Quality of Life

(Banja Luka)

VOLUME **16** ISSUE **3-4** July 2025 ISSN 1986-602X
The Republic of Srpska, Bosnia and Herzegovina EISSN 1986-6038

UDC 614

A Multidisciplinary Journal of Food Science,
Environmental Science and Public Health

APEIRON
ΑΠΕΙΡΟΝ

VOLUME 16
ISSUE 3-4

Quality of Life
(Banja Luka)

Izdavač/Published by Panevropski univerzitet "Apeiron" Banja Luka/ Pan-European University "Apeiron" Banja Luka
Urednik izdavača/Editor of University Publications Aleksandra Vidović, Bosnia and Herzegovina

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Technical Editor/Layout Sretko Bojić
Web design Marko Milovanović
Printed by Markos design & print studio, Banja Luka
Printed in 300 copies

Aim and Scope

Quality of Life publishes original research papers and reviews and aims to provide a forum for the rapid dissemination of significant novel research in the various disciplines encompassing the Science and technology of food, Public health engineering, Sanitary inspection and control, Environmental and public health. Topics covered by the journal include:

- Dietetics; Nutrition principles applied to foods
- Food Technology; Production and preservation of foodstuffs; Food preservation technique
- Industrial microbiology; Science and technique of applied microbiology; Applied mycology
- Public Health, environment and hygiene
- Hygiene of air, water, soil; Pollution and its control
- Water; Sanitation; Water treatment
- Sewage; Treatment, disposal, utilization of sewage
- Urban hygiene; Wastes; Refuse; Rubbish; Garbage; Collection and disposal of town wastes
- Measures against industrial and other nuisances
- Occupational health hazards; Occupational health and hygiene
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- Related topics

UDC 614 Quality of Life is registered with the Ministry of Science and Technology of the Republic of Srpska by serial registration code 07.030-053-160-4/10, date 03.03.2010.
Quality of Life (ISSN 1986-602X) is an international journal published two times a year.

Indexed in:

ebscohost.com
crossref.org
esjindex.org
citefactor.org/contact
road.issn.org
scholar.google.com
cosmosimpactfactor.com
doisrpska.nub.rs
erihplus.nsd.no

UDC 614

ISSN 1986-602X (Print)
ISSN 1986-6038 (Online)

Quality of Life

Vol. 16(2025) No. 3-4 (73-128)

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Annual subscription is 30 EUR
Full-text available free of charge at http://www.qol-au.com

Dear readers,

it is my pleasure to present you the new issue of Quality of Life magazine and to thank you for your trust.15 years ago, we published the first issue of the journal Quality of Life. During that time, we have faced various challenges that have served as guides for progress and improvement in the work and publication of this journal. In the future, we will strive to continue working to provide our readers with quality and interesting research papers from various disciplines that include food technology, health engineering, sanitary inspection and control, environment and public health.

The journal Quality of Life was registered in the Register of Public Media in 2010 by the Decision of the RS Ministry of Education and Culture. Over the past years, this journal has published a large number of original scientific research papers, communications and review papers. Quality of Life is published twice a year by Pan-European University "Apeiron" Banja Luka. All the papers published so far have undergone a thorough review by the editorial board and the reviewers, made up of experts from both RS/B&H, the surrounding and other countries, from proven and recognized university and research institutions. As a result of a professional approach to selecting and reviewing papers, and raising the quality of the journal, Quality of Life was classified in the first category of journals in 2019 by the Ministry of Education and Culture. We are proud to say that Quality of Life has been well received by the scientific and the general public in a relatively short period, which gives the editorial board a strong motivation for further work. The editorial team would like to thank our many reviewers who helped to maintain the journal standard; our many authors who submitted their best work to the journal; and, most importantly, our readers for your continuing support. I assure all our readers that our consistent efforts will be aimed toward increasing the visibility, impact, editorial cycle time, citations and overall quality of our journal. We very much look forward to strengthening the reputation of our publications, and we want to attract more higher-quality submissions.

As always, I would like to thank the authors for their contributions to this issue of the magazine, and express great gratitude to all the reviewers who participated in the editorial process by providing valuable feedback to the editors and authors in a timely manner. We also extend our heartfelt thanks to the numerous authors who submitted their best papers to the magazine, and most importantly, to our readers for their continued support.

In the spirit of continuous improvement, any constructive input on streamlining our processes is very welcome. Please help us grow by citing articles that you read in Quality of Life. We look forward to receiving your contributions in the near future.

Editors

Quality of Life (2025) **16**(3-4):77-82

DOI: 10.7251/QOL2503077A **UDC:** 616-083.9:[614.253.8-055.1/.2

Original scientific paper

Patient Satisfaction in the Primary Health Care

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Abstract: Countries like Bosnia and Herzegovina have significant challenges in their health systems. Evaluation of the quality of family medicine services is the basis for the development of quality in primary health care. Patient satisfaction assessments, using the EUROPEP questionnaire, reflect patients' expectations and experiences. **Objective:** Using the EUROPEP questionnaire to assess satisfaction with primary health care services and identify key areas for improvement. **Methods:** The research was conducted using the EUROPEP questionnaire in Zenica during 2024. Patients were selected using a random sample method during a visit to the primary health care. In total, thirteen primary health care clinics of the Zenica Health Centre participated in the study. **Results:** In a representative sample from 2024, 44.45% of respondents were male, while 52.77% were female. In the research, the results show that the respondents are satisfied, the highest score is 4.64, and the lowest score is 4.04. The lowest-rated aspects were the ability to contact doctors by phone and patient wait times for appointments. Healthcare personnel exhibited the highest level of willingness to provide assistance. The analysis identified a high ceiling effect, suggesting that patient satisfaction is high but leaves little room for further improvement. **Conclusion:** Healthcare providers should focus on improving lower-rated aspects, particularly doctor accessibility via phone and appointment waiting times, to enhance the overall patient experience. Due to the high ceiling effect, it is recommended that the study be repeated with a larger sample. It is necessary to validate the questionnaire with an extended rating scale.

Key words: primary health care, patient satisfaction, EUROPEP questionnaire.

INTRODUCTION

Countries around the world are going through significant challenges in the transition of their healthcare systems, such as: declining health standards, lack of financial resources, weak organizational and management structures, and a mismatch between healthcare needs and demands. Accordingly, it is necessary to introduce activities of continuous monitoring and collection of feedback on patient satisfaction in order to build a better quality control system and improve the quality of the healthcare system.(Pilav A., 2017).

Negative ratings by health service users can indicate areas for improvement. Users of primary health care (PHC) services have clear insights into the quality of care provided. Health care professionals often respond to patients based on perceived needs and experiences(Jung HP, 1997; Richards, 1999). The results of regular application of satisfaction analysis can be used in health systems for quality improvement (Grol, 2000).

Collecting feedback has the potential to become a common method of work because patients feel that their opinion is valued. At the same time, it indicates to healthcare providers that it is necessary to change the parameters of work if they want to provide improved quality services(Cilović Lagarija Š., 2020). The EUROPEP Patient Satisfaction Questionnaire measurement instrument can serve as a key tool for assessing the quality of healthcare services(Stylianou et al., 2024).

Objective

The aim of the study is to use the EUROPEP questionnaire and descriptive methods to investigate: 1) patient satisfaction with PHC services in Zenica, 2) determine which aspects of PHC work they are satisfied with, 3) which service parameters need to be improved.

MATERIALS AND METHODS

This study is a cross-sectional survey using the verified measurement instrument EUROPEP. The instrument questionnaire consists of 23 items divided into five aspects: 1. medical approach, 2. availability and organization of services, 3. health, 4. communication with the patient, 5. general satisfaction. EUROPEP has a scale from 1 to 5 **with 1 representing the lowest or worst option and 5 representing the highest or best option**. It corresponds to the Likert scale according to which the respondents rated the parameters. *1 Very Poor*, Poor 2, Fair 3, Good 4, Excellent 5.

Examination

The research was conducted in the Zenica Primary Health Care Centre in 2024. The sample consisted of 180 patients who agreed to complete the survey. All respondents provided informed consent prior to participation.

The survey was anonymous and the data were kept confidential. Inclusion criteria: patients were over 18 years of age and had agreed to participate in the study. Exclusion criteria: patients who refused to complete the survey.

Statistics

The data were processed using the Statistical Package for the Social Sciences (SPSS ver. 27) and MS EXCEL. Basic descriptive statistical methods were used. The confidence level of the tests was 95% in certain statistical analyses. Descriptive statistical analysis (arithmetic mean, standard deviation) was used. Cronbach's alpha (α) is a statistical test that measures the reliability of a scale or questionnaire and was used to assess the reliability of the EUROPEP questionnaire instruments.

Table 1. Display of reliability statistics

Cronbach's Alpha	Cronbach's Alpha Based on Standardized Items
0.957	0.958

RESULTS AND DISCUSSION

EUROPEP can serve as a key tool for assessing the quality of health services and even acts as a predictor of health outcomes (Stylianou et al., 2024)

The paper(Spasojevic et al., 2015). Quality measurements provide insight into the state of PHC for several reasons: objective assessment of the quality of services provided, detection of weaknesses in the system, and connection with patient satisfaction.

The EUROPEP questionnaire is a standardized measurement instrument used to assess patient satisfaction in primary care. The items in the questionnaire are grouped into the following categories: medical approach (expertise, explanations, respect for patients), availability and organization of services (waiting time, scheduling appointments), health care (continuity of care, support in solving problems), communica-

tion with the patient, and general satisfaction.

Medical approach

Medical approach were rated by respondents as follows: (1) Does the doctor spend enough time talking to you? (4.27, SD = 0.888), (2) Does the doctor show interest in your complaints? (4.29, SD = 0.798), (3) Does he/she make it easier and help you cope with your complaints? (4.36, SD = 0.747), (4) Does the doctor include you in making decisions about your health? (4.28, SD = 0.788), (9) Do you have the impression that the doctor does his/her job well? (4.40, SD = 0.812), (10) Does he/she perform an examination (pulse, blood pressure, etc.)? (4.26, SD = 0.906).

Patients rated doctors' expertise and dedication highly, with the average score in this segment between 4.26 and 4.40. The lowest score was given to physical examinations (4.26), while the most positive score was given to thoroughness of work (4.40). Standard deviations indicate relative agreement among respondents.

General satisfaction

General satisfaction were rated by the respondents as follows: (6) Does the doctor keep your information confidential? (4.61, SD = 0.670), (18) How willing is the nurse at the clinic to help you? (4.64, SD = 0.581), (23) Rate the speed of the doctor and nurse in emergency cases. (4.57, SD = 0.713).

Patients are very satisfied with key aspects of health care. Data confidentiality (4.61) and the nurse's willingness to help (4.64) receive the highest ratings with the least variability, showing high consistency in positive experiences.

The results indicate significant patient satisfaction. Similar trends were noted in Bulgaria (Dimova et al., 2017)where patients highly rated the confidentiality of medical data and the speed of response to emergency situations.

Healthcare

Healthcare was rated by respondents as follows: (7) Does he try to alleviate your problems as quickly as possible? (4.42, SD = 0.739), (8) Does it help you feel better and return to your daily activities as soon as possible? (4.40, SD = 0.779), (14) Does it help you to overcome emotional problems related to your health condition? (4.25, SD = 0.940), (11) Are you satisfied with the offer of preventive health services? (4.21, SD = 0.900).

Patients are satisfied with the support provided by their doctors, especially the speed of problem resolution (4.42) and assistance in returning to daily activities (4.40). Emotional support (4.25) and preventive services (4.21) have slightly lower scores and greater variability in responses.

Communication with the patient

Communication with the patients were rated by the respondents as follows: (5) Does the doctor listen carefully when you explain your problems? (4.47, SD = 0.734), (12) Are you satisfied with the explanation of the reasons for the requested tests and the planned treatment? (4.30, SD = 0.785), (13) Does your doctor inform you about everything you wanted to know about your symptoms and illnesses? (4.35, SD = 0.829), (15) Does he help you understand how important it is to follow advice and instructions? (4.28, SD = 0.882), (16) Assess whether the doctor remembers what was said during previous visits. (4.18, SD = 0.951), (17) Does he explain what you can expect during a visit to a specialist or hospital treatment? (4.18, SD = 0.925).

Doctors' communication skills are generally rated highly, especially attentive listening (4.47). Recalling previous visits (4.18) and informing about specialist examinations (4.18) have the lowest scores and the highest variability of responses, indicating a potential area for improvement.

Service organization

Organization of services were rated by respondents as follows: (19) Can you schedule an appointment with your doctor at a time that suits you? (4.40, SD = 0.852), (20) Is it easy to reach the clinic staff by phone? (4.39, SD = 0.778), (21) What is the possibility of talking to the doctor by phone? (4.04, SD = 1.148), (22) Rate the waiting time for an appointment in the waiting room of the clinic. (4.35, SD = 0.788).

The ratings indicate satisfaction with the availability of services, especially with scheduling appointments (4.40) and contacting staff (4.39). The lowest rating was given to the ability to speak directly to a doctor by phone (4.04), with the highest variability in responses (SD = 1.148), indicating different patient experiences. The highest level of dissatisfaction among respondents was recorded in a study(Gavran Larisa, 2013). The results pertain to patient wait times for doctor's appointments. It was stated that the waiting time was up to 60 days.

Studies in Bulgaria (Dimova et al., 2017) found that waiting times in waiting rooms and telephone availability received the lowest ratings (2.8 on a scale of 5). Such results are comparable to our research results. Similar problems were identified in Norway and Bulgaria, where telephone availability was often rated below average(Bjertnaes et al., 2011; Dimova et al., 2017). Problems with waiting times for examination in waiting rooms were identified as key challenges.

Tablica 2. Analysis of answers to the EUROPEP questionnaire

Variables	MEAN Arithmetic mean	SD Standard deviation
Medical approach		
1. Does the doctor spend enough time talking to you?	4.27	0.888
2. Does the doctor show interest in your complaints?	4.29	0.798
3. Does it make it easier and does it help you deal with your complaints?	4.36	0.747
4. Does the doctor include you in making decisions about your health?	4.28	0.788
9. Do you have the impression that the doctor does his job thoroughly?	4.40	0.812
10. Does he perform an examination (pulse, blood pressure, etc.)?	4.26	0.906
General satisfaction		
5. Does the doctor listen carefully when you present your problems?	4.47	0.734
12. Are you satisfied with the explanation of the reasons for the requested tests and planned treatment?	4.30	0.785
13. Does your doctor inform you about everything you wanted to know about your symptoms and illnesses?	4.35	0.829
15. Does it help you understand how important it is to follow advice and instructions?	4.28	0.882
16. Assess whether the doctor remembers what was said during previous visits?	4.18	0.951
17. Does it explain what you can expect during a specialist appointment or hospital treatment?	4.18	0.925
Healthcare		
6. Does the doctor keep your information confidential?	4.61	0.670
18. How willing is the nurse at the clinic to help you?	4.64	0.581
23. Rate the speed of doctors and nurses in emergencies?	4.57	0.713
Communication with the patient		

7.Does he try to alleviate your problems as soon as possible?	4.42	0.739
8. Does it help you feel better and return to your daily activities as soon as possible?	4.40	0.779
11. Are you satisfied with the offer of preventive health services (systemic examinations, health checks even when you are not sick, vaccinations)?	4.21	0.900
14. Does it help you overcome emotional problems related to your health condition?	4.25	0.940
Service organization		
19. Can you make an appointment with your doctor at a time that suits you?	4.40	0.852
20. Is it easy to reach the clinic staff by phone?	4.39	0.778
21. What is the possibility of a telephone conversation with the doctor?	4.04	1,148
22. Rate the waiting time for an examination in the ambulance waiting room.	4.35	0.788

In Norway (Bjertnaes et al., 2011)identified a significant high ceiling effect using the EUROPEP questionnaire. Research (Stylianou et al., 2024) from Cyprus identified this methodological problem of high ceiling effects, too. The results of this research indicate a skewed distribution towards the "excellent" option. The high ceiling effect was potentially an indicator of lower questionnaire response. There is wide variation in the criteria used to assess the effects of high ceilings(Stylianou et al., 2024). A high ceiling effect indicates high scores on the measurement scale. The result approaches the upper limit of the measuring instrument, which makes it impossible to reliably measure the parameters. In order to avoid this effect, it is necessary to increase the number of respondents and change the scale, the weight of the test from 1-5 in the original EUROPEP questionnaire to the distribution of the scale 1-7. For these changes, it is desirable to make a new pilot study and verify the scale changes. Using these methods ensures that the instruments adequately measure the full range of variables.

CONCLUSION

The lowest rating was given to the possibility of a direct telephone conversation with a doctor (4.04), with variability in responses (SD = 1.148), which indicates a wide range of ratings and patient experiences. The possibility of telephone consultations was rated with a low satisfaction rating.

The nurse's readiness was rated the highest, 4.64 variability in responses (SD = 0.581), which indicates a low range of ratings, that is, patients' experiences are equal on the given item.

Also, the presence of a high ceiling effect was confirmed, which indicates that the answers given by the patients are concentrated at the upper limit of the scale. To improve the validity of future research, we recommend conducting a pilot study using an expanded EUROPEP scale (from 1 to 7), allowing for a more precise assessment of patient satisfaction and better differentiation of subtle variations.

Integrating the EUROPEP questionnaire into routine health system evaluations could provide valuable insights for ongoing quality improvement. Efforts should be directed towards optimizing workflow and reducing patient wait times. Improving the work environment in primary healthcare is essential for retaining healthcare professionals. This includes better working conditions, free continuing education and professional development. The opening of new family medicine clinics in all communities needs to be encouraged to increase the quality of health care at the primary level. Attention needs to be paid to rural areas, where access to health services is limited. Increasing the number of health workers, improving transport and introducing telemedicine can reduce inequalities in health care and reduce congestion. Decision-makers should consider potential solutions to reduce waiting times.

Thank You
We would like to thank the staff of the Health Centre Zenica for the contribution from the PHC for their kind help in conducting the survey.

Conflict of Interest
The author declares that there is no conflict of interest.

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Recived: February 27, 2025
Accepted: March 25, 2025

DOI: 10.7251/QOL2503083V **UDC:** 577.213.3:615.381.065
Original scientific paper

Comparison of DNA Yield and Quality From Two Blood Extraction Kits

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Abstract: A high-quality isolated sample of deoxyribonucleic acid (DNA) is essential for the success and reliability of various molecular genetic analyses. This paper presents the results of a comparative analysis of the concentration and quality of extracted DNA from 200 samples of human blood using two different commercial kits: Qiagen DNeasy Blood & Tissue Kit and High Pure PCR Template Preparation Kit – Roche Diagnostics**.** Concentration and quality of isolated DNA were measured by spectrophotometric (NanoDrop One) and fluorometric methods (Qubit 2.0). The average concentration of extracted DNA by the Qiagen kit was 45.06ng/µl±17.75, while with the Roche kit it was 20.97ng/µl±8.8. The quality of DNA was presented as the ratio of absorbance at 260nm and 280nm. The obtained mean value of $A_{260/280}$ for the Qiagen kit was 1.8, and for the Roche kit, $A_{260/280}$ was 1,66. The concentration obtained by the Qiagen kit was 2.15 times higher than the concentration obtained by the Roche kit. Concentration measured by NanoDrop was 2,33 times higher than values obtained by Qubit for the same samples. Both kits showed good quality and concentration of isolated DNA, but the Qiagen kit has superior performance in both analyzed categories. Measurement with Qubit was more specific than measurement with NanoDrop.

Keywords: DNA isolation, Roche, Qiagen, DNA quality.

INTRODUCTION

Purity and concentration of DNA isolates are key parameters for the success of experimental and diagnostic techniques such as PCR, sequencing, quantitative PCR analysis, and CGH array. A properly performed isolation process directly affects the purity of the isolated DNA as well as the concentration of DNA in the isolate. A careful isolation process reduces the presence of unwanted molecules and substances that can interfere with the accuracy of the results. This becomes especially important in experiments where high specificity of identification and analysis of target nucleic acids is required (Lojo-Kadrić, 2018). Maintaining the integrity of the molecule during isolation is another important factor. A properly preserved structure of the DNA molecule is crucial for preserving the genetic information during further experimental steps. This is especially important to the optimization of polymerase chain reactions (PCR), where quality samples contribute to increasing the efficiency and reliability of these reactions. The quality and concentration of the isolate are necessary to achieve consistency and reproducibility of results and the validity of scientific research. Overall, achieving a high standard of isolation of nucleic acids plays a key role in providing a reliable foundation for various molecular analyses. There are several techniques available for DNA extraction, including organic extraction, the salting-out method, extraction using silica-based columns, and automatic isolation. In most clinical laboratories, silica column-based methods are the preferred approach due to their convenience and efficiency. The selection of an extraction method depends on the nature and quantity of the samples, as well as the specific molecular assays planned for downstream analysis (Lojo-Kadrić, 2018). Despite differences among methods, all DNA isolation protocols share common steps: cell lysis, separation

of DNA from debris and proteins, precipitation, purification, and finally resuspension in a suitable buffer. Silica column-based techniques utilize kits that contain columns filled with a silica matrix, which selectively binds DNA under specific conditions. Other cellular components are washed away, resulting in a purified DNA sample suitable for further analysis. This method is used to isolate DNA from various samples (Mardan-Nik, 2019; Psifidi, 2015; Sarnecka, 2019; Tagliaferro, 2021; Tolosa, 2007).

MATERIALS AND METHODS

Patients

In this work, data on concentrations and purity of isolated DNA taken from the protocols and databases of the Medical Genetics Department of the University Clinical Center of the Republic of Srpska (UCCRS) in Banja Luka were used. Consent for the use of data for the preparation of this paper was given by the Ethics Committee UCCRS under number 01-19-498-2/23. Personal data of all patients were anonymized according to the recommendations of the ethics committee.

The study included a total of 200 patients, all of whom were healthy individuals with normal leukocyte counts. DNA isolation was performed using two different commercially available extraction kits to allow for comparative analysis.

Isolation of DNA

Isolation of DNA was performed from venous blood obtained by venipuncture of 2 ml of blood in vacutainers with 3.8% Na-citrate as an anticoagulant. Genomic DNA was isolated from the patients' lymphocytes with two different commercial kits. In the first hundred analyzed patients (Group A), DNA isolation was performed with Qiagen DNeasy® Blood & Tissue Kit (Qiagen, 2023), and in the second 100 (Group B) with High Pure PCR Template preparation Kit - Roche diagnostics (Roche, 2023) according to the manufacturer's recommended protocols (Qiagen, 2023, Roche 2023). Isolation procedures of both kits are presented in **Table 1**.

Table 1. Isolation procedures by Qiagen DNeasy® Blood & Tissue Kit and ROCHE High Pure PCR Template preparation Kit

Qiagen	Roche
200 µl blood	200 µl blood
20 µl proteinase K	40 µl proteinase K
200 µl AL buffer, vortex	200 µl BB, vortex
Incubation 56°C 10 minutes	Incubation 70°C 10 minutes
200 µl ethanol (96-100%), vortex transfer to the filter tube	100 µl isoprpanol, vortex, transfer to filter tube
CF, 1 min. 8000 rpm	CF 1min. 8000 rcf
500 µl AW1 buffer	500 µl Inhibitor removal buffer
CF, 1 min. 8000 rpm	CF 1min 8000 rcf
500 µl AW2 buffer	500 µl Wash buffer
CF 3 min 14000 rpm	CF 1 min. 8000 rcf
200 µl AE buffer	500 µl Wash buffer
Incubation 1 min.	CF 1 min. 8000 rcf
CF, 1 min. 8000 rpm	CF 10 sec. 13000 rcf
Purified Template DNA	200 µl Elution Buffer
	CF 1min 8000 rcf
	Purified Template DNA

In the isolation process, all recommendations for good laboratory practice have been implemented.

Quantification of DNA

The concentration of DNA in the isolate was determined using both spectrophotometric and fluorometric methods.

NanoDrop One, Thermo Scientific, was used for spectrophotometric measurement of DNA concentration. DNA concentration was obtained by measuring the absorbance at 260 nm, while the purity of isolated DNA was shown through the absorbance ratio $A_{260/280}$ and $A_{260/230}$. DNA concentration is expressed in ng/μL.

Samples with an $A_{260/280}$ ratio of 1.8-2.0 were considered pure, while ratios below 1.6 may indicate the presence of contaminants like proteins and phenol. Additionally, the $A_{260/230}$ ratio can also be used to assess purity, with values greater than 1.5 generally indicating a pure sample. Lower values indicate the presence of unwanted organic compounds like Trizol, phenol, and Guanidine in the sample.

To measure the DNA concentration by the fluorometric method on the Qubit 2.0 Fluorometer, Life Technologies, Invitrogen, a kit for measuring the concentration of double-stranded DNA with high sensitivity (Qubit™ dsDNA HS) was used. This kit enables highly specific binding of the probe to double-stranded DNA so that the absorbance obtained from individual nucleotides and RNA molecules is not detected in the measurement.

Analysis of results

Microsoft Office Excel 2021 was used for data analysis, including the calculation of mean values and standard deviations, as well as for generating graphical representations of the results.

RESULTS AND DISCUSSION

Concentration of DNA isolated from blood with a commercial kit - Qiagen DNeasy® Blood & Tissue Kit

This group of 100 patients (Group A) had DNA isolated from whole blood with a commercial Qiagen kit. Isolation was performed for genomic DNA sequencing using the next-generation sequencing method (NGS). Sequencing requires high concentrations and high-quality DNA.

In this group, there were 54 male subjects and 46 female subjects. The median age was 39 years.

The average concentration of DNA in the isolate obtained from peripheral blood leukocytes was 45.06 ng/μL, with a standard deviation of 17.75. The lowest value obtained was 20.3 ng/μL, and the highest value of the isolate was 134.6. The purity of the $A_{260/280}$ sample is 1.8, while the $A_{260/230}$ is 1.37.

Observed by gender, the average concentration of DNA in the isolate obtained with the Qiagen kit is 43.11 ng/μl for men and 47.05 ng/μl for women.

The mean value of the DNA concentration in the isolate obtained with the Qiagen kit, observed by age distribution and gender, measured by NanoDrop, is shown in **Table 2**.

Table 2. The mean value of the DNA concentration in the isolate obtained in Group A (Qiagen), observed by age and sex

Sex \ Age	0-10	11-20	21-40	>41
Women	50.5 ng/μL	45.7 ng/μL	42.8 ng/μL	48 ng/μL
Men	42.15 ng/μL	43.88 ng/μL	43.58 ng/μL	45.75 ng/μL

Concentration of DNA isolated from blood with a commercial kit - High Pure PCR Template Preparation kit (Roche Diagnostics)

In this group of 100 healthy patients (Group B), DNA was isolated with a commercial Roche kit for routine diagnostics and examination of certain gene variants.

In this group, there were 14 male respondents and 86 female respondents. The median age was 35.3 years. The main study participants were women of reproductive age tested for inherited thrombophilia.

The average concentration of DNA in the isolate obtained from peripheral blood leukocytes was 20.97 ng/μL, while the standard deviation is 8.8. The lowest value obtained was 6.9 ng/μL, and the highest value of the isolate was 48.3 ng/μl. The purity of the $A_{260/280}$ sample is 1.66, while the $A_{260/230}$ is 1.25. Although the purity of samples obtained with the Roche kit is lower than recommended, in clinical routine diagnostics, it has been shown that there is no influence on the results of routine genotyping.

The mean concentration of DNA in the isolate obtained with the Roche kit, observed by patient gender, is 20.88 ng/μL for women and 20.12 ng/μL for men.

Observed by age groups and by sex, the determined DNA concentrations, measured by NanoDrop, are shown in **Table 3**.

Table 3. The mean value of the DNA concentration in the isolate obtained in Group B (Roche), observed by age and sex

Sex \ Age	0-10	11-20	21-40	>41
Women	12.35 ng/μL	25 ng/μL	22.1 ng/μL	17.3 ng/μL
Men	/	/	23 ng/μL	19.33 ng/μL

Comparative quantitative and qualitative analysis of isolated DNA from two different kits

To assess which commercial kit provides a higher yield and greater purity of the DNA isolate, the results have been summarized and presented in **Table 4.** The analysis was performed by gender as well as by age groups to determine whether gender and age have an influence on the yield of DNA in the isolate.

In comparing the results obtained by DNA isolation with the Qiagen and Roche High Pure PCR Template Preparation kits, significant differences are observed in the mean values of DNA concentration and isolate purity. The Qiagen kit showed a significantly higher average DNA concentration (45.06 ng/μL) compared to the Roche kit (20.97 ng/μL), suggesting its greater efficiency in isolating DNA from peripheral blood. Also, the purity of the isolate was higher with the Qiagen kit, which is an indication of less contamination with unwanted materials in the sample. Analysis of the results by gender and age revealed variations in DNA isolate concentrations. For instance, when using the Qiagen kit, women generally exhibited higher average DNA concentrations across most age groups compared to men; however, these differences were not statistically significant. In contrast, the Roche kit showed less pronounced gender-based differences.

Table 4. Comparison of DNA concentration and purity (A260/A280, A260/A230) between Qiagen and Roche kits, stratified by gender and age

	Mean DNA conc. (ng/μL)	Mean $_{260/280}$	Mean $A_{260/230}$	Women (ng/μL)	Men (ng/μL)
Qiagen	45.06±17.75	1.8	1.37	47.05	43.11
ROCHE	20.97±8.8	1.66	1.25	20.88	20.12

In Group A higher value for DNA concentration was noticed for women than for men, but the calculated p-value was above 0.05 (p=0.262), so that does not indicate statistical significance. In group B, there are no differences in DNA concentration between men and women. This suggests that biological sex differ-

ences do not affect the quality or quantity of DNA isolated from male and female samples.

In **Figure 1**, the distribution of concentration between patients is shown. There are more homogeneous results in group A than in group B. Only a few samples from Group B are near the mean value from Group A, and more than 10% of samples were under 10 ng/μL. In group A, there were no samples with values under 20 ng/μL, but there were a significant number of samples above 50 ng/μL.

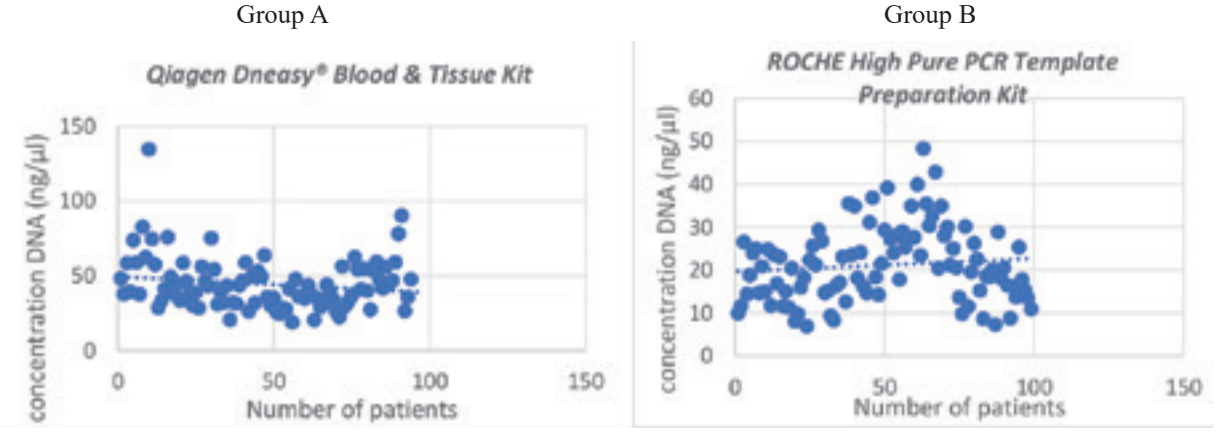

Figure 1. Comparative Analysis of Isolation Methods: Distribution of concentration of DNA in Group A and in Group B

Comparative analysis of measured concentrations of isolated DNA from blood on a spectrophotometer and a fluorometer

Since DNA concentration was measured using two different devices (each based on a distinct measurement principle), the results for the same samples were compared, and the coefficient of difference was calculated to quantify the discrepancy in measurements between the two devices.

In this section, 30 DNA samples isolated from blood using the Roche kit were randomly selected. Samples obtained by Roche kits used for routine diagnostics in the Department for Medical Genetics were saved for 5 years in a refrigerator at -20 °C. Samples isolated by the Qiagen kit were sent to the cooperating laboratory and were not available for measuring by Qubit 2.0 during this study. The average DNA concentration measured with the Qubit 2.0 fluorometer was 7.47 ng/μL, whereas the same samples measured with the NanoDrop One spectrophotometer showed an average concentration of 17.28 ng/μL. These results are summarized in **Table 5.**

Table 5. Comparative presentation of measured concentrations from the same samples by spectrophotometric (NanoDrop) and fluorometric methods (Qubit 2.0)

	Mean DNA conc. (ng/μL)
NanoDrop One	17.28
Qubit2.0	7.47

The coefficient of difference between the concentrations measured by Qubit and NanoDrop is 2.33. The formula for the obtained conversion factor is:

$$k=C_N/C_Q=2,33$$

k - conversion factor
C_N – Mean value of concentration measured on the NanoDrop One spectrophotometer
C_Q - Mean value of concentration measured on Qubit 2.0 fluorometer

The significant difference in concentration of DNA from the results obtained using different measurement methods (7.47 ng/μL – fluorimetry on Qubit 2.0 compared to 17.28 ng/μL –spectrophotometry on NanoDrop) which led to a coefficient difference of 2.33, indicates a mismatch between the two methods.

Fluorimetry, especially through devices like the Qubit system, provides a precise way to measure the concentration of DNA and RNA. This method uses fluorogenic dyes that selectively bind to nucleic acids and emit fluorescence. Concentration is determined based on fluorescence intensity, providing high accuracy, especially at lower concentrations. A Qubit system often allows specificity for DNA or RNA, improving measurement precision. Spectrophotometry, which includes devices like the Nanodrop spectrophotometer, uses light absorption by nucleic acids to determine concentration. DNA and RNA absorb light at characteristic wavelengths (eg, 260 nm). Measurement is performed directly on very small samples (eg, 1-2 μL), thus reducing the need for a large amount of sample. However, this method can be sensitive to contamination and requires careful sample preparation. Both fluorometry and spectrophotometry are reliable methods for measuring DNA and RNA concentrations. The choice between them depends on several factors, including the required sensitivity, the specific goals of the experiment, and the availability of laboratory equipment.

CONCLUSION

Both tested commercial kits (Qiagen DNeasy® Blood & Tissue Kit and High Pure PCR template preparation kit - Roche diagnostics) showed high quality of the obtained DNA isolate. The concentrations and purities of the isolates were satisfactory for further genomic analyses. The commercial kit Qiagen DNeasy® Blood & Tissue Kit showed better performance in terms of the values of the measured concentrations and the purity of the isolates compared to the High Pure PCR template preparation kit - Roche diagnostics. The values of the measured concentrations with the Qiagen kit are 2.15 times higher than those of the Roche kit. Roche High Pure PCR Template Preparation Kit shows more consistent concentration values among individual samples.

The Qiagen kit is the better choice for sample preparation for sequencing and microarray processes, while the Roche kit, due to consistent concentrations, is the choice for routine genotyping in clinical practice. The concentrations measured by the fluorimetric method (Qubit 2.0) and the spectrophotometric method (NanoDrop) show a significant difference. NanoDrop shows higher concentrations compared to those measured on Qubit 2.0. The conversion coefficient between the DNA concentration values measured using the Qubit 2.0 fluorometer and the NanoDrop spectrophotometer was calculated to be 2.33.

Conflict of interest
The authors declare no conflict of interest.

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Recived: June 10, 2025
Accepted: July 3, 2025

DOI: 10.7251/QOL2503090R **UDC:** 796.4:[601.4:575:796.01

Original scientific paper

Genetically Predisposed Physical Characteristics and Performance Factors in Athletes: A Cross-Sectional Study

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Abstract: Genetic predispositions, along with a supportive social environment, personal motivation, perseverance, dedication, and susceptibility to injuries, all play a role in shaping sports performance and success. In this study, physical characteristics (height, weight, body mass index - BMI) were compared between active males and females in three sports disciplines (field and track athletics, football, and basketball) and inactive control goup. Research on the correlation between genetically predisposed physical characteristics and success in sports activities was conducted through a survey at sports clubs in Banja Luka, Bosnia and Herzegovina. A total of 118 respondents (63 female and 55 male) answered the 16-question questionnaire. The highest were male basketball players, with an average height 13 cm higher than the average male height in Bosnia and Herzegovina. Average BMI was 23.16±3.14, 23.99±1.82, and 23.61±1.14 for male athletes, football players, and basketball players, respectively. In the female group, the BMIs were 20.8±2.07, 20.76±2.36, and 22.175±2.25 for athletics, football, and basketball, respectively. The control group had a BMI of 25.59±3.81 for males and 21.74±2.4 for females. Endurance is the main quality declared by athletes who practice long-term sports, while strength is the main quality in athletes who are active in short-term disciplines. The highest rate of injuries was noted among female football players at 92%. The most frequent injury was injury to the meniscus, tendons, and hamstrings. A positive family history of sports activity showed a significant influence on sports success. Every sport brings numerous health benefits in the form of better immune resistance, maintenance of body mass index (BMI), mental and psychological stability, self-confidence, better social contacts, and more.

Keywords: sport genetics, BMI, endurance, strength, sport success.

INTRODUCTION

Sports performance and success in sports activities are influenced by genetic predispositions, as well as a stimulating social environment, personal motivation, perseverance, commitment, and a tendency towards injuries (Posthumus, 2009; Posthumus, 2010). There is abundant scientific evidence on the influence of genes on physical characteristics such as: height, arm length, muscle mass, muscle structure, heart size, lung size and volume, joint flexibility (Pickering, 2019); but also on physiological characteristics such as: resting heart rate, blood pressure, air flow in the lungs, muscle strength, muscle endurance, speed, aerobic endurance, etc. (**Table 1**).

Table 1. Genetic Influence on Physical Characteristics (Kolač, 2011)

Characteristic	Genetic Influence
Height and arm length	High
Waist circumference	Low to moderate
Muscle size	High
Muscle structure (fast and slow-twitch fibers)	High
Number of mitochondria per gram of muscle	Low

Heart size	High
Lung size and volume	High
Activity of muscle enzymes involved in energy production	Low to moderate
Resting heart rate	High
Blood pressure	Moderate
Airflow in the lungs	Moderate
Muscle strength	High
Muscular endurance (e.g., push-ups)	Moderate to high
Speed	Moderate
Balance	Low
Joint flexibility	High
Reaction time	Low to moderate
Movement precision	Low to moderate
Aerobic endurance	Moderate to high
Anaerobic threshold	Moderate

Body height is 97% hereditary, body mass 85%, and muscle fiber composition 97%. In addition to morphological characteristics, there are also functional abilities. Speed is up to 97% hereditary, reflex reaction 95%, aerobic power 92%, and anaerobic power 90%. Based on these percentages, it was concluded that genetics has a great influence on predispositions, and it is important for an athlete to have predetermined sports predispositions, but success itself does not depend only on genetics. One of the conditions for success is training that can improve these genetic predispositions. Flexibility can be improved by up to 20%, endurance by 8-20%, and speed, at least, by only 5% (Aranitović, 2018).

Sports performance can also be affected by diet and the use of supplements. The influence from the environment is also important, so a supportive family in which parents and other family members play sports represents a significant social influence for achieving sports success. There are numerous examples of people who try harder than others, pay attention to diet, lifestyle, and rhythm, use illegal substances, but do not achieve success like someone who does not pay attention to other factors besides training. In athletics, if a person who does not have a genetic predisposition for speed enters the training, he can improve that characteristic by training only up to 5%. An individual who wants to engage in, for example, bodybuilding, if he is born with wide hips, small calves, small arm circumference or small shoulder circumference, will not be able to improve these characteristics even in 15/20 years and will not be able to sculpt his body in the way he would like. However, every sport brings numerous health benefits in the form of better immune resistance, maintenance of body mass index (BMI), mental and psychological stability, self-confidence, better social contacts, and more. In research on the influence of genetic factors on sports predispositions, the presence of certain genetic markers for certain morphological characteristics or functional abilities is sought out. A Genome-Wide Association Study (GWAS) is a research method used to identify genetic variations associated with specific traits or diseases by analyzing the entire genome of a large group of people. It essentially scans the genomes of individuals, looking for common genetic variants that occur more frequently in those with a particular trait or disease compared to those without it.

This paper is part of a pilot project aimed at enhancing public awareness of the importance and impact of genetic testing on individuals who intend to actively and professionally engage in sports. In this regard, key genes related to sports genetics will be identified.

To date, over 150 genetic markers have been discovered (Varilas-Delgado, 2022; Epstein, 2014). The most important ones are: angiotensin I-converting enzyme (ACE) and ACTN gene (for endurance), actinin alpha 3 (ACTN3) (Fang, 2013) and PPARγ (for power and muscle strength) (Ahmetov, 2013), Hypoxia Inducible Factor 1 Subunit Alpha (HIF1A gene) (Kaznar, 2019), Peroxisome proliferator activated receptor alpha (PPARA) (Ahmetov, 2013), COL51A gene (collagen and flexibility) (Collins, 2010; September, 2009; Releigh, 2009), ACSL1 gene (aerobic capacity) and numerous others.

Hypertrophic cardiomyopathy is the most common cause of sudden death on sports fields. HCM occurs in the general population with a prevalence of about 0.2%. The annual mortality from HCM is 3-4%, while in children it is over 6%. In HCM, three gene mutations are most common: cardiac beta-myosin heavy chains – MYH7 gene (first identified), cardiac protein C – MYBPC3 gene, myosin-related, and cardiac troponin T – TNNT2 gene (Baraković,2007).

MATERIAL AND METHODS

Survey

Research on the correlation of genetic predispositions and success in sports activities was conducted in the form of a survey in sports clubs in Banja Luka, Bosnia and Herzegovina. A survey of 16 questions was created. The questions related to height, weight, type of sports discipline, frequency and type of injuries, type of diet, as well as existing infectious diseases (coronavirus, Epstein-Barr virus, coxsackie virus). The respondents were active athletes from mainly three sports disciplines: basketball, football, and athletics, as well as a control group of respondents who were not actively involved in sports. Respondents provided information about their greatest sporting success, family sport history, hereditary diseases (cardiovascular, metabolic, malignant diseases), and they also made a self-assessment about their best sporting quality: endurance or strength.

Respondents

118 respondents answered the survey, 63 women (44 active and 16 inactive) and 55 men (41 active and 14 inactive). In summary, 85 are actively involved in sports, and the other 30 represent a control group of respondents who are not actively involved in sports.

Respondents were classified by three main sports disciplines: field and track athletics (22 females and 13 males), basketball (11 males and 3 females), and football (15 males and 13 females).

Seven women respondents declared: CrossFit, fitness, dance, volleyball, and handball as sports disciplines. Six men respondents declared some rare sport disciplines: badminton and whitewater kayaking, as well as karate, water polo, handball, and swimming. Those respondents were not statistically analyzed.

Analysis of results

All collected data were classified in a Microsoft Office Excel 2021 database. IBM SPSS 22 was used to analyze the results, calculate the BMI and the mean value of physical characteristics, and create graphs as well as descriptive statistics for the calculation of standard deviation (SD), range, p-value, confidence interval, and coefficient of correlation. Student's t-test and ANOVA test were performed for the parametric test of normally distributed data. Statistically significant p-value was all values under 0.05 ($p \leq 0.05$).

RESULTS AND DISCUSSION

Results of Descriptive Statistics

Body height and body weight

The average body height (BH) of female basketball players, field and track athletes (F&T athletes), and football players was 178.7±3.2 cm, 169±7.1 cm, and 169.9±5.9 cm, respectively. The body weights (BW) in those groups were 71±9.6 kg, 59.47±7.02 kg, and 60±7.9 kg. The average height for women in Bosnia and Herzegovina is 167.5 cm, similar to that of our group of female F&T athletes and football players. However, basketball players are taller than the state average by about 11.1 cm (https://www.ncdrisc.org/).

The average height of the male F&T athletes, football and basketball players who filled out the survey, was 180.9±6.8 cm, 184.4±5.7 cm, and 195.54±9.2 cm, respectively. Men in this category weigh an average of 76.2±14.3 kg, 81.6±7.37 kg, and 90.54±10.9 kg. In the male group, basketball players are 13 cm higher than the average of 182.5 cm for Bosnia and Herzegovina. Football players tend to be slightly higher than average in the general population (https://www.ncdrisc.org/). (Table 2).

In a group of junior basketball players, age 13, the average height for boys was 177.5±8.4 cm and for girls 164±10.5 cm. According to the WHO, the average height of teenage boys and girls at the age of 13 is 156.7±7.4 cm and 156.4 ± 6.9 cm, respectively (Height-for-age boys and girls, WHO,2023). In our group of tested boys, height was 20.8 cm higher than average from WHO data, and girls were 7.6 cm higher than their peers.

BMI and diet

Body mass index (BMI) indicates body size. Normal BMI is from 18.5 to 24.9 kg/m^2. Under 18.5 is underweight, and above 24.9 is overweight.

Body mass index was calculated using the formula:

$$BMI=Weight(kg)/(Height(m))^2$$

Calculated BMI is shown in every sports group and compared between each sports discipline and the control group.

All participants in the survey had an omnivorous diet. Active sportsmen and women follow a diet recommended by a nutritionist with a balanced intake of nutrients.

A confidence interval (CI), for a confidence level value 95%, was calculated. Mean value, standard deviation (SD), CI, and range are shown in **Table 2** for measured physical characteristics in every group.

Table 2. Descriptive statistical results of physical characteristics in three sports disciplines and the control group in men and women

Men	n	BH (cm) Mean±SD	Range	95% CI	BW (kg) Mean±SD	Range	95% CI	BMI (kg/m^2) Mean±SD	Range	95% CI
Field and Track Athletes	13	180.9±6.8	172-192	176.9-184.87	76.2±14.3	55-102	67.87-84.53	23.16±3.14	18-27.7	21.33-25
Football Players	15	184.4±5.7	175-195	181.3-187.4	81.6±7.37	70-94	77.6-85.6	23.99±1.89	21-26.9	22.96-25.01
Basketball Players	11	195.54±9.2*	180-207	189.7-201.4	90.54±10.9	76-101	83.6-97.5	23.61±1.14	21.6-25.3	22.9-24.33

Control group	14	185.29±8.3	175-205	180.6-190	87.71±13.6	67-110	80.09-95.32	25.59±3.81	19.6-34	23.45-29.73
Women		BH (cm) Mean±SD	Range	95% CI	BW (kg) Mean±SD	Range	95% CI	BMI (kg/m^2) Mean±SD	Range	95% CI
Field and Track Athletes	22	169±7.1	164-180	165.8-172.2	59.47±7.02	51-84	56.3-62.6	20.8±2.07	19-26.8	19.9-21.7
Football Players	13	169.9±5.9	162-178	166.5-173.3	60±7.9	50-73	55.4-64.59	20.76±2.36	17.7-25.9	19.38-22.13
Basketball Players	3	178.7±3.2*	175-181	174.8-182.6	71±9.6*	60-78	59.3-82.7	22.17±2.25	19.6-23.8	19.44-24.9
Control group	16	170.56±5.1	160-181	167.9-173.23	63.44±9.13	47-84	58.7-68.2	21.74±2.4	17.7-26.8	20.5-23

BH- body height, BW – body weight, BMI – body mass index, CI – confidence interval; * $p<0.05$

Track and Field Athletes

There were 22 females in the athletics category. The mean value of BMI in active athletes was 20.8±2.07 kg/m^2.

50% of the whole group reported injuries: meniscus, tendons, hamstring/quadriceps, and lower back, with 17.6%, 14.7%, 8.82%, and 8.82%, respectively.

In this group, 13 women participated in the marathon or half marathon. Ten of them were aged 35 to 50 years, and three were 50+ years. Medals from the National, Balkan, and European championships were won by 5, 2, and 2 athletes, respectively. All participants with medals were 18 to 25 years old. A family history of sports activities and other active family members was reported by 14/22 (61%) respondents, of whom 11 won medals and had significant sports success. This indicates the importance of positive social influence on sports success. However, in the control group, there were 10 respondents no longer active in sports, but who were active and during the active period, had sports success, and did not have a family history of sports activities. Participants in this group have strong personal motivation, perseverance, and dedication.

Endurance as a main quality was reported by 61.76%, and 38.24% reported strength. All women who ran in out marathon/half-marathon reported endurance as the main quality.

In this group of 13 male athletes. The mean value of BMI was 23.16±3.14 kg/m^2. Injuries were reported by 46% of sportsmen: meniscus 23%, tendons 15.4%, and hamstring 7.7%.

Family history of sports activity was 53.18% (7/13), but eleven athletes won medals.

The main quality in 46% of respondents was endurance, and strength was reported by 53.8% of athletes.

Football

Thirteen junior female football players were included in this study. The mean value of BMI was 20.76±2.36 kg/m^2.

Injuries were reported in 92% of female football players: 54% hamstring/quadriceps, 31% meniscus and ligaments, and 7.7% discus hernia.

Strength was reported as the main quality in 54% of players, and endurance in 46% of cases. In this group were reported other difficulties during training (breathing, weakness, and fatigue).

Active family members in sport were reported in 8/13 girls (61%), and 6 of them noted sports success.

In the male football group were 15 sportsmen with a mean value of BMI 23.99±1.89 kg/m^2.

47.6% of football players reported injuries: 14.2% had injuries of tendons and ankle joints, and 9.5% each had meniscus and hamstring injury. Nine football players noticed other difficulties as breathing, weakness, and heart rate, during the training process.

Endurance as the main quality was chosen by 57% of sportsmen in this group, and 43% declared strength as the main quality.

Half respondents declared about sports success, and all of them had a stimulating social environment.

Basketball

In this group of respondents, there were 11 male elite basketball players, 3 female basketball players, and 13 child respondents (aged 13 years) who practice basketball (5 girls and 4 boys).

The survey was completed by elite basketball players who play in the ABA league in KK "Igokea", aged 18-32, with an average age of 22.3. The average BMI in this group was 23.61±1.14kg/m^2.

Based on the questionnaire, 55.5% of players declared endurance as the main quality, and 44.5% strength. No one noted any sports injuries.

In the female group, although a small group, the results are shown. BMI was 22.17±2.25 kg/m^2. All respondents noted injuries to the meniscus. The main quality was endurance.

In children's group BMI for boys was 18.7±1.7, and for girls, 18.6±3.7. Five young basketball players declared endurance as the main quality, and 4 noted strength.

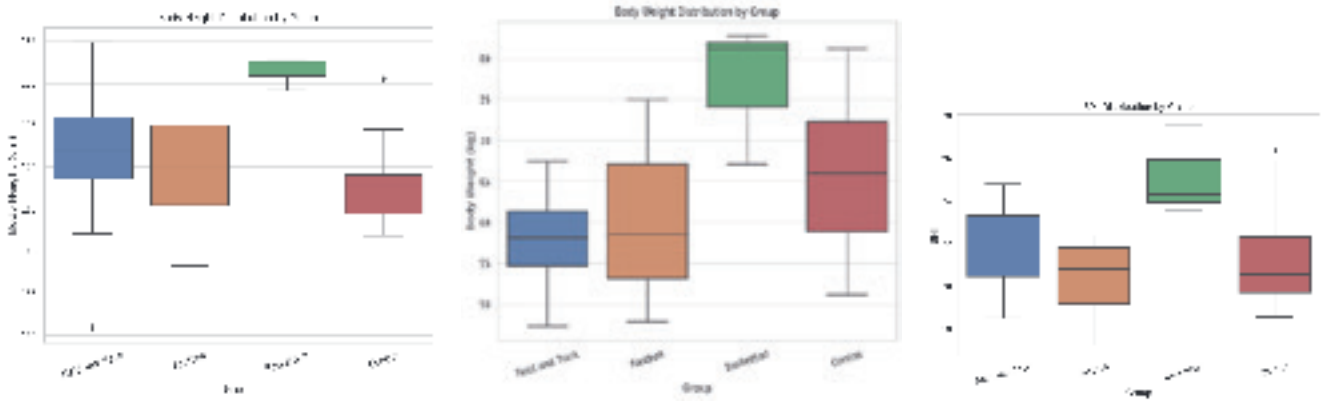
Control group

In the control group (inactive group), there were 30 respondents, 14 men and 16 women. The control group had an average height of 185.29±8.3 cm for men and 170.56±5.1 cm for women. The BW was 87.71±13.56 kg for males and 63.44±9.13 kg for females. The corresponding BMIs are 25.59±3.81 kg/m^2 for men and 21.74±2.4 for women. The highest BMI in the male group was 34, and in the female group was 26.8. The smallest BMI was 19.6 for males and 17.7 for females. All subjects had at least one chronic disease (hypertension, diabetes mellitus type 2, insulin resistance, cardiovascular diseases).

Comparative presentation of genetically conditioned traits in observed sports disciplines

The ANOVA test is used to compare arithmetic means of body height, body weight, and BMI in females (**Figure 1**) and in males (**Figure 2**) in four different groups.

Figure 1. Comparative presentation of mean height (Figure 1A), weight (Figure 1B), and BMI (Figure 1C) in the group of female F&A athletes, football and basketball players, and the control group

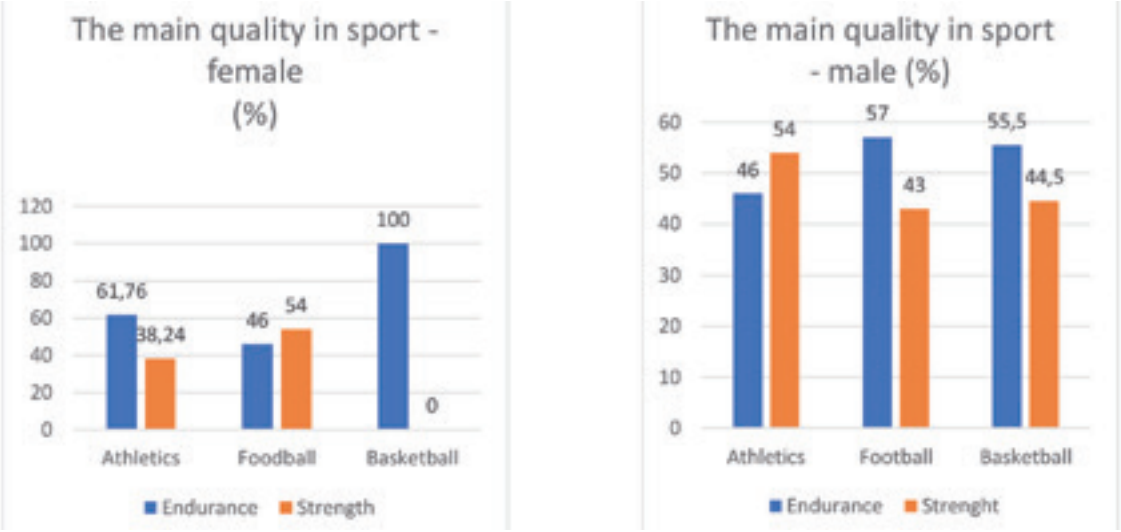


A B C

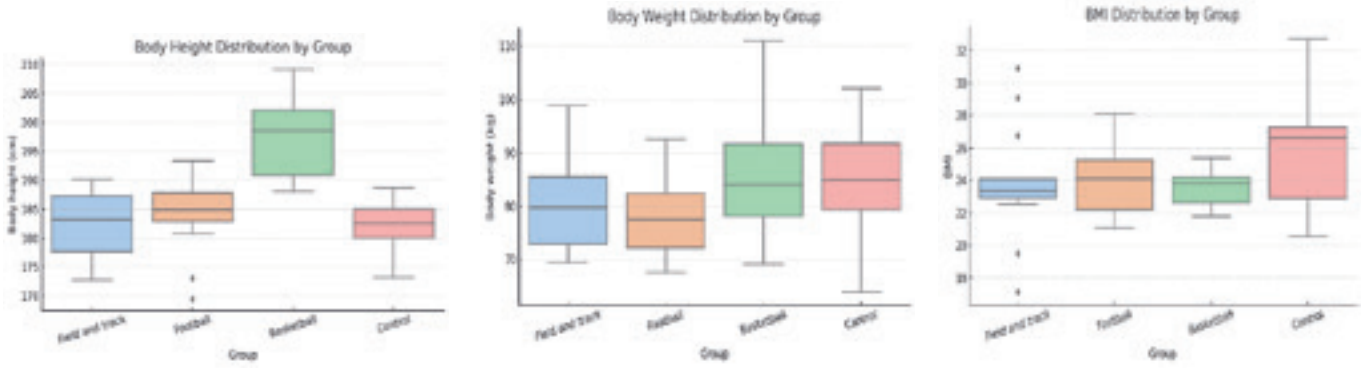
In four observed groups, statistically significant differences are observed in category BW with $p<0.05$. Although there is no statistically significant difference between the groups in BMI, it is important to note that 10 women from the control group were active in sport for many years, and even though they are not active for more than 5 years, their history of sport activity had a positive influence on the maintenance of health BMI values.

In the male group, there is a statistically significant $p<0.05$ in height for basketball players and other groups.

Figure 2. Comparative presentation of mean height (Figure 2A), weight (Figure 2B) and BMI (Figure 2C) in the group of male F&A athletes, football and basketball players, and the control group



A B



A **B** **C**

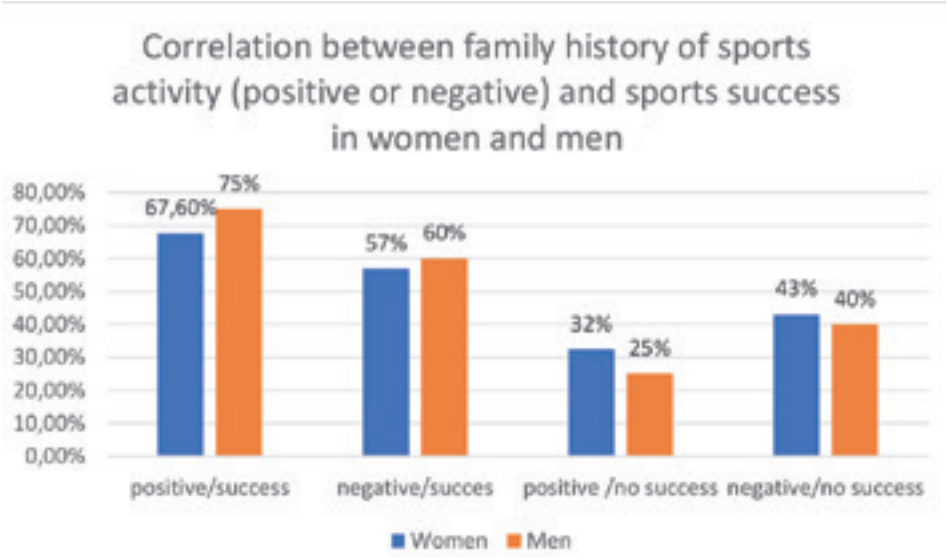
The analysis of height in three sports disciplines indicates that the tallest participants were in basketball, both in females and males. That is the result of positive selection by height for this sport. But, based on analyses of the other two disciplines and the control group can be concluded that sports activity, especially in young age, could help to achieve genetically predisposed height (Dubois,2012).

Sports activity has a big influence on the value of BMI. Even in those who are no longer active in sports, their past activities have a positive effect on BMI values. The biggest variation in BMI was in the male control group. There is an increase in BMI, which, once more time, elucidates that physical activity is crucial for the maintenance of healthy values of BMI.

The main quality (endurance and strength) of the three sports disciplines

In this study, respondents self-assessed their main quality. The results are shown in **Figure 3**.

Figure 3. Comparative presentation of self-assessment about the main quality (endurance or strength) between female (Figure 3A) and male (Figure 3B) F&T athletes, football, and basketball players



Endurance was the main quality for the male football and basketball players and female basketball and field and track athletes. Strength was the main quality of female football players and male field and male field and track athletes. Endurance is the key quality in long-duration sports, while strength plays a major role in short-duration activities.

It could be interesting to compare the results of genetic testing for genetic markers of endurance and strength, and self-assessment of these respondents in some future projects.

Influence of a positive social environment on sports success

In the female sports group, 37 women had positive family sports activity, and 25 of them (67.6%) had significant sports success in international or national competitions. The other 21 female participants had no positive family history, but 12 of them (57%) had success in sports.

In the male group, 24 men had positive family sports activity, and 18 of them (75%) had significant sports success in international or national competitions. The rest of the 20 sportsmen had no positive family history, but 12 of them (60%) had won medals. (**Figure 4**)

Figure 4. Correlation between family history of sports activity (positive or negative) and sports success in women and men

Based on these results, it is obvious that a positive social environment had a significant influence on sports success, but personal motivation, dedication, and determination could not be ignored.

Infection diseases

In the survey, respondents reported being aware of infectious diseases such as coronavirus, Epstein-Barr virus, and Kaposi's sarcoma-associated herpesvirus (KSHV), also known as Kocsakyi virus. Viral infections can cause health problems, which can reduce physical and athletic performance, but on the other hand, it was expected that active athletes had a stronger immune response and lower rates of viral infections.

In this research, only fourteen responders (11.8%) got COVID-19. That is a significantly lower rate than 42.4% in the common population (in 2022) (https://www.unicef.org/bih/media/7481).

Mononucleosis caused by EBV was reported in 3 athletes (2.52%). Mononucleosis is not uncommon in Bosnia and Herzegovina — it regularly ranks among the 5 to 10 most common infectious diseases. In this research, EBV infection was quite rare in the specific population of sportsmen and women.

CONCLUSION

Based on the results presented in this study, several conclusions were drawn. The tallest male participants are basketball players; both females and males are significantly taller than the average in the general population in Bosnia and Herzegovina. This is a sign that positive selection by height was present in basketball. Sports activity at a young age could help to achieve genetically predisposed height. Sports activity has a big influence on BMI, even after stopping sports. The highest BMI was noted in the control inactive men group. A strong correlation was found between a family history of engaging in sports and the personal athletic success of the participants in all three examined sports categories, highlighting the importance of a stimulating and supportive environment for athletic achievements. The highest percentage of injuries was found among female football players. The most frequent injury in all three sports disciplines was injury of the meniscus and tendons. None of the participants from the three sports categories reported chronic health problems, while all individuals in the control group reported certain health issues (hypertension, insulin resistance, or diabetes). Genetic testing is important for determining athletic predispositions at an early age, as well as for the early detection of hereditary diseases that may lead to fatal outcomes during intense physical activity. Based on such findings, it would be possible to implement protective and preventive genetic testing, especially for those young sportsmen who plan to pursue professional activity in sports.

Conflict of interest
The authors declare no conflict of interest.

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Recived: June 10, 2025

Accepted: July 14, 2025

DOI: 10.7251/QOL2503100H **UDC:** 159.922.8:341.485-053.6
Professional paper

Adolescents' Perceptions and Experiences of Psychological Violence

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Abstract: Psychological violence among adolescents and young adults represents a significant challenge for both public health and the educational system, with long-term consequences for mental well-being. This study aimed to examine the prevalence and forms of psychological violence among individuals aged 12 to 24, as well as their awareness and personal experiences related to this issue. The research was conducted on a sample of 75 participants aged between 12 and 24 years, using a structured questionnaire that included demographic data, experiences with psychological violence, and familiarity with protective responses. The results revealed that a considerable number of participants had been exposed to various forms of psychological violence, including verbal abuse, social exclusion, and rejection. The study provides insight into the extent of psychological violence in this age group and indicates the need for further support measures. The discussion emphasizes the importance of involving students, parents, and teaching staff in efforts aimed at fostering emotional literacy and non-violent communication. Further research and programmatic development are recommended to enhance supportive strategies in school environments.

Keywords: adolescents, psychological violence, prevention, awareness, emotional literacy.

INTRODUCTION

Adolescence is a developmental period marked by profound emotional, cognitive, and social transformations, but also by increased vulnerability to various forms of violence—among which psychological violence is especially concerning (Gajić & Topalović, 2021). This type of violence includes verbal abuse, social exclusion, manipulation, cyberbullying, and emotional neglect, often leaving lasting consequences on the emotional development of young people and their interpersonal relationships (Zhao et al., 2023).

Psychological violence is frequently covert and normalized through everyday interactions, which makes its recognition and prevention particularly complex. This complexity requires a multisectoral approach in which education plays a vital role (Kecojević, Živković & Petrović, 2024). Various forms of health-related and psychosocial education are recognized as potential means to support adolescents in understanding and addressing such experiences (Smith & Robinson, 2020).

Educational programs that support emotional literacy, assertive communication, and tolerance may contribute to building safer and more inclusive school environments (Stanojević & Milojević, 2022). Although not always physically visible, psychological violence undermines the dignity and emotional security of adolescents. It can take the form of verbal humiliation, rumor-spreading, exclusion, threats, and manipulation through digital media. Adolescents often face difficulties distinguishing conflict from violence, while their social surroundings may trivialize such behaviors by labeling them as "jokes" or "peer pressure."

Early warning signs—such as social withdrawal, anxiety, emotional instability, or academic underperformance—require sensitive and coordinated responses by teachers, parents, and professional staff

(UNICEF, 2023). In this context, the family has a protective role. Children raised in emotionally stable families with open communication and mutual respect are more resilient and less likely to engage in or suffer from psychological violence (Petković, Radošević & Ilić, 2023).

Parents serve as key role models and shape their children's coping strategies through consistent support and dialogue. Their collaboration with educational and health professionals strengthens the foundation for early recognition and prevention (Bradshaw, Waasdorp & O'Brennan, 2021). Likewise, schools, as the primary social environment for adolescents, should uphold clear anti-violence policies and promote positive classroom climates. Educators play a significant role in recognizing risky behaviors and fostering nonviolent communication through structured programs and continuous professional development (UNICEF, 2023; Bradshaw et al., 2021).

In this system, the role of health and social educators may be especially relevant in promoting psychosocial well-being and offering support to students. Through group work, workshops, and interactive learning, such professionals can help adolescents recognize risk, seek support, and build protective factors (WHO, 2022).

Given the complexity of psychological violence, this study aimed to analyze its prevalence, forms, and consequences among adolescents, with particular attention to their reactions, sources of violence, and awareness of available protective mechanisms. The findings may contribute to a deeper understanding of the emotional and social effects of psychological violence and inform the development of future educational and preventive interventions.

PARTICIPANTS AND RESEARCH METHODOLOGY

This study was designed as a quantitative, descriptive, cross-sectional survey conducted using an online questionnaire. Data collection took place between May 15 and June 6, 2024. The survey was distributed through school staff and youth organizations using closed communication channels—such as school Viber groups, email lists, and educational platforms (e.g., Google Classroom)—ensuring targeted reach to adolescents and young individuals. The survey link was not posted publicly on social media.

A total of 75 participants aged 12 to 24 years were included, covering both adolescent and early post-adolescent age groups. Respondents were recruited from diverse educational institutions and socio-economic settings across the Central Bosnia Canton, aiming to reflect a variety of backgrounds.

Participation was voluntary and based on prior informed consent. All responses were collected anonymously and treated as confidential, in line with applicable ethical standards and institutional research guidelines.

Purposive sampling was used, facilitated by cooperation with schools and local youth organizations. Inclusion criteria were: (1) age between 12 and 24 years; (2) current school enrollment or classification as part of the youth population; and (3) voluntary agreement to participate. The sampling strategy aimed to include participants from different municipalities, educational levels, and socio-economic contexts.

Although random sampling was not employed, efforts were made to achieve demographic and contextual diversity. Therefore, the findings may be indicative of broader trends among youth in the region, while acknowledging the limitations of non-probability sampling.

The data collection instrument was a structured, study-specific questionnaire comprising 20 questions, grouped into three thematic sections:

1. Demographic characteristics (age, gender, educational status);
2. Experiences with psychological violence (forms, frequency, context, and sources of exposure);
3. Awareness and perceptions related to psychological violence and available protective mechanisms.

4. Most items were close-ended, with several using a Likert scale to assess perceptions and awareness levels. The questionnaire was designed for ease of completion, requiring approximately 10 minutes.
5. A previously validated instrument was not used; rather, the questionnaire was developed for the purposes of this study based on the research objectives and a review of relevant literature. A pilot test with a small group of participants was conducted beforehand to assess the clarity and structure of the items.

RESEARCH RESULTS

Table 1. Demographic Data of Respondents

Table 1.1. Age Structure of Respondents

Age Group	Number of Respondents	Percentage (%)
12–14 years	12	16.0
15–17 years	31	41.3
18–20 years	18	24.0
21–24 years	14	18.7
TOTAL 12–17 years	43	57.3
TOTAL 18–24 years	32	42.7

Table 1.2. Gender Structure of Respondents

Gender	Number of Respondents	Percentage (%)
Male	45	60.0
Female	30	40.0

Table 1.3. Educational Status of Respondents

Educational Status	Number of Respondents	Percentage (%)
Completed primary school	25	33.3
Currently attending high school	34	45.3
Completed high school	8	10.7
Currently attending university	8	10.7

The demographic characteristics of the respondents (Tables 1.1 to 1.3) are presented across three categories: age, gender, and educational status. According to the age distribution, 57.3% of respondents were between 12 and 17 years old, while 42.7% were between 18 and 24 years. The largest subgroup was aged 15–17 years (41.3%), while the smallest was 12–14 years (16.0%).

As shown in Table 1.2, 60% of respondents were male and 40% female.

Regarding educational status (Table 1.3), the majority of respondents were currently attending high school (45.3%), followed by those who had completed primary school (33.3%). Equal proportions of respondents (10.7%) reported either completing high school or currently attending university. These figures reflect the expected educational distribution for the age range represented in the sample.

QUANTITATIVE RESEARCH RESULTS

Table 2. Frequency of Experienced Psychological Violence

Frequency of Psychological Violence	Frequency (n)	Percentage (%)
Never	11	14.7%
Rarely	19	25.3%
Occasionally	26	34.7%
Often	9	12.0%
Very often	10	13.3%
Total	75	100.0%

The distribution of self-reported experiences with psychological violence is shown in Table 2. Out of 75 participants, 11 (14.7%) stated they had never been exposed to psychological violence, while 19 (25.3%) reported rare exposure. The most common answer was occasional exposure (n = 26; 34.7%). Furthermore, 9 participants (12.0%) indicated they often experience psychological violence, and 10 (13.3%) reported very frequent exposure. These results point to a notable presence of psychological violence within the studied group and suggest the need for further examination of support systems available to adolescents.

Table 3. Forms of Psychological Violence Among Adolescents

Form of Psychological Violence	Frequency (n)	Percentage (%)
Rejection	16	21.3%
Terrorizing	21	28.0%
Ignoring	19	25.3%
Isolation	12	16.0%
Exploitation	7	9.3%

Note: Respondents could select more than one form of psychological violence; therefore, total percentages exceed 100%.

As illustrated in Table 3, the most frequently reported form of psychological violence was terrorizing (n = 21; 28.0%), referring to emotional intimidation or threat-based behavior. This was followed by ignoring (n = 19; 25.3%) and rejection (n = 16; 21.3%), both indicative of social exclusion and passive aggression. These findings reflect the diversity of psychological violence manifestations encountered by adolescents in everyday social contexts.

Table 4. Adolescent Reactions to Psychological Violence

Reaction to Psychological Violence	Frequency (n)	Percentage (%)
Withdrawal	26	34.7%
Aggression towards others	20	26.7%
Avoidance of the perpetrator	15	20.0%
Seeking help	14	18.7%

Note: Respondents could choose more than one reaction; therefore, total percentages exceed 100%.

As shown in Table 4, the most frequently reported response to psychological violence was withdrawal (n = 26; 34.7%), indicating a tendency toward emotional avoidance or internalized coping. Aggressive behavior toward others (n = 20; 26.7%) was also mentioned, suggesting that some adolescents externalize their emotional responses. Other reactions included avoiding the perpetrator (20.0%) and seeking help (18.7%). These self-reported responses reflect the range of emotional and behavioral strategies used by adolescents when facing psychological stress.

Table 5. Impact of Psychological Violence on Adolescents' Social Relationships

Impact on Social Relationships	Frequency (n)	Percentage (%)
Social withdrawal / avoidance	41	54.7%
Conflict reactions / aggression	20	26.7%
Seeking help / support reliance	14	18.7%

Note: Respondents could select more than one response; therefore, total percentages exceed 100%.

According to Table 5, over half of the respondents (n = 41; 54.7%) reported some form of social withdrawal or avoidance following experiences of psychological violence. Conflict-related reactions such as aggression (26.7%) and help-seeking behaviors (18.7%) were also reported. These findings suggest that psychological violence may influence adolescents' social functioning in multiple ways. The relatively low proportion of adolescents who sought help may warrant further investigation into the accessibility and perceived effectiveness of available support mechanisms.

Table 6. Sources and Settings of Experienced Psychological Violence

Category	Frequency (n)	Percentage (%)
Perpetrator – Family member	46	61.3%
Perpetrator – Teacher	15	20.0%
Setting – School	26	34.7%
Setting – Internet	24	32.0%
Setting – Family home	16	21.3%
Setting – Public space	9	12.0%

Note: Respondents were allowed to select multiple responses; therefore, the total percentage exceeds 100%.

As shown in Table 6, participants reported a variety of sources and environments in which they experienced psychological violence. The most frequently identified source was a family member (n = 46; 61.3%), followed by teachers (n = 15; 20.0%). Regarding settings, the most commonly mentioned were school (n = 26; 34.7%) and the internet (n = 24; 32.0%), with some respondents also reporting the family home (21.3%) and public spaces (12.0%). These results indicate the presence of psychological violence across multiple interpersonal and contextual domains.

The use of a multiple-response format allows for a more nuanced understanding of the complexity and overlap between perpetrators and environments in which psychological violence occurs. Since participants could select more than one response, totals may exceed 100%.

Table 7. Reactions and Level of Awareness Among Adolescents Regarding Psychological Violence

Reaction and Awareness	Frequency (n)	Percentage (%)
Sought help	14	18.7%
Aware but did not react	41	54.7%
No protective reaction	20	26.6%

According to Table 7, 18.7% of adolescents reported that they sought help when faced with psychological violence. Over half of the participants (54.7%) stated they were aware of the situation but did not take any action. Additionally, 26.6% of respondents reported no protective reaction. These findings suggest varying levels of awareness and response among adolescents, with some indicating uncertainty or lack of access to protective resources.

DISCUSSION

This study, based on a descriptive analysis of 75 adolescents and young adults aged 12 to 24, identified multiple personal experiences of psychological violence across various settings. The most represented age group was 15 to 17 years, which corresponds to a developmental stage characterized by emotional sensitivity and intense social interaction.

The findings show that schools (34.7%) and online platforms (32.0%) are the most frequently reported settings in which psychological violence occurs, while family members were identified as the most common sources of such violence (61.3%). These results suggest that psychological violence is not limited to a single specific environment, but occurs across the various interpersonal spaces that adolescents interact with daily.

Similar patterns have been recorded in studies from the region. For example, Petrović et al. (2020) reported that over 60% of adolescents in Serbia experienced verbal and emotional violence in school settings.

Kobal Grum (2021) emphasized the importance of family emotional support in mitigating the effects of school-based violence. Although direct comparisons are limited, these findings provide valuable context for understanding the presence of psychological violence in the social environments of young people in Bosnia and Herzegovina.

Only 18.7% of respondents reported seeking help, while the majority indicated awareness of psychological violence but took no further steps. Although this study did not explore the reasons behind such behavior, the results indicate variability in adolescents' response mechanisms and call for further research.

Differences in reactions based on gender were also observed: for example, 26.7% of male respondents reported aggressive reactions. Although the sample was not large enough to draw definitive gender-based conclusions, these findings may point to different response patterns that deserve attention in future studies.

Overall, the data highlight the presence of psychological violence in school, family, and digital contexts. This study does not aim to evaluate educational strategies or preventive frameworks but provides a snapshot of the respondents' personal experiences. These insights can serve as a basis for future research and support the development of context-specific programs for adolescents.

In addition, regional data from the Balkan Epidemiological Study on Child Abuse and Neglect (BECAN, 2018), conducted across nine Balkan countries including Bosnia and Herzegovina, reported lifetime exposure rates to psychological violence among school-aged children at 64.6% based on self-reports. This prevalence closely mirrors the findings of this study and supports the conclusion that psychological violence is a persistent concern among adolescents in this context.

CONCLUSION

Psychological violence among adolescents is a significant and growing public health and social issue, with long-term consequences for emotional, mental, and relational well-being. This study, conducted on a sample of 75 participants aged 12 to 24 from the Central Bosnia Canton, revealed that more than half of respondents had been exposed to at least one form of psychological violence. Emotional withdrawal and social isolation were the most frequently reported reactions, indicating early symptoms of emotional distress.

The study also found that psychological violence most commonly occurred in school environments and through online interactions. A substantial number of respondents also reported experiencing such violence within their own families, highlighting the urgency of addressing domestic dynamics that may compromise adolescent mental health. Moreover, only a small portion of participants stated that they had access to support or knew how to seek help, emphasizing the need to strengthen existing protective systems and improve awareness of available services.

Although this research did not assess specific prevention strategies or the role of health education as a structured intervention, the findings underline systemic shortcomings in awareness, access to help, and institutional response. The study complements findings from regional literature and confirms the widespread nature of psychological violence, as demonstrated in similar contexts such as Serbia and Slovenia.

The value of this study lies in its empirical overview of adolescents' experiences with psychological violence and their behavioral and emotional responses. The findings point to the need for targeted, intersectoral interventions that include education, mental health, and social welfare systems.

Based on the results, the following strategic actions are recommended:

• Strengthening school-based programs aimed at fostering emotional literacy, peer support, and non-violent communication;

• Improving psychosocial support services through school and community-based mental health initiatives;

• Establishing effective municipal-level referral and protection mechanisms;

• Introducing structured family education programs focusing on positive communication and parenting skills.

Ultimately, this study calls for integrated and sustained efforts to address psychological violence as a public health priority. Adolescents have the right to safe environments that promote psychological security and personal development. Ensuring that right requires coordinated action, stronger prevention frameworks, and evidence-based policies adapted to local needs.

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Recived: June 09, 2025

Accepted: July 07, 2025

DOI: 10.7251/QOL2503108B **UDC:** 628.1.033:546.815(497.6Banja Luka)

Professional paper

Impact of the Technological Purification Process on the Drinking Water Quality in the Water Supply System of Banja Luka

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Abstract: This paper analyzes the impact of the technological purification process on the drinking water quality in the water supply system of the City of Banja Luka. Microbiological and physico-chemical parameters of raw water samples from the River Vrbas and purified water samples from reservoirs collected during three time periods: 1977, 2006 and 2025 were used. The research has showed a significant microbiological load of raw water, especially the presence of coliform bacteria and enterococci, which indicates fecal contamination of the source. Despite this, the analysis of the results has determined that the water conditioning process, which includes ozonation, filtration and chlorine disinfection, successfully eliminates contaminants and ensures the microbiological and physicochemical safety of drinking water. The results confirm that the purified water meets the requirements of the Regulation on the health safety of drinking water across analyzed years. Based on these findings, the need for continuous monitoring of raw water quality and technical improvement of treatment plants is emphasized in order to ensure the long-term safety of the water supply.

Key words: drinking water, conditioning, microbiological analysis, physicochemical parameters, water supply of Banja Luka and the River Vrbas.

INTRODUCTION

Water is the fundamental condition for life and existence of all living beings, representing a key medium for the development of biological processes. Due to its unique physico-chemical properties, water has enabled the development and maintenance of life, as well as a continuous circulation in the biosphere known as the hydrological cycle.

According to the World Health Organization, only 2.5% of the world's total supplies represent freshwater, while most of this portion is captured in glaciers, snowpack and difficult-to-reach groundwater, which further highlights the significance of managing available water resources.

In modern urban environments, supplying the population with safe drinking water is a public health priority. Due to the increasing pressures of anthropogenic activities and climate change that cause variability in the raw water quality, the water treatment and conditioning are becoming increasingly complex. In this context, monitoring and evaluating the efficiency of purification processes are key tools in ensuring the health and safety of end users (Figure 1).

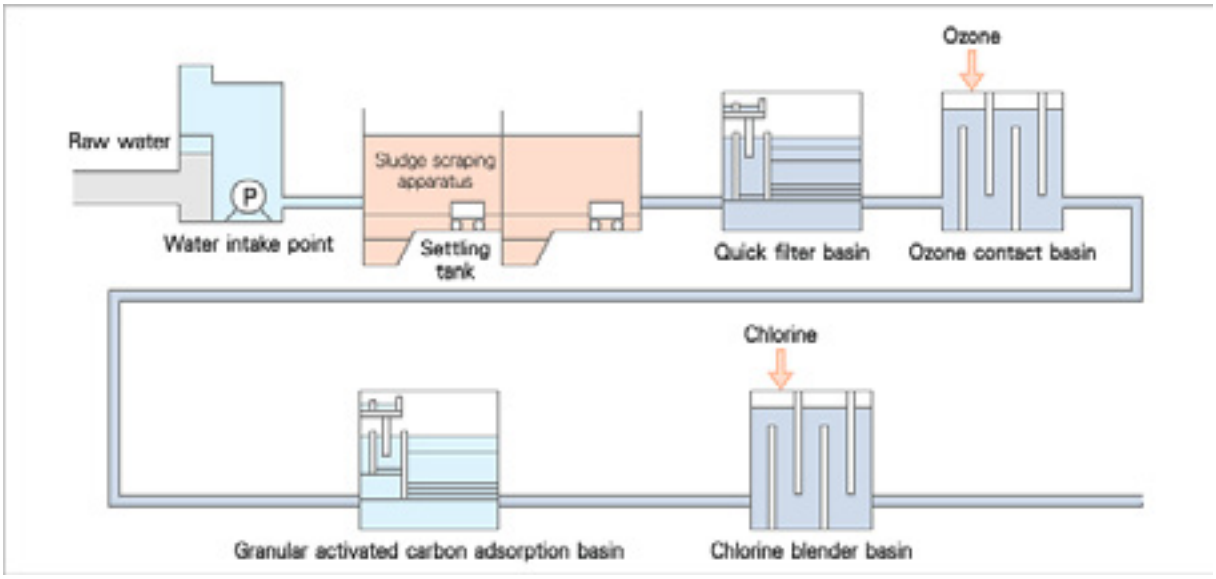

Figure 1. Flowchart of advanced water purification process (https://www.toshiba-clip.com/en/detail/p=147)

Climate changes alter the pH of water significantly and disrupt the carbonate system balance. Therefore, the corrosiveness of water is affected which can lead to increased concentrations of metals such as iron and manganese in underground sources. *(https://www.eea.europa.eu/en/newsroom/news/state-of-water)*.

Water, as a universal solvent, plays a key role in maintaining life and the stability of biological systems. According to the World Health Organization (WHO, 2016), the minimum daily water intake required to maintain basic physiological functions in an adult is between 2 and 3 liters, depending on individual and climatic factors. Water participates in the transport of nutrients, the elimination of metabolic waste, the thermoregulation and osmotic balance.

The health and safety of drinking water are directly related to the prevention of infectious diseases. According to WHO data, about 80% of diseases in developing countries are caused by poor water quality and inadequate sanitation. Populations consuming water contaminated with faeces are particularly at risk, as this enables the spread of enteric pathogens such as Escherichia coli, Salmonella spp., Shigella spp., and hepatitis A virus.

The drinking water quality is defined by physicochemical (pH, turbidity, electrical conductivity, presence of ammonia, nitrates, chlorides, etc.) and microbiological parameters (total coliform bacteria, E. coli, enterococci). The control of these parameters is regulated by national legislation, such as the Regulation on the water safety for human consumption (Official Gazette of the Republic of Srpska, No. 88/17, 97/18, 93/23 and 96/24), as well as international standards (e.g. European Union (EU) Directive 2020/2184 and WHO guidelines).

Water quality monitoring encompasses all phases of the water supply system: from the source through processing to distribution. In that sense, it is important to separate operational monitoring conducted by water supply company from independent health control conducted by accredited laboratories. An efficient monitoring system must include properly determined sampling points, standardized analysis methods and continuity in testing.

Technological water treatment processes include a series of physicochemical and biological treatments. The main treatments include:

- coagulation and flocculation - removal of suspended matter,
- sedimentation and filtration - mechanical purification,
- disinfection - most often using chlorine, ozone or UV radiation.

Within the water supply system of the City of Banja Luka, the main water source is the River Vrbas, which is, due to urbanization and insufficient sewage infrastructure, subject to variations in quality. A modern water processing plant applies ozonation as an oxidation pre-reaction, then filtration through multilayer filters, and final disinfection with chlorine. This system enables efficient removal of microbiological contaminants and reduction of organic matter, thus improving the water supply safety.

The significance of regular monitoring and optimization of the purification process is reflected in the fact that climate change and anthropogenic impacts are increasingly leading to sudden changes in the quality of surface water sources. Therefore, it is necessary to harmonize local systems with international standards and recommendations (npr. Sustainable Development Goal 6 – SDG6: Clean water and sanitation for all).

THE ROLE OF WATER IN THE BODY

The role of water in the human body is vital and diverse. It is part of all tissues and biological fluids in the human body. On average, it constitutes 2/3 of an adult's body weight, and all life processes in the body are linked to its presence (Antonić, 2017). Water transports nutrients, maintains the normal structure of all tissues, and eliminates the final metabolic products – the matter and energy turnover in the human system. Due to the unusual physicochemical properties of water, substances with different physicochemical properties can coexist in biological fluids (Antonić, 2017). Due to the certain chemical inertness of water, substances dissolved in biological fluids can preserve their individual properties while passing through the organism, which is the course of biochemical processes in the organism (Antonić, 2015).

HYGIENIC-EPIDEMIOLOGICAL SIGNIFICANCE OF WATER

The hygienic significance of water is reflected, first of all, in the necessity of water for maintaining both personal and general hygiene, for cooking and dish washing, sports, recreation, as well as for satisfying a number of modern man's life needs.

Drinking water can also be contaminated with toxic metals and non-metals, radionuclides, poisons, as well as microorganisms. Contamination can be either accidental or intentional. The deficit of certain substances (iodine, fluorine, etc.) plays a significant epidemiological role in the development of caries, goiter, etc. For the reasons aforementioned, drinking water must meet standards and be subject to ongoing health controls (Kristoforović-Ilić, 2002).

If sufficient quantities of hygienically correct water are available to meet the hygienic and other needs of the population, then it is easier to maintain personal and general hygiene, which directly and indirectly affects the health status of the population. Water can fulfill its basic hygienic role only if it is available in sufficient quantities and if its physical, chemical and microbiological properties do not adversely affect health, and if it does not have such organoleptic properties that limit its use.

The world population growth along with the development of human civilization leads to a constant increase in the use of water resources. Water is taken from rivers, lakes, springs and aquifers for irrigation, domestic use, urban and industrial purposes. The amount of natural water consumed has increased by multiple orders of magnitude in the past 100 years. This is due to the increased growth rate of water demand, recently estimated to be about 2.5 times greater than the population growth rate (Stanojević, 2022).

According to WHO estimates in 2021, over 2 billion people lived in countries facing water shortages, and this problem is expected to worsen in some regions as a result of climate change and population

growth. In 2022, globally, at least 1.7 billion people used drinking water contaminated with faeces. Microbial contamination of drinking water resulting from fecal contamination poses the greatest risk to the drinking water safety. Microbiologically contaminated drinking water can transmit diseases such as diarrhea, cholera, dysentery, typhoid and polio, and is estimated to cause approximately 505.000 deaths from diarrhea each year. In 2022, 73% of the world's population (6 billion people) used safely managed drinking water supply services – that is, the one that is located in a facility, available when needed and free from contamination (WHO, 2023).

Supplying the population with hygienically safe water arises as one of the basic prerequisites for good health. WHO has included drinking water quality in 12 basic indicators of the health status of the population, which confirms its role in health protection and promotion. For these reasons, WHO experts are constantly engaged in developing methods for improving and harmonizing drinking water quality standards and continuous evaluation in terms of the impact of water on human health.

The United Nations General Assembly, with its resolution number 64/292 from 2010, recognized the right to clean and accessible drinking water as one of the basic human rights (Antonić, 2017).

Given the growing trend of pollution and variability in the quality of surface waters, this work is designed to determine the efficiency of drinking water conditioning devices by comparing microbiological and physicochemical results from raw water of the River Vrbas and purified water across different periods.

DRINKING WATER MONITORING

Production, processing, transport and quality control of drinking water is a multi-phase system (Dalmacija, 2015). Water quality monitoring includes the control of four segments:

- source
- water processing and disinfection
- reservoirs and
- distribution network.

In each of the above segments, water contamination is possible, which represents a potential danger to end users. That is why an adequate system for controlling the water safety is the main goal of water safety monitoring. Control of all segments of the System is essential for safeguarding consumer health and monitoring the operation of purification and disinfection plants, as well as the basic raw material. Timely detection of contamination occurring in the first stages of the system prevents contamination of the entire system and endangering the population health. The goal is to present a method of systematic drinking water quality control based on regulations and experiences, WHO recommendations, as well as EU (European Union) regulations (Dalmacija, 2015).

Drinking water quality control is carried out at two levels:

1. Continuous water quality control carried out by the Public Utility Company for Water Processing and Distribution, the so-called "operational control", should ensure adequate processing and disinfection of raw water and distribution of drinking water to the end consumer,
2. Public health drinking water quality control which involves testing physical-chemical, chemical and microbiological, parasitological, biological and radiological integrity (Dalmacija, 2015).

Establishing drinking water quality monitoring implies a system of activities that ensure that sampling and laboratory testing are in accordance with defined quality standards and the required level of reliability, i.e. that the safety and quality control postulates are met (Dalmacija, 2015).

Given that the ultimate goal is to preserve the population health, the main task of establishing drinking water quality monitoring is to deliver hygienically safe water. In order to achieve such a task, it is neces-

sary that data obtained are valid – accurate, precise, complete, representative, comparable and compatible (Dalmacija, 2015).

Quality assurance is ensured by:

- adequate selection of measurement points
- sampling frequency and proper sampling
- sample preparation and transport
- appropriate training of personnel.

Quality assessment consists of:

- report on the measurement results obtained and
- assessment of the measurement results obtained in relation to the maximum permitted concentrations.

Operational water quality control is carried out in accordance with predefined quality standards, with the Public Utility Companies defining the control programme themselves.

Monitoring pursuant to the Law on the Protection of Population Health from Communicable Diseases and the Rulebook on the Hygienic Safety of Drinking Water falls under the jurisdiction of health organizations authorized to control the drinking water quality, i.e. the Public Health Institute. Measurement points - places where samples are taken are

- sources,
- places conditioned by technological procedures for water processing,
- reservoirs and distribution network (Dalmacija, 2015).

DRINKING WATER PREPARATION PROCEDURES

Before assessing whether a certain water needs to be processed, in addition to getting acquainted with test results at a given moment, it is necessary to understand or predict the trend of water quality changes. Raw water can change quality during the day, season, year or over a longer period. Drinking water preparation can be more or less complex depending on the quality of the raw water, as well as the possibility of removing existing obstacles. Sometimes numerous procedures are to be combined to achieve the pollution elimination in the best way, while selecting the optimal processing method, both in terms of investment and exploitation costs. Figure 2 shows the basic water treatment technological lines. The selection of the technological lines depends on the quality of the raw water. The technological lines show procedures from the simplest (only disinfection) ones to the most complex ones. It should be emphasized that each stage of preparation can be improved by introducing a specific procedure with a performance (primarily) at only one parameter. Recently, a particularly significant increase has been observed in the total organic content in water, as well as the concentration of mobile bioresistant organic compounds (pesticides, organochlorine compounds, etc.), both in surface and groundwater.

Conventional water preparation procedures cannot remove such compounds and for these reasons, there has been a need for innovation in older plants, and for the development of new ones - the need to define new water purification procedures (Dalmacija, 2015).

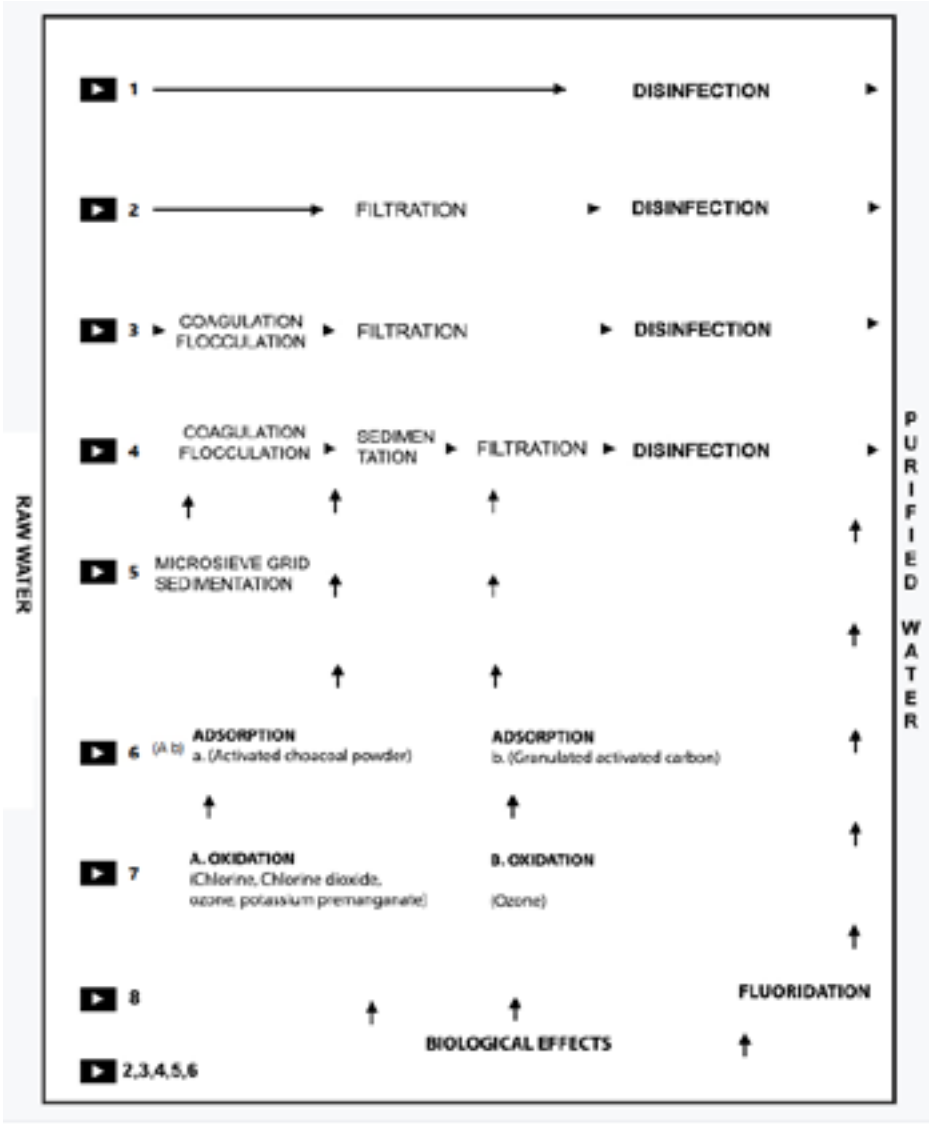

Figure 2. Water treatment lines depending on the raw water quality

1. It is applied when the raw water quality corresponds to the quality prescribed by the drinking water standard

2. It is applied for the processing of waters containing only suspended matter that can be removed by filtration (concentration of suspended particles below 10 mg/l).

3. It is applied if, in addition to suspended matter, the water also contains colloidal matter (usually less than 20-40 mg/l)

4. It is applied in waters with a larger amount of suspended and colloidal matter compared to the previous case

5. It contains additional processes to the previous lines in case of using surface water from which it is necessary to remove a wide range of suspended matter, plankton, algae, etc.

6. and 7. These lines include additional water treatment phases that can be included in any of the previous technological lines, and are used in case of increased content of toxic organic substances or increased concentration of dissolved organic and inorganic substances.

8. It is applied if there is a lack of fluorine in the water (Dalmacija, 2015).

Water Supply System in Banja Luka

The development of the water supply system of every larger and urban settlement is always a complex technical-technological, economic and social **undertaking**. In the development of the water supply system in Banja Luka, it is possible to indicate certain characteristic stages, recognizable for providing drinking water. Namely, there are four periods:

- Gravity-flow water system "Subotica"
- exploitation of the underground aquifer in Novoselija,
- artificially enriched underground aquifer in Novoselija through infiltration,
- preparing drinking water from the River Vrbas using water conditioning device.

Today, the water supply system of Banja Luka is of a regional character because, in addition to Banja Luka, it provides drinking water supplies to the Municipality of Čelinac and part of the Municipality of Laktaši. The main water source is the Vrbas watercourse, upstream at the Novoselija site, on the right bank of the River Vrbas. The natural flow regime of the River Vrbas is characterized by significant oscillations in water levels and flow rates. A turndown ratio of minimum and maximum flow rates obtained by measuring profiles is greater than 1:1000 (Antonić, 2017). Not enough work has been done to regulate the water regime of the River Vrbas. The hydropower facilities built in the middle reaches of the Jajce I and Jajce II hydroelectric power plants have not had an impact on improving the water regime, while the situation is somewhat more favorable with the construction of the Bočac reservoir. Of the settlements, only Banja Luka, Jajce and Bugojno have a more complete sewage system. All of these sewage systems were introduced into the watercourses without any treatment. This shows that the Vrbas basin flow, as well as the Vrbas itself, are wastewater recipients and drinking water sources at the same time (Antonić, 2017).

In the region of Banja Luka, there is only one small wastewater treatment plant in the settlement of Zeleni vir, which is in operation and has a capacity of 500 equivalent inhabitants (EC), *(https://vodovod-bl.com)*.

The conditioning device has a capacity of 600 L/s in the first phase, and 1200 L/s in the second phase. In preparing documentation, connecting new 2x600 L/s has been taken into account so that the water conditioning device will have a final phase capacity of 2400 L/s. Today, Banja Luka is supplied from three sources:

1. source of ''Subotica" – gravity water supply,
2. well-based water intake structure ''Stari pogon",
3. open water intake structure and water conditioning ''Novi pogon", (Antonić, 2017).

The total reservoir space of the water supply system Banja Luka is 26,350 cubic meters, and it consists of 11 pumping stations that provide drinking water supply to the entire city. Pumping stations are located at key points in the water supply network and, in addition to enabling a uniform water supply, they are also key to maintaining pressure in the network, as well as avoiding leaks. There are over 40,000 connections in the region of Banja Luka, out of which 30,000 connections are for individual consumers, over 30,000 for residential buildings and 5,000 for bussinesses, skilled trades and institutions (*https://vodovod-bl.com*).

Conditioning process in the water supply system in Banja Luka

At the water intake, raw water of the River Vrbas is pumped and transported through a 1000 mm steel pipeline to a separation tower at 179 m above sea level to ensure further gravity flow. From the separation tower, the water goes to sedimentation tanks where clarification occurs. In the further preparation phase in the new factory facility, ozone is included as a new form of disinfection. Then the water goes to a filter station for filtering and then to a clean water tank where final disinfection with chlorine is performed. The water is then transported through pipelines towards the city using a pumping station. (*https://vodovod-bl.com*).

METHODS

Sampling methods

The sampling methods for water analysis are designed and defined by the standards ISO 5667-5:2007 Guidelines for sampling of drinking water from treatment works and piped distribution systems and ISO 5667-1:2004 Guidelines on the design of sampling programmes and sampling techniques.

Water samples for microbiological analysis were taken in clean 250 mL glass bottles, previously sterilized in a dry sterilizer at temperatures of 160-180 °C for 1 hour, previously closed with cork stoppers covered with aluminum foil. In the same bottles intended for taking chlorinated water, 0.15 mL of 5% sodium thiosulfate solution was poured to reduce chlorine. Before collecting water from the water supply, the tap was ignited with a flame and the water ran for 3 to 5 minutes. When the bottle was filled with water to ¾ of its volume, it was carefully closed with a stopper, the protective cap was replaced and tied with a rope.

Samples for physicochemical analysis were collected in chemically clean 1-liter bottles. When collecting water samples at the sampling point, the water temperature was measured and the organoleptic properties were determined.

The samples were delivered to the laboratory on the same day and, as a rule, immediately processed for microbiological and physicochemical testing.

Methods, instruments and techniques used in the research

Laboratory tests of the quality of both raw water and drinking water were carried out in accredited laboratories of sanitary chemistry and sanitary microbiology of the Public Health Institute of the Republic of Srpska Banja Luka. When determining the microbiological and physicochemical characteristics of raw water and drinking water samples, basic analyses were performed and BAS EN ISO methods were used for testing the hygienic safety of untreated and treated water.

Determination of microbiological parameters

Raw water

- for the determination of all coliform bacteria by the MPN method, the following were used: one 50 mL tube (LAP) 5X10 mL LAP and 5 tubes of 5 mL LAP each (preliminary test), the media were incubated for 24-48 h at 36 ºC;
- all liquid media that showed a change (turbidity, acidity) after 48 hours at a temperature of 36 ºC were plated onto appropriate solid media for further identification (confirmatory test);
- for confirmed coliform bacteria and E. coli, the most probable number (MPN) is determined.

Table 1. MPN values per 100 mL of sample and 95 % confidence interval

Number of tubes with a positive reaction		MPN	Limit values	
1 50-mL test tube	*5 10-mL test tubes*	(per 100 mL)	*lower limit*	*upper limit*
0	0	<1		
0	1	1	<1	4
0	2	2	<1	6
0	3	4	<1	11
0	4	5	1	13
0	5	7	2	17
1	0	2	<1	6

1	1	3	<1	9
1	2	6	1	15
1	3	9	2	21
1	4	16	4	40
1	5	>18		

Purified water

- To determine coliform bacteria, 100 mL of the sample water to be tested is filtered using a membrane filter; the minimum volume for filtration is 10 mL of sample; after filtration, the membrane filter is placed on homogeneous coliform agar (CCA) and the Petri dish is inverted to ensure that there is no air underneath; incubation follows at 36 ± 2 ºC for 21 ± 3 h; after which colonies suspected of being coliform bacteria other than E.coli are identified and confirmed using the oxidase test; if necessary (colonies too small for the oxidase test or located next to other colonies), subcultures are made and incubated on non-selective agar at 36 ± 2 ºC for 21 ± 3 h.
- To determine the number of colonies at 22 ºC, the pour plate method is applied by placing a certain amount of test sample ≤ 2 mL per Petri dish and adding 15-20 mL of dissolved medium, followed by mixing by gentle rotation, after which the media are left to solidify. The time between the addition of the test sample and the medium must not exceed 15 minutes, at least one incubation plate is incubated at each temperature; one set facing downwards is incubated at 36 ± 2 ºC, 44 ± 4 h, and the other at 22 ± 2 ºC, 68 ± 4 h. The plates are examined immediately after removal from the incubator and the colonies present in 1 mL of sample are counted for each incubation temperature.
- The determination of Enterococcus is performed by placing the filter membrane on Slanetz Bartley medium after filtration and incubating at 36 ± 2 ºC for 44 ± 4 h. If typical colonies are present, the membrane and colonies are transferred with sterile forceps to a bile-esculin-azide agar medium that has been preheated to 44 ºC and after incubation at 44 ± 0.5 ºC for 2 h, the results are read immediately.

Determination of physicochemical parameters

Table 2. Physico-chemical parameters

PHYSICOCHEMICAL PARAMETERS	TEST METHODS FOR THE PERIODS OF 1977 AND 2006	TEST METHODS FOR THE PERIOD OF 2025
Water temperature°C	***Thermometers with divisions of 0,1 ºC***	***Thermometers with divisions of 0,1 ° C***
Color-degree on the Co-Pt scale	*Colorimetric*	*Colorimetric*
Smell	*Organoleptic*	*Organoleptic*
MTurbidity	*Turbidimetric*	*Turbidimetric*
pH-value	*Potentiometric*	Potentiometric
$KMnO_4$ mg/L consumption	Titrimetric	Titrimetric
Electrical conductivity μScm^{-1} at 293,16 K (20 ° C)	Conductometric	Conductometric
Ammonia	Colorimetric (Nesler method)	Electrochemical/spectrophotometric
Nitrates and nitrites	Colorimetric (Brucin method)	Spectrophotometric
Chlorides	Titrimetric by the Mohr method	Titrimetric by the Mohr method
Sulfates	Titrimetric BaC_2O_4	Not sought
Total alkalinity	Titrimetric with HCl	Not sought
Carbonate hardness	Titrimetric with HCl	Not sought
Total hardness	Complexometric	Not sought

Residue after evaporation	Gravimetric	Not sought
Loss on ignition	Gravimetric	Not sought
Residue on ignition	Gravimetric	Not sought
Residual chlorine	Colorimetric O-toluidine method	Photometric
Dissolved oxygen	Titrimetric according to Winkler	Not sought
Biological oxygen demand– BPK_5	Titrimetric according to Winkler	Not sought

A brief description of the determination of physical and chemical parameters

- Water temperature is determined during water sampling at the collection site; the measurement is performed by placing the thermometer in water directly and reading the temperature only after a period of time that ensures constant values.
- Odor is determined organoleptically at room temperature and at 40ºC; the water sample is poured into an Erlenmeyer flask, covered with a watch glass and placed on a water bath; when the desired temperature is reached, the flask is removed from the bath, uncovered and tested for odor immediately.
- As a standard method for color measurement, the platinum-cobalt method based on a visual comparison of the sample with specially colored discs of certain values according to the Pt-Co scale is applied.
- Turbidity is measured with an optical turbidimeter, i.e. by photoelectric measurement of the intensity of light transmitted through the suspension. The sample of water for testing is poured into a cuvette and the result is read on the display of the apparatus. Turbidity measured in this way is expressed using nephelometric units (NTU), results are usually in the range of 0-40 NTU. For the periods 1977 and 2006, a conductometric test method was also used, the values of which are comparative to those of 2025, but were expressed in different units (SiO_2/L).
- Determination of pH values of the samples is performed using a pH meter. Prior to performing measurements, the probe should be washed thoroughly with distilled water. The probe is immersed in water directly and the value is read on the display.
- Electrical conductivity measurement is performed using a probe that consists of two electrodes inside the unit, these establish electrical contact with the water and enable the measurement of electrical conductivity. The probe is immersed in water directly and the value is read on the display.
- Spectrophotometric determination of nitrates is carried out by direct transfer of water into quartz cuvettes of at least 1 cm, followed by measurements performed at 220 nm and 275 nm on spectrophotometer. A blank test is also performed using distilled water. Since dissolved organic matter can absorb at 220 nm, while NO_3^- does not absorb at 275 nm, the second measurement performed at 275 nm is used for nitrate correction.
- Spectrophotometric determination of nitrite is determined by the formation of a reddish-pink azo color at pH 2,0-2,5 in the reaction of diazotized sulfanilamide with N-(1-naphthyl)-ethylenediamine dihydrochloride (NED dihydrochloride). A 50 mL sample of the test water is poured into an Erlenmeyer flask, and 2 mL of colored reagent is added. The absorbance of the resulting solution is measured on a spectrophotometer at 543 nm using glass cuvettes.
- Determination of ammonia is carried out using the phenanthate method by pouring 25 mL of test water into an Erlenmeyer flask and gradually adding 1 mL of phenol solution followed by 1 mL of Na-nitroprusside solution (as a catalysts). This is followed by addition of hypochlorite solution (2,5 mL) for oxidation. The sample is covered with parafilm and left at room temperature

(22-27 °C) for at least one hour for the color development. Measurement on a spectrophotometer at 640 nm with cuvettes of 1 cm or larger is performed. A test using distilled water as a blank is performed in parallel.

- Chlorides are determined by Mohr's method, by adding 1 mL of potassium chromate indicator into 100 mL of the sample, followed by titrating in a solution of $AgNO_3$ (0,02 mol/L) until the color of the sample changes to reddish-brown.
- Consumption of $KMnO_4$ - the permanganate index is determined by heating 25 mL of the test water sample and 5 mL of sulfuric acid in a boiling water bath (10 min). This is followed by addition of potassium permanganate (5 mL) and reheating for 10 minutes after which Na-oxalate solution (5 mL) is added and the mixture left until discoloration is observed. The discolored mixture is titrated with permanganate until a light pink color detected.
- Residual chlorine is determined during water sampling at the site using a chlorine photometer. The sample is poured into a cuvette and a few drops of the reagent are added. The sample is measured in a photometer, and the value is read off the display.

RESULTS

This chapter presents the testing results of microbiological and physicochemical parameters of raw and treated water in the periods April/May 1977, 2006 and 2025. The analyses were performed on samples taken at the water intake locations of the River Vrbas and from the reservoir following the conditioning process. The interpretation of the results was carried out according to the Regulation on the health safety of water for human consumption (Official Gazette of the Republic of Srpska, No 88/17, 97/18, 93/23 and 96/24)

Microbiological characteristics of raw water

Table 3. Microbiological Characteristics of Raw Water – The River Vrbas, April 1977, 2006 And 2025

MICROBIOLOGICAL PARAMETERS		TEST RESULTS April 1977	TEST RESULTS April 2006	TEST RESULTS April 2025
Coliform bacteria	/100mL	21000	more than 161	MPN>18
Among coliform bacteria, 2025 E.coli was identified	/100mL	E. coli	E. coli	MPN>18
Fecal coliform bacteria	/100mlL	not sought	found	not sought
Total aerobic mesophilic bacteria count/2025 colony count at 37 ºC	/1mL	1400	200	100
Colony count at 22 ºC	/1mL	not sought	not sought	220
Faecal streptococci/Enterococci	/100mL	0	found	>80
Proteus species	/100mL	0	0	not sought
Sulfite-reducing clostridia	/100mL	not sought	50	20
Pseudomonas aeruginosa	/100mL	not sought	0	not sought

Table 4. Microbiological Characteristics of Raw Water – The River Vrbas, May 1977, 2006 And 2025

MICROBIOLOGICAL PARAMETERS		TEST RESULTS May 1977	TEST RESULTS May 2006	TEST RESULTS May 2025
Coliform bacteria	/100mL	8800	more than 161	MPN>18
Among coliform bacteria, 2025 E.coli was identified	/100mL	E. coli	E. coli	MPN>18
Fecal coliform bacteria	/100mL	not sought	found	not sought
Total aerobic mesophilic bacteria count/2025 colony count at 37 ºC	/1mL	150	50	100

Colony count at 22 ºC	/1mL	not sought	not sought	220
Faecal streptococci /Enterococci	/100mL	0	found	>80
Proteus species	/100mL	0	0	not sought
Sulfite-reducing clostridia	/100mL	not sought	40	20
Pseudomonas aeruginosa	/100mL	not sought	0	not sought

The test results indicate the presence of a significant number of coliform bacteria, including fecal forms such as Escherichia coli, which confirms the sanitary-fecal contamination of the River Vrbas.

The number of aerobic mesophilic bacteria at 37 ºC was high in earlier analyses (in 1977, it was 1400 CFU/mL), while in 2025, it was 100 CFU/mL.

The number of colonies at 22 ºC in 2025 was 220 CFU/mL, while it had not been monitored before.

The presence of Enterococcus is also significant (>80/100 mL in 2025), while sulfite-reducing clostridia ranged from 0 to 50/100 mL.

The presence of other pathogens (Proteuss spp. and P. aeruginosa) was not detected in all the periods studied.

Physico-chemical characteristics of raw water

Table 5. Physico-Chemical Parameters of Raw Water – The River Vrbas, April 1977, 2006 and 2025

PHYSICOCHEMICAL PARAMETERS	TEST RESULTS April 1977	TEST RESULTS April 2006	TEST RESULTS April 2025
Water temperature at ºC	9,2	9,3	11,7
Color-degree on the Co-Pt scale	0	0	5
Smell	without	without	without
Turbidity	1000 mg SiO_2/L	5 mg SiO_2/L	2,56 (NTU scale)
pH-value	8,2	7,66	8,06
$KMnO_4$ mg/L consumption	21,5	7,3	< 0,5
Electrical conductivity µS/c^m-1	-	410	298
Ammonia	0,00	0,00	< 0,10
Nitrites as N mg/L	0,001	0,003	0,02
Nitrates as N mg/L	1,00	1,00	< 2,0
Chlorides mg/L	10	10	< 5,0
Sulfates mg/L	21,7	34,8	not sought
Total alkalinity $CaCO_3$/L	36	210,1	not sought
Carbonate hardness $CaCO_3$/L	10,8	210,1	not sought
Total hardness $CaCO_3$/L	12,46	228,1	not sought
Evaporation residue mg/L	448	250	not sought
Loss on ignition mg/L	152	61	not sought
Residue on ignition mg/L	296	189	not sought
Residual chlorine g/L	0	0,00	not sought
Dissolved oxygen	14,44	11,95	not sought
Biological oxygen demand-BPK_5	-	2,13	not sought

Table 6. Physico-Chemical Characteristics of Raw Water – The River Vrbas, May 1977, 2006 and 2025

PHYSICOCHEMICAL PARAMETERS	TEST RESULTS May 1977	TEST RESULTS May 2006	TEST RESULTS May 2025
Water temperature at ºC	12,4	10,3	13,4
Color-degree on the Co-Pt scale	0	0	5
Smell	without	without	without
Turbidity	20 mg SiO_2/L	5 mg SiO_2/L	3,01 (NTU scale)
pH-value	8,2	7,86	7,84
$KMnO_4$ mg/L consumption	11,89	6,8	< 0,5
Electrical conductivity µS/cm^{-1}	-	398	381
Ammonia mg/L	0,00	0,00	< 0,10
Nitrites as N mg/L	0,002	0,002	0,02
Nitrates as N mg/L	1,00	1,00	< 2,0
Chlorides mg/L	8	12	< 5,0
Sulfates mg/L	65,3	33,9	not sought
Total alkalinity $CaCO_3$/L	36	200,0	not sought
Carbonate hardness $CaCO_3$/L	9,59	200,0	not sought
Total hardness $CaCO_3$/L	10,08	231,3	not sought
Evaporation residue mg/L	270	249	not sought
Loss on ignition mg/L	112	63	not sought
Residue on ignition mg/L	158	186	not sought
Residual chlorine g/L	0	0,00	not sought
Dissolved oxygen	11,44	12,06	not sought
Biological oxygen demomd-BPK_5	-	1,92	not sought

The temperature of the raw water (the River Vrbas) varied from 9.20 ºC to 13.42 ºC depending on the sampling period. Smell and colour were not significant, while turbidity decreased significantly over the years, from 1000 SiO_2/L in 1977 to 2.56 NTU in 2025.

pH-value remained stable (7.66-8.20), and the $KMnO_4$ consumption decreased, indicating better oxidative capacity of the water in the recent period.

Electrical conductivity was within the prescribed values, and the concentrations of ammonia, nitrate and nitrite remained low.

In 2025, the chloride concentration was less than 5 mg/L, while sulfates and hardness were not analyzed.

The water was well saturated with oxygen (1977:14.44 mg/L), which indicates the capacity for natural self-purification in the river.

Microbiological characteristics of purified water

Table 7. Microbiological Characteristics of Drinking Water-Reservoir, April 1977, 2006 and 2025

MICROBIOLOGICAL PARAMETERS		TEST RESULTS April 1977	TEST RESULTS April 2006	TEST RESULTS April 2025
Coliform bacteria	/100mL	0	0	0
Among coliform bacteria, 2025 E.coli was identified	/100mL	0	0	0
Fecal coliform bacteria	/100mL	not sought	0	not sought
Total aerobic mesophilic bacteria count/2025 colony count at 37 ºC	/1mL	0	0	0
Colony count at 22 ºC	/1mL	not sought	not sought	0

Faecal streptococci/for 2025 Enterococci	/100mL	0	0	0
Proteus species	/100mL	0	0	not sought
Sulfite-reducing clostridia	/100mL	not sought	0	not sought
Pseudomonas aeruginosa	/100mL	not sought	0	not sought

Table 8. Microbiological Characteristics of Drinking Water – Reservoir, May 1977, 2006 and 2025

MICROBIOLOGICAL PARAMETERS		TEST RESULTS May 1977	TEST RESULTS May 2006	TEST RESULTS May 2025
Coliform bacteria	/100mL	0	0	0
Among coliform bacteria, 2025 E.coli was identified	/100mL	0	0	0
Fecal coliform bacteria	/100mL	not sought	0	not sought
Total aerobic mesophilic bacteria count/2025 colony count at 37 ºC	/1mL	0	0	0
Colony count at 22 ºC	/1mL	not sought	not sought	0
Faecal streptococci/for 2025 Enterococci	/100mL	0	0	0
Proteus species	/100mL	0	0	not sought
Sulfite-reducing clostridia	/100mL	not sought	0	not sought
Pseudomonas aeruginosa	/100mL	not sought	0	not sought

The test results showed that all samples of purified water from the reservoir were microbiologically correct. In all the years tested (1977, 2006 and 2025), coliform bacteria, including Escherichia coli, were not detected. There were no Enterococci, Pseudomonas aeruginosa, sulfite-reducing clostridia, or other microbiological indicators of contamination.

Physico-chemical characteristics of purified water

Table 9. Physico-Chemical Characteristics of Drinking Water – Reservoir, April 1977, 2006 and 2025

PHYSICOCHEMICAL PARAMETERS	TEST RESULTS April 1977	TEST RESULTS April 2006	TEST RESULTS April 2025
Water temperature at ºC	8,6	9,5	11,7
Color-degree on the Co-Pt scale	0	0	< 2,5
Smell	without	without	without
Turbidity	0 mg SiO_2/L	0 mg SiO_2/L	< 0,02 (NTU scale)
pH-value	8,1	7,96	7,70
$KMnO_4$ mg/L consumption	4,4	4,1	< 0,5
Electrical conductivity µS/cm^{-1}	-	325,5	317
Ammonia mg/L	0,00	0,00	< 0,10
Nitrites as N mg/L	0,003	0,00	< 0,01
Nitrates as N mg/L	1,00	1,00	< 2,0
Chlorides mg/L	12	12	< 5,0
Sulfates mg/L	26,9	28,3	not sought
Total alkalinity $CaCO_3$/L	40	38	not sought
Carbonate hardness $CaCO_3$/L	11,2	11,9	not sought
Total hardness $CaCO_3$/L	11,76	12,26	not sought
Evaporation residue mg/L	268	211	not sought
Loss on ignition mg/L	158	70	not sought
Residue on ignition mg/L	110	141	not sought
Residual chlorine g/L	0,5	0,5	0,48

Table 10. Physico-Chemical Characteristics of Drinking Water – Reservoir, May 1977, 2006 and 2025

PHYSICOCHEMICAL PARAMETERS	TEST RESULTS May 1977	TEST RESULTS May 2006	TEST RESULTS May 2025
Water temperature ºC	8,6	9,5	13,4
Color-degree on the Co-Pt scale	0	0	< 2,5
Smell	without	without	without
Turbidity	0 mg SiO_2/L	0 mg SiO_2/L	< 0,02 (NTU scale)
pH-value	8,1	7,96	7,43
$KMnO_4$ mg/L consumption	4,4	4,1	< 0,5
Electrical conductivity µS/cm^{-1}	-	325,5	404
Ammonia mg/L	0,00	0,00	< 0,10
Nitrites as N mg/L	0,003	0,00	< 0,01
Nitrates as N mg/L	1,00	1,00	< 2,0
Chlorides mg/L	12	12	< 5,0
Sulfates mg/L	26,9	28,3	not sought
Total alkalinity $CaCO_3$/L	40	38	not sought
Carbonate hardness $CaCO_3$/L	11,2	11,9	not sought
Total hardness $CaCO_3$/L	11,76	12,26	not sought
Evaporation residue mg/L	268	211	not sought
Loss on ignition mg/L	158	70	not sought
Residue on ignition mg/L	110	141	not sought
Residual chlorine g/L	0,5	0,5	0,47

The temperature of the treated water ranged from 8.6 ºC to 13.4 ºC.

Smell and colour were absent, and turbidity was reduced to values below 0.02 NTU.

pH-values were stable (7.43-8.1). $KMnO_4$ consumption was significantly lower in 2025 (< 0.5 mg/L), indicating a high degree of oxidizability after treatment.

Residual chlorine was stable and in accordance with recommendations (0.47-0.50 mg/L). Ammonia, nitrite and nitrate concentrations were low, and chlorides showed a decrease in concentration (< 0.5 mg/L). Other parameters (hardness, alkalinity, sulfates) in compliance with applicable regulations were not analyzed.

Conclusions based on the results:

1. Raw water of the Vrbas shows signs of fecal contamination and significant variability in physicochemical parameters, but with a decrease in load in recent years.
2. The water conditioning process in the Banja Luka Water Supply System effectively removes microbiological and most physicochemical pollutants.
3. Purified water meets all applicable microbiological and physicochemical standards prescribed by the Regulation.

These results confirm the validity and functionality of the existing technological line for water purification, as well as the need for its constant improvement in accordance with changes in raw water quality.

CONCLUSION

According to analyses in three time periods (1977, 2006 and 2025), the raw water of the River Vrbas still represents a valuable, although challenging, source for the drinking water production. Progress has been noted in reducing the microbiological and chemical load of raw water, but continuous water quality monitoring and preventive measures aimed at preserving the quality of the source are still required.

On the other hand, the water conditioning in the Banja Luka Water Supply System has proven to be highly efficient and stable. Through the application of modern methods – including ozonation, filtration and chlorination – a high degree of contamination elimination has been achieved, thus ensuring safe drinking water supply for end users.

All tested purified water samples met the criteria of the Regulation on the health safety of drinking water, both in microbiological and physicochemical terms. This confirms that the existing water treatment system in Banja Luka is in compliance with modern standards and can meet the challenges of modern urban supply.

Given the increased hydrological oscillations and climate change that may affect the quality of surface waters, these activities are recommended:

1. continuous 24-hour monitoring of raw water quality,
2. improving measures to protect water sources from fecal and industrial contamination,
3. regular technological improvement in processing plants and
4. additional education of personnel and strengthening laboratory capacities.

Maintaining a high-quality drinking water is of crucial significance for protecting population health and preserving ecological stability in a broader social context.

Climate change gives rise to changes in chemical composition of water. In order to sustain a safe and stable water supply, it is crucial to develop strategies for chemical monitoring and prompt actions to the changes (*https://www.eea.europa.eu/en/newsroom/news/state-of-water*).

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Recived: May 31, 2025
Accepted: July 23, 2025

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