have the potential to damage all life activities of the living beings and change this metabolic structure [15, 16]. According to the literature review, there are no detailed morphological measurement studies for *R. decussatus* in the Yumurtalık Lagoon, which is located in the northeastern part of the Mediterranean and is very rich in terms of both fish and invertebrate species [17, 18]. Therefore, giving the morphological characteristics of the mussel in terms of length, width, height and weight is important for the comparison of mussel morphology in future studies. Although there are a few studies which attempt to determine the distribution areas of the *R. decussatus* species on the Yumurtalık coast [17, 19, 20], there are not enough studies on morphological characteristics and heavy metal accumulation together. This study firstly aims to examine shell composition of the Yumurtalık coast and depending on this, investigates shell evaluations about some morphological parameters and heavy metal levels of *R. decussatus* sampled from the Yumurtalık coast.

2. MATERIALS AND METHODS

This study was carried out at the Yumurtalık Coastline of İskenderun Bay in the spring and autumn seasons of 2018 (Figure 1). The temperature and salinity levels of the sea water in the spring and autumn seasons were measured by YSI EcoSense. A total of 602 shells were collected from the sea coast and sediment of the sea (about 0-15 m). Samples were stored in labelled plastic container for being subjected to morphometric measurements. Species names were updated according to the checklist of species-group taxa of the Taxonomic Database on Marine Mollusca [21]. For heavy metal analysis, the samples kept in the deep freezer were taken out and washed by removing salt water. The muscle was completely removed from the shell and other parts, taken into jars to be dried, and then dried in an oven at 70 ºC for 48 hours. The dried samples were divided into small pieces by a microwave homogenizer and homogenized. Nitric acid and hydrogen peroxide were used for mussel muscle samples (1:1). All samples were completed in 10 ml polypropylene tubes with distilled water and the levels of heavy metals (Zn, Cu, Cd, Pb) were measured using inductively coupled plasma mass spectrometry (ICP-MS) [22]. Standard solutions of the analyzer were calibrated with chemicals prepared from Merck. Standard reference material (SRM, Dorm-2) was used to analyze the accuracy and precision of our results. Allometry was examined for morphometry (LWR) [23] and shape indices of individual bivalves were determined [24]. Shell length (maximum antero-posterior distance), shell height (maximum distance from hinge to ventral margin), shell inflation (maximum distance between outer edges of two valves), roundness index [RI=SL/SH] and cup index [$CI=SI/(SL*SH)^{0.5}$] of individual organisms were measured accurately to 0.01 mm using digital calipers [24]. The total weight of individual mussels and their shell weight were determined by digital balance (precision of 0.001 g). Ricker's [25] length-weight equation and Pauly's *t*-test [23] were applied. Independent samples *t*-test was applied for evaluation of heavy metal analysis in muscle tissue by using the SPSS software (SPSS Statistics V 27.0.1.0, IBM, Corp., USA).

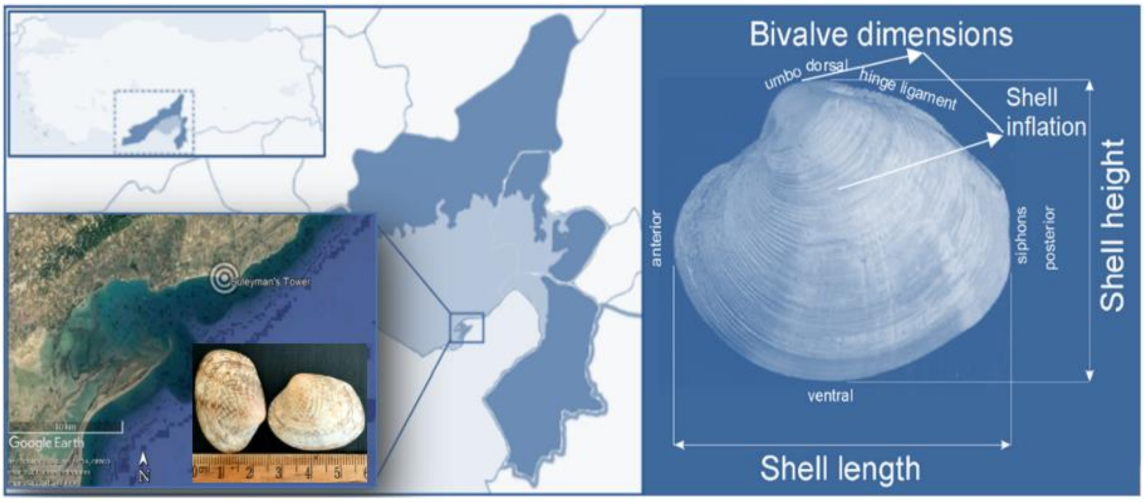

Figure 1. In the left side; The sampling site (Yumurtalık Coastline in İskenderun Bay) and *Ruditapes decussatus,* in the right side; bivalve dimensions can be viewed at wileyonlinelibrary.com

3. RESULTS AND DISCUSSION

Morphometric analysis and measurement of the heavy metal accumulation in the muscle tissue of *R. decussatus* were carried out at the Yumurtalık Coastline in İskenderun Bay. The mean sea water temperatures were measured to be 23.45±1 and 19.77±1 in spring and autumn, respectively. The salinity levels, however, were 37.10±0.16 and 35.65±0.33 ppt in spring and autumn, respectively. Biotope characteristics of the sample area were determined as gravelly, sandy, muddy and silt bottoms. All the 602 shells were collected from the Yumurtalık coast. A classification of them showed that they belonged to Anomioidea (0.99%), Arcidae (1.99%), Cardiidae (5.81%), Chamidae (1.32%), Glycymerididae (11.29%), Lucinidae (1.49%), Mytilinidae (2.82%), Nassariidae (1.16%), Ostreidae (11.46%), Pectinidae (0.99%), Pteriidae (1.99%), Semelidae (0.99%), Strombidae (0.83%), Spondylidae (0.83%), Tellinidae (1.22%), Veneridae (1.34%) and other (53.48%) families. These results were in line with the findings of previous systematic studies [17,19,20]. The descriptive statistics of the body size measurement of *R. decussatus* are presented in Table 1. For both seasons, the arithmetic means and standard error of measurements of the SL, TW, SH, SI, SW, RI, CI were obtained as 29.05±1.01 cm, 8.59±0.84 g, 19.97±1.13 mm, 14.53±0.70 mm, 3.65±0.10 g, 1.52±0.07 and 0.62±0.01, respectively. The minimum and maximum values of the SL, TW, SH, SI, SW, RI, and CI of both seasons were calculated as 21.50-39.00 mm, 0.80-22.15 g, 10.50-28.70 mm, 9.11-20.90 mm, 3.08-4.66 g, 1.03-2.14 and 0.58-0.67 respectively. The arithmetic means and standard error values of Bivalvia measurements according to the seasons were found to be 32.45±1.10 mm, 15.57±1.04 g, 22.35±1.20 mm, 16.9±0.70 mm, 4.02±0.11 g, 1.48±0.08 and 0.62±0.00 for spring and 25.65±0.70 mm,1.60±0.18 g, 17.60±1.60 mm, 12.16±0.40 mm, 3.27±0.02 g, 1.56±0.11 and 0.61±0.00 for autumn. The minimum and maximum values were calculated as 28.50-39.00 mm, 7.12-22.15 g, 17.30-28.70 mm, 13.9-20.90 mm, 3.37-4.66 g, 1.06-1.90 and 0.60-0.67 for spring and 21.50-28.0 mm, 0.80-2.75 g, 10.50-25.50 mm, 9.11-14.21 mm, 3.08-3.38 g, 1.03-2.14 and 0.58-0.67 for autumn. The coefficient R^2 of determination for bivalves was found to be 0.879 for both seasons. Similarly, the values of a and b were found as 0.011 and 2.971. The growth type of Bivalvia specimens was determined to be isometric growth. A positive correlation was reported in the relationship between the total weight and shell length (P<0.01; 0.8795; Figure 2). A similar situation was observed between the cup index and roundness index, as well (P<0.01; 0.9898; Figure 2). In general, descriptive features in bivalves were measured mostly in the spring season (Figure 3), except for the roundness index (1.48±0.08) and b (2.540) value (Table 1). The growth type of Bivalvia specimens was determined as negative allometry in spring and isometric growth in autumn (Table 1). *R. decussatus* has a wide distribution in the world and an important ecological and economic value in terms of its place in the trophic chain of lagoons and marine systems. One may study it as a bioindicator in these areas in terms of its place in the culture of shellfish [6]. Therefore, the morphometric indices and length-weight relationships of *R. decussatus* have been estimated widely in several areas as an indicator to compare the differences between ecosystems (Table 1). Environmental factors such as water temperature and nutrient availability are primary factors for the development of Bivalvia while salinity and photoperiod have a secondary effect [26].

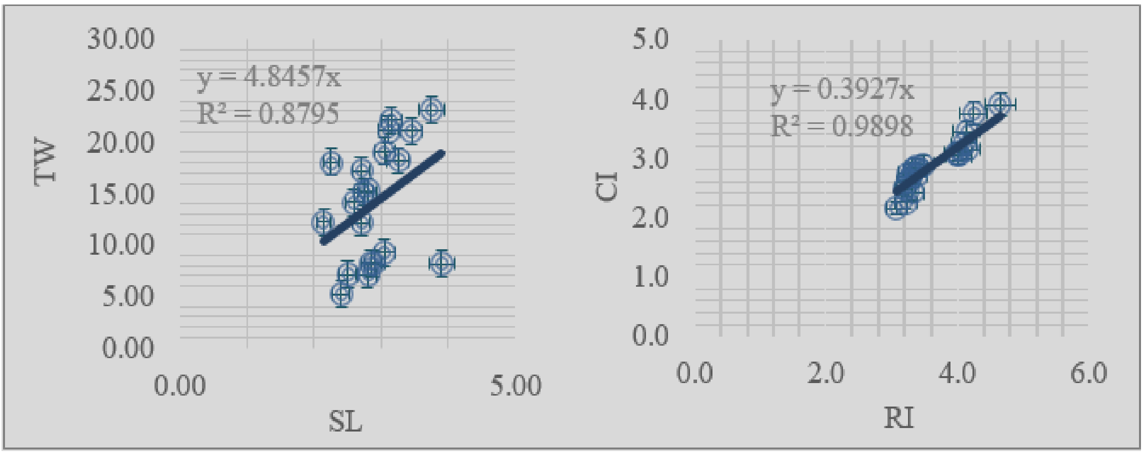

Figure 2. The relation of *R. decussatus*'s total weight (TW, g), shell length (SL, cm), cup index (CI) and roundness index (RI).

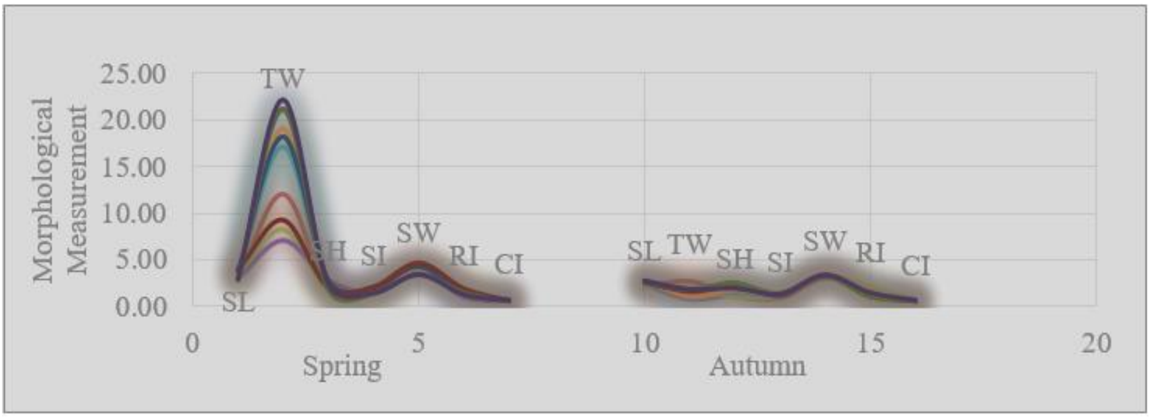

Figure 3. Morphological traits of *R. decussatus*'s in spring and autumn season

Table 1. The shell length (mm), total weight (g), shell height (mm), shell inflation (mm), shell weight (g), roundness index and cup index with growth parameters (LWR) of *Ruditapes decussatus* (n=18).

	Descriptive statistics							LWR			
Season	SL x̅ ±SEM Min-Max	TW x̅ ±SEM Min-Max	SH x̅ ±SEM Min-Max	SI x̅ ±SEM Min-Max	SW x̅ ±SEM Min-Max	RI x̅ ±SEM Min-Max	CI x̅ ±SEM Min-Max	*a*	*b*	R^2	*GT*
Spring	32.45±1.10 28.50-39.00	15.57±1.04 7.12-22.15	22.35±1.20 17.30-28.70	16.90±0.70 13.90-20.90	4.02±0.11 3.37-4.66	1.48±0.08 1.06-1.90	0.62±0.00 0.60-0.67	0.005	2.540	0.877	-A
Autumn	25.65±0.70 21.50-28.0	1.60±0.18 0.80-2.75	17.60±1.60 10.50-25.50	12.16±0.40 9.11-14.21	3.27±0.02 3.08-3.38	1.56±0.11 1.03-2.14	0.61±0.00 0.58-0.67	0.003	2.888	0.863	I
In this study (∑) x̅ ±SEM Min-Max	29.05±1.01 21.50-39.0	8.59±0.84 0.80-22.15	19.97±1.13 10.50-28.70	14.53±0.70 9.11-20.90	3.65±0.10 3.08-4.66	1.52±0.07 1.03-2.14	0.62±0.01 0.58-0.67	0.011	2.971	0.879	I
In other studies											
Moroccan Coast [27]	25.86-49.36	5.30-22.01	20.29-33.94	14.18-24.23	ns	ns	ns	ns	ns	ns	ns
Lake Timsah, Egypt [28]	26.36±1.51 11.70-40.60	3.95±0.99 0.31-10.51	17.84±1.73 8.40-27.00	11.7±1.32 4.90-17.3	2.66±0.67 0.23-6.71	ns	ns	0.000	2.864	0.980	I
Pag Bay - Eastern Adriatic Sea [29]	31.9±5.5 17.7-43.5	ns	ns	ns	ns	ns	ns	ns	2.910	0.965	I
Port Said-Egypt [30]	≦16-35.9	≦0.70-5.80	≦0.70-2.25	ns	≦0.70-5.50	ns	ns	0.091	3.350	0.857	+A
Lake Timsah, Egypt [31]	22.00	0.86-1.15	ns	ns	1.27-1.65	ns	ns	3.55	2.81	0.979	I
Aquaculture, Turkey [32]	32.10±3.01	11.46±1.57	33.67±1.61	ns	8.88 ± 1.37	ns	ns	ns	ns	ns	ns
Galicia, N.W. Spain [33]	48.79±2.42	26.47±3.99	34.92±1.71	23.27± 1.26	14.17±2.24	ns	ns	ns	ns	ns	I

LWR: total length and weight relationship, x̅ ±SEM: arithmetic mean± standard error of the mean, Min-Max: the range of variability of the linear and mass indices, *a* and *b*, coefficients of the equations; R^2: coefficient of determination, *GT*: growth type (I: isometric growth, -/+A: negative or positive allometry), ns: not specified.

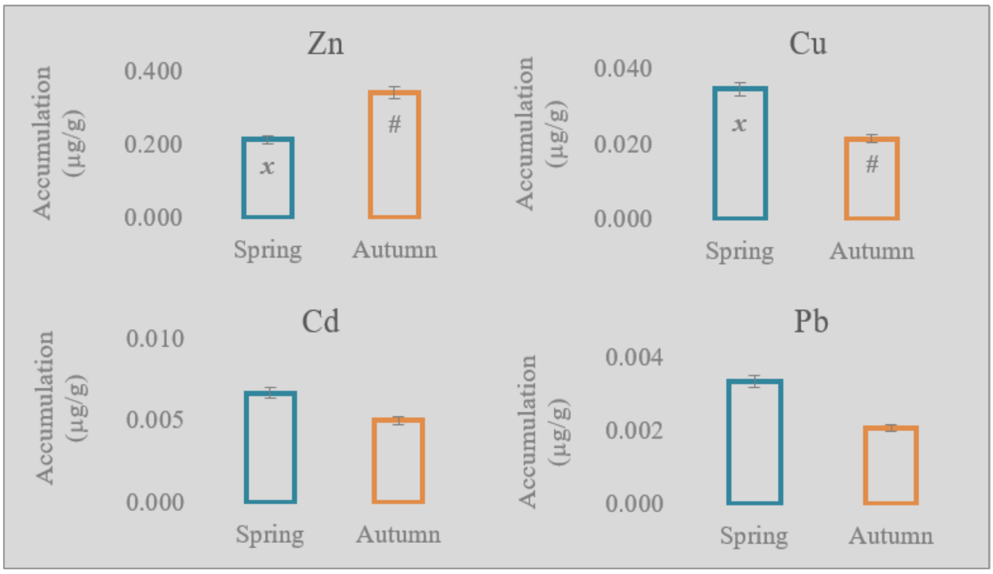

Figure 4. Accumulation level (µg/g) of metals in muscle tissue of *R. decussatus*. The sign of cross and sharp (*x, #*) in bars within season indicate statistically significant differences (P<0.05).

In our study, negative allometry (b=2.540, R^2=0.877) and isometry (b=2.888, R^2=0.863) were observed in the Yumurtalık coast in the spring and autumn seasons, respectively. The growth characteristics of Bivalvia species sampled from the Urdaibai Estuary (North Spain) also showed isometric characteristics (*b*=2.900, R^2=0.903) [34]. The dominance of isometries and negative allometries over positive allometries is an important phenomenon in bivalve growth types. This may be related to the creature's continuous egg-laying strategy. A similar situation was observed in different studies (e.g.: [31]). In a study by Sherif [30], it was reported that the shell growth of bivalve *V. decussatus* was mainly affected by temperature. In another study conducted with *R. decussatus*, it was reported that shell development slowed down in the winter season [35]. Descriptive measurement values of bivalves in the Yumurtalık coast were calculated mostly in the spring season, and found to be 23.45±1 ºC and 37.10±0,16 ppt. It has been reported that bivalves develop well when the water temperature is 20-24 ºC and the salinity is between 32-40 ‰ [36]. When Bivalvia samples from different regions are compared with the Bivalvia samples in our study in terms of the height-weight relationship and growth patterns, we can conclude that this species has average morphometric characteristics and this area is ecologically suitable for the species. The average Zn, Cu, Cd, and Pb concentrations in the muscle tissue of *R. decussatus* in this study and some species belonging to the Veneridae family in different localities are given in Table 2.

Table 2. Compare with the average Zn, Cu, Cd, and Pb concentrations in muscle tissue of *R. decussatus* in this study and other species belonging to Veneridae family in different study (µg/g dry weight).

Species	Zn	Cu	Cd	Pb	References Year
Ruditapes decussatus Yumurtalık Coast, Turkey	0.278±0.001	0.028±0.001	0.006±0.000	0.003±0.000	In this study
In other studies					
Circe scripta Region: Daya Bay, China	9.79±0.82	0.90±0.03	0.07±0.03	0.34±0.10	[37] 2020
Gafrarium divaricatum Region: Daya Bay, China	10.64±0.52	1.10±0.59	1.14±0.25	0.24±0.13	[37] 2020
Ruditapes decussatus Region: Sardinian (Calich) Coast, Italy	16.00±0.85	1.2±0.05	0.010±0.0013	0.059±0.0063	[38] 2018
Ruditapes philippinarum Region: Laizhou Bay (Southern), China	53.47±30.21	12.13±3.40	1.98±0.86	1.06±0.28	[39] 2017
Ruditapes decussatus Region: Alexandria coast, Egypt	ns	ns	2.063±0.26	8.090±0.19	[40] 2013
Paphia undulata, Region: Alexandria coast, Egypt	ns	ns	0.903±0.10	3.120±0.17	[40] 2013
Tapes decussata Region: Egyptian Mediterranean coast	14.13±0.02	3.46±0.32	0.115±0.002	0.29±0.003	[41] 2012
Paphia undulata Region: Egyptian Mediterranean coast	9.72±0.06	1.92±0.32	0.133±0.002	0.14±0.003	[41] 2012
Venerupis decussata Region: Egyptian Mediterranean coast	8.35±0.06	1.54±0.12	0.036±0.003	0.05±0.004	[41] 2012
Gafrarium pectinatum Region: Egyptian Mediterranean coast	51.83±0.02	11.85±0.32	0.131±0.001	0.43±0.002	[41] 2012
Tapes decussata Region:Varano Lagoon, Italy	ns	ns	0.22±0.06	0.14±0.006	[42] 2001

The levels of Zn, Cu, Cd, and Pb found in muscle tissue of *R. decussatus* indicated statistically significant differences for only zinc and copper accumulation within seasons ($P<0.05$; Figure 4). The arithmetic means and standard error values of heavy metals in the muscle tissue for the sum of the two seasons were reported to be 0.278±0.001 µg Zn/g, 0.028±0.001 µg Cu/g, 0.006±0.000 µg Cd/g, 0.003±0.000 µg Pb/g (Table 2). Copper and zinc are trace elements that are normally required for metabolic cellular activities, but cadmium and lead are non-essential heavy metals that pose a threat to life even at low concentrations [38]. Essential metal levels (Zn-Cu) and non-essential metal levels (Cd-Pb) in the investigated coastal bivalves were found to be in the range of 0.19-0.35 µg Zn/g; 0.019-0.039 µg Cu/g and 0.004-0.009 µg Cd/g; 0.0019-0.005 µg Pb/g. These concentrations were observed below the concentrations determined in the species in China, Italy, and Egypt (Table 2). Heavy metals cause accumulation in the tissues of bivalves far above normal levels. At non-lethal toxic stressor levels for bivalves, situations such as escaping from the environment (withdrawal by siphoning) and closing of the valve to maintain its internal balance have been observed; in contrast, inhibition of byssal yarn production, impaired burrowing behavior in the dune field, inhibition of respiration, inhibition of filtration rate, inhibition of protein synthesis and suppressed growth have been observed in cases where toxicity exposure was fatal [43]. It has been reported that small organisms show more heavy metal accumulation than others [44] but *V. senegalensis* was reported in their sensitivity to heavy metal accumulation and their resistance to metal in another study [45]. *R. decussatus* has a high filtration capacity and is therefore viewed as an important biological model for an indication of environmental pollution and contamination [6]. The molybdenum intolerance of *V. senegalensis* belonging to Veneridae was investigated, and it was concluded that molybdenum was not toxic at the levels encountered in the marine environment. In addition, the lethal and non-lethal effects of the copper were examined, and it was reported that *V. senegalensis* had a high tolerance to heavy metals and a high recovery rate [45]. As we can see in Table 2, where comparisons were made with the species belonging to the Veneridae family sampled from different regions, heavy metal levels did not seem to be at high levels in the samples collected from the Yumurtalık coastal area. This was a very important and positive situation for the area from an ecological point of view. Some bivalve catcher was found to be naturally important in the predation of shorebirds of some species belonging to Veneridae family [46]. It is suggested that the average heavy metal levels in the wet tissue of bivalves should be below 50.0 mg $kg^{-}1$ for Zn, 1.0 mg $kg^{-}1$ for Cu and Cd, and 0.50 mg $kg^{-}1$ for Pb [47]. Accordingly, we can conclude that the metal pollution in the area where the samples were collected was not at a high level.

4. CONCLUSION

In this study, in which morphological characteristics and muscle tissue heavy metal concentrations of *R. decussatus* were examined, statistically significant differences were found only for zinc and copper accumulation over the seasons. As a consequence of these values, this species has average morphometric properties and this area is ecologically suitable for the species. In addition to its ecological importance, the species *R. decussatus* has an important socio-economic role in all regions. Nevertheless, intense fishing pressure can lead to the extinction of the natural population stock. The coastal area of Yumurtalık is very important in terms of fish species and shorebird richness. A commercial evaluation of the area within ecological disciplines in crustacean cultivation, in which this species is included, may be of importance for development.

CONFLICT OF INTEREST

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

REFERENCES

[1] Márquez F, Robledo J, Escati-Peñaloza G, Van der Molen S. Use of different geometric morphometrics tools for the discrimination of phenotypic stocks of the striped clam *Ameghinomya antiqua* (Veneridae) in north Patagonia, Argentina. Fish Res 2010; 101(1-2): 127-131.

[2] Schöne BR. A ‘clam-ring’ master-chronology constructed from a short-lived bivalve mollusc from the northern Gulf of California, USA. The Holocene 2003; 13(1): 39-49.

[3] Gosling E. Bivalve molluscs: Biology, Ecology and Culture. UK: John Wiley & Sons, 2008.

[4] Krantz DE, Williams DF, Jones DS. Ecological and paleoenvironmental information using stable isotope profiles from living and fossil mollusks. Palaeogeogr. Palaeoclim Palaeoecol 1987; 58(3-4): 249-266.

[5] Wanamaker Jr. AD, Kreutz KJ, Schöne BR, Maasch KA, Pershing AJ, Borns HW, Introne DS, Feindel S. A late Holocene paleo-productivity record in the western Gulf of Maine, USA, inferred from growth histories of the long-lived ocean quahog (*Arctica islandica*). Int J Earth Sci 2009; 98(1): 19-29.

[6] Bebianno MJ, Geret F, Hoarau P, Serafim MA, Coelho MR, Gnassia-Barelli M, Romeo M. Biomarkers in *Ruditapes decussatus*: a potential bioindicator species. Biomark 2004; 9(4-5): 305-330.

[7] Caill-Milly N, Bru N, Mahé K, Borie C, D'Amico F. Shell shape analysis and spatial allometry patterns of Manila clam (*Ruditapes philippinarum*) in a mesotidal coastal lagoon. J Mar Biol 2012; 1: 1-11.

[8] Farag EA, Dekinesh SI, El-Odessy HM. Taxonomical studies on the edible bivalve mollusks inhabiting the coastal zones of Alexandria, Egypt. Pak J Biol Sci 1999; 2(4): 1341-1349.

[9] Jara-Jara R, Abad M, Pazos AJ, Perez-Paralle ML, Sanchez JL. Growth and reproductive patterns in *Venerupis pullastra* seed reared in waste water effluent from a fish farm in Galicia (N.W. Spain). J Shellfish Res 2000; 19(2): 949-956.

[10] Freneix S, Saint Martin JP, Moissette P. Bivalves hétérodontes du Messinien d’Oranie (Algérie occidentale). Bull Mus Natl Hist Nat 1987; 4(9): 415-453.

[11] Rayment WJ. *Venerupis corrugata*. Pullet carpet shell. In: Tyler-Walters H, Hiscock K editors. Marine Life Information Network: Biology and Sensitivity Key Information Reviews. Plymouth: Mar Biol Assoc UK, 2007.

[12] Parache A, La palourde. Pêch Marit Paris 1982; 61: 496-507.

[13] Poppe GT, Goto Y. European seashells. Vol 1 (Polyplacophora, Caudofoveata, Solenogastra, Gastropoda). Wiesbaden: Verlag Christa Hemmen, 1991.

[14] Özsuer M, Sunlu U. Temporal Trends of Some Trace Metals in *Lithophaga lithophaga* (L., 1758) from Izmir Bay (Eastern Aegean Sea). Bull Environ Contam Toxicol 2013; 91(4): 409-414.

[15] Hu H. Exposure to metals. Occupational and Environmental Medicine 2000; 27(4): 983-996.

[16] Kayhan FE, Muşlu MN, Koç ND. Bazı Ağır Metallerin Sucul Organizmalar Üzerinde Yarattığı Stres ve Biyolojik Yanıtlar. J Fish Sci 2009; 3(2): 153-162.

[17] Bakır BB, Öztürk B, Doğan A, Önen M. Mollusc fauna of Iskenderun Bay with a checklist of the region. Turkish J Fish Aquat Sci 2012; 12(1): 171-184.

[18] Başusta N, Kumlu M, Gökçe MA, Göçer M. Seasonal change and productivity index of species by trawled in Yumurtalık Bay. Ege J Fish Aquat Sci 2002; 19(1): 1-1.

[19] Demir M. Shells of Mollusca collected from the seas of Turkey. Turkish J Zool 2003; 27(2): 101-140.

[20] Öztürk B, Doğan A, Bakir BB, Salman A. Marine molluscs of the Turkish coasts: an updated checklist. Turkish J Zool 2014; 38(6): 832-879.

[21] Marine Species Identification Portal. Taxonomic Database on Marine Species. http://species-identification.org/about.php. Accessed on August 2020.

[22] Plessi M, Bertelli D, Monzani A. Mercury and selenium content in selected seafood. J Food Compos Anal 2001; 14(5): 461-467.

[23] Pauly D. Some simple methods for the assessment of tropical fish stocks. FAO Fish Technol Pap 1984; 234: 1-52.

[24] O'Meley CM. Effects of Shell Abrasion and Aerial Exposure on the Performance of Pacific Oysters *Crassostrea gigas* (Thunberg, 1793) Cultured in Tasmania, Australia. PhD, University of Tasmania, Australia, 1995.

[25] Ricker WE. Computation and interpretation of biological statistics of fish populations, Bull Fish Res Bd Can 1975; 191: 1-382.

[26] Pouvreau S, Gangnery A, Tiapari J, Legarde F, Garnier M, Bodoy A. Gametogenetic cycle and reproductive effort of the tropical blacklip pearl oyster, *Pinctada margaritifera* (Bivalvia: Pteriidae), cultivated in Takapoto atol (French Polynesia). Aquat Living Resour 2000; 13: 37-48.

[27] Amane Z, Tazi L, Idhalla M, Chlaida M. Morphometric analysis of European clam *Ruditapes decussatus* in Morocco. Aquac Aquar Conserv Legis 2019; 12(5): 1623-1634.

[28] Mohammad SH, Belal AAM, Hassan SSZ. Growth, age and reproduction of the commercially clams *Venerupis aurea* and *Ruditapes decussatus* in Timsah Lake, Suez Canal, Egypt. Indian J Geo-Mar Sci 2014; 43(4): 589-600.

[29] Jurić I, Bušelić I, Ezgeta-Balić D, Vrgoč N, Peharda M. Age, growth and condition index of *Venerupis decussata* (Linnaeus, 1758) in the Eastern Adriatic Sea. Turkish J Fish Aquat Sci 2012; 12(3): 613-618.

[30] Sherif, RA. Allometry, population dynamics, shell growth and age determination of the bivalve *Venerupis decussatus* collected from Port Said, Egypt. Proc 6th Con Biol Sci (Zool) 2010; 6(10): 1-23.

[31] Kandeel, K. Length-Weight Relationships and Monthly Variations in Body Weights and Condition Indices of Two Clam's Species; *Venerupis aurea* and *Tapes decussata* in Lake Timsah, Egypt. Catrina: Int J Environ Sci 2008; 3(1): 111-124.

[32] Dincer T. Differences of Turkish clam (*Ruditapes decussates*) and Manila clam (*Ruditapes philippinarum*) according to their proximate composition and heavy metal contents. J Shellfish Res 2006; 25(2): 455-459.

[33] Ojea J, Pazos AJ, Martinez D, Novoa S, Sanchez JL, Abad M. Seasonal variation in weight and biochemical composition of the tissues of *Ruditapes decussatus* in relation to the gametogenic cycle. Aquac 2004; 238(1-4): 451-468.

[34] Urrutia MB, Ibarrola I, Iglesias JIP, Navarro E. Energetics of growth and reproduction in a high-tidal population of the clam *Ruditapes decussatus* from Urdaibai Estuary (Basque Country, N. Spain). J Sea Res 1999; 42(1): 35- 48.

[35] Garcia F. Interprétation des stries valvaires pour l'évaluation de la croissance de *Ruditapes decussatus* L. Oceanol Acta 1993; 16(2): 199-203.

[36] Loosanoff V, Davis HC. Rearing of bivalve larvae. Adv Mar Biol 1963; 1: 1-136.

[37] Yuan Y, Sun T, Wang H, Liu Y, Pan Y, Xie Y, Huangb H, Fan Z. Bioaccumulation and health risk assessment of heavy metals to bivalve species in Daya Bay (South China Sea): Consumption advisory. Mar Pollut Bull 2020; 150: 110717.

[38] Esposito G, Meloni D, Abete MC, Colombero G, Mantia M, Pastorino P, Prearo M, Pais A, Antuofermo E, Squadrone S. The bivalve *Ruditapes decussatus*: A biomonitor of trace elements pollution in Sardinian coastal lagoons (Italy). Environ Poll 2018; 242: 1720-1728.

[39] Liu J, Cao L, Dou S. Bioaccumulation of heavy metals and health risk assessment in three benthic bivalves along the coast of Laizhou Bay, China. Mar Poll Bull 2017; 117(1-2): 98-110.

[40] Abdallah MAM. Bioaccumulation of heavy metals in mollusca species and assessment of potential risks to human health. Bull Environ Contam Toxicol 2013; 90(5): 552-557.

[41] El Nemr A, Khaled A, Moneer AA, El Sikaily A. Risk probability due to heavy metals in bivalve from Egyptian Mediterranean coast. The Egyptian J Aquat Res 2012; 38(2): 67-75.

[42] Storelli MM, Marcotrigiano GO. Heavy Metal Monitoring in Fish, Bivalve Molluscs, Water. Bull Environ Contam Toxicol 2001; 66: 365-370.

[43] Aberkali HB, Trueman ER. Effects of environmental stress on marine bivalve molluscs. Adv Mar Biol 1985; 22: 101-198.

[44] Boyden CR. Trace metals content and body size in molluscs. Nat 1974; 251: 311-314.

[45] Abbott OJ. The toxicity of ammonium molybdate to marine invertebrates. Mar Poll Bull 1977; 8(9): 204-205.

[46] Johannessen OH. Length and weight relationships and the potential production of the bivalve *Venerupis pullastra* (Montagu) on a sheltered beach in western Norway. Sarsia 1973; 53(1): 41-48.

[47] Mol S, Çakalp Z, Çırpıcı E. Su Ürünlerinde İz Element Birikimi ve İnsan Sağlığına Etkileri, Hasad Gıda Dergisi 2005; 244: 1-1.

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

2022, 11(2), pp. 50-56, DOI:10.18036/estubtdc.1052090

RESEARCH ARTICLE

MOLECULAR INVESTIGATION OF CANINE HEPATOZOONOSIS IN BATMAN AND VAN PROVINCES OF TÜRKİYE

Bekir OGUZ [1, *], M. Serdar DEGER [1], Saeed EL-ASHRAM [2, 3]

[1] Department of Parasitology, Faculty of Veterinary Medicine, University of Van Yuzuncu Yil, Van, Turkey
[2] Faculty of Science, Kafrelsheikh University, Kafr el-Sheikh, Egypt
[3] College of Life Science and Engineering, Foshan University, Foshan, Guangdong, China

ABSTRACT

Canine hepatozoonosis is a tick-borne protozoan disease spread by hard ticks of the Ixodidae family. Although this illness has been seen in numerous locations in Türkiye, its existence in the Batman and Van provinces has yet to be confirmed. The purpose of this research was to look into canine hepatozoonosis in stray dogs from two distinct areas in Türkiye using conventional polymerase chain reaction (PCR). Between 2019 and 2021, blood samples were collected from 197 stray dogs in Batman and Van provinces in Türkiye. A unique 486–520 bp segment of the 18S rRNA gene of *Hepatozoon* spp. was amplified using PCR. According to the PCR findings, none of the 197 stray dogs tested positive for *Hepatozoon* spp. This research offers epidemiological data on the prevalence of canine hepatozoonosis in Türkiye, which may be useful in future studies with larger sample sizes and dogs of varied origins.

Keywords: Batman, Canine hepatozoonosis, PCR, Stray dogs, Türkiye, Van

1. INTRODUCTION

In America, Latin America, Europe, Asia, and Africa, *Hepatozoon canis* and *H. americanum* cause canine hepatozoonosis, a tick-borne disease. *H. canis* is a more frequent species than *H. americanum* [1]. Hard ticks of the Ixodidae family transmit both species. Although *Rhipicephalus* (*Boophilus*) *microplus* and *Rhipicephalus turanicus* are the main vectors of *H. canis*, other tick species, such as *Amblyomma ovale*, *Haemaphysalis longicornis*, *Haemaphysalis flava*, and *Rhipicephalus* (*Boophilus*) *microplus* and *Rhipicephalus turanicus* have been reported to carry the parasite in recent years [2-8]. *A. maculatum* is the vector tick that transmits *H. americanum* [9]. Ticks operate as vectors, physically or physiologically transmitting various infections of parasitic, bacterial, or viral origin from one vertebrate host to another. Furthermore, certain infections are spread both vertically and horizontally. *H. canis* and *H. americanum* transfer their sprozoites to intermediate hosts by vector ingestion, which is a horizontal transmission mode [10]. In Türkiye, only three tick vector studies on canine hepatozoonosis in dogs have been undertaken [11, 12, 13]. According to these investigations, *R. sanguineus* sensu lato might be considered a possible *H. canis* vector in our country. In general, canine hepatozoonosis is asymptomatic. Although fever is a frequent symptom in both species, *H. americanum* infection has been associated with more serious clinical symptoms, such as lameness and mucopurulent eye discharge [14]. Canine hepatozoonosis has been recorded in a few clinical case investigations and epidemiological surveys in Türkiye, and has been linked to *Hepatozoon canis* and *Hepatozoon* sp. MF [1, 11, 13, 15-25]. Canine hepatozoonosis has been diagnosed using blood smears, serology, and molecular methods [17, 25, 26]. Of these, DNA-based approaches are the most preferred among them because of their remarkable sensitivity and specificity in distinguishing between *H. canis* and *H. americanum*. Previous research from several provinces of Türkiye, such as Diyarbakir,

*Corresponding Author: bekiroguz@yyu.edu.tr
Received: 02.01.2022 Published: 26.07.2022

Kayseri, Konya, Karaman, Sivas, and Samsun, found canine hepatozoonosis. However, to the best of the authors' knowledge, canine hepatozoonosis has not been studied in Van province. It has yet to be discovered in Batman province. The purpose of this study was to use genetic approaches to evaluate the existence of *Hepatozoon* species in dogs residing in the Batman and Van province of Türkiye.

2. MATERIAL AND METHODS

2.1. Animals

During 2019–21, 197 reportedly asymptomatic stray dogs from municipal animal care and rehabilitation centers in Batman (97 sample) and Van (100 sample) provinces were investigated (Figure 1). Regulations define the responsibilities of these centers, which include actions for gathering and collecting stray animals, providing health treatment (including endo and ecto-parasitic applications), sterilization, and immunization. The age and gender of the animals were recorded (data not shown). Animal ethics, method and sampling approval was obtained from the University of Van Yuzuncu Yil Animal Ethics Committee (approval number: 2021/12-10). For DNA isolation, blood samples were collected from the cephalic veins and placed in sterile tubes containing EDTA.

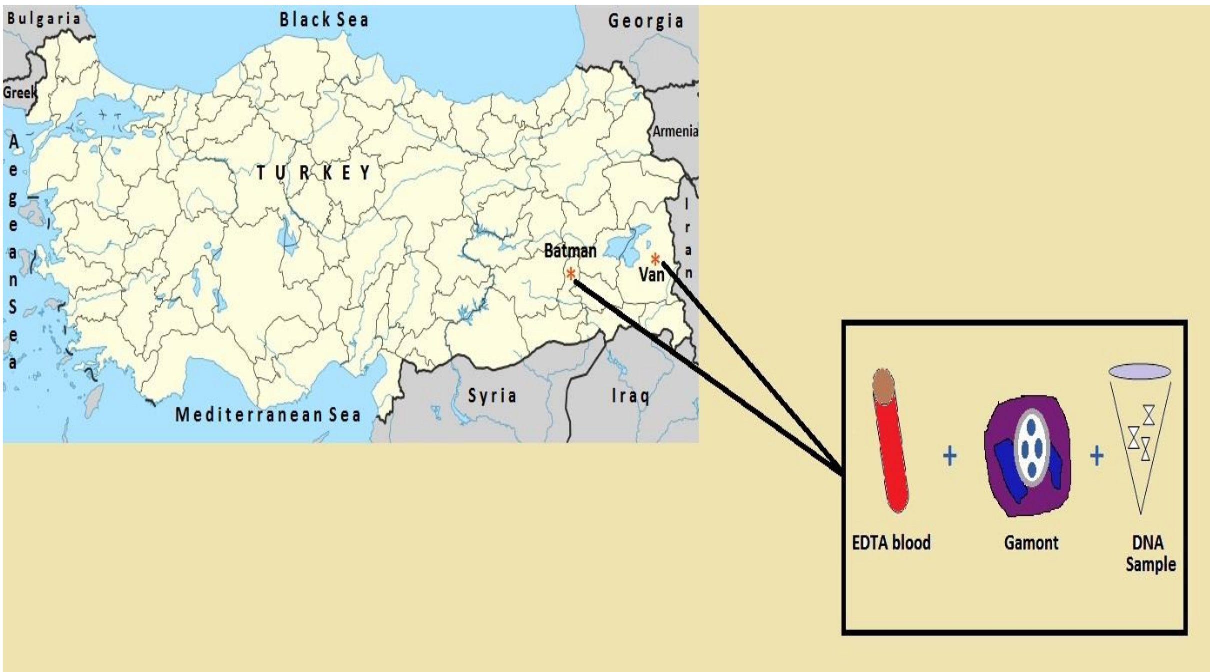

Figure 1. Map of Türkiye, locations Batman and Van. (https://tr.pinterest.com)

2.2. DNA Extraction and PCR

Using the Blood Genomic DNA Isolation Kit (Cat no: MG-GDNA-01-250, Hibrigen, Türkiye), total genomic DNA was extracted from 200 µl blood samples according to the manufacturer's instructions. Until used, genomic DNA was stored at -20 °C. The BJ1 (5′-GTCTTGTAATTGGAATGATGG-3′) and BN2 (5′-TAGTTTATGGTTAGGACTACG-3') primers were used to amplify the 486-520 bp region of the 18S rRNA gene of *Hepatozoon* spp. using a PCR technique [27]. The peculiarity of these primers is that they are specific for all *Babesia* species and can amplify blood parasites, such as *Hepatozoon* spp., *Hemolivia mauritanica*, and *Theileria* spp [23, 24, 28]. The PCR was performed in a final volume of 25 µL, which included 7.5 µL of DNase-and RNase-free sterile distilled water (Biobasic, Canada), 10 L of 5X MyTaq Reaction buffer, 1 µL of each primer (20 pmol), 5 µL of template DNA (100-200 ng), and 0.5 μL of Taq DNA polymerase (1.25 IU) (MBI Fermentas, Lithuania).The PCR conditions were as follows: 5 min at 95 °C(initial denaturation), 35 cycles of 60 s at 94 °C, 60 s at 55 °C, 2 min at 72 °C, and finally 5 min at 72 °C(final extension) [27]. The PCR products were separated on 1.5% agarose gels, stained with ethidium bromide, and photographed using an UV transilluminator.

3. RESULTS

According to the PCR data, none of the 197 stray dogs tested positive for *Hepatozoon* spp. This study's positive control sample was amplified properly (Figure 2). All dogs were treated for ectoparasites.

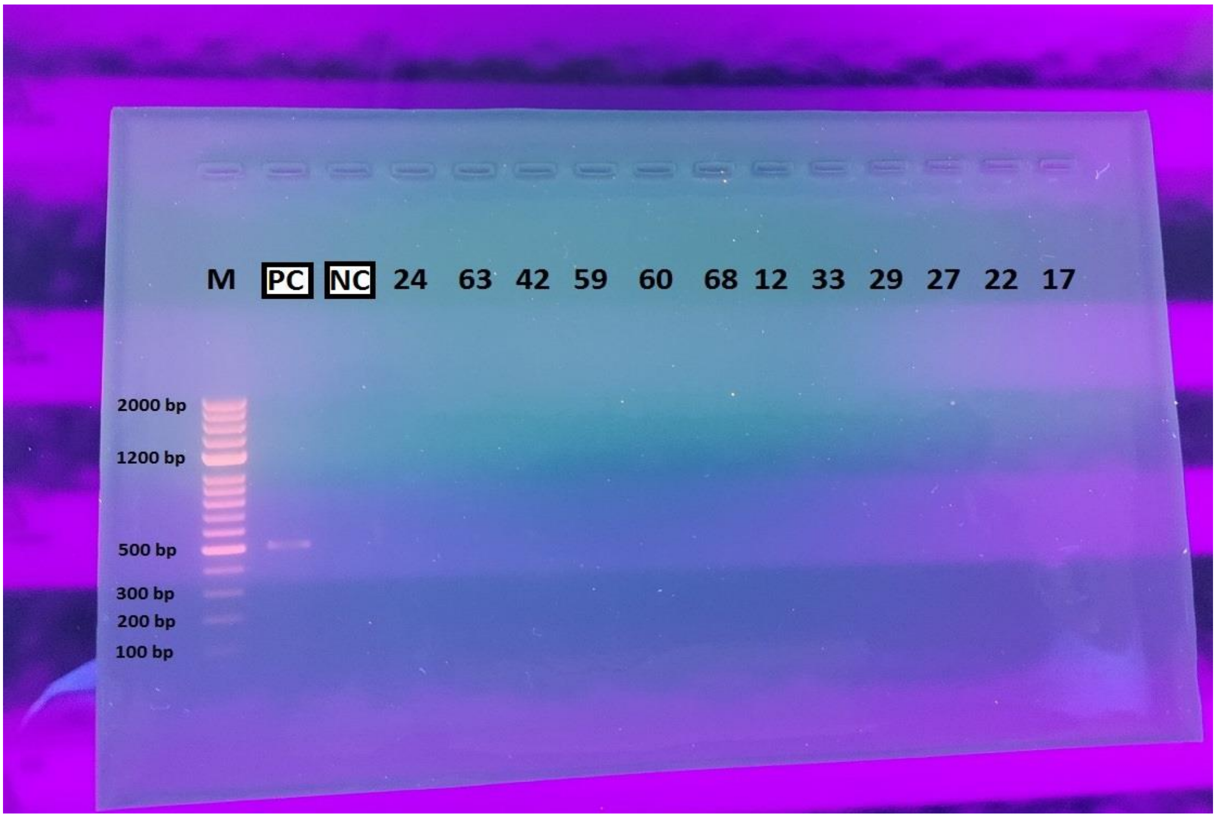

Figure 2. The single-PCR products of *Hepatozoon* species. M: Marker 24/63/42/59/60/68/12/33/29/27/22/17: samples, PC: Positive control, NC: Negative control

4. DISCUSSION

Babesiosis, anaplasmosis, dirofilariosis, hepatozoonosis, bartonellosis, ehrlichiosis, borreliosis, and leishmaniosis are the most commonly diagnosed canine vector-borne illnesses in dogs [29, 30]. These infections are spread by blood-feeding arthropods such as ticks, fleas, mosquitoes, and sand flies [31]. Ticks and fleas are the most common arthropods in dog, cat, and human vector-borne diseases [32]. *Hepatozoon* spp. were not discovered in any of the stray dogs in our investigation. However, the existence and prevalence of *Hepatozoon* spp. in dogs in several provinces of Türkiye has been reported. The prevalence of canine hepatozoonosis in dogs in Türkiye ranges between 0.5 and 54.3% [13, 17, 18, 20, 22-25]. Bolukbas et al. [20] and Aslantas et al. [24] reported a *H. canis* PCR prevalence of 0.5% in shelter dogs in Samsun and Hatay. According to Aktas and Ozubek et al. [13], *H. canis* was found in 54.3% of stray dogs in a Diyarbakir shelter. They stated that all canines in the Samsun research were administered anti-endo and anti-ectoparasitic medications. All of the dogs in the Hatay study were clinically healthy and free of ectoparasites. However, Aktas et al. [19] in Diyarbakir stated that no tick or flea management therapy was provided to the animals. In a recent survey conducted in five different parts of Sivas province, Ulas was the most prevalent region for canine hepatozoonosis (67.85%). The prevalence of canine hepatozoonosis was found to be quite low in another part of the province (Susehri) (3.84%). The difference in infection rates between studies could be due to the use of ectoparasiticides on a regular basis.

Batman province, which is located in the Tigris Section of the Southeastern Anatolia Region, has a continental climate. The summers are hot and dry, while the winters are mild and rainy [33]. The province of Van is located in the Upper Murat-Van Section of the Eastern Anatolia Region, in the closed basin of Lake Van. Van experiences a continental climate, and the winters are cold and long [34]. To date, no research on canine hepatozoonosis has been published from Türkiye's Van province. In Batman province, however, a study on canine hepatozoonosis was conducted [24]. According to the findings, none of the 50 canines tested positive for *Hepatozoon* DNA. *Hepatozoon* infection was not discovered in our current investigation, which is consistent with earlier research. The existence of *H. canis* has been confirmed in research conducted in several provinces in the Eastern and Southeastern Anatolia areas. For example, Aktas and Ozubek [13] found a PCR frequency of 54.3% for *H. canis* in Diyarbakir (the nearest city to Batman). In a study conducted in Erzurum, 377 kilometers from Van, Aktas et al. [19] discovered a 42.8% *H. canis* PCR infection rate. Guven et al. [22]; however, discovered a lower *H. canis* prevalence of 5.3% in the same area. These differences between our results and previous studies in the same area could be explained by different sampling periods and animal origins, as well as the spread of arthropod vectors in different parts of Türkiye [35, 36]. In regions where *R. sanguineus* sensu lato is the major tick species, *Hepatozoon canis* is often seen. In research done in the Eastern Anatolia Region, the presence of *R. sanguineus* s.l. in the province of Van was also documented [37]. The existence of the vector tick species in the Van province increases the risk of infection, but in our present investigation, we found no positive findings for this species. There has been no research on the prevalence and distribution of related tick species in the Batman province. We hypothesize that this somewhat validates the study's negative findings. Furthermore, hepatozoonosis may have a different transmission mechanism than tick-borne infections, such as *Theileria*, *Babesia*, and *Anaplasma* [31, 38, 39]. This may have a direct impact on the prevalence and occurrence of these diseases.

5. CONCLUSION

In our study, dogs in Batman and Van provinces were not infected with *Hepatozoon* spp. According to earlier investigations, two genotypes (*H. canis* and *Hepatozoon* spp. MF) have been discovered in dogs in Türkiye [1, 22, 25]. More research is needed to better understand the pathogenicity and frequency of emerging new genotypes. Additionally, studies using hunting dogs, sheepdogs, or wild canids are essential to determine the parasite's existence.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

ACKNOWLEDGEMENTS

We thank Prof. Armagan Erdem UTUK and Assist. Prof. Ufuk EROL for providing positive DNA of *Hepatozoon* spp. The authors also thank Veterinarians Onur KILIC and Omer SELCIN for kind help during sample collection.

AUTHOR CONTRIBUTION

B.O designed and carried out the experiments. B.O and M.S.D researched literature, analyzed the data and wrote the manuscript. S.A edited the language and helped with the review process of the final manuscript.

REFERENCES

[1] Aydin MF, Sevinc F, Sevinc M. Molecular detection and characterization of *Hepatozoon* spp. in dogs from the Central part of Turkey. Ticks Tick Borne Dis, 2015; 6(3): 388-392.

[2] Murata T, Inoue M, Taura Y, Nakayama S, Abe H, Fujisaki K. Detection of *Hepatozoon canis* oocyst from ticks collected from the infected dogs. J Vet Med Sci, 1995; 57(1): 111-112.

[3] Forlano M, Scofield A, Elisei C, Fernandes KR, Ewing SA, Massard CL. Diagnosis of Hepatozoon spp. in Amblyomma ovale and its experimental transmission in domestic dogs in Brazil. Vet Parasitol, 2005; 134(1-2): 1-7.

[4] Baneth G. Perspectives on canine and feline hepatozoonosis. Vet Parasitol, 2011; 181(1): 3-11.

[5] de Miranda RL, de Castro JR, Olegário MM, Beletti ME, Mundim AV, O'Dwyer LH, Eyal O, Talmi-Frank D, Cury MC, Baneth G. Oocysts of Hepatozoon canis in Rhipicephalus (Boophilus) microplus collected from a naturally infected dog. Vet Parasitol, 2011; 177(3-4): 392-396.

[6] Giannelli A, Lia RP, Annoscia G, Buonavoglia C, Lorusso E, Dantas-Torres F, Baneth G, Otranto D. Rhipicephalus turanicus, a new vector of Hepatozoon canis. Parasitology, 2017; 144 (Suppl 6): 730-737.

[7] Orkun O, Nalbantoglu S. Hepatozoon canis in Turkish red foxes and their ticks. Vet Parasitol Reg Stud Reports, 2018; 13: 35-37.

[8] Léveillé AN, Baneth G, Barta JR. Next generation sequencing from Hepatozoon canis (Apicomplexa: Coccidia: Adeleorina): complete apicoplast genome and multiple mitochondrion-associated sequences. Int J Parasitol, 2019; 49: 375-387

[9] Mathew JS, Ewing SA, Panciera RJ, Woods JP. Experimental transmission of Hepatozoon americanum Vincent-Johnson et al., 1997 to dogs by the Gulf Coast tick, Amblyomma maculatum Koch. Vet Parasitol, 1998; 80: 1-14.

[10] Inci A, Duzlu O. Vectors and Vector-Borne Diseases. Erciyes Üniv Vet Fak Derg, 2009; 6(1): 53-63.

[11] Aktas M, Ozubek S, Sayın Ipek DN. Molecular investigations of Hepatozoon species in dogs and developmental stages of Rhipicephalus sanguineus. Parasitol Res, 2013; 112: 2381-2385.

[12] Aktas M. A survey of ixodid tick species and molecular identification of tick-borne pathogens. Vet Parasitol, 2014; 200(3-4): 276-283.

[13] Aktas M, Ozubek S. A survey of canine haemoprotozoan parasites from Turkey, including molecular evidence of an unnamed Babesia. Comp Immunol Microbiol Infect Dis, 2017; 52: 36-42.

[14] Kramer. Canine hepatozoonosis-a summary for the practitioner. CVBD World Forum, 2009; 6: 1-12.

[15] Tuzdil AN. Biz de ilk defa görülen bir Hepatozoon canis vakası. Türk Bay Cem Mec, 1933; 13: 35.

[16] Voyvoda H, Pasa S, Uner A. Clinical Hepatozoon canis infection in a dog in Turkey. J Small Anim Pract, 2004; 45: 613-617.

[17] Karagenc TI, Pasa S, Kirli G, Hosgor M, Bilgic HB, Ozon YH, Atasoy A, Eren H. A parasitological, molecular and serological survey of Hepatozoon canis infection in dogs around the Aegean coast of Turkey. Vet Parasitol, 2006; 135: 113-119.

[18] Duzlu O, Inci A, Yildirim A, Onder Z, Ciloglu A. The investigation of some tick-borne protozoon and rickettsial infections in dogs by Real Time PCR and the molecular characterizations of the detected isolates. Ankara Univ Vet Fak Derg, 2014; 61: 275-282.

[19] Aktas M, Ozubek S, Altay K, Balkaya I, Utuk AE, Kırbas A, Simsek S, Dumanli, N. A molecular and parasitological survey of Hepatozoon canis in domestic dogs in Turkey. Vet Parasitol, 2015; 209: 264-267.

[20] Bolukbas CS, Pekmezci D, Gurler AT, Pekmezci GZ, Guzel M, Acici M, Umur S. Molecular survey of Hepatozoon canis in dogs from Samsun Province of Northern part of Turkey. J Etlik Vet Microbiol, 2016; 27: 104-107.

[21] Guo H, Sevinc F, Ceylan O, Sevinc M, Ince E, Gao Y, Moumouni PFA, Liu M, Efstratiou A, Wang G, Cao S, Zhou M, Jirapattharasate C, Ringo AE, Zhenh W, Xuan X. A PCR survey of vector-borne pathogens in different dogpopulations from Turkey. Acta Parasitol, 2017; 62: 533-540.

[22] Guven E, Avcioglu H, Cengiz S, Hayirli A. Vector-borne pathogens in stray dogs in Northeastern Turkey. Vector Borne Zoonotic Dis, 2017; 17(8): 610-617.

[23] Orkun O, Nafiye K, Sursal N, Cakmak A, Nalbantoglu S, Karaer Z. Molecular characterization of tick-borne blood protozoa in stray dogs from central Anatolia region of Turkey with a high-rate Hepatozoon infection. Kafkas Univ Vet Fak, 2018; 24(2): 227-232.

[24] Aslantas O, Celebi B, Usluca S. Investigation of vector-borne diseases in dogs. Harran Üniv Vet Fak Derg, 2020; 9(2): 154-160.

[25] Erol U, Altay K, Ataş AD, Özkan E, Şahin ÖF. Molecular Prevalence of Canine Hepatozoonosis in Owned-Dogs in Central Part of Turkey. Isr J Vet Med, 2021; 76(2): 71-76.

[26] Attipa C, Hicks CAE, Barker EN, Christodoulou V, Neofytou K, Mylonakis ME, Siarkou VI, Vingopoulou EI, Soutter F, Chochlakis D, Psaroulaki A, Papasouliotis K, Tasker S. Canine tick-borne pathogens in Cyprus and a unique canine case of multiple co-infections. Ticks Tick Borne Dis, 2017; 8(3): 341-346.

[27] Casati, SS, Sager, HH, Gern, LL, Piffaretti, JCJ-C. Presence of potentially pathogenic Babesia sp for human in Ixodes ricinus in Switzerland. Ann Agric Environ Med, 2006; 13(1): 65.

[28] Gökçe T. Investigations of hard tick species (Acari:Ixodidae) parasitizing in Corum region and presence of Babesia in these ticks.Msc, Hitit Üniversitesi, Çorum, Turkey, 2017.

[29] Andersson MO, Tolf C, Tamba P, Stefanache M, Waldenström J, Dobler G, Chițimia-Dobler L. Canine tick-borne diseases in pet dogs from Romania. Parasit Vectors, 2017; 10(1): 155.

[30] Maggi RG., Krämer F. A review on the occurrence of companion vector-borne diseases in pet animals in Latin America. Parasites Vectors, 2019; 12: 145.

[31] Dantas-Torres F, Otranto D. Hepatozoonosis. In: Marcondes CB, editors. Arthropod Borne Diseases. Springer; Cham, Switzerland: 2017; 215–222.

[32] Nguyen VL, Colella V, Greco G. et al. Molecular detection of pathogens in ticks and fleas collected from companion dogs and cats in East and Southeast Asia. Parasites Vectors, 2020; 13: 420.

[33] Alaeddinoglu F. The Functional Characteristics and Primary Problems of the Batman City. Eastern Geography Review, 2011; 15(24): 19-42 .

[34] Kalelioglu E. Characteristics of the climate of Van Plain (Van Ovasının iklim özellikleri). Ankara Üniv. Dil Tarih-Coğrafya Fakültesi Dergisi, 1991; 35, 155-166.

[35] Dantas-Torres F, Latrofa MS, Weigl S, Tarallo VD, Lia RP, Otranto D. Hepatozoon canis infection in ticks during spring and summer in Italy. Parasitol Res, 2012; 110 (Suppl 2): 695-698

[36] Pacifico L, Braff J, Buono F. et al. Hepatozoon canis in hunting dogs from Southern Italy: distribution and risk factors. Parasitol Res, 2020; 119: 3023-3031.

[37] Deger MS, Bicek K, Oguz B. Infestation rate and distribution of hard ticks on cattle in the Eastern Anatolia Region of Turkey. Sci Parasitol, 2016; 17: 76-82.

[38] Smith TG. The genus hepatozoon (Apicomplexa: Adeleina). J Parasitol, 1996; 82: 565-585.

[39] Taylor MA, Coop RL, Wall RL. Veterinary Parasitology. 4th. ed. Blackwell Publishing, Oxford, 2016.

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

2022, 11(2), pp. 57-65, DOI: 10.18036/estubtdc.1121512

RESEARCH ARTICLE

INVESTIGATION FOR CONTAMINATIONS OF RAW MILK ON MILK COLLECTION UNITS IN NEVSEHIR PROVINCE

Serkan TEKİN [1], **Zeliha LEBLEBİCİ** [2]*

[1] Department of Biology, Faculty of Arts and Sciences, 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

In this study, it was aimed to investigate Aflatoxin M_1 and heavy metal pollution in raw milk samples taken from milk collection centers operating in Nevsehir province. As a result of the analyzes made on raw milk samples; The amount of Aflatoxin M_1 was determined in the range of 0.013-0.150 ppb, in 7 raw milk samples remained below the measurement limit. A limit of 0.05 ppb has been determined for Aflatoxin M1 in the Turkish Food Codex and the amount of Aflatoxin M_1 was determined above the legal limit in 15% of the raw milk samples taken. As a result of heavy metal analysis with ICP-MS in raw milk samples, Mg 41,96±1,88 ppm; Ca 79,83±4,30 ppm; Mn 0,0115±0,007 ppm; Fe 0,176±0,003 ppm; Ni 0,0283±0,002 ppm; Cu 0,0181±0,003 ppm; Zn 1,56±0,005 ppm; As 0,0046±0,000 ppm; Cd 0,001549±0,002 ppm and Pb 0,00048±0,000 ppm were found, respectively. According to our study results, it was determined that there was no risk human health risk in terms of heavy metal.

Keywords: Raw Milk, Heavy Metal, Aflatoxin M_1, Nevsehir

1. INTRODUCTION

Milk is a vital basic food that can meet the needs of the organism in all mammals due to the wide variety of nutrients it contains [1].

Milk has a very important place in human nutrition. It can threaten human health if it is not stored and processed under minimum technical and hygienic conditions.

According to the Turkish Food Codex Contaminants Regulation; "All kinds of foreign substances, such as animal hair, insect fragments, which are not intentionally added to the food but are found in the food as a result of the production, manufacture, processing, preparation, processing, packaging, packaging, transportation or preservation, including the primary production stage of the food, or environmental contamination is defined as contaminants [2].

If an evaluation is made in terms of raw milk; Veterinary drugs, mycotoxins, pesticides, detergent residues used in cleaning and disinfection, and heavy metals, not naturally present in the composition of milk, but contaminate the milk from various sources, can be given as examples for contaminants [2].

The presence of Aflatoxin M_1 in raw milk is an important element that threatens public health. Among animal products, aflatoxin is mostly found in milk and dairy products. There are two main reasons for aflatoxin contamination of milk and dairy products. The first is that Aflatoxin B_1 and Aflatoxin B2, which are taken by lactating animals as a result of consuming feeds contaminated with molds, are metabolized in the animal body and passed into milk as Aflatoxin M_1 and Aflatoxin M_2. The second is

*Corresponding Author: zleblebici@nevsehir.edu.tr
Received: 26.05.2022 Published: 26.07.2022

caused by the contamination of aflatoxin-synthesizing molds and the production of aflatoxins during the transportation, processing and storage processes after milking [3].

There is no significant change in the amount of Aflatoxin M_1 during pasteurization of milk or processing into other dairy products, and Aflatoxin M_1 passes into dairy products at various rates [4]. Due to the reasons stated, legal regulations have been made by the countries regarding the maximum amount of Aflatoxin M_1 that can be found in milk and dairy products that are frequently consumed by almost all age groups, especially children.

Rapid developments in industry and technology not only make life easier for people, but also increase production and consumption, disrupt natural balances, destroy resources, and pollute air, water and soil. All living things are adversely affected due to environmental pollution, and exposure of plants and animals to toxic pollutants in various ways indirectly threatens human health. Heavy metals and pesticides can be given as examples of these toxic pollutants that contaminate the food chain with environmental pollution [5].

In terms of mineral matter, milk is an important source of Ca and P; Heavy metal contamination can occur in raw milk from the pastures where the animal is fed, from the feed, from the water it drinks, from the containers used to preserve the milk [5-7]. The main elements in metallic contamination arising from metal containers and operating water used during technological processes or for the preservation of milk and its products; metals such as copper, zinc, iron, tin, lead, arsenic, cadmium [8].

In our country, milk processing facilities mainly supply raw milk to be processed from collection centers. Although there are scientific studies on raw milk produced on farms in our country, it is not sufficient and the necessary precautions cannot be taken effectively.

In this study, it is aimed to evaluate the raw milk in terms of contaminants by investigating the presence of Aflatoxin M_1 and heavy metal pollution in raw milk samples taken from milk collection centers operating in Nevşehir province. Our study will have a unique value with the results obtained in terms of the lack of available scientific data on the examination of raw milk collected in Nevşehir province in terms of contaminants.

2. MATERIALS AND METHOD

2.1. Material

In this study, one approved milk collection center operating in each district of Nevşehir was determined. 7 raw milk samples were used as material.

2.2. Aflatoxin M_1 Analysis

A total of 28 raw milk samples were taken and analyzed for Aflatoxin M_1 analysis in sterile sample containers in accordance with the sampling rules from the milk collection centers determined in quarterly periods between January and December 2021.

In Aflatoxin M1 analysis; 10 ml of each sample was weighed. It was homogenized by adding 80 ml of 35°C water. The obtained homogenates were incubated in a water bath at 35°C for 30 minutes. After homogenization, the samples were centrifuged at 4000 rpm for 15 minutes at room temperature and the dilution process was started. In the dilution process, the extract was filtered through whatman no:4 filter paper. After the filtration process was completed, 50 ml of the extract was taken and the adsorption process was started. The milk sample is passed directly through the immunoaffinity column. Mobile phase content Methanol / Acetonitrile / Water (2:3:5 v/v/v/) was used as the running fluid for HPLC.

Aflatoxin M1 mobile phase prepared in this way was kept in an ultrasonic bath for about 15 minutes. The samples were analyzed by reversed phase liquid chromatography (RP-HPLC) [9]

2.3. Determination of Heavy Metal Analysis

Between January and December 2021, a total of 28 raw milk samples were taken from the same milk collection centers determined in quarterly periods, and heavy metal and mineral substance analyzes were carried out in the Inductively Coupled Plasma-Mass Spectrometer (ICP-MS).

The samples were dissolved in acidic medium by wet burning (microwave oven) method. The metal (Mg, Ca, Mn, Fe, Ni, Cu, Zn, As, Cd and Pb) contents of the samples were measured with the ICP-MS device using certain standards [10].

3. RESULTS

3.1. Aflatoxin M_1 Results

The maximum value that can be found for Aflatoxin M_1 in raw milk is specified as 0.05 ppb in the Turkish Food Codex Contaminants Regulation [2]. The legal limit for Aflatoxin M_1 in China and the USA is 500 ng/L [11].

The lowest value for Aflatoxin M_1 was 0.013±0.003 ppb in the Ürgüp district during July-September 2021; the highest value was determined as 0.150±0.006 ppb in the Gulsehir district during the October-December period. Aflatoxin M_1 level was below the measurement limit of 0.0088 ppb in 25% of the raw milk samples taken.
In 15% of the raw milk samples taken, the maximum value that can be found for Aflatoxin M_1 in raw milk reported in the Turkish Food Codex Contaminants Regulation and the EU 1881/2006 commission regulation was found to be above 0.05 ppb [2, 12].

Aflatoxin M_1 levels of analyzed raw milk samples are given in Table 1.

Table 1. Aflatoxin M_1 levels (ppb)

Districts	January-March 2021	April-June 2021	July-September 2021	October-December 2021
Acıgöl	**ND**	**0,016**	**ND**	**0,030**
Avanos	**0,026**	**0,037**	**0,020**	**0,016**
Gülşehir	**0,078**	**0,120**	**0,093**	**0,150**
Derinkuyu	**ND**	**0,029**	**0,036**	**0,041**
Hacıbektaş	**ND**	**0,030**	**0,027**	**0,027**
Kozaklı	**ND**	**0,036**	**0,043**	**0,025**
Ürgüp	**ND**	**ND**	**0,013**	**0,034**
NOTE: ND (Not Detectable - Limit of Measurement is below 0.0088 ppb.)				

Statistical evaluation of aflatoxin M_1 analysis results was made using SPSS 15.0 package program. Shapiro-Wilk test was applied to determine whether the obtained findings had a normal distribution, and it was determined that the data did not have a normal distribution. The findings were evaluated by applying the Kruskal Wallis H test to examine whether the difference between the sampled districts was significant. When the aflatoxin M_1 analysis results are evaluated, there is a statistically significant difference between the districts according to the 5% significance level ($p<0,05$). When the aflatoxin M1 analysis results were evaluated, there was no statistically significant difference between collection times.

Aflatoxin M_1 concentration percentages of the analyzed raw milk samples are given in Figure 1.

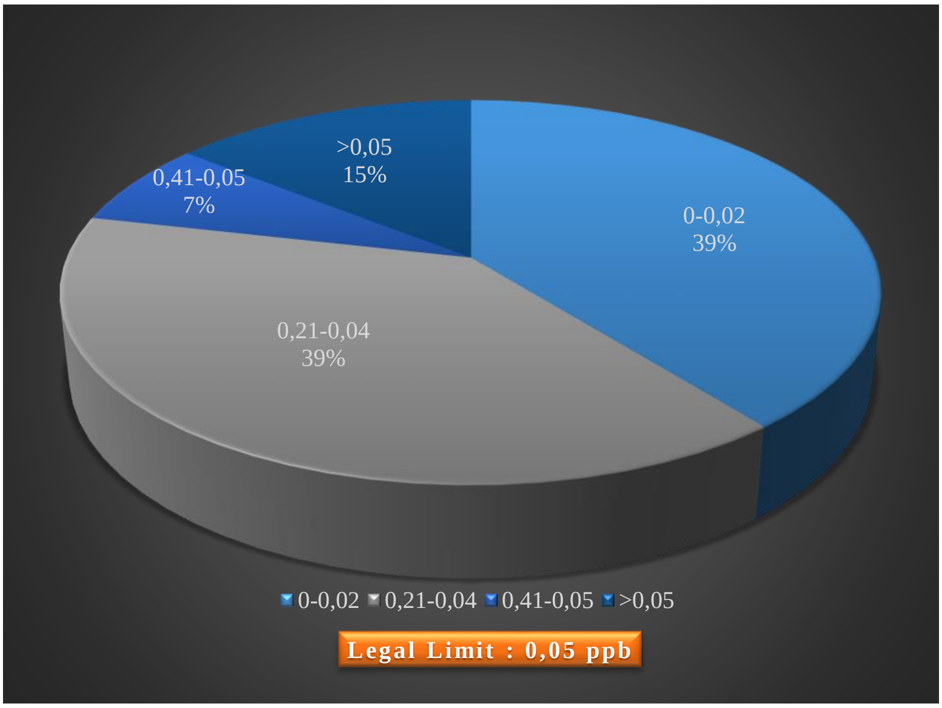

Figure 1. Aflatoxin M_1 Concentration Percentages of Analyzed Raw Milk Samples

3.2. Heavy Metal Results

As a result of heavy metal and mineral substance analysis with ICP-MS; The contents of mean heavy metals in raw milk samples were found that 41.96±1.88 ppm for Mg; 79.83±4.30 ppm for Ca; 0.0115±0.007 ppm for Mn; 0.176±0.003 ppm for Fe; 0.0283±0.002 ppm for Ni; 0.0181±0.003 ppm for Cu; 1.56±0.05 ppm for Zn; 0.0046±0.000 ppm for As; 0.001549±0.002 ppm for Cd and 0.000488±0.000 ppm for Pb (Table 2 and 3).

Table 2. Heavy metal levels (ppm)

	Mg	Ca	Mn	Fe	Ni
Acıgöl	$39,63 \pm 3,00^b$	$76,28 \pm 3,70^b$	$0,01245 \pm 0,009^b$	$0,146 \pm 0,002^b$	$0,040 \pm 0,004^d$
Avanos	$43,21 \pm 3,27^b$	$83,79 \pm 5,50^{bc}$	$0,011375 \pm 0,009^b$	$0,202 \pm 0,009^c$	$0,031 \pm 0,005^{bc}$
Gülşehir	$42,46 \pm 5,67^b$	$81,70 \pm 3,46^{bc}$	$0,010875 \pm 0,007^b$	$0,163 \pm 0,009^b$	$0,024 \pm 0,009^b$
Derinkuyu	$31,48 \pm 1,13^a$	$60,37 \pm 2,73^a$	$0,008375 \pm 0,005^a$	$0,130 \pm 0,009^a$	$0,021 \pm 0,007^a$
Hacıbektaş	$43,79 \pm 2,19^b$	$187,20 \pm 7,5^d$	$0,0112 \pm 0,008^b$	$0,218 \pm 0,006^d$	$0,027 \pm 0,003^b$
Kozaklı	$42,48 \pm 2,02^b$	$79,49 \pm 4,57^b$	$0,0114 \pm 0,009^b$	$0,178 \pm 0,003^b$	$0,034 \pm 0,002^c$
Ürgüp	$50,66 \pm 2,51^c$	$96,01 \pm 4,08^c$	$0,0152 \pm 0,010^c$	$0,193 \pm 0,000^c$	$0,023 \pm 0,005^b$

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

Tablo 3. Heavy metal levels (ppm)

	Cu	Zn	As	Cd	Pb
Acıgöl	0,0199±0,009^{c}	1,52±0,421^{b}	0,0116±0,011^{d}	0,004465±0,007^{c}	0,0006±0,000^{d}
Avanos	0,0187±0,004^{b}	1,66±0,892^{bc}	0,0057±0,004^{c}	0,001525±0,001^{bc}	0,0005±0,000^{c}
Gülşehir	0,0180±0,003^{b}	1,65±0,714^{bc}	0,0038±0,003^{b}	0,001075±0,001^{b}	0,0003±0,000^{b}
Derinkuyu	0,0134±0,009^{a}	0,95±0,183^{a}	0,0036±0,001^{b}	0,0007±0,000^{a}	0,0003±0,000^{b}
Hacıbektaş	0,0186±0,002^{b}	1,88±1,621^{c}	0,0024±0,001^{b}	0,00135±0,001^{bc}	0,0007±0,000^{d}
Kozaklı	0,0182±0,006^{b}	1,43±0,744^{b}	0,0018±0,001^{a}	0,00095±0,000^{b}	0,0004±0,000^{c}
Ürgüp	0,0199±0,006^{c}	1,80±1,051^{c}	0,0031±0,003^{b}	0,000775±0,000^{ab}	0,0002±0,000^{a}

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

Statistical evaluation of heavy metal results was made using SPSS 15.0 package program. ANOVA test was used for statistical comparison of the means. The statistical significance of the results obtained was evaluated at the level of $p<0.05$ and $p<0.01$ and expressed using different letters in the Tables 2 and 3.

When the results are examined; the lowest values were determined in Derunkuyu district for Mg, Ca, Mn, Fe, Ni, Cu, Zn and Cd elements. The lowest value was determined in Kozaklı district for As. The lowest value was determined in Ürgüp district for Pb.

The highest values were determined in Ürgüp district for Mg, Ca, Mn, Fe, Cu and Zn elements. The highest values were determined in Kozaklı district for Pb and Ni. The highest values were determined in Acıgöl district for As and Cd.

4. DISCUSSION AND CONCLUSION

In a study conducted in 40 long-life milk samples in our country; the mean amount of Aflatoxin M_1 has been reported as 0.0029±0.000 µg/L [13].

Temamoğulları and Kanıcı determined that in Şanlıurfa with the ELISA method; In 38 raw milk samples and 12 UHT milk samples, the average Aflatoxin M1 amounts were determined as 56.74±40.32 ng/kg and 43.1±23.19 respectively.

Hussain and Anwar [15] reported that in 2008, 99.4% of 168 raw milk samples exceeded EU limits for Aflatoxin M1 content.

Almeida Picinin et al. [16] were determined, in three different climatic conditions, with the ELISA method, on 129 raw milk samples in Brazil; They found the mean amount of aflatoxin M_1 to be 0.0195±0.0021 µg/l. They stated that all of the samples were in compliance with the Brazilian legal limit (0.5 µg/L), and 18 samples (13.95%) exceeded the Codex Alimentarius and EU limits (0.05 µg/L).

In the study of Fallah and his colleagues in which they examined 88 raw milk samples in Iran by TLC; They found the amount of aflatoxin M_1 in the range of 0.013-0.394 µg/L and an average of 0.052±0.006 µg/L [17].

Li et al.; They found the average Aflatoxin M_1 amount of 5650 raw milk samples collected from main milk producers in China during 2016 to be 36.8±43.6 ng/L. They stated that only 63 of the samples exceeded the EU limits [18].

In a study by Duarte and his colleagues in pasteurized and UHT milk; They reported the mean amount of aflatoxin M_1 as 23.4±24.0 ng/L [19].

The findings obtained as a result of Aflatoxin M_1 analyzes in this study; It was found higher than the findings of Almeida Picinin et al., Duarte et al.; It is similar to the study of Kabak and Özbey. [13,16,19]. On the other hand, the mean amount of Aflatoxin M_1 was found to be lower than the findings of Fallah et al., Temamogullari and Kanici, Li et al., Hussain and Anwar [14-15,17-18].

In the study, Aflatoxin M_1 was detected in 75% of raw milk samples and 15% were not in compliance with the Turkish Food Codex Contaminants Regulation. It points out the importance of producing, transporting and storing the feed consumed by animals in suitable conditions, and raising the awareness of producers and consumers on these issues.

Heavy metal results obtained in our study and literature data are shown in Table 4.

Table 4. Findings Obtained as a result of Heavy Metal Analysis Performed by ICP-MS in Our Study and Comparison with Literature Data

Heavy metals (ppm)		National Food Composition Database (ppm) [20]	Özturan (ppm) [21]	Özrenk (ppm) [22]	Birghila et. al. (ppm) [23]	Lindmark-Mansson et. al (ppm) [24]	Licata et.al. [25]	Gövercin [26]	İstanbulluoğlu [27]	Rana et. al. [28]	Çakır [29]
Mg	41,96±18,88	90	107,33	45,601	214						
Ca	79,83±78,30	980	1258,48	568,104		1140					
Mn	0,0115±0,0079		0,022	0,066	0,08	LOD					
Fe	0,176±0,122	0,2	0,640	0,309	0,72	0,4					
Ni	0,0283±0,028		0,034	0,189	0,04						
Cu	0,0181±0,013		0,079	0,182	0,17	0,1	0,00198				
Zn	1,56±0,85		1,406	3,003	0,98	4,4	2,016				
As	0,0046±0,005						0,0379	<0,003	0,00068	0,156±0,009	
Cd	0,001549±0,0028				0,000004		0,00002		≤0,00016		0,00009±0,00009
Pb	0,000488±0,0004				0,00012		0,00132	<0,01			0,008±0,009

When compared with the literature data, it was observed that the mineral substances obtained in this study were low. It is known that reasons such as breed, species, lactation status, seasonal conditions, poor and malnutrition cause mineral substance changes in milk.

When the heavy metal findings were examined in the study, it was seen that, they were not at a level to risk to human health.

Since milk is a sensitive product; should not be forgotten that pastures where animals are fed, water consumed by animals, various environmental and industrial pollutants, pesticides, veterinary drugs used in animal treatment, detergents used in cleaning and disinfection can contaminate milk.

Control of pollutants that may occur in milk; It is important for both human and public health and milk technology

ACKNOWLEDGMENT

This study received support from Nevşehir Hacıbektaş Veli University Scientific Research Projects Coordination Unit project no ABAP-20F42. We thank NEU BAP unit for their support.

CONFLICT OF INTEREST

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

REFERENCES

[1] Yetişemiyen A, Süt Üretimi, Süt Hayvancılığı, Sütün Oluşumu ve Sağımı. Süt Teknolojisi. Editör/ Yetişemiyen A, Ankara, 2013.

[2] T.C. Cumhurbaşkanlığı Mevzuat Bilgi Sistemi Türk Gıda Kodeksi Bulaşanlar Yönetmeliği. https://www.mevzuat.gov.tr/mevzuat?MevzuatNo=15692&MevzuatTur=7&MevzuatTertip=5

[3] Blanco JL, Carrioon BA, Liria N, Diaz S, Garcia ME, Dominguez L, Suarez G, Behavior of Aflatoxins During Manifacture and Storage of Yoghurt. Milchwiss, 1993; 48: 385-387.

[4] Badea M, Micheli L, Messia MC, Candigliota T, Marcani E, Mottram T, Velasco-Garcia M, Moscone D, Palleschi G, Aflatoxin M1 Determination in Raw Milk Using a Flow –Injection Immunoassay System. Anal Chim Acta, 2004; 520: 141-148.

[5] Vural H, Ağır Metal İyonlarının Gıdalarda Oluşturduğu Kirlilikler. Çevre Dergisi, 1993; 8: 3-8.

[6] Sağlam N, Cihangir N, Ağır Metallerin Biyolojik Süreçlerle Biyosorbsiyonu Çalışmaları. Hacettepe Üniversitesi Eğitim Fakültesi Dergisi, 1995; 11: 157-161.

[7] Kahvecioğlu Ö, Kartal G, Güven A, Timur S, Metallerin Çevresel Etkileri-I. Metalurji Dergisi, 2004; 136: 47-53.

[8] Metin M, Süt Teknolojisi. Sütün Bileşimi ve İşlenmesi. Ege Üniversitesi Basımevi:Yayın No:33, İzmir, 2001.

[9] TS EN ISO 14501, Süt ve Süt Tozu-Aflatoksin M1 Muhtevası Tayini-İmmunoaffiniti Kromatografi ile Temizleme ve Yüksek Performanslı Sıvı Kromatografi ile Tayini. 2008.

[10] Committee on Food Analysis, Trace elements, As, Cd, Hg, Pb and other elements. Determination by ICP-MS after pressure digestion. No:186, 14, 2007.

[11] Li S, Min L, Wang P, Zhang Y, Zheng N, Aflatoksin M1 Contamination in Raw Milk from Major Milk-Producing Areas of China during Four Seasons of 2016. Food Control, 2017; 82: 121-125.

[12] An Official Website of the European Union. https://eur-lex.europa.eu/legal-content/EN/ALL/?uri=celex%3A32006R1881

[13] Kabak B, Özbey F, Aflatoxin M1 in UHT Milk in Consumed in Turkey and First Assessment of its Bioaccessibility Using an in Vitro Gestion Model. Food Control, 2012; 28: 338-344.

[14] Temamoğulları F, Kanıcı A, Short Communication: Aflatoxin M1 in Dairy Products Sold in Şanlıurfa, Turkey. J Dairy Sci, 2014; 97: 162-165.

[15] Hussain I, Anwar J, A Study on Contamination of Aflatoxin M1 in Raw Milk in the Punjab Province of Pakistan. Food Control, 2008; 19: 393-395.

[16] Almeida Picinin LC, Oliveira Pinho Cerqueira MM, Vargas EA, Quintão Lana AM, Maia Toaldo I, Bordignon-Luiz M., Influence of Climate Conditions on Aflatoxin M1 Contamination in Raw Milk From Minas Gerais State, Brazil. Food Control, 2013; 31: 419-424.

[17] Fallah AA, Rahnama M, Jafari T, Siavash Saei-Dehkordi S, Seasonal Variation of Aflatoxin M1 Contamination in Industrial and Traditional Iranian Dairy Products. Food Control, 2011; 22: 1653-1656.

[18] Li S, Min L, Wang P, Zhang Y, Zheng N, Aflatoksin M1 Contamination in Raw Milk from Major Milk-Producing Areas of China during Four Seasons of 2016. Food Control, 2017; 82: 121-125.

[19] Duarte SC, Almeida AM, Teixeira AS, Pereira AL, Falcão AC, Pena A, Lino CM, Aflatoxin M1 in Marketed Milk in Portugal: Assessment of Human and Animal Exposure. Food Control, 2013; 30: 411-417.

[20] T.C. Tarım ve Orman Bakanlığı, Ulusal Gıda Kompozisyon Veri Tabanı. http://www.turkomp.gov.tr/food-17

[21] Özturan K, Erzurum ve Çevresinde Üretilen Süt ve Süt Ürünlerinin Mevsimlere Göre Mineral Madde ve Ağır Metal İçeriği. Atatürk Üniversitesi Sağlık Bilimleri Enstitüsü, Doktora Tezi, Erzurum, 2010.

[22] Özrenk Ö, Van İli ve İlçelerinde Üretilen İnek Sütlerinin Ağır Metal Kirlilik Düzeyi ve Bazı Mineral Madde İçerikleri. Yüzüncü Yıl Üniversitesi Fen Bilimleri Enstitüsü, Doktora Tezi, Van, 2002.

[23] Birghila S, Dobrinas S, Stanciu G, Soceanu A, Determinatipon of Major and Mınor Elements in Milk Through ICP-AES. Environ Eng Manag J, 2008; 7: 805-808.

[24] Lindmark-Mansson H, Fonden R, Pettersson HE, Composition of Swedish Dairy Milk. Int Dairy J, 2003; 13: 409-425.

[25] Licata P, Trombetta D, Cristani M, Giofre F, Martino D, Calo M, Naccari F, Levels of Toxic and Essential Metals in Samples of Bovine Milk From Various Dairy Farms in Calabria, Italy. Environ Int, 2004; 30: 1-6.

[26] Gövercin İ, İzmir İlinde Sütlerde Bazı Ağır Metal (Kurşun, Kadmiyum, Arsenik, Civa, Bakır, Çinko) Düzeylerinin Belirlenmesi. Ege Üniversitesi Fen Bilimleri Enstitüsü, Doktora Tezi, İzmir, 2010.

[27] İstanbulluoğlu H, Piyasada Satılan Süt ve Süt Ürünlerinde Ağır Metal Kirliliği. GATA Halk Sağlığı Anabilim Dalı Başkanlığı, Tıpta Uzmanlık Tezi, Ankara, 2011.

[28] Rana T, Sarkar S, Mandal T, Bhattacharyya K, Roy A, Kol L, Contribution of Arsenic from Agricultural Food Chain to Cow Milk in Highly Arsenic Prone Zone in Nadia District of West Bengal in India. Internet J Veterin Med, 2007; 4: 1-6.

[29] Çakır EO, Türkiye'nin Farklı Bölgelerinden Toplanan Süt Örneklerinde Bazı Metal Düzeyleri. Ankara Üniversitesi Sağlık Bilimleri Enstitüsü Doktora Tezi, Ankara, 2009.