OPEN ACCESS

ISSN 2587-3458
e-ISSN 2587-3466

Category A

OH&RM ONE HEALTH & RISK MANAGEMENT

THE SCIENTIFIC JOURNAL OF THE
MOLDAVIAN BIOSAFETY AND BIOSECURITY ASSOCIATION

VOLUME 5, ISSUI 4/OCTOBER 2024

The Moldovan Association for Biosafety and Biosecurity (MDBBA) is a scientific and practical, instructive and educational, non-governmental, apolitical and non-profit professional organization, created in 2017.

The main objective of the association is the development of good practices and culture in the field of biosafety and biosecurity and the promotion of knowledge within professional and research-innovation groups.

Biosafety – includes security principles, technologies and rules to be followed to prevent unintended exposure to pathogens and toxins or their accidental release/leakage.

"Protection of personnel, population from unintended exposure to pathogens/biohazardous material".

Biosecurity - includes a wide spectrum of measures (biosecurity policies, regulatory regime, scientific and technical measures) applied in an organized framework, necessary to minimize risks (prevention of actions, terrorist attacks by the intentional release of pathogens or toxins as well as loss, their theft or misuse).

"Protection and prevention of theft, intentional misuse of pathologies/biohazardous material".

Risk management – is a decision-making process in which the results of risk assessment (the process of estimating workplace hazards) are integrated with economic, technical, social and political principles to generate strategies for risk reduction.

CONTENTS –TABLE DES MATIÈRES

Quarterly edition
Languages of publication: English, French
Founder: Asociaţia de Biosiguranţă şi Biosecuritate din Republica Moldova

Category A
Scopus®

ISSN 2587-3458 (Print)
e-ISSN 2587-3466 (Online)
Edited by: Typography "Print-Caro", Edition: 300 ex.
Registered at the Ministry of Justice with no. 476676, 05th of July, 2017

Harnessing multidisciplinary expertise to combat antimicrobial resistance: a *One Health* approach for sustainable solutions

Gloria CÓRDOBA,
MD MPH PhD in Public Health and Epidemiology,
Senior scientific advisor for ICARS,
Denmark, Copenhagen

Antimicrobial resistance (AMR) poses a threat to global health, thus requiring a collaborative effort from professionals across diverse disciplines to achieve optimal health outcomes for humans, animals, plants, and the environment.

Therefore, it is essential to recognize that -effective planning and implementation of sustainable solutions will only be achievable when multidisciplinary research teams, including expertise beyond health sciences, such as implementation scientist and economists, are regarded as a primary source of high-quality evidence rather than merely a supplementary or unconventional approach to scientific research.

For example, a multidisciplinary and *One Health* approach involves not only creating a comprehensive system for monitoring AMR trends in humans, animals, and the environment but also identifying the most cost-effective methods, as well as understanding the factors that motivate various stakeholders to change their behavior and provide high-quality, timely data. Besides, integrating findings from cost-effectiveness studies into broader AMR programs, enables stakeholders to make informed decisions that would optimize health outcomes while minimizing economic burdens.

The *One Health and Risk Management* journal promotes interdisciplinary collaboration and supports innovative research within The *One Health* Field, particularly regarding AMR. Thus, I would like to sincerely congratulate the colleagues from Republic of Moldova for their remarkable achievement in publishing the fourth issue of this national journal. This initiative encourages collaboration, stimulate innovative research, as well as contribute significantly to the overall global efforts in tackling the AMR-related challenges. Enjoy reading a good compilation of multidisciplinary work.

OH&RM ONE HEALTH & RISK MANAGEMENT

VOL. 5, ISSUE 4
2024

RESEARCH ARTICLE – ARTICLES DE RECHERCHE

BIOLOGICAL SCIENCES

OPEN ACCESS

HAEMATOCOCCUS PLUVIALIS DERIVED ASTAXANTHIN – A POWERFUL BIOACTIVE COMPOUND FOR VEGETABLE OILS

Ludmila RUDI[1], Ecaterina PLINGAU[2], Vera MISCU[1]

[1]Institute of Microbiology and Biotechnology, Technical University of Moldova, Chisinau, Republic of Moldova

[2] Doctoral School of Natural Sciences, Moldova State University, Chisinau, Republic of Moldova

Corresponding author: Ludmila Rudi, e-mail: ludmila.rudi@imb.utm.md

DOI: 10.38045/ohrm.2024.4.01 CZU: 582.263:577.1:665.3

Keywords: *Astaxanthin, Haematococcus pluvialis, bioactive compound, antioxidant, vegetable oil, conjugated dienes.*

Introduction. *The beneficial effects on health result from the protective action of astaxanthin, (AXT) a powerful antioxidant capable of scavenging free radicals and protecting cells from oxidative stress. The study aimed to evaluate the protective role of astaxanthin derived from Haematococcus pluvialis in reducing the thermal oxidation of fatty acids in vegetable oils.*

Material and methods. *Astaxanthin, obtained by extraction from the biomass of Haematococcus pluvialis, at a concentration of 0.26-0.29 mg/mL, was added to olive, sunflower, almond, walnut, sesame, and poppy seed oils. The progression of oxidation was monitored based on the formation of conjugated dienes. The formation of conjugated dienes was monitored spectrophotometrically.*

Results. *Astaxanthin reduced the content of conjugated dienes in sesame, almond, and walnut oils by 30-34%. A strong antioxidant effect of AXT was noted in the case of poppy seed oil, for which the formation of conjugated dienes was decreased by 42% and the oxidation was delayed by 60 min when exposed to high temperatures compared to native oil. For sunflower oil, which exhibited a high degree of thermal oxidation, addition of AXT reduced the formation of conjugated dienes by 22% during the experiment.*

Conclusions. *Astaxanthin from Haematococcus pluvialis significantly reduced the formation of conjugated dienes, indicating that it does not act as a prooxidant in various vegetable oils.*

Cuvinte-cheie: *Astaxantina, Haematococcus pluvialis, compus bioactiv, antioxidant, ulei vegetal, diene conjugate.*

ASTAXANTINA DERIVATĂ DIN *HAEMATOCOCCUS PLUVIALIS* – UN PUTERNIC COMPUȘ BIOACTIV PENTRU ULEIURILE VEGETALE

Introducere. *Beneficiile astaxantinei (AXT) pentru sănătate rezultă din acțiunea sa protectoare. Acest antioxidant puternic neutralizează radicalii liberi și protejează celulele împotriva stresului oxidativ. Studiul a avut ca scop evaluarea rolului protector al astaxantinei, obținute din Haematococcus pluvialis, în reducerea efectelor oxidării termice asupra acizilor grași din uleiurile vegetale.*

Material și metode*. Astaxantina, obținută prin extragere din biomasa de Haematococcus pluvialis, a fost adăugată la uleiurile: de măsline, floarea-soarelui, migdale, nuci, susan și de mac în concentrația de 0,26-0,29 mg/ml. Evoluția procesului oxidativ a fost monitorizată în baza formării dienelor conjugate. Formarea dienelor conjugate a fost înregistrată spectrofotometric.*

Rezultate*. Astaxantina a redus cu 30-34% conținutul dienelor conjugate în uleiurile de susan, migdale și de nuci. Astaxantina a demonstrat un efect antioxidant semnificativ pentru uleiul de mac, expus temperaturilor înalte, diminuând formarea dienelor conjugate cu 42% și amânând oxidarea cu 60 de minute, în comparație cu uleiul netratat. Pentru uleiul de floarea-soarelui, care a prezentat un grad ridicat de oxidare termică, adăugarea de AXT a redus formarea dienelor conjugate cu 22%.*

Concluzii*. Astaxantina derivată din Haematococcus pluvialis a redus semnificativ formarea dienelor conjugate în diverse uleiuri vegetale, indicând lipsa efectului prooxidant.*

INTRODUCTION

Astaxanthin (AXT) is a xanthophyll carotenoid with a unique molecular structure. This distinctive structure imparts strong antioxidant properties to AXT, allowing it to function both inside and outside the cell membrane (1). It has been widely studied for its health benefits, including alleviating diabetes mellitus, neurodegenerative and cardiovascular diseases, hepatic disorders, and providing protection against various cancers. These effects stem from its ability to scavenge free radicals and shield cells from oxidative stress (2, 3).

One of the most recognized unconventional sources of astaxanthin is the unicellular green alga *Haematococcus pluvialis*, which can accumulate 1.5-3% pigment on a dry cell weight basis (4). Astaxanthin, produced by *H. pluvialis*, is the main natural source for human consumption. Due to its high price and limited sources, AXT is not well-known to consumers and is undervalued by food manufacturers. The antioxidant potential of AXT allows food technologists to offer a wide range of functional foods (5, 6). Incorporating astaxanthin into oils is a promising alternative to using this pigment (7).

Haematococcus pluvialis, a unicellular green alga, is a major natural source of astaxanthin, primarily used for human consumption (4). Despite its high price and limited sources, astaxanthin's antioxidant potential makes it valuable in developing functional foods (5, 6). Incorporating astaxanthin into oils enhances oxidative stability, offering a promising alternative for health-promoting food products (7).

Vegetable oils have long been regarded as functional foods and nutraceuticals, offering a variety of beneficial effects on human health (8). For example, olive oil is an essential component of the Mediterranean diet and plays a crucial role in reducing the incidence of cardiovascular diseases, including myocardial infarction and stroke. Oleic acid is the most abundant monounsaturated fatty acid in olive oil, with its concentration ranging from 56% to 84% of the total fatty acid content. Tocopherols, hydrophilic and lipophilic phenols, and other minor constituents account for 1-2%. All these components help boost heart health (9). Walnut oil is widely used in traditional medicine and has become a popular dietary supplement in many countries. Walnut-rich nutrition is viable to prevent declining cholinergic function in the brain and reduce oxidative stress in neurons by activating antioxidant enzymes like superoxide dismutase and glutathione peroxidase (10). Research is being conducted to identify the mechanism of action of the oil as a valuable supplement in treatment of multiple sclerosis (11). In addition to its antioxidant activity, walnut oil significantly decreases serum tumor necrosis factor-α, interleukin-6, and IL-1β levels, improving the anti-inflammatory ability and generating anti-inflammatory compounds by restoring bacterial balance (12). The protective effects of sesame oil are manifested through the reduction of proinflammatory cytokines (11). Poppy seed oil can improve the plasma lipid profile and the antioxidant status of hepatocytes (13). Based on its fatty acid profile, almond oil is also a nutraceutical product with notable antiatherosclerotic, antihepatotoxic, and regenerative effects in humans (14, 15). Functional foods are defined as foods consumed as part of a normal diet that contain biologically active components (whether added or naturally present) with the potential to improve health and/or reduce the risk of disease (16). Vegetable oils are an excellent source of bioactive compounds that can be utilized in the nutraceuticals and functional foods field (17).

Incorporating natural bioactive into traditional foods to create new functional foods (such as oils, beverages, baked goods, and dairy products) is a rapidly growing global market (18). In the commercial segment of functional foods, the category of vegetable oils is experiencing the fastest growth. Consequently, a recent study has focused on the potential of astaxanthin as a natural additive of health-promoting compounds, useful for production of functional foods. Thus, the combination of linseed oil and astaxanthin can mitigate the effects of oxidative stress and reduce inflammatory processes, positioning itself as a functional food to prevent cardiovascular diseases (19).

Vegetable oils have been studied as solvents and stabilizers for astaxanthin obtained from natural sources. Vegetable oil may be one of the factors responsible for the increased bioavailability of astaxanthin, thus defining the application areas of the final product (20).

Incorporating natural astaxanthin into vegetable oil leverages astaxanthin's potent antioxidant properties, without pro-oxidant effects. This combination can substantially inhibit the oxidation of oils. Considering astaxanthin's potential to improve the oxidative stability of vegetable oils, *this study aimed* to evaluate its antioxidant effect when added to various vegetable oils.

MATERIAL AND METHODS

The biomass of unicellular green alga *Haematococcus pluvialis* CNMN-AV-05 strain, deposited in the National Collection of Nonpathogenic Microorganisms (Technical University of Moldova, Institute of Microbiology and Biotechnology, Chisinau, Moldova) served as a source of astaxanthin. The microalga was cultured in a mineral medium with the following composition (in g/L): $NaNO_3$ – 0.3; KH_2PO_4 – 0.02; K_2HPO_4 – 0.08; NaCl – 0.02; $CaCl_2$ – 0.05; $MgSO_4 \cdot 7H_2O$ – 0.01; $ZnSO_4 \cdot 7H_2O$ – 0.0001; $MnSO_4 \cdot 5H_2O$ – 0.0015; $CuSO_4 \cdot 5H_2O$ – 0.00008; H_3BO_3 – 0.0003; $(NH_4)6Mo_7O_{24} \cdot 4H_2O$ – 0.0003; $FeCl_3 \cdot 6H_2O$ – 0.0175; $Co(NO_3)2 \cdot 6H_2O$ – 0.0002; EDTA – 0.0075; at a temperature of 26°C, under constant illumination of 28 µmol m-2 s-1 with periodic stirring for the first ten days of cultivation. Astaxanthin production was induced by excessive illumination at 56 µmol $m^{-2}s^{-1}$ for 72 hours.

H. pluvialis biomass consisting of aplanospores (red cysts) was separated from the culture liquid by centrifugation for 5 min at 1500 g, and the cell pellet was disrupted by microwave treatment at 450 W for 120 seconds. To extract astaxanthin, 10 mg of the prepared biomass was mixed with 5 mL of 96% ethanol. The extraction process involved continuous stirring at room temperature for 30 minutes.

The astaxanthin content was determined spectrophotometrically at 478 nm. A calibration curve was prepared using synthetic astaxanthin with 98% purity (Merck KGaA, Darmstadt, Germany) over a concentration range of 0.5 to 4.0 µg/mL (n=7). The linearity of the calibration curve was confirmed with an r^2 value of 0.999.

Vegetable oils from sunflower, almond, poppy seeds, and walnut kernels were manufactured by "INDUSTRY INVESTMENT" SRL, Chisinau, Republic of Moldova. Sesame oil was produced by "Condiprod-Com" SRL, Ukraine, and olive oil-by "Helcom," Portugal.

To solubilize astaxanthin in oils, 100 mL of an oil suspension with 1.0 g of MW-treated biomass was prepared and stirred at room temperature for 180 minutes. The oils were then separated from the remaining biomass by decantation. The amount of astaxanthin in oils was determined spectrophotometrically and recalculated according to the calibration curve.

The presence of astaxanthin in oils was confirmed by recording absorption spectra with the determination of the specific maximum of astaxanthin at a wavelength of 482-484 nm.

In this study, lipid oxidation was induced by maintaining the samples at a temperature of 60°C in the dark. The formation of conjugated dienes was monitored using spectrophotometry. Vegetable oils were diluted with hexane at a ratio of 1:600 (v/v). The absorbance of these diluted oil samples was measured at 234 nm. Changes in absorbance values were recorded as indicators of diene formation (17).

The investigations were conducted in three independent experiments. The results were statistically analyzed by calculating the arithmetic mean, standard error, and confidence interval, using the parametric t-test with a significance level of $p<0.05$.

RESULTS

Astaxanthin obtained from *H. pluvialis* biomass was supplemented with vegetable oils in concentrations of 0.26-0.29 mg/mL. The temperature of 60℃, chosen as the inductor of the oxidation process, caused a slow process of fatty acid oxidation when exposed to an oxidizing factor.

Figure 1 shows the spectra of oils before and after solubilization of natural astaxanthin in them.

Vegetable oils with AXT exhibited absorption peaks at 482-484 nm, indicating the solubilization of astaxanthin in oils. These recorded absorption peaks are characteristic of the pigment. The content of astaxanthin in oils ranged from 0.26 mg/mL in the case of sesame oil and olive oil (fig. 1D, 1B), 0.72 mg/ml in sunflower oil and almond oil (fig. 1A, 1F) to 0.287-0.29 mg/mL in poppy seed oil and wal nut oil (fig. 1E, 1C). In most cases, the solubilization of astaxanthin in oils did not depend on the degree of unsaturation.

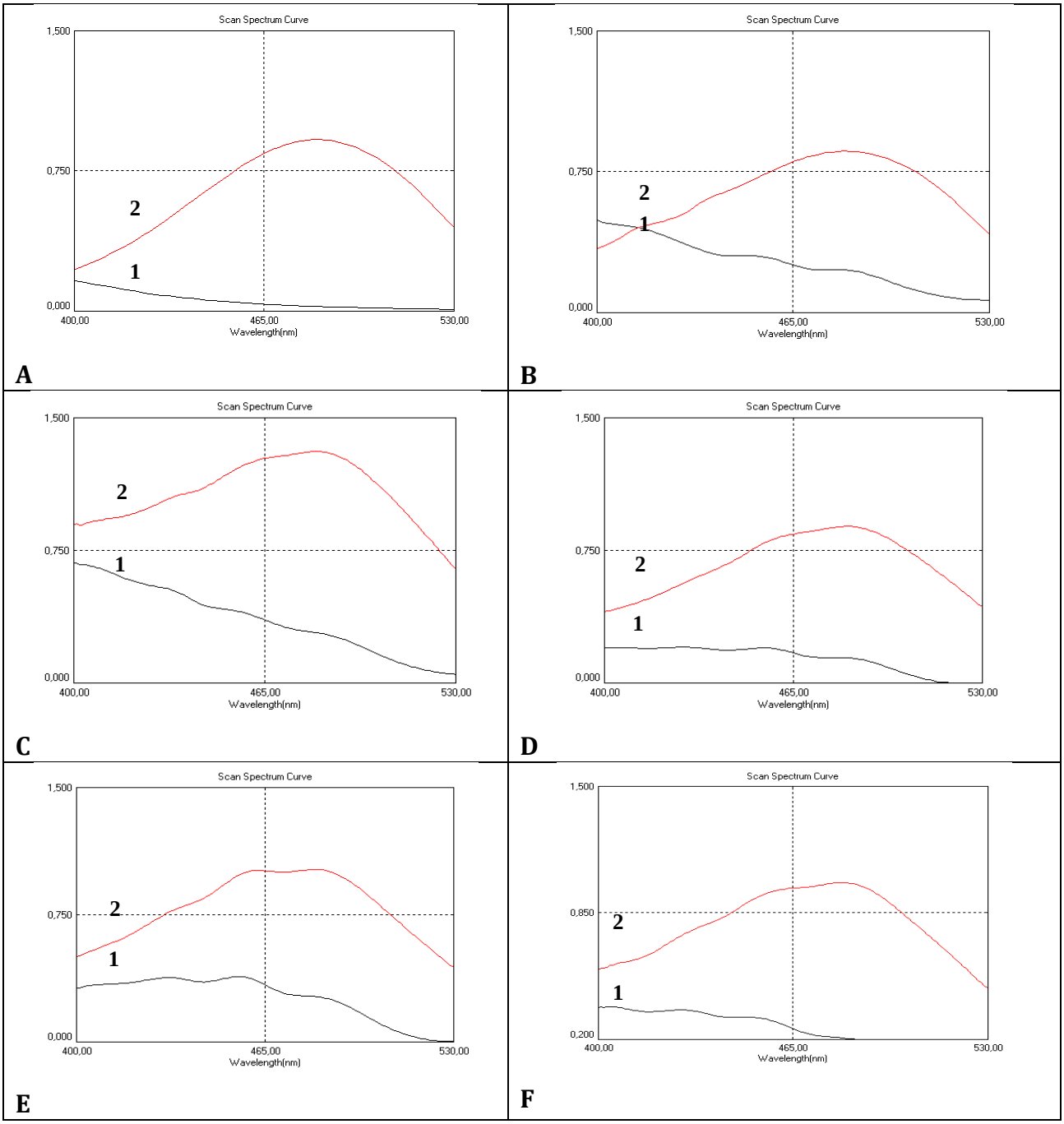
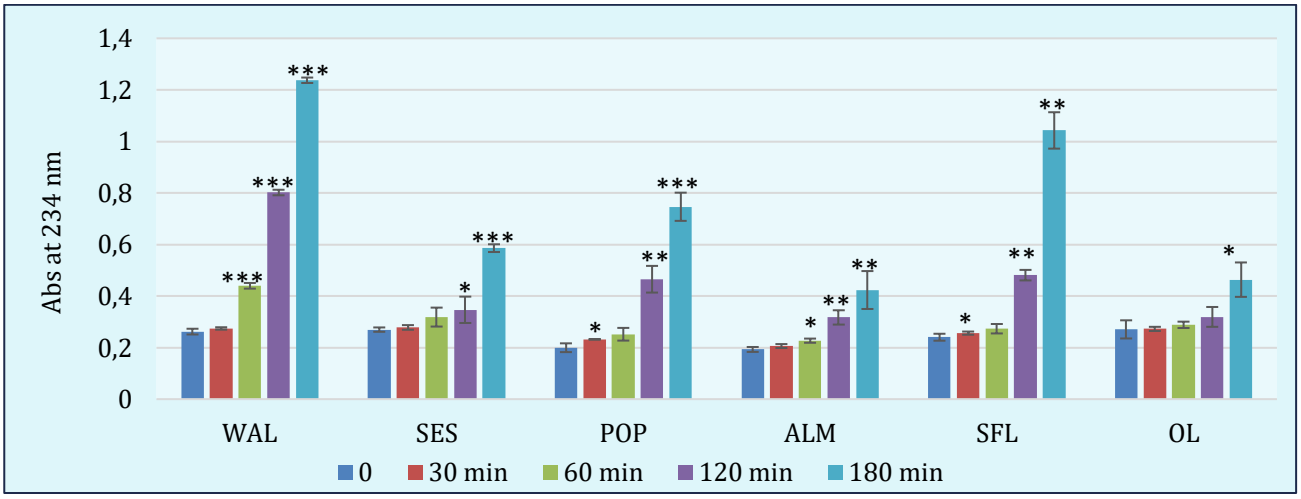


Figure 1. Spectra of oils used to solubilize natural AXT from *H. pluvialis*: A – sunflower oil; B – olive oil; C – walnut oil; D – sesame oil; E – poppy seed oil; F – almond oil; 1 – spectrum of native vegetable oil; 2 – spectrum of oil with solubilized natural astaxanthin.

Native vegetable oils and oils with solubilized natural astaxanthin were subjected to lipid oxidation. Vegetable oils and their mixtures with AXT that have not passed the oxidation test are called the zero variant. The change in the absorbance at 234 nm of oils in their native form was analyzed. Within 30 min, the absorbance at 234 nm for the oils under study did not change significantly. In poppy seed oil, the content of conjugated dienes increased by 16% ($p<0.05$). After 60 min, the stability of the olive oil was assessed. The content of conjugated dienes in sesame and almond oils increased by 18%, and in sunflower oil – by 14%. The absorption value of poppy seed oil increased by 26% at 234 nm. In the case of walnut oil, the absorption increased by 68% ($p<0.001$).



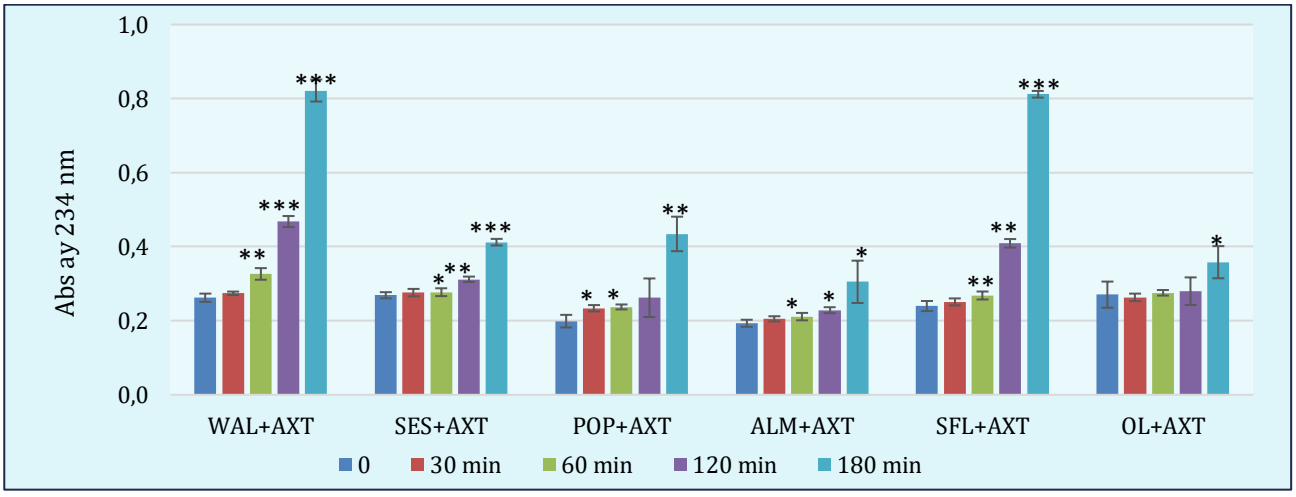
Note: n=3, "0"- native oils, * p<0.05; ** p<0.01; *** p<0.001

Figure 2. Changes in absorption data at 234 nm of vegetable oils subjected to a temperature of 60°C.

After 120 min of oil incubation at 60°C, the olive oil remained the most stable, with an 18% increase in absorbance at 234 nm. In sesame and almond oils, the absorption value increased by 28% and 64%, respectively. In the case of sunflower and poppy seed oils, the absorbance at 234 nm was doubled. A tripling of the content of conjugated dienes was recorded for walnut oil.

The duration of 180 min heat stress further induced lipid oxidation, which in the case of walnut and olive oils increased by another 45-54% compared to the content of dienes determined after an oxidation time of 120 min. The absorption value of sesame and poppy seed oils increased by 60-70%. The content of conjugated dienes in sunflower oil increased by more than 100%. Almond oil slowed the oxidation process, increasing the absorbance at 234 nm by 33%.

Compared to the zero variant, vegetable oils subjected to moderate heat stress accumulated conjugated dienes differently. Thus, the content of conjugated dienes in walnut and sunflower oils increased more than four times. In poppy seed oil, the content of conjugated dienes increased by 3.7 times (p<0.001). In sesame and almond oils, absorbance at 234 nm increased by 2.17 (p<0.001) – 2.19 (p<0.01) times. Olive oil turned out to be the least susceptible to the oxidation process caused by a temperature of 60°C, the increase in the absorbance value at 234 nm was 1.7 times (p<0.05).



Note: n=3, "0"- native oils, * p<0.05; **p<0.01; *** p<0.001

Figure 3. Changes in absorption data at 234 nm of vegetable oils with natural astaxanthin subjected to a temperature of 60°C.

Table 1. Fatty acid content (average values) in selected oils (8).

The type of oil	Unsaturated fatty acids, % total fatty acids				Saturated fatty acids, % total fatty acids	
	18:1n9	18:2n6	18:3n3	n3 + n6	18:0	16:0
Sunflower	28	62	-	62	3	6
Olive	66	16	2	18	2	16
Walnut	11	66	10	76	3	7
Sesame seeds	42	41	-	41	6	10
Poppy seeds	12	74	-	74	2	10
Almond	67	23	-	23	3	7

Astaxanthin, derived from microalgae *Haematococcus pluvialis*, has been added to vegetable oils to monitor the pigment's ability to stop fatty acid oxidation. During a 30-minute exposure at 60°C, the absorbance at 234 nm for oils with the addition of AXT changed similarly to native vegetable oils. The content of conjugated dienes in poppy seed oil increased by 17% ($p<0.05$). Heat stress exposure of oils with AXT for 60 min resulted in absorption stability at 234 nm for sesame, olive, and almond oils and a 24% ($p<0.01$) increase in walnut oil. Sunflower and poppy seed oils, supplemented with AXT, slightly changed their absorbance at 234 nm. After 120 min incubation at 60°C, the excess of conjugated dienes in sunflower and walnut oils was determined, while the absorption value increased by 70% and 80%, respectively.

In oils with AXT, for which the oxidation process started at a temperature regime of 60°C, the accumulation of conjugated dienes occurred slowly. The absorption value of poppy seed oil increased by 31% ($p<0.01$) at 234 nm. In the presence of astaxanthin, no doubling of conjugated dienes content was observed during the 120-minute heat stress accumulation period. Keeping vegetable oils with the addition of AXT for 180 min at a temperature of 60°C led to the oxidation of fatty acids with an increase in the absorbance value at 234 nm by 53% ($p<0.001$) in sesame oil and by 58% ($p<0.05$) in almond oil. In walnut and sunflower oils, the absorption value increased by more than threefold. Olive oil was stable, content of conjugated dienes was higher by 32% ($p<0.05$).

DISCUSSIONS

Vegetable oils examined as lipid carriers for astaxanthin can be utilized to produce nutraceuticals or AXT-based functional foods (21). The varied content of fatty acids in oils diversifies and enhances the nutraceutical component of oily astaxanthin preparations, defining their application areas. Among the identified fatty acids in walnut oil (about 77%), α-linoleic acid (ω-3) predominated at 66% (tab. 1).

Poppy seed oil is characterized by having more than 70% linoleic acid, and sunflower oil has over 62%. On the contrary, both olive and almond oils are dominated by oleic acid (66-67% of the total fatty acids). Sesame oil contains equal parts of oleic acid and linolenic acid. The content of polyene acids in vegetable oils is one factor that largely determines the oxidation process with the formation of conjugated dienes (22, 23). Among the vegetable oils subjected to a long-term test (63°C), rapeseed oil, containing up to 60% monounsaturated fatty acids, was the least susceptible to oxidation. Among the oils selected for the study, the most resistant to thermal oxidation were olive and almond oils with an oleic acid content of more than 65%, and sesame seed oil with over 40% oleic acid (tab. 1).

In another study of the thermal stability of vegetable oils, depending on their content of polyenoic fatty acids, the thermal stability of native sunflower oil was compared with that of sunflower oil with added oleic acid. The oils were exposed to 180-190°C for 8 hours over three days. The oil enriched with oleic acid showed better thermal stability than native sunflower oil (24).

When comparing oils that are rich in polyunsaturated omega-6 and omega-3 fatty acids, such as sunflower, walnut, and poppy seed oils, the oxidetion of these oils occurred at different rates. Walnut oil was the most prone to degradation processes, likely due to the presence of 10% linolenic acid. In our case, walnut oil had the highest values of formed dienes. Some studies have established that a determining factor in the intensity of oxidative processes in this oil is the palmitic acid content (25). Therefore, the technological conditions

affecting the fatty acid composition of this oil, are important (26).

Native sunflower and poppy seed oils similarly accumulated oxidative degradation products of fatty acids. In the oxidation process of vegetable oils at 180°C, sunflower oil exhibited the highest levels of oxidative degradation products (27). Poppy seed oil is considered stable against thermal-oxidative stress (28). The inherent antioxidants of the oils, those added for product preservation, and the extraction method applied were not considered (29). This assumption is supported by results showing the addition of astaxanthin, which significantly reduced the oxidation process in walnut, sunflower, and poppy seed oils, with the latter showing the most favorable result: a reduction in conjugated diene content by 42% ($p<0.01$).

The lowest values of diene content in native vegetable oils subjected to oxidation were found in olive, sesame, and almond oils (fig. 2). Olive oil contains the lowest levels of polyunsaturated fatty acids, followed by almond and sesame oils (tab. 1). Olive oil is considered one of the most resistant vegetable oils to high temperatures. These properties are due to its low polyunsaturated acid content (30) as well as the presence of antioxidant components, the content of which depends on the extraction techniques applied (31). Almond oil has also proven to be resistant to hyperthermic conditions. Studies have shown that this property depends on the origin of the almonds (32). In the experiment, sesame oil demonstrated stability against thermal oxidation, a known characteristic of the oil (33). Adding astaxanthin to olive oil, almond, and sesame oils did not alter their antioxidant properties, as they continued to exhibit low levels of conjugated dienes (fig. 3).

Astaxanthin in the studied oils delayed the oxidation of fatty acids. In native oils, the accumulation of conjugated dienes begins after 60 minutes of hyperthermia (fig. 2), whereas in oils with added astaxanthin, this process starts after 120 minutes (fig. 3).

Adding pigment extracts from *H. pluvialis* to sunflower oil, significantly increased ($p<0.05$) its oxidation stability during short-term exposure of 180°C (34). Astaxanthin donates a hydrogen atom to peroxyl radicals due to the presence of dihydroxyl groups in the β-ionone rings (35). In several edible oils, including sunflower, olive, and mustard oils, subjected to thermal oxidation at 50-70°C, astaxanthin remains an active component. However, a temperature of 180°C leads to the degradation of the antioxidant (20).

Products of microalgae origin are being studied as vegetable oil protectors. Microalga *Nannochloropsis oculata* in powder form added to soybean oil inhibited its oxidation (36). A positive dependence of the oxidative process in oils on the concentration of the added microalgae product has been demonstrated. Thus, 5.0% microalgal powder increased more than twice the stability index of soybean oil subjected to oxidation at temperatures of 120 and 130°C. Microalga *N. oculata* has been proposed as an antioxidant additive to vegetable oils to prevent the generation of free radicals during processing at high temperatures. Microalga *Chlorella vulgaris*, added to olive oil in concentrations of 0.5, 1.0, and 1.5%, significantly delayed the formation of oxidative degradation products by 20-33% compared to industrial antioxidants. The antioxidant effect of microalgae was even higher than that of β-carotene used as a control stabilizer (37). Microalgae-derived antioxidants are starting to gain popularity in the processing of functional and organic foods. Astaxanthin is included in the list of these antioxidants (9, 38).

CONCLUSIONS

1. Astaxanthin from *Haematococcus pluvialis* significantly reduced the formation of conjugated dienes, indicating that it does not act as a prooxidant in various vegetable oils. The addition of astaxanthin notably enhanced the thermal stability of oils, particularly those high in oleic acid. For example, the formation of conjugated dienes decreased by 42% in poppy seed oil and by 30-34% in sesame, almond, and walnut oils.
2. Olive oil showed the smallest increase in conjugated dienes when supplemented with astaxanthin, even under high-temperature conditions, demonstrating that astaxanthin can modify the functional properties of oils without acting as a pro-oxidant. Future studies should examine the long-term stability of enriched oils under various conditions and assess their sensory and nutritional impacts. Exploring the combination of astaxanthin with other antioxidants could provide insights into developing more effective antioxidant systems for food applications.

CONFLICT OF INTERESTS

The authors of the article deny the existence of any conflict of interest in the publication of this material.

FUNDING ACKNOWLEDGEMENT

This research was funded by the Government of Republic of Moldova, Ministry of Education and Research, Research Subprogram 020101 "InBioS-Innovative biotechnological solutions for agriculture, medicine and environment". Institutional financing contract no. 4/FI of 22 February 2024.

REFERENCES

1. Yuan JP, Peng J, Yin K, Wang JH. Potential health-promoting effects of astaxanthin: A high-value carotenoid mostly from microalgae. *Molecular Nutrition & Food Research*. 2011;55:150–165. doi:10.1002/mnfr.201000414
2. McNulty HP, Byun J, Lockwood SF, Jacob RF, Mason RP. Differential effects of carotenoids on lipid peroxidation due to membrane interactions: X-ray diffraction analysis. *Biochimica et Biophysica Acta.* 2007;1768(1):167-174. doi:10.1016/j.bbamem.2006.09.010
3. Regnier F, Bastias J, Rodriguez-Ruiz V, Caballero-Casero N, Caballo C, Sicilia D, Fuentes A, Maire M, Crepin M, Letourneur D, Gueguen V, Rubio S, Pavon-Djavid G. Astaxanthin from *Haematococcus pluvialis* Prevents Oxidative Stress on Human Endothelial Cells without Toxicity. *Marine Drugs*. 2015;13:2857-2874. doi:10.3390/md13052857
4. Sarada R, Tripathi U, Ravishankar GA. Influence of stress on astaxanthin production in *Haematococcus pluvialis* grown under different culture conditions. *Process Biochemistry.* 2002; 37(6):623-627. doi:10.1016/S0032-9592(01)00246-1
5. Stachowiak B, Szulc P. Astaxanthin for the Food Industry. *Molecules*. 2021;26(9):2666. doi:10.3390/molecules26092666
6. Yang Y, Kim B, Lee JY. Astaxanthin Structure, Metabolism, and Health Benefits. *Journal of Human Nutrition & Food Science.* 2013;1:1003:1-1003:11. Available from: https://www.jscimedcentral.com/public/assets/articles/nutrition-1-1003.pdf [Accessed: July 30th 2024].
7. Xu J, Gao H, Zhang L, Chen C, Yang W, Deng Q, Huang Q, Huang F. A combination of flaxseed oil and astaxanthin alleviates atherosclerosis risk factors in high fat diet fed rats. *Lipids in Health and Disease*. 2014;13:63. doi:10.1186/1476-511X-13-63
8. Morya S, Menaa F, Jimenez-Lopez C, Lourenço-Lopes C, BinMowyna MN, Alqahtani A. Nutraceutical and Pharmaceutical Behavior of Bioactive Compounds of Miracle Oilseeds: An Overview. *Foods*. 2022;11(13):1824. doi:10.3390/foods11131824
9. Nocella C, Cammisotto V, Fianchini L, D'Amico A, Novo M, Castellani V, Stefanini L, Violo F, Carnevale R. Extra virgin olive oil and cardiovascular diseases: benefits for human health. *Endocrine Metabolic & Immune Disorders Drug Targets.* 2018;18(1):4-13. doi:10.2174/1871530317666171114121533
10. Liao J, Feng L, Chen Y, Li M, Xu H. Walnut oil prevents scopolamine-induced memory dysfunction in a mous model. *Molecules*. 2020;25(7):1630. doi:10.3390/molecules25071630
11. Faraji F, Hashemi M, Ghiasabadi A, Davoudian S, Talaie A, Ganji A, Mosayebi G. Combination therapy with interferon beta-1a and sesame oil in multiple sclerosis. *Complementary Therapies in Medicine.* 2019;45:275-279. doi:10.1016/j.ctim.2019.04.010
12. Miao F, Shan C, Shad SAH, Akhtar RW, Geng S, Ning D, Wang X. The protective effect of walnut oil on lipopolysaccharide-induced acute intestinal injury in mice. *Food Science & Nutrition.* 2020;9(2):711-718. doi:10.1002/fsn3.2035
13. Fotschki B, Opyd P, Juskiewicz J, Wiczkowski W, Jurgonski A. Comparative effects of dietary hemp and poppy seed oil on lipid metabolism and the antioxidant status in lean and obese zucker rats. *Molecules*. 2020;25(12):2921. doi:10.3390/molecules25122921
14. Ahmad Z. The uses and properties of almond oil. *Complementary Therapies in Clinical Practice.* 2010;16(1):10-12. doi:10.1016/j.ctcp.2009.06.015
15. Sarkar SK, Miyaji T, Sasaki J, Biswas SN, Ali S, Salam A. Fatty acid composition, physicochemical and antioxidant properties of almond seed (*Terminalia catappia* L.) oil and its therapeutic uses. *Journal of Global Biosciences*. 2020;9(5):7419-7433. Available at: www.mutagens.co.in/jgb/vol.09/05/090511.pdf [Accessed: January 05th 2024]
16. Gul K, Singh AK, Jabeen R. Nutraceuticals and functional foods: the foods for the future world. *Critical Reviews in Food Science and Nutrition*. 2016; 56(16):2617–2627. doi:10.1080/10408398.2014.903384
17. Suárez M, Gual-Grau A, Ávila-Román J, Torres-Fuentes C, Mulero M, Aragonès G, Isabel Bravo F, Muguerza B. Oils and oilseeds in the nutraceutical and functional food industries. In: Lafarga T, Bobo G, Aguiló-Aguayo I, eds. *Oil and Oilseed Processing*. 2021. doi:10.1002/9781119575313.ch11
18. Lourenço SC, Moldão-Martins M, Alves VD. Antioxidants of natural plant origins: From sources to

food industry applications. *Molecules*. 2019; 24:4132. doi:10.3390/molecules24224132
19. Xu J, Rong S, Gao H, Chen C, Yang W, Deng Q, Huang Q, Xiao L, Huang F. A combination of flaxseed oil and astaxanthin improves hepatic lipid accumulation and reduces oxidative stress in high fat-diet fed rats. *Nutrients*. 2017;9(3):271. doi:10.3390/nu9030271
20. Rao AR, Sarada R, Sarada R, Ravishankar GA. Stabilization of astaxanthin in edible oils and its use as an antioxidant. *Journal of the Science of Food and Agriculture.*2007;87:957–965.doi:10.1002/jsfa.2766
21. Yang L, Gu J, Luan T, Qiao X, Cao Y, Xue C. Influence of oil matrixes on stability, antioxidant activity, bioaccessibility and bioavailability of astaxanthin ester. *Journal of the Science of Food and Agriculture*. 2021;101(4):1609-1617. doi:10.1002/jsfa.10780
22. Miraliakbari H, Shahidi F. Oxidative stability of tree nut oils. *Journal of Agricultural Food and Chemistry*. 2008;56:4751–4759. doi:10.1021/jf8000982
23. Maszewska M, Florowska A, Dluzewska E, Wroniak M, Marciniak-Lukasiak K, Zbikowska A. Oxidative Stability of selected Edible Oils. *Molecules.* 2008;23(7):1746. doi:10.3390/molecules23071746
24. Ali MA, Najmaldien AHA, Latip RA, Othman NH, Majid FAA, Salleh LM. Effect of heating at frying temperature on the quality characteristics of regular and high-oleic acid sunflower oils. *Acta Scientiarum Polonorum. Technologia Alimentaria*. 2013; 12(2):159-167. Available at: https://www. food. actapol.net /volume12/ issue2/3_2_2013. pdf [Accessed: June 03th 2024].
25. Ampofo J, Grilo FS, Langstaff S, Wang SC. Oxidative Stability of Walnut Kernel and Oil: Chemical Compositions and Sensory Aroma Compounds. *Foods*. 2022;11:3151. doi:10.3390/foods11193151
26. Li H, Han J, Zhao Z, Tian J, Fu X, Zhao Y, Wei C, Liu W. Roasting treatments affect oil extraction rate, fatty acids, oxidative stability, antioxidant activity, and flavor of walnut oil. *Frontiers in Nutrition*. 2023;9:1077081. doi:10.3389/fnut.2022.1077081
27. Amin MAl, Ali Abbas M, Shamsul AM, Aktarun N, Sook Chin C. Oxidative degradation of sunflower oil blended with roasted sesame oil during heating at frying temperature. *Grain & Oil Science and Technology.* 2023;6(1):34-42. doi:10.1016/j.gaost.2022.11.004
28. Cibulková Z, Čertík M, Dubaj T. Thermooxidative stability of poppy seeds studied by non-isothermal DSC measurements. *Food Chemistry.* 2014; 150:296-300. doi:10.1016/j.foodchem.2013.11.011
29. Dąbrowski G, Czaplicki S, Konopka I. Composition and quality of poppy (*Papaver somniferum* L.) seed oil depending on the extraction method. *LWT-Food Sciences and Technology*. 2020;134:110167. doi:10.1016/j.lwt.2020.110167
30. Lee J, Lee Y, Choe E. Temperature dependence of the autoxidation and antioxidants of soybean, sunflower, and olive oil. *European Food Research and Technology*. 2007;226:239–246. doi:10.1007/s00217-006-0532-5
31. Conte L, Milani A, Calligaris S, Rovellini P, Lucci P, Nicoli MC. Temperature Dependence of Oxidation Kinetics of Extra Virgin Olive Oil (EVOO) and Shelf-Life Prediction. *Foods.* 2020;9(3):295. doi:10.3390/foods9030295
32. Sidhu AR, Naz S, Mahesar SA, Kandhro AA, Khaskheli AR, et al. Effect of storage at elevated temperature on the quality and stability of differrent almond oils: a comprehensive study. *Food Materials Research.* 2023;3:30. doi:10.48130/FMR-2023-0030
33. Abou-Gharbia H.A, Shehata A, Shahidi F. Effect of processing on oxidative stability and lipid classes of sesame oil. *Food Research International.* 2000; 33:331-340. doi:10.1016/S0963-9969(00)00052-1
34. Wang L, Yang B, Yan B, Yao X. Supercritical fluid extraction of astaxanthin from *Haematococcus pluvialis* and its antioxidant potential in sunflower oil. *Innovative Food Science and Emerging Technologies*. 2012;13:120–127. doi:10.1016/j.ifset.2011.09.004
35. Naguib YM. Antioxidant activities of astaxanthin and related carotenoids. *J Agric Food Chem.* 2000;48:1150-4. doi:10.1021/jf991106k
36. Lee YL, Chuang YC, Su HM, Wu FS. Freeze-dried microalgae of Nannochloropsis oculata improve soybean oil's oxidative stability. *Applied Microbiology and Biotechnology.* 2013;97:9675–9683. doi:10.1007/s00253-013-5183-4
37. Alavi N, Golmakani MT. Improving oxidative stability of virgin olive oil by addition of microalga Chlorella vulgaris biomass. *Journal of Food Science and Technology*. 2017;54:2464–2473. doi:10.1007/s13197-017-2689-2
38. Begum H, Yusoff FM, Banerjee S, Khatoon H, Shariff M. Availability and Utilization of pigments from Microalgae. *Critical Reviews in Food Science and Nutrition*. 2016;56(13):2209-2222. doi:10.1080/10408398.2013.764841

Ludmila RUDI, SCOPUS ID: 55681134100; WoS Researcher ID: AAY-3219-2020
Vera MISCU, SCOPUS ID: 55681134100

Date of receipt of the manuscript: 09/06/2024
Date of acceptance for publication: 29/09/2024

OH&RM ONE HEALTH & RISK MANAGEMENT

VOL. 5, ISSUE 4
2024

MEDICAL SCIENCES

OPEN ACCESS

ENHANCING SEXUAL AND REPRODUCTIVE HEALTH LITERACY AMONG LOCAL AND REFUGEE YOUTH IN MOLDOVA: A COMMUNITY-ENGAGED DIGITAL HEALTH INITIATIVE

Camille THOMAS[1], Svetlana COCIU[2], Angela CAZACU-STRATU[2,3], Vesel QASIMI[3], Maria CHAVEZ[3], Serghei CEBANU[2], Sara L NAM[4]

[1]Association INTERSOS Moldova, Independent Consultant, Toronto, Canada
[2]*Nicolae Testemitanu* State University of Medicine and Pharmacy, Chisinau, Republic of Moldova
[3]Association INTERSOS Moldova, Chisinau, Republic of Moldova
[4]Association INTERSOS Moldova, Independent Consultant, Annecy, France

Corresponding author: Svetlana Cociu, e-mail: svetlana.cociu@usmf.md

DOI: 10.38045/ohrm.2024.4.02 CZU: [612.6+613.88+314.745.22](478)

Keywords: *adolescents, sexual and reproductive health, digital app, healthcare access, qualitative and quantitative study.*

Introduction. *Despite progress in sexual and reproductive health (SRH) in Moldova, adolescents still face barriers accessing accurate health information and services. To address these issues, a digital resource called the YK App was developed to enhance health literacy and healthcare access for Moldovan and refugee youth.* ***Material and methods.*** *Three regional focus group discussions were conducted with 50 youths aged 15-24, including Ukrainian refugees. These discussions, held in multiple languages, explored participants' SRH knowledge, information sources, barriers, and enablers. Qualitative data and socio-demographic profiles were analyzed thematically and descriptively.* ***Results.*** *Participants rely strongly on social media for SRH information, but identified significant gaps in their awareness, particularly on contraception, sexually transmitted infections, puberty and menstruation. While healthcare professionals are seen as credible sources, shame and fear of rejection often prevent direct involvement with healthcare services. Anonymity, confidentiality, and use of digital platforms were identified as key facilitators for accessing SRH information.* ***Conclusions.*** *The findings emphasize the potential of a digital, youth-focused SRH resource that adolescents can use independently. Key elements should include clear, easy-to-understand formats such as audio and video content, and collaboration with health professionals and official health organizations to ensure credibility.*

Cuvinte-cheie: *adolescenți, sănătate sexuală și repro-ductivă, aplicație digi-tală, acces la asistență medicală, studiu cali-tativ și cantitativ.*

ÎMBUNĂTĂȚIREA NIVELULUI DE CUNOȘTINȚE ÎN DOMENIUL SĂNĂTĂȚII SEXUALE ȘI REPRODUCTIVE AL TINERILOR AUTOHTONI ȘI AL CELOR REFUGIAȚI ÎN REPUBLICA MOLDOVA: O INIȚIATIVĂ DIGITALĂ COMUNITARĂ

Introducere. *Deși au fost realizate progrese în domeniul sănătății sexuale și reproductive (SSR) în Republica Moldova, adolescenții continuă să se confrunte cu dificultăți în accesarea informațiilor corecte și a serviciilor de sănătate. Pentru a răspunde acestor provocări, a fost dezvoltată YK App, o resursă digitală menită să sporească nivelul de alfabetizare în domeniul sănătății și accesul la asistență medicală pentru tinerii moldoveni și cei refugiați.* ***Material și metode.*** *S-au selectat 3 grupuri-țintă regionale, constituite din 50 de tineri cu vârste între 15-24 ani, inclusiv refugiați ucraineni. Discuțiile, desfășurate în mai multe limbi, au explorat cunoștințele participanților despre SSR, sursele de informații, barierele și factorii facilitatori. Datele calitative și profilurile socio-demografice au fost analizate tematic și descriptiv.* ***Rezultate.*** *Tinerii se informează preponderent pe rețelele sociale despre SSR, însă cunoștințele lor vizând , în special, problemele legate de contracepție, infecții cu transmitere sexuală, pubertate și menstruație prezintă lacune substanțiale. Deși specialiștii din domeniul sănătății sunt recunoscuți ca fiind niște surse credibile, rușinea și teama de respingere îi descurajează pe tineri în a se implica direct în soluționarea problemelor cu care se confruntă. Anonimatul, confidențialitatea și platformele digitale au fost identificate ca principale bariere în accesul la informații.* ***Concluzii.*** *Datele obținute relevă potențialul impactului pe care îl pot exercita resursele digitale SSR asupra tinerilor. Conținuturile formulate clar, ușor de înțeles (audio și video), colaborarea cu profesioniști din domeniul sănătății și cu organizațiile oficiale vor servi drept elemente-chei în asigurarea credibilității.*

INTRODUCTION

Sexual and reproductive health is a lifelong concern for both women and men, from infancy to old age, with significant implications for health in later stages of life (1). Recent figures from the UN Population Fund indicate that 1,8 billion adolescents, aged 10-19 years comprise over 16% of the global population (2). However, many young people face limitations due to social norms, cultural attitudes, institutional and structural barriers, and violations of their fundamental rights based on age. This issue is underscored in a report by the High Commissioner for Human Rights on youth and human rights (2).

The Republic of Moldova faces significant demographic and social challenges, particularly concerning its youth population. According to the National Bureau of Statistics (3), 17% of Moldovans are between 10 and 24 years old, and 11% are adolescents aged 10 to 19. Also, there is a high adolescent birth rate of 22.4 per 1,000 women aged 15 to 19, with more than 12% of these young women being married before turning 18 years old.

Recognizing these issues, the Moldovan government has prioritized youth-friendly health services. This focus is particularly crucial given the health crises in neighboring regions and the recent influx of Ukrainian refugees. As of April 14, 2024, nearly 120,000 refugees from Ukraine have been recorded in Moldova by the UNHCR, with the majority being female (60%) and children (52%) (4). This places additional pressure on Moldova's healthcare and social services, highlighting the urgent need for effective youth-focused interventions and support systems.

In the Republic of Moldova, sexual and reproductive health rights were recognized as a priority in 1994 after the International Conference on Population and Development in Cairo. The National Reproductive Health Strategy (2005-2015) mandated a life skills-based education course including SRH topics in schools, but only some SRH topics are covered so far in schools in the optional health education courses (5). An assessment of sexuality education conducted in 2021 found that there was full compliance of the integration of a comprehensive sexuality education into the national curriculum among only 28% of schools. Although this was more than a doubling from 12% in 2017, it still represents a considerable gap in meeting the informational needs on sexuality education for young people (6, 7). The authors' recommendations include the need align comprehensive sexuality education program in schools programs to international standards; strengthen referral and cooperation with youth-friendly services and invest in literacy regarding the benefits of sexuality education.

In response to meeting the SRH needs of adolescents and young people including refugees, the non-governmental organization, INTERSOS, in partnership with Neovita and *Nicolae Testemitanu* State University of Medicine and Pharmacy, Department of Preventive Medicine and other institutions, is implementing the "Community Participatory Digital Health Initiative". The initiative aims to identify ways to make SRH healthcare, services and information more accessible to adolescents and young adults, and to reduce barriers such as cost, geography, language, and anonymity. At the outset, a stakeholder meeting was held in Chisinau in May 2023, to consult with health implementers, donors, UN agencies, youths, non-governmental organizations and health providers to discuss the potential of an online application (the YK App) to achieve this aim. The potential for an App was agreed on, and the need to involve adolescents and young adults in the design and content of the App was agreed.

The *aim of the study* was to explore the adolescents' SRH needs in terms of knowledge and healthcare and develop a YK App among local and refugee youth.

MATERIAL AND METHODS

Study population

In total 50 young people, including refugees aged 15-24 were recruited. The recruitment process involved volunteers from the Neovita Youth Klinic Network and *Nicolae Testemitanu* State University of Medicine and Pharmacy.

Study tools

This was a qualitative study, but quantitative data were collected fully anonymously to describe the socio-demographic profile of respondents. Focus Group Discussions (FGD) were led by using the research study guide developed collaboratively by researchers from *Nicolae Testemitanu* State University of Medicine and Pharmacy of the

Republic of Moldova, INTERSOS, Neovita, and international SRH specialists. The guide covered questions related to young peoples' opinions about access to sexual and reproductive health information and services; previous experiences with and perspectives on using digital health technologies to increase health literacy and health care access; and recommendations for the creation of a successful mobile health App for young people. For the quantitative part - all participants completed a short socio-demographic information to allow us to describe some characteristics of the participants. Recognizing the potential biases of having both older and younger adolescents and youths from different background in the same group discussions, each FGD started with an ice-breaker activity. At the end of the FDG all the participants completed a short anonymous questionnaire to assess potential factors that may have either inhibited some participants from contributing to the discussion or may have caused them to provide acquiescent responses due to social acceptance or similar biases.

Data collection

Two experienced researchers with SRH expertise led 3 FGDs in each region (North, Center and South). Each FGD was composed of 8-16 participants and lasted between 90 to 120 minutes. FGDs were conducted in Romanian, Russian, or both languages. Each participant received a unique code which was used in discussions to anonymize the transcript. The FGDs were coded, audio-recorded with the agreement of the participants, manually transcribed, translated from Romanian and Russian to English for analyses.

Data analysis

Quantitative analysis was conducted using Excel 2020. Qualitative analyses were performed thematically, a coding scheme was built up inductively from the dataset and was applied to interview transcripts. New codes were added if a novel theme emerged that was not captured by the current coding scheme. The coding process was dynamic and collaborative; workshops were conducted with the researchers who collected the data in order to validate the themes.

Ethics approval

All participants were informed that their involvement would remain confidential and anonymous, as well as entirely voluntary. Signed informed consent was obtained from all participants over the age of 18. For participants under 18 years old, the signed informed consent was obtained from the guardians and verbal assent was obtained from the participants, and confirmed prior to each FGD. Ethics approval was also obtained.

RESULTS

Socio-demographic characteristics

Participants of both genders were included in equal proportions (tab. 1). Half of the participants were aged 15-16 years old (50%), and 28% were aged 17-18 years old, and 22% were aged 19-24 years old (fig. 1).

Table 1. Participants distribution by regions and gender.

Country regions	Female		Male		Total	
	N	%	N	%	N	%
FGD 1 (South)	<5	*10*	<5	*6*	8	*16*
FGD 2 (Centre)	8	*16*	8	*16*	16	*32*
FGD 3 (North)	12	*24*	14	*28*	26	*52*
Total	25	*50*	25	*50*	50	*100*

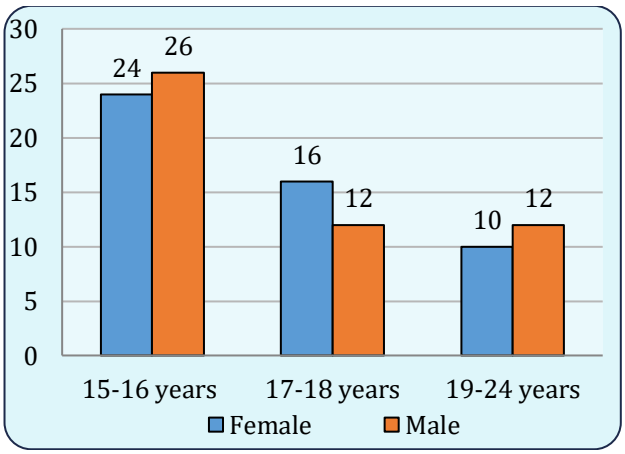


Figure 1. Age group distribution of participants, %.

Two thirds (72%) of the participants were Moldovan citizens, and 22% were refugees or asylum seekers. For fist language, one third of the participants (36%) spoke Romanian, 44% – Russian, and 16% – Ukrainian. Nearly all participants spoke Russian (82%) and one third also spoke English. The analysis of participants' educational levels reveals that a significant portion (56%) completed secondary education, followed by 22% with primary education and 16% with higher education. Among the refugee participants (n=11), one-third reported living in Moldova for two years, while another third had been there for more than one year (tab. 2).

Table 2. Socio-demographic characteristics of the respondents.

Statute	N	%	Education	N	%
Other	<5	4	No formal education	<5	6
Citizen of Moldova	36	72	Primary education	11	22
Refugee	10	20	Secondary education	28	56
Resident	<5	2	Higher education	8	16
Asylum seeker	<5	2			
Mother tongue			**Length of residence in Moldova (referring to refuges)**		
Russian	22	44	1 years.	<5	36
Romanian	18	36	1,5 years	<5	18
Ukrainian	8	16	2 years	<5	36
Other (list)	<5	4	11 years	<5	9
Most common languages spoken			**Employment status**		
English	17	34	Employed	8	24
Russian	41	82	Unemployed	25	76
Romanian	21	42			

Popular Apps and websites used by young people

When asked about the most popular Apps or websites which young people use, nearly all participants mentioned social media, including Instagram, TikTok, Facebook, YouTube, WhatsApp, and Telegram. Social media was seen not only as a platform for entertainment and communication, but also as educational, particularly in the areas of sexual and reproductive health: (*"In Instagram, you can also communicate with acquaintances, friends, as well as find entertaining videos and interesting facts" (Male, aged 19-24); "Personally, I use websites containing information about SRH, YouTube, and other related sites with articles covering various aspects of SRH. On YouTube, I follow different channels run by doctors, gynecologists, urologists, etc., providing information and answers." (Male, aged 19-24).*

The features that made certain Apps most appealing were explored throughout discussions. A common theme was the ability to find information easily and concisely on social media. Several participants enjoyed short videos as a medium to obtain information: *"I could say about TikTok that it is one of the most accessible platforms. The fact that there are short videos makes the information concrete, strictly on the subject, and if you have a specific question, you can receive an answer in a very short time." (Female, aged 19-24)*. Some participants discussed the ease of accessibility and navigation as other important features. Several participants cited using Google or other search engines, particularly for homework or when searching for health-related information. One participant suggested that as people age, they might be more inclined to use search engines or health-specific sites: *"We could categories information by age; I think for the younger ones, it would be TikTok and Instagram, and as we age, Facebook becomes more prevalent. Additionally, individuals over 18 tend to search on Google or various sites managed by doctors or hospitals, for example, in Romania, the website of Regina Maria Hospital provides information on various health aspects." (Female, aged 19-24).*

In terms of health-specific Apps, several female participants use Apps to track their menses, including 'Flo' App or other Apps of daily step counts or sleep monitoring.

Topics young people want to know more about

Different SRH topics that young people felt were most important and wanted to learn more about were explored. Sex, sexually transmitted infections, and contraception were commonly discussed topics. As noted by one participant, these topics are considered taboo and ones which many young people did not know much about *("This topic is considered taboo among young people of our age. Adolescents often seek information about contraception, [...] the band STI, and their first sexual experience." (Female, aged 15-18); "They may seek information about contraceptive methods and how to use them, such as emergency contraception like the morning-after pill, which many may not be familiar with." (Female, aged 15-18).*

Discussions particularly emphasized a significant knowledge gap regarding different forms of contraception and their correct application, including the use of emergency contraception. One participant discussed wanting to know more about *"what to do to terminate a pregnancy in the early hours" (Female, aged 15-18),* and another discussed wanting to *"explore the potential use of emergency contraception after each sexual encounter" (Female, aged 19-24).* This suggests a need for more education and information on contraception, as well as pregnancy termination.

Several participants expressed wanting to know more about sexually transmitted infections, such as HIV and syphilis, and how to protect against these. Of note, not a single participant discussed condoms, thus highlighting a gap in knowledge around safer sex practices ('dual contraception') to avoid both unwanted pregnancies and STI transmission.

Both male and female participants discussed puberty as an important topic. They were interested in understanding the ways in which the body changes during puberty and adolescence. One participant noted that it was important for young people to have a good understanding of puberty *"so that later on, many questions and problems don't arise" (Male, aged 15-18).*

Some male participants highlighted the need for specific information on male puberty, including around *"nocturnal eliminations and morning erections"*. One male noted that boys received less teaching on this topic as compared to girls, but, like girls, required information on this topic (*"Considering that this topic hasn't been adequately addressed in our education system, especially with boys, there seems to be a small disparity where boys are less informed compared to girls. This highlights the need for more comprehensive education for both genders." (Male, aged 19-24)).*

Several females discussed menstruation, with one participant saying it was *"one of the most sought-after subjects"* (Female, aged 15-18). Many females felt this was an important topic, but one that was embarrassing to discuss with parents *("I think there were questions when I was younger about menstruation when I didn't have it yet, and it was interesting to me how and what it would be like when it appeared. What to do with it because I was embarrassed to ask my mom." (Female, aged 15-18)).* Another female participant discussed the importance of "*personal hygiene of one's intimate area"*; again, it was noted that this was not a topic which could be easily discussed with parents.

A few participants from both genders said that SRH was not a topic in which everyone was interested. One noted that SRH remained an important topic for young people, even for those who were uninterested: *("Unfortunately, not many want to know about this; many understand it quite subjectively, and not everyone wants to be aware. However, it is a very important topic for defense against infections." (Male, aged 15-18)).*

Sources of SRH information

The sources and methods through which young people obtain information about sexual and reproductive health (SRH) were discussed.

Most participants discussed health professionals, including doctors, nurses, and gynecologists, as a key source of SRH information. In general, the information received from health professionals was seen as a trustworthy, due to their specialist training and education. One participant discussed that although the internet could provide "vast amounts" of information, a doctor or health specialist was need for reliable information. Similarly, information coming from local health clinics, the Ministry of Health, or international health bodies, such as the World Health Organization, was also seen as credible *("If the information is published on the website of a gynecologist or another medical professional, I consider it reliable. However, if the information is written on sites like 'Mail.ru' by some man who talks nonsense, I do not consider it correct because he is not a licensed doctor." (Female, aged 15-18)).* Of note, one participant shared an example of an experience of health professionals being sources of misinformation: *"... there are also doctors spreading misinformation on websites, such as claims that vaccines chip you or make you infertile. We strive to bring correct information, even though they position themselves as influential and experienced individuals." (Female, aged 19-24).*

Many participants highlighted social media as a popular source for SRH information. Most acknowledged that not all information on social media was accurate, however many felt that they could still use social media to find reliable information. Some pointed to certain "influencers" who were felt to have credible reputations *("Social networks that we often use are in the middle;*

we often find accurate information, but there are also cases of false data." (Female, aged 19-24); "... for example, on platforms like Telegram or Instagram, there is a diversity of opinions. Different individuals can express their views, but there are those with a certain reputation, and we often listen to them." (Male, aged 19-24)).

Participants also discussed that social media allowed them to subscribe to SRH information from credible sources, such as health professionals *("I use Instagram because I can subscribe to pages managed by gynecologists or other individuals discussing sexual education, hygiene, menstruation, pregnancy, etc. The information is presented concisely, clearly, and comprehensively." (Female, aged 19-24)).* Search engines, particularly Google, were also noted to be a common source of SRH information. One participant described Google as a platform with *"a wealth of information which is easily accessible" (Male, aged 19-24).*

Some felt that social media was not a credible source and that *"the internet does not always tell the truth"*. Several young men expressed concern with the anonymity of those positing on the internet. *One participant felt that publications were more credible than information online, as it could be verified ("On the internet, anyone can be whoever they want, often not revealing their true identity. This creates a false sense of security." (Male, aged 15-18)).*

A common theme across all FGD was that corroborating information from multiple sources made it more credible. Many participants felt that social media enabled them access to multiple sources of information, allowing them to draw their own conclusions *("We choose where to gather information. For instance, I am subscribed to reliable sources where I have seen that accurate information is posted, including interviews with doctors. However, I am aware that alongside these, there are many unreliable sources." (Female, aged 19-24); "For example, I listen to information from various places, and in the end, I draw my conclusions, but the most credible sources for me are Instagram and bloggers." (Female, aged 15-18)).* In the male only group, porn was mentioned as a source of SRH information, but this was not elaborated on.

Participants reported having received some education on SRH in school settings, typically integrated within biology classes, with an emphasis on puberty. However, they expressed discomfort in discussing sexual and reproductive health (SRH) with teachers, viewing the topic as particularly taboo when addressed with older adults. Some participants also commented that often the teachers who provided the SRH curriculum were older in age, with limited ability to provide up-to-date and accurate information (*"I think students find it uncomfortable to talk to teachers [...] Often, they are put in situations to discuss these topics with the class, and most of them are from the post-Soviet era and may not be as open to discussing these matters. It's difficult for them as they are not experts in the field and can only provide general information that students already know. They may struggle to provide more recent information." (Female, aged 19-24)).* Some participants discussed that when they received SRH teaching from younger teachers, they found it more relatable and had more positive experiences.

Older siblings or peers with similar experiences were often cited as a reliable source of information, as they had "gone through similar situations". A few participants also mentioned their parents, or specifically their mother, as a source of information. However, most participants said that they were embarrassed to discuss SRH topics with parents *("... teenagers usually don't learn from their parents. It's not easy for parents to talk to their children about this. The information mainly comes from other sources, like friends or the internet ..." (Male, aged 15-18).* Although participants felt comfortable talking to their peers or older siblings, researchers leading the FGDs noted that the information from these sources was not always accurate.

Barriers to accessing SRH services or reliable information

Participants were asked about things that make it difficult for young people to access SRH services or information.

Many participants expressed feeling fear and shame in discussing SRH, which is considered a taboo topic. In particular, participants worried about being dismissed by health professionals, teachers, or older adults, which was a barrier to seeking care or information ("*It's something intimate, and when you want to address it with a doctor or gynecologist and ask something, you feel ashamed to tell them everything. You wonder, what will the doctor think about you? You're not sure if they'll understand the emotions you're experien-*

cing now." (Female, aged 15-18)). For this reason, many participants said they preferred to seek information from their peers or people closer to their age, where they felt free to discuss more openly.

Many participants described not knowing where to seek reliable SRH information. They noted frequent misinformation online; as a result, they said they struggled to *"differentiate and determine what is correct and true"*. Several participants discussed the importance of finding accurate information that could help clarify common misconceptions, such as: *"the misconception that using a tampon means you're not a virgin or that you can't get pregnant during menstruation" (Female, aged 19-24); "A problem I have encountered is that when adolescents search for information about masturbation on the internet, there is a multitude of misinformation, such as claims that it causes blindness or hair loss. Due to this, I believe that young people need to know where to turn for accurate information and have reliable sources available." (Male, aged 19-24).*

Participants explained that sometimes when the information was from what was considered a reliable source, the way it was presented was often difficult to understand, for example in an academic paper or medical website. This was in contrast to social media, where information was presented concisely and clearly *("A significant issue is that reliable sites with accurate medical data or those based on scientific articles are often complex and take time to understand. In contrast, social media platforms provide information briefly, making it easier to comprehend, though not necessarily evidence-based ..." (Female, aged 19-24)).*

Some participants noted that less information was available for males. They discussed that teaching in schools often focused more on girls, and that there was more information online for women than men. Participants also discussed that males were less likely to discuss SRH topics amongst themselves as compared to females, which further exacerbated the gendered information gap *("Considering that this topic hasn't been adequately addressed in our education system, especially with boys, there seems to be a small disparity where boys are less informed compared to girls ..." (Male, aged 19-24)).*

Some female participants reflected that men lack knowledge about female SRH topics, such as menstruation. Equally, there is a gap in female knowledge around topics such as male condoms. In general, FGDs highlight that SRH topics remain gendered, and are not often discussed between males and females *("On the internet, there is more information available for women who are concerned about their health. There are forums where they discuss these topics, but men do not talk about it much, only with each other. There is such a difference in women; they paraphrase the word 'menstruation' into 'guests from Krasnodar', 'red days', and they feel intimidated if they experience leaks [during menstruation]. Additionally, men feel intimidated to buy pads and contraceptives because they believe others will judge them." (Female, aged 15-18)).*

However, one male participant expressed a desire for specific websites for men in order to improve their understanding of female SRH topics, so that *"men can understand and not shame women" (Female, aged 15-18).*

Enablers to SRH information and services

Participants were asked about things that make it easier or could improve access to access SRH services and information for young people.

Several participants discussed that confidentiality was important and could make it easier for young people to access information and services. Several participants felt that there should be online platforms where young people could anonymously chat with a specialist *("Not everyone feels comfortable discussing their problems face-to-face. Maybe they don't want their face to be seen." (Male, aged 19-24)).*

Some participants also discussed that they saw the use of websites and Apps as an opportunity to improve access to SRH information and services. These were seen as anonymous, accessible, and convenient ways to access information. Nearly all participants reported having daily access to the internet on their phones or computers, which they used to search for information.

Some participants wanted more sexual education lessons in schools. A common theme was that participants felt more comfortable receiving SRH information from people closers to their age. Additionally, many participants explained that they would prefer to have health professional or health specialists discuss sensitive SRH topics in schools. They also expressed that they felt more comfort-

table discussing these topics with the younger teachers *("Teachers are already adults, and while they share practical experiences, it's challenging for us to talk to them about this topic as we don't feel comfortable discussing such matters with older individuals." (Male, aged 15-16)).*

Awareness and experiences of SRH services

Awareness of SRH services was touched on only briefly during the FGDs and will be explored in more depth in the KAP surveys. Participants noted a few youth-friendly SRH clinics, including Neovita, which provided access to specialists, free HIV testing, and free condoms.

In the other FGDs, few participants discussed having sought SRH care themselves, other than for mandatory medical examinations in school. These examinations are typically a source of anxiety, particularly for females, as there is a fear that peers will compare their puberty development amongst each other, and that peers will know who is sexually active. One participant described a "higher level of tension" between the teachers and students during the mandatory medical examination at school.

Only one participant elaborated on an experience accessing SRH outside of mandatory exams. She reported having had a negative experience with a gynecologist at a youth-friendly clinic; as a result, she stopped visiting the clinic: *"For example, when I was in middle school, I reached out to the Youth-Friendly Centre, which had access to a gynecologist. However, the communication style there was vulgar, and after the first session, I left. This occurred in my local area." (Female, aged 19-24).*

Given that very few participants had accessed SRH services, this example of a negative experience at a youth clinic is particularly concerning.

Interest in a health App and concerns

Overall, participants felt there was a need for a SRH App. When asked which features would be most useful, a common theme was the option to anonymously ask questions or discuss with a specialist. As discussed above, specialists were seen as credible sources of information, and participants felt that anonymity could help improve access. A few participants also suggested having hotlines. Some participants requested that it would be useful to have contact information for local clinics *("... we desire an interactive application with the ability to ask questions and receive personalized answers, getting to know about existing centers." (Male, aged 19-24)).*

Many discussed the importance of using easily understandable terms and presenting statistical data clearly. Some highlighted the importance of ease of navigation on the platform *("I view the creation of an application very positively, with clear and accessible statistical data, easy to understand." (Female, aged 19-24)).*

Several participants suggested having audio and video content, including doctors or specialists discussing SRH topics. One participant requested links to *'reliable TikTok channels'*. Many others suggested having 'personal stories' and mini-interviews'.

Participants agreed that the App should have information for both males and females, and suggested having separate sections for the different genders. Participants felt that the App should be available in different languages, including English, and should be freely available.

Most did not express safety concerns about a potential App, although this may be due to fatigue and time constraints towards the end of the interviews. Participants noted that data privacy was important. A few of the male participants discussed that data leaks or hackers could be a concern, but seemed to think this was low risk: *"Information leaks. It wouldn't be good if the person who shared their life story had their account hacked, and someone else found out." (Male, aged 19-24); "Generally, I don't think there are serious concerns. It's good to be anonymous, but I don't think anyone will have serious worries that something might happen for which to be concerned, so I don't think there are concerns." (Male, aged 15-18).*

Participants suggested that the best way to raise awareness about a new SRH App would be through advertising on social media advertising, on television, in schools, and through youth training sessions.

A few participants noted that the App should be free, to promote access for all groups. Nearly all FGD participants had access to personal phone or computer, with daily internet access, and felt this was the case for most young people in Moldova. Some suggested that those without phones could use schools, youth centers, or public libraries to access the App anonymously.

Feedback from participants after conducting FGD

In general, most of participants reported feeling uncomfortable discussing SRH matters to some extent, this was higher among younger participants (15-17 age group with 36% compared to 26% in 18-24 years), but a notable portion in each group felt very comfortable discussing the topics freely (fig. 2). In total, 62% of participants reported feeling 'very uncomfortable' discussing any of the topics (38% female, 24% male), and a further 18% reported feeling 'somewhat uncomfortable' (6% female and 12% male).

Half of all the participants found the conducted warm-up exercises prior to each FGD helpful and felt more comfortable talking openly as a result, 38% of participants indicated that they perceived the warm-up activities as somewhat helpful, and they felt somewhat more comfortable expressing themselves during the group discussion and 12% of participants reiterated that they found the warm-up activities helpful, leading to an increased comfort level when talking openly during the group discussion.

Nonetheless, when asked how honestly, they responded to the questions, 74% of participants reported 'always' contributing with truthful ideas and answers; only 8% said that 'almost all of the time' they shared ideas that agreed with what others were saying, even if they did not really agree with them. This suggests that although participants felt uncomfortable discussing sensitive SRH topics, most still provided honest answers (tab. 3).

Participants listed language, nationality, and the age of other participants as issues that affected how they contributed to the discussion. Overall, participants emphasized the importance of well-planned and inclusive warm-up activities to create a supportive environment conducive to meaningful group discussions. They felt that conversations were enjoyable, informative, and expressed a positive view towards the initiative, suggesting a desire for more frequent organization, particularly for the younger ones ones *("...It was a very interesting conversation; ...very friendly; ... such activities are very important and should be organized as frequently as possible").*

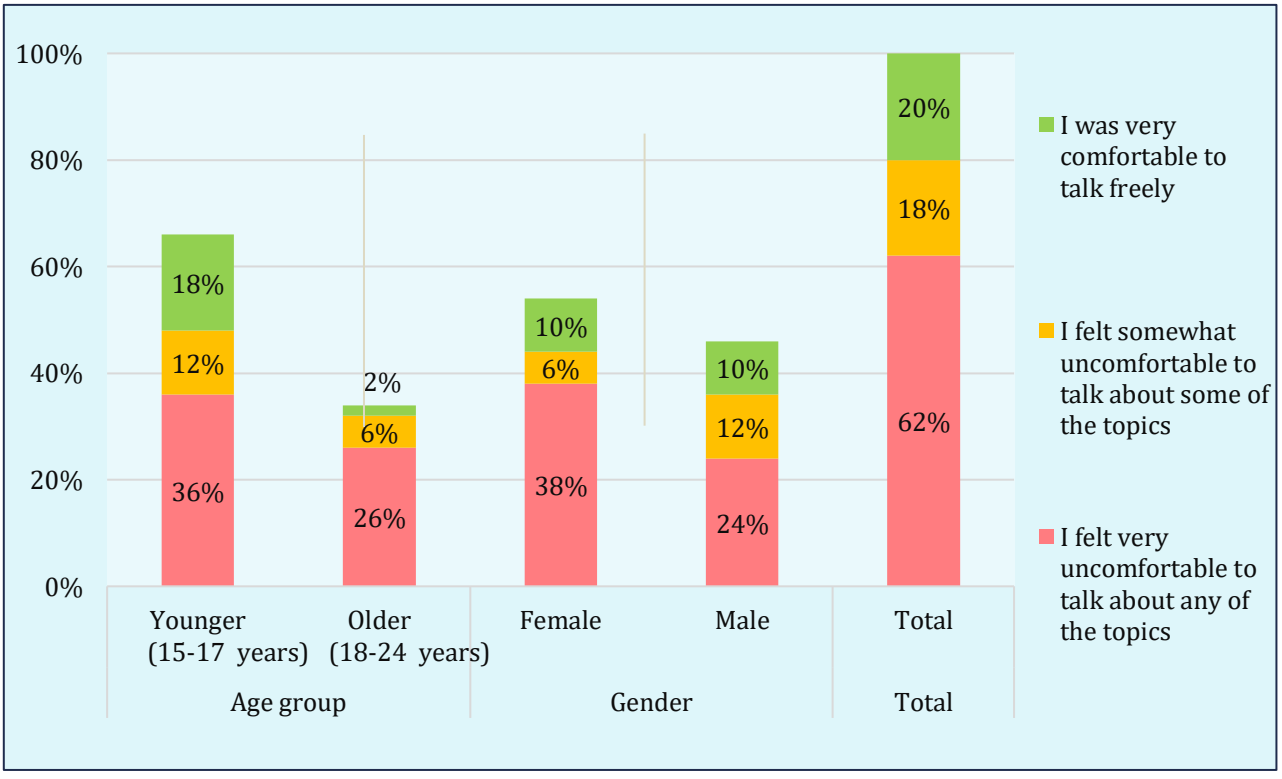


Figure 2. FGD participants' ranking on level of comfort in talking freely during the discussion distributed by age group and gender, % (N=50).

Table 3. FGD participants' ranking on how honestly they responded to the questions.

Questions	N	%
What other people think of me was not a worry - when I contributed to the discussion, it was always with my truthful ideas and answers.	37	74
I was very worried about what other people might think of me - almost all of the time, I shared ideas or answers that agreed with other people in the group even if I did not really agree with what they were saying.	4	8
I was somewhat worried about what other people would think about me - for about half the time I was able to say what I really thought, and half the time I agreed with what others were saying even if I didn't really agree with what they were saying.	9	18
Total	**50**	**100**

DISCUSSIONS

The results obtained from the focus group discussions conducted in the three regions of the country revealed that young people primarily rely on social media and various websites for accessing information on sexual and reproductive health. Their strong voice expressed the need for the development of an SRH App, with priority topics including safe sex, puberty, menstruation, and contraception, including emergency contraception. Barriers to finding accurate SRH information were also discussed, with young people expressing difficulty in identifying reliable sources and evaluating the trustworthiness of online information. Health professionals were generally considered credible sources, but shame, embarrassment, and fear of dismissal often inhibited young people from accessing SRH healthcare. Anonymous chats with SRH specialists or doctors were suggested as a potential solution to reduce these barriers.

The results also indicated that young people value the vast amount of information available on social media and often rely on 'reputable influencers' or reliable sites to draw their own conclusions. This highlights the importance of equipping young people with the skills to assess the reliability and accuracy of online information.

Other studies have found that including educational elements, interactive elements to Apps can reinforce fundamental knowledge on SRH, being effective in raising knowledge and attitudes of young generation in relation to sexual health education (8, 9, 10).

Most young people aged 15-24 have access to a personal smartphone and use it to access the internet daily. However, public spaces such as schools, libraries, and youth centers may provide access to a SRH App or website for those without personal phones (11). With an internet penetration rate of 65% and more cell phone connections than people in the country, the YK App has great potential to reach a wide range of users across the country. However, ensuring the YK App can function (at least partially) offline will increase its potential utility in Moldova, given that an estimated 35% of the population does not have internet access (11).

Interventions delivered via mobile devices have been shown to successfully improve people's health. Compared to conventional interventions, people benefit from mHealth by being enabled to quickly access information at low thresholds and independently of time and place, as well as at low cost. mHealth interventions can also meet the need for anonymity, which is especially crucial when it comes to sexuality (8). However, it will be crucial that the YK App is accompanied by investments in quality, trustworthy, and patient-centered services that engage young people beyond simply providing text-based information.

Actively involving young people in the development and promotion of the app will not only increase the app's relevance and usability, but also empower young people to take charge of their own health in a collaborative and informed way (9, 12).

Overall, digital apps in this field have the potential to empower individuals, improve health outcomes, and contribute to public health initiatives by making information and services more accessible and user-friendly.

When discussing the limitations of the study, it's

essential to acknowledge any factors that may have impacted the results or the generalizability of the findings. The following limitations should be highlighted: the sample's higher proportion of Russian and Ukrainian speakers may not fully represent Moldova's youth, potentially impacting the generalizability of the findings, particularly for underrepresented ethnic and linguistic groups. Additionally, self-reported data on sensitive SRH topics may be subject to social desirability bias, despite efforts to promote honesty through anonymity. Variability in digital literacy and internet access, particularly among refugees and rural youth, could impact the feasibility and usability of the proposed YK App. Lastly, recruitment through specific networks may have introduced selection bias, as participants may be more interested in SRH issues or more experienced with digital technologies.

CONCLUSIONS

1. The findings from the conducted research suggest that the development of a youth-focused SRH App or website could be a valuable resource for young people in Moldova, including refugees and vulnerable populations. SRH apps allow health information to be disseminated quickly, at low thresholds and in a practical and cost-effective manner. Moreover, they allow for anonymous usage independently of time and place. The App should prioritize topics such as puberty, menstruation, contraception, and STI prevention, and present information in clear, easily understandable formats, free from medical jargon. Including personal stories or interviews and providing opportunities for anonymous access to health professionals could improve engagement and credibility.
2. Schools and youth clubs could play a role in strengthening SRH education and teaching young people how to identify reliable sources of information. Future research should consider segregating young people by gender and age to ensure they are free to share their views. Individual discussions with vulnerable groups, such as refugees and Roma, may also create a more comfortable space for them to share their unique barriers to access.
3. To strengthen the conclusions, consider incorporating evidence from similar studies or initiatives that have successfully implemented youth-focused SRH Apps or websites. This could provide additional support for the recommendations and highlight potential challenges or considerations.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ETHICAL APPROVAL

This research is part of the "Community Participatory Digital Health Initiative" international project funded by the European Union, implemented in the Republic of Moldova by INTERSOS, in partnership with Neovita and *Nicolae Testemitanu* State University of Medicine and Pharmacy, Department of Preventive Medicine. Ethics approval was obtained from *Nicolae Testemitanu* State University of Medicine and Pharmacy of the Republic of Moldova, decision no 2 from October, 12, 2023.

ACKNOWLEDGEMENTS

The work reported in this publication was funded by the European Union. The authors gratefully acknowledge all members of INTERSOS Moldova and "Nicolae Testemiţanu" SUMPh for their work on the project overall and for the contributions of project documentation used in this manuscript. Special thanks are extended to the INTERSOS team for their exceptional leadership in data collection and critical reviews and guidance to the research project, including Alina Armasaru, Mariana Rosca. Many thanks to the National Resource Centre in Youth Friendly Health Services (NEOVITA), Republic of Moldovan, including Galina Lesco for their work in SRH topics. Lastly, recognition is given to ECHO for identifying the need to strengthen SRH services for vulnerable refugee and host adolescents in Moldova, in the context of the Russian-Ukrainian war.

REFERENCES

1. Schnitzler L, Paulus ATG, Roberts TE, Evers SMAA, Jackson LJ. Exploring the wider societal impacts of sexual health issues and interventions to build a framework for research and policy: a qualitative study based on in-depth semi-structured interviews with experts in OECD member countries. *BMJ Open*. 2023;13(1). doi:10.1136/bmjopen-2022-066663
2. United Nations General Assembly. *General Assembly.*; A/HRC/39/33; 2018. Available at: https://documents.un.org/doc/undoc/gen/g18/193/07/pdf/g1819307.pdf (Accessed in May 27, 2024).
3. National Bureau of Statistics. Statistical Databank. Available at: https://statbank.statistica.md/PxWeb/pxweb/en/20 Populatia si procesele demografice/ (Accessed in June, 7, 2024).
4. IOM-UN Migration. Ukraine & Neighboring Countries 2022-2024. 2 Years of Response. Published online 2024. Available at: https://www.iom.int/sites/g/files/tmzbdl486/files/documents/2024-02/iom_ukraine_neighbouring_countries_2022-2024_2_years_of_response.pdf (Accessed in May 27, 2024).
5. UNESCO. *Moldova Comprehensive Sexuallity Eductation Profile.*; 2023. Available at: https://education-profiles.org/europe-and-northern-america/moldova/~comprehensive-sexuality-education (Accessed in June 15, 2024).
6. UNFPA. Country case studies on UNFPA's global programme on out-of-school CSE - Moldova. Available at: https://www.unfpa.org/resources/moldova-country-case-studies-out-school-comprehensive-sexuality-education (Accessed in May 27, 2024).
7. Sirbu L, Lesco G. GC. Comparison of progress in CSE in Moldova measured by SERAT - from 2017 to 2022. *16th Eur J Public Heal*. 2023;33(Supplement_2):ii165. doi:10.1093/eurpub/ckad160.421
8. Hubert C, Estrada F, Campero L, et al. Designing digital tools capable of reaching of reaching disadvantaged adolescents and improving their sexual health: a Mexican experience. *J Health Care Poor Underserved*. 2021;32(2):62-84. doi:10.1353/hpu.2021.0051
9. Patel A, Louie-Poon S, Kauser S, Lassi Z, Meherali S. Environmental scan of mobile apps for promoting sexual and reproductive health of adolescents in low- and middle-income countries. *Front Public Heal*. 2022;10:993795. doi:10.3389/fpubh.2022.993795
10. Quiala Portuondo J, Portuondo Hernández Y, Franco Chibás A, Moreaux Herrera D, Guilarte Guindo P. Salud sexual reproductiva. Intervención educativa en jóvenes Sexual reproductive health. [Educational intervention in young persons.] *Rev Inf Cient*. 2016;95(4):571-580. Available at: https://revinfcientifica.sld.cu/index.php/ric/article/view/88/2287 (Accessed in June 15, 2024).
11. DATAREPORTAL. DIGITAL 2024: MOLDOVA. Available at: https://datareportal.com/reports/digital-2024-moldova (Accessed in June, 7, 2024).
12. Muehlmann M, Tomczyk S. Mobile apps for sexual and reproductive health education: a systematic review and quality assessment. *Curr Sex Heal Reports*. 2023;15(2):77-99. doi:10.1007/s11930-023-00359-w

Svetlana COCIU, WoS Researcher ID: GNM-7830-2022

Date of receipt of the manuscript: 24/07/2024
Date of acceptance for publication: 29/09/2024

OH&RM ONE HEALTH & RISK MANAGEMENT

VOL. 5, ISSUE 4
2024

MEDICAL SCIENCES

OPEN ACCESS

OPTIMIZING THE EFFICACY OF COVID-19 PATIENT SATISFACTION SURVEYS IN INDIA: A DELPHI-BASED VALIDATION PROCESS

Shazina SAEED[1,2], Karuna Nidhi KAUR[1], Manmohan SINGHAL[2], Mohd SHANNAWAZ[1], Farah NIAZI[1], Bhavna KUMAR[2], Aanchal Anant AWASTHI[3]

[1]Amity Institute of Public Health and Hospital Administration, Amity University, Noida, India
[2]School of Pharmaceutical & Population Health Informatics, DIT University, Mussoorie-Diversion Road, Makkawala, Dehradun, Uttarakhand, India
[3]Amity Institute of Applies Sciences, Amity University, Uttar Pradesh, India

Corresponding author: Manmohan Singhal, e-mail: manmohan.singhal@dituniversity.edu.in

DOI: 10.38045/ohrm.2024.4.03 CZU: 616.98:578.834.1(540)

Keywords: *COVID-19 Pandemic, e-Delphi method, healthcare services, patient satisfaction, questionnaire validation.*

Introduction. *The COVID-19 pandemic in India increased healthcare costs and reduced patient satisfaction. This study aimed at designing and validating a new questionnaire to assess patient satisfaction with health care services amidst COVID-19 in India.* ***Material and methods.*** *An e-Delphi method was employed to design as well as validate a self-report questionnaire. The questionnaire included a total of 18 close-ended questions which were to evaluate patient level satisfaction in patients with the disease. A panel of experts, including healthcare professionals, researchers, and public health specialists, approved an instrument in a series of structured rounds.* ***Results.*** *The questionnaire developed in the study appears to measure patient experiences during episode of hospitalization and was implemented as a pivotal instrument for policymakers or healthcare providers focusing on the most relevant aspects that should be considered, which help achieve better understanding how they can respond more effectively to meet the respective needs. Further, as the questions had been framed in a contextual manner, considering linguistic and socio-cultural parameters of Indian population, they were designed to assess region-based healthcare practices, thereby enhancing relevance to contextuality.* ***Conclusions.*** *This study will address an important gap in the assessment of patient satisfaction during a health care crisis, as well highlighting the use of Delphi Technique for validation and obtaining valid patient perspective data that can be used further to make policies and understand perception towards improvement is survey-based studies.*

Cuvinte-cheie: *pandemia COVID-19, metoda e-Delphi, servicii de asistență medicală, satisfacția pacienților, validarea chestionarului.*

OPTIMIZAREA EFICACITĂȚII SONDAJELOR DE SATISFACȚIE A PACIENȚILOR PRIVIND PANDEMIA DE COVID-19 ÎN INDIA – UN PROCES DE VALIDARE BAZAT PE METODA DELPHI

Introducere. *Pandemia de COVID-19 din India a cauzat scumpirea serviciilor medicale și scăderea satisfacției pacienților. Acest studiu a avut ca scop elaborarea și validarea unui nou chestionar pentru evaluarea satisfacției pacienților privind serviciile medicale în contextul pandemiei COVID-19 din India.* ***Material și metode.*** *A fost folosită metoda e-Delphi pentru a elabora și valida un chestionar de autoraportare, care a inclus în total 18 întrebări închise ce urmau să evalueze satisfacția pacienților cu această maladie. Un grup de experți, care include profesioniști din domeniul sănătății, și evaluatori din domeniul sănătății publice au ratificat instrumentul printr-o serie de runde structurate.* ***Rezultate.*** *Chestionarul elaborat în studiu măsoară experiențele pacienților în timpul episodului de spitalizare și a fost implementat ca instrument esențial pentru factorii de decizie sau prestatorii de servicii medicale, concentrându-se pe aspectele cele mai relevante care ar trebui luate în considerare, care ajută la o mai bună înțelegere a modului în care aceștia pot răspunde mai eficient pentru satisfacerea nevoilor respective. Mai mult, deoarece întrebările au fost încadrate într-o manieră lingvistică și socio-culturală ai populației indiene, , sporind astfel relevanța contextualității.* ***Concluzii.*** *Acest studiu va elucida lacunele importante în evaluarea satisfacției pacienților în timpul unei crize de îngrijire medicală, evidențiind, de asemenea, utilizarea tehnicii Delphi pentru validare și pentru a obține date valabile privind perspectiva pacientului care pot fi utilizate în continuare pentru a elabora politici și a înțelege percepția de îmbunătățire a studiilor efectuate în baza chestionarelor.*

INTRODUCTION

Patient satisfaction surveys are important to conduct for assessing the caliber of medical service. During the COVID-19 pandemic, their importance was further carried out so that one can find new insights into patient's experiences in such situations. These surveys are an important aspect of understanding and sustaining patient footfall, along with understanding the healthcare frameworks while being flexible and reasonable especially when the healthcare domain is rapidly evolving.

Organizations, such as the Agency for Healthcare Research and Quality (AHRQ) and the National Quality Forum (NQF) have demonstrated the importance of patient satisfaction assessments (1). The World Health Organization (WHO) has always highlighted the absolute importance of care quality to an optimal level and provides frameworks for its evaluation (2). Nevertheless, in recent times, during the pandemic these surveys have become even more important owing to the presence of multidimensional challenges such as resource limitations, interruptions in healthcare delivery, and the adaptation of rapidly evolving protocols. The insights thus committed from patient feedback help to do the gap analysis in these services as well as identify potential areas for enhancement. Moreover, the pandemic has highlighted the necessity of give personalized patient approaches, thus ensuring that healthcare approaches will be in sync with specific desires and requirements of patients (3). Surveys help to gauge the level of understanding the information they have been given, enabling any necessary changes to communication strategies. They aid in evaluating the efficacy of educational resources prepared for COVID-19 prevention, symptoms, and immunization (4).

The pandemic highlighted the critical need of psychosocial assistance for patients, irrespective of their demographic variables. Healthcare discrepancies can sometimes get worse during times of crisis. Patient – experience based surveys help to design policies that provide equitable access to healthcare resources, especially to the most vulnerable and marginalized populations. Knowing that the dynamic nature of COVID-19 demands strong and flexible adaptive healthcare systems, such surveys will help to assess how much an organization has altered to accommodate new requirements, such as the introduction of telemedicine, visitation laws, and modifications to the way care is delivered (5, 6).

This study aims to address all the above concerns regarding overall patient satisfaction. However, there is a need in India to develop a validated questionnaire to assess this accurately. Current instruments may not fully capture the bottlenecks in patient care, particularly given the distinct conditions and challenges faced during the pandemic and its aftermath on the Indian healthcare sector.

Patient Satisfaction Surveys

Patient satisfaction surveys have rapidly evolved since the 1950s, when they primarily focused on basic aspects of care, such as communication and cleanliness (7). Over the recent years, these surveys have expanded its horizons to other disciplines, such as technical competence of healthcare providers, interpersonal skills, responsiveness to patient needs, and the degree of patient involvement in decision-making (8). For example, the services marketing model views healthcare as a service, emphasizing the importance of meeting patient expectations to achieve desired satisfaction (9). The expectation disconfirmation theory refutes the same (10). Other models, such as the patient-centered care framework and the Donabedian model of quality, highlight the central role of patient perceptions in evaluating healthcare quality (7, 11).

Delphi Technique

It was in the 1950's, that the goal of the Delphi technique, was created by RAND Corporation researchers. They brought together experts to reach a point of consensus on certain problems through controlled and structured communication. It was initially used on military technology forecasting, but thereafter due to its strategies like group think and the dominance of outspoken individuals, it has then been widely used in many other disciplines including healthcare (12).

The Delphi process is a systematic approach to communication that incorporates several core ideas to match a certain objective and efficient consensus-building among experts. Essentially, the method maintains participant anonymity, thus reducing the impact of peer pressure and assists them to sincere beliefs. It uses a repetitive process with many rounds of changing and improving the questionnaires, controlled feedback

methods are used to support this process. Following each round, all the experts receive summaries of the group's responses, which allows them to revisit and amend their own responses considering the larger group perspective. The Delphi method's primary component is its dependence on an expert panel (13).

Delphi proves to be a useful system for facilitating professional discussions and reaching agreements on delicate issues, gaining popularity as a reliable method in the healthcare sector. Its sustained effectiveness across various research and operational disciplines will be ensured by recognizing both its advantages and disadvantages while embracing contemporary trends.

Questionnaire Validation in Healthcare Research

Validating questionnaires for healthcare research or surveys is a meticulous process that ensures the accuracy and reliability of the data collected. During the validation process, a rigorous analysis of the instrument is conducted to ensure it accurately reflects the selected variables. A validated questionnaire is a standardized approach to collection of data, thus ascertaining that the research findings are creditable and comparable across different studies and populations (14). While evaluating construct validity, statistical methods like factor analysis are done so that one can confirm the accuracy of the questionnaire and its correlation to the theoretical constructs that it aims to measure (15). On the other hand, criterion validity examines the methods correlation with established benchmarks within the field, 'thus acting like a litmus test for its accuracy (16). Moreover, statistical measures such as Cronbach's alpha are used to determine the consistency among the items within the questionnaire, thereby acting as the internal consistency and reliability tool for the instrument (17). Through validated questionnaires, researchers can robustly capture patient-reported outcomes, adverse events, risk perceptions, and other critical data, thereby enhancing the quality and reliability of their findings. A validated questionnaire not only raises the caliber of research but also informs more precise clinical interventions and policies, grounding them in solid empirical evidence.

The e-Delphi method

The present study utilized the e-Delphi methodology to develop and validate a questionnaire aimed at evaluating Patient Satisfaction Surveys related to healthcare delivery systems during the COVID-19 pandemic in India. As part of this methodology, a cohort of experts, consisting of accomplished public health professionals, was recruited to provide critical feedback by sharing their valuable insights and knowledge to finalize the questionnaire. A unique aspect of this study is that it substitutes a single representative's opinion with the collective diversity of the group. Traditionally, the Delphi method was paper-based for gathering information from the expert cohort, but it has since been replaced by a digital alternative known as "e-Delphi methods." The digital platform enabled the maintenance of anonymity and facilitated faster response times.

The primary aim of this research was to develop a survey designed to evaluate patient satisfaction with healthcare delivery systems during the COVID-19 pandemic in India. To validate and improve the questionnaire, the study also utilized the Delphi Technique to gather a panel of experts, including researchers, public health officials, and healthcare professionals. This approach ensures that the instrument reflects the diverse perspectives and experiences relevant to the Indian healthcare landscape. To enhance its effectiveness, the questionnaire was tailored to capture the cultural and contextual nuances of the Indian population. By addressing these objectives, the study seeks to fill a critical gap in assessing patient satisfaction during the COVID-19 pandemic in India, highlighting the importance of a validated questionnaire in obtaining accurate and meaningful data for healthcare improvement.

MATERIAL AND METHODS

Design

The study followed the Delphi methodology, consisting of three rounds of expert discussions to finalize the questionnaire. Instead of the traditional method of correspondence, an online survey protocol was used for the e-Delphi method, supported by virtual meetings and email to facilitate discussions and collect data.

In this study, a 75% consensus threshold was proposed as the standard for reaching agreement. It was planned that a consensus of 75% or greater on each question would be considered acceptable for the study. The flow of the Delphi process is shown in Figure 1.

Three rounds of the Delphi process

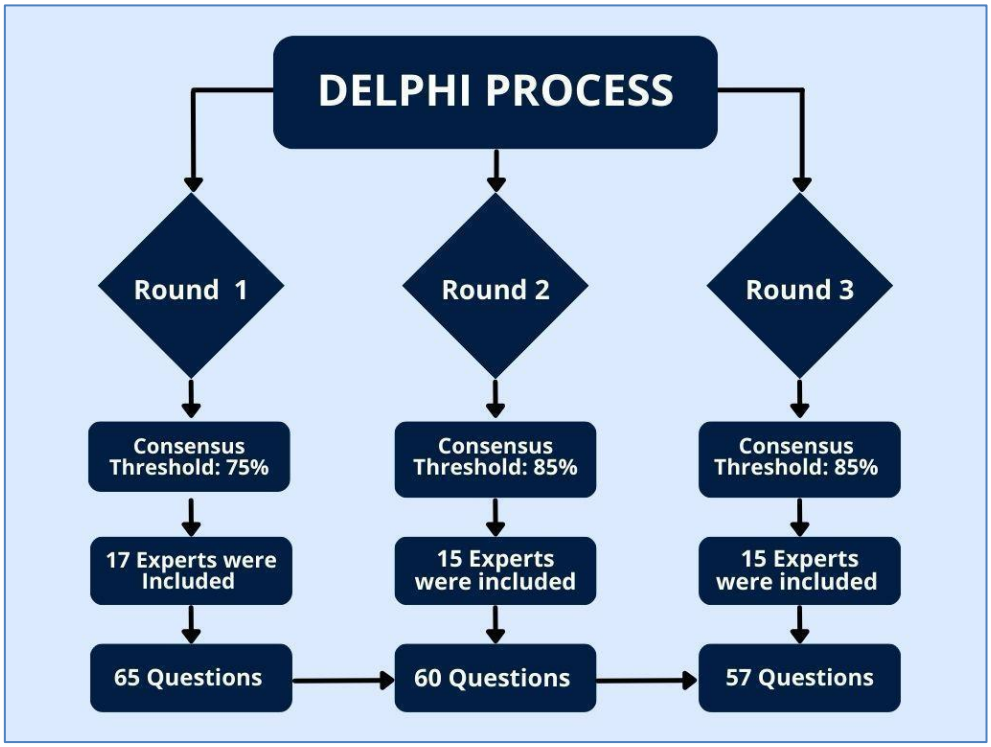


Figure 1. The figure illustrates the progression of the Delphi process across three rounds.

Note: Each round is represented by a distinct column, showcasing the key activities and changes in thresholds.

Participants

The cohort of experts for this study was carefully selected, consisting of individuals with involvement in the Public Health field. An electronic invitation was sent to all the experts to participate in the modified e-Delphi study, which included study details and consent information. A follow-up phone call provided the opportunity to answer any further questions and offer clarifications related to the project. The participants were initially blinded to other members to encourage the expression of neutral opinions, particularly in the first round.

The criteria for selecting the expert cohort were as follows: (i) Public Health Experts with a minimum of 10 years of clinical experience in either non-communicable disease management or the healthcare sector, along with experience in conducting research related to COVID-19 and its impact on patient care and satisfaction; (ii) professionals with a background in research methodology, questionnaire development, or validation studies to ensure methodological rigor and validity; (iii) individuals who have access to an email account; and (iv) those who are willing to participate. This diverse panel could provide an impartial reflection of current knowledge and perceptions in both the health and technology spheres.

Data collection

Three rounds of data were collected for the study, August and November of 2023 in the form of an online survey, an online group meeting and email communication. Ethical Clearance was obtained by Institutional Ethics Committee of Amity University (AUUP/IEC/2023/4).

Round 1: Introductory Research and Assessment

During the first phase of the Delphi procedure, experts were recruited in the study. The questionnaire was shared with them through the google forms to collect the survey data. The initial draft of the questionnaire had a total of 65 closed-ended questions that were designed to assess and evaluate the patient satisfaction levels among COVID-19 survivors who had been hospitalized. Structurally, the questionnaire encompassed three main sections: the socio-demographic pro-

file, details of hospital services provided with details of vaccination, and utilization of the validated satisfaction scale (PSQ-18) (5).

The preliminary questionnaire items were formulated in English and shared with each Delphi participant via email. The questionnaire was evaluated by the experts, and they provided feedback on the inclusion or exclusion of the question based on its relevance to the study. Each question was separately evaluated based on; whether the question is relevant on a dichotomous scale, whether the question was coherently worded (also on a dichotomous scale), and whether it needed rephrasing (open question). The questionnaire had an option to add comments next to each question. And propose any additional questions and make general comments.

After a four-week rigorous collection of data and follow up with the expert panel, the agreement rate was subsequently calculated for each question, along with the proportion of experts who thought that the question was relevant, worded coherently. Only after gaining 75% of the panelist's approval, were the questions included in the next round.

Round 2: Consensus-based finalization of the questionnaire

In the second round, all the approved questions from the first round were incorporated and some new questions were also included based on the expert's advice and critical feedback. After incorporating all the changes in the electronic survey forms, it was once again shared with the experts to once again allow them to assess the relevance, clarity and for changes in the verbatim of the questions. This entire process took four weeks to complete. At the end of this stage, the panelists were also asked to rate each question on a Likert scale where a score of 1- indicated irrelevance and 5 indicated relevance. The degree of agreement with each question in the final questionnaire was then evaluated, and all drafting and general remarks were compiled.

Responses rating questions as 4 or 5 on the Likert scale were defined recommendations for inclusion. The predetermined threshold for agreement was set at 85% of the panel.

Round 3: Final feedback and consensus on questionnaire

The questionnaire was further revised in round 3 and formatted as per the suggestions of the experts and then shared with all of them via email for individual reviewing. Each question was evaluated on the basis that if the question was recommended to be included in the final questionnaire, or if it required any revisions or needs to be excluded (with appropriate reasoning). Consensus was defined as 85% of the experts agreed on the final inclusion of the questions in the questionnaire. The consensus for the third round took approximately four weeks to collect and analyze.

In each Delphi round, the consensus rate of the experts on the inclusion of the questions were evaluated by calculating both the proportion and mean. Initially, a cut-off level for consensus was set at 75% in the round one. Subsequently, in round two, the experts rated their level of agreement for each question on a scale ranging from 1 – 5 (1 = the question can be excluded; 5=question is relevant to the questionnaire). At this round, the level of agreement threshold was set at 85%. In the third round, the agreement threshold was set at 85%.

The consensus among the experts, in conjunction with their critical feedback and suggestions for improvement, bolstered the internal and content validity of the questionnaire. A statistical software package known as SPSS was employed to conduct the statistical analysis.

RESULTS

The outcomes of the study indicate that a significant majority of the expert panel – 15 out of 17 members, representing 88.2% – recognized the necessity for a standardized patient satisfaction survey instrument specifically tailored for the assessment of healthcare services related to COVID-19. Five out of seventeen experts (29.4%) expressed that a new patient satisfaction survey questionnaire should be formulated, however a larger majority of 70.5 % suggested that question related to COVID-19 healthcare delivery can be added to the otherwise standard patient satisfaction questionnaire (PSQ-18), suggesting that certain items could be selectively incorporated as needed.

In the first round, thematic evaluation was done for the questions to be included in the survey for assessing the patient satisfaction related to healthcare delivery system among COVID-19 hospitalized survivors. The themes included the accessibility of healthcare services, clinical hazards

and resultant outcomes, caregiver cognizance, attitudes, and responsiveness towards patients afflicted with COVID-19, the socio-demographic attributes of the participants, as well as the phenomenon of vaccine hesitancy.

In the next round, the above-mentioned themes were applied, and an initial questionnaire was made after incorporating the questions identified in the initial round. In this round about 85% of the experts agreed upon the comprehensiveness of the questionnaire. However, 62% of the experts thought that the questionnaire was not feasible as it was too lengthy, in contrast to the above, over 47% among them regarded that almost every question was "important" or "essential".

In the third round of evaluation, the panel scrutinized the subsequent draft for precision and delineated a fundamental set of inquiries, encompassing sociodemographic aspects. An impressive 85% of the panel members designated all inquiries as "clear," leading to the exclusion of 8 questions from the initial pool of 65.

During the iterative Delphi rounds, the expert panel achieved a consensus on multiple critical aspects, including the significance of employing Likert scales for response measurement and the necessity of incorporating inquiries pertinent to COVID-19. In conclusion, a consensus was established on 57 distinct questions. Nonetheless, persistent discord was evident concerning the potential inclusion of focus group interviews with the younger demographic, specifically individuals aged 18 to 22 years.

The final version of the newly developed questionnaire, based on the findings from the Delphi survey, includes 57 questions related to COVID-19, healthcare provision during the pandemic, vaccination ambivalence, and sociodemographic attributes. It is methodically designed to utilize a 5-point Likert scale to quantify patient satisfaction indices, as depicted in Figure 1.

DISCUSSIONS

The development of a standardized patient satisfaction survey questionnaire tailored for COVID-19 healthcare delivery represents a significant advancement in understanding patient experiences during the pandemic. A similar strategy was adopted by Wu, Meng-San et al. in their study conducted in Liverpool, UK (18). Another study from India (19) also evaluated patient satisfaction during the hospital stay of COVID-19 patients, but only in terms of psychometric evaluation, thus making it different from ours. Internationally, since the onset of the pandemic, several studies across the globe have been pioneers in using the Delphi process for questionnaire validation (19). However, this method has not been widely used in India. Our study utilized the e-Delphi method, which facilitated a structured and iterative process among a diverse panel of experts, including healthcare professionals, researchers, and public health officials. The consensus-driven approach ensured that the final questionnaire not only reflects the multifaceted nature of patient satisfaction but also aligns with the cultural and contextual nuances of the Indian healthcare system.

The results from the Delphi rounds indicate a strong agreement among experts regarding the necessity of a dedicated questionnaire for assessing patient satisfaction in the context of COVID-19. With 88.2% of the panel recognizing the need for such an instrument, it is evident that there is a critical gap in existing patient satisfaction measures that this study aims to fill. The identification of key themes – such as access to care, clinical risks, caregiver responsiveness, and vaccine hesitancy – highlights the unique challenges faced by patients during the pandemic and underscores the importance of capturing these dimensions in the survey. Another study from India (19) had highlighted the themes as daily need facilities, treatment and hospital appropriate facilities, thus having very different conclusions. Apart from the themes, their method of validation was purely systematic by conducting several qualitative interviews and evaluating it on a psychometric scale.

The iterative nature of the Delphi process allowed for continuous refinement of the questionnaire. In the second round, the incorporation of feedback led to a draft that over 85% of panelists agreed to include with all essential elements. However, the suggestion from nearly 62% of experts to shorten the questionnaire reflects a common challenge in survey design: balancing comprehensiveness with feasibility. The final version, consisting of 57 questions, successfully navigates this challenge by focusing on critical areas while ensuring that the instrument remains manageable for respondents. To the best of our knowledge, there is no questionnaire based on patient satisfaction re-

lated to COVID-19 which is so detailed and robust in nature. Moreover, the use of a 5-point Likert scale for quantifying each response enhances the questionnaire's ability to effectively summarize the data collected. This choice of survey design aligns with best practices in conducting surveys, allowing for nuanced responses that can inform healthcare providers about specific areas needing improvement. This is also the methodology followed by most studies (20). The emphasis on clarity and coherence in question wording, as evidenced by the elimination of 8 questions in the final round, further strengthens the validity of the instrument.

The implications of this study extend beyond the immediate context of COVID-19. By establishing a validated questionnaire, healthcare providers can gain valuable insights into patient experiences, which can inform quality improvement initiatives and policy decisions. Additionally, the findings underscore the importance of engaging diverse stakeholders in the development of healthcare assessment tools, ensuring that they are relevant and effective in addressing the needs of the population.

CONCLUSIONS

1. The questionnaire serves as an essential tool for evaluating and improving healthcare delivery to individuals, as it encapsulates the intricate experiences of patients during the COVID-19 pandemic. Opting for the e-Delphi technique, ensured the preservation of robust methodology while accurately depicting the cultural and healthcare-specific contexts of India.
2. The questionnaire has emerged as an indispensable resource for policymakers and healthcare practitioners, serving a profound understanding of patient needs. It has helped to foster resiliency within the healthcare system, thereby arming the healthcare fraternity to recognize and manage health crises in the future. Future research should focus on the implementation of this questionnaire in clinical settings and its potential impact on enhancing patient care and satisfaction.

LIMITATIONS

Despite the methodological strengths of this study, certain limitations are present. A primary concern is the relatively small size of the expert panel, which was selected based on domain expertise. This limitation may constrain the diversity of perspectives and experiences represented, potentially affecting the questionnaire's generalizability within the broader healthcare context. Furthermore, the questionnaire, developed specifically to capture patient experiences during the COVID-19 pandemic, is tailored to the circumstances of that period. Consequently, its relevance, while aligned with the context of the pandemic, may not encompass the subtleties of evolving public health scenarios or the spectrum of future patient experience.

CONFLICT OF INTERESTS

There is no conflict of interest.

ETHICAL APPROVAL

The Institutional Ethical Clearance (IEC) was obtained by Amity University, Noida, India (IEC approval number: AUUP/IEC/2023/4).

ACKNOWLEDGMENT

Our profound gratitude is extended to all specialists who participated in the e-Delphi process; their invaluable perspectives and contributions were integral to the formulation and endorsement of the patient satisfaction survey instrument. We wish to convey our appreciation to public health officials, researchers, and healthcare practitioners for their commitment, time, and expertise in advancing healthcare delivery throughout the COVID-19 crisis. A particular acknowledgment is due to the institutions and organizations that fostered and supplied the essential resources for this investigation. Ultimately, we express our thanks to the patients whose experiences and feedback significantly influenced our work, underscoring the importance of patient-centered care in these unprecedented conditions.

REFERENCES

1. Agency Healthcare Research and Quality (AHRQ). A for. Published online August 22, 2023. Available at: https://www.ahrq.gov/cahps/index.html (Accessed 10.09.2024).
2. Dixon J. Improving the quality of care in health systems: towards better strategies. *Isr J Health Policy Res.* 2021;10(1):15. doi:10.1186/s13584-021-00448-y
3. Prakash U, Venkatesan K, Sudesh D, et al. Evaluation of cancer patient satisfaction during COVID-19 pandemic: A survey conducted at a tertiary care center in India. *J Cancer Res Ther.* 2021; 17(6):1540-1546. doi:10.4103/jcrt.JCRT_1720_20
4. Gotthardt CJ, Haynes SC, Sharma S, Yellowlees PM, Luce MS, Marcin JP. Patient Satisfaction with Care Providers During the COVID-19 Pandemic: An Analysis of Consumer Assessment of Healthcare Providers and Systems Survey Scores for In-Person and Telehealth Encounters at an Academic Medical Center. *Telemed J E Health*. 2023;29(8): 1114-1126. doi:10.1089/tmj.2022.0460
5. Soklaridis S, Lin E, Lalani Y, Rodak T, Sockalingam S. Mental health interventions and supports during COVID- 19 and other medical pandemics: A rapid systematic review of the evidence. *Gen Hosp Psychiatry*. 2020;66:133-146. doi:10.1016/j.genhosppsych.2020.08.007
6. Murphy L, Markey K, O' Donnell C, Moloney M, Doody O. The impact of the COVID-19 pandemic and its related restrictions on people with pre-existent mental health conditions: A scoping review. *Arch Psychiatr Nurs*. 2021;35(4):375-394. doi:10.1016/j.apnu.2021.05.002
7. Donahedian A. Ann Arbor, MI. *The definition of quality and approaches to its assessment.* Health Administration Press: 1980. Available at: https://psnet.ahrq.gov/issue/definition-quality-and-approaches-its-assessment-vol-1-explorations-quality-assessment-and (Accessed 10.08. 2024).
8. Yellen E, Davis GC, Ricard R. The measurement of patient satisfaction. *J Nurs Care Qual*. 2002;16(4): 23-29. doi:10.1097/00001786-200207000-00005
9. Afrashtehfar KI, Assery MKA, Bryant SR. Patient Satisfaction in Medicine and Dentistry. *Int J Dent*. 2020;2020:6621848. doi:10.1155/2020/6621848
10. Zhang J, Chen W, Petrovsky N, Walker RM. The expectancy-disconfirmation model and citizen satisfaction with public services: a meta-analysis and an agenda for best practice. *Public Admin Rev*. 2022;82:147-159. doi:10.1111/puar.13368
11. Serrano CI, Shah V, Abràmoff MD. Use of Expectation Disconfirmation Theory to Test Patient Satisfaction with Asynchronous Telemedicine for Diabetic Retinopathy Detection. *Int J Telemed Appl*. 2018;2018:7015272. doi:10.1155/2018/7015272
12. Shang Z. Use of Delphi in health sciences research: A narrative review. *Medicine (Baltimore).* 2023; 102(7):e32829. doi:10.1097/MD.0000000000032829
13. Dalkey N. An experimental study of group opinion. *Futures.* 1969;1(5):408-426. doi:10.1016/s0016-3287[69]80025-x
14. Schreuder N, de Hoog Q, de Vries ST, Jager PL, Kosterink JGW, van Puijenbroek EP. Patient-reported adverse events of radiopharmaceuticals: development and validation of a questionnaire. *Drug Saf*. 2020;43(4):319-328. doi:10.1007/s40264-019-00895-2
15. Van Loey NE, Hofland HW, Hendrickx H, Van de Steenoven J, Boekelaar A, Nieuwenhuis MK. Validation of the burns itch questionnaire. *Burns*. 2016;42(3):526-534. doi:10.1016/j.burns.2015.08.001
16. Nemer McCoy R, Blasco PA, Russman BS, O'Malley JP. Validation of a care and comfort hypertonicity questionnaire. *Dev Med Child Neurol*. 2006;48(3): 181-187. doi:10.1017/S0012162206000405
17. Contreras-Yáñez I, Lavielle P, Clark P, Pascual-Ramos V. Validation of a risk perception questionnaire developed for patients with rheumatoid arthritis. *PLoS One*. 2019;14(7):e0219921. doi:10.1371/journal.pone.0219921
18. Wu MS, Watson R, Hayat F, et al. What do people hospitalised with COVID-19 think about their care? Results of a satisfaction survey during the first wave of COVID-19 in Liverpool. *Future Healthc J.* 2021;8(1):e70-e75. doi:10.7861/fhj.2020-0260
19. Wunadavalli LT, Satpathy S, Satapathy S, et al. Patient Satisfaction Scale for Hospitalized COVID-19 Patients: Development and Psychometric Properties. *J Patient Exp*. 2022;9: 23743735221086762. doi:10.1177/23743735221086762
20. Agarwal A, Ranjan P, Rohilla P, et al. Development and validation of a questionnaire to assess preventive practices against COVID-19 pandemic in the general population. *Prev Med Rep*. 2021; 22:101339. doi:10.1016/j.pmedr.2021.101339

Shazina SAEED, SCOPUS ID: 57193236455; WoS Researcher ID: ABI-1699-2020
Karuna Nidhi KAUR, SCOPUS ID: 57224586399; WoS Researcher ID: JVO-3468-2024
Manmohan SINGHAL, SCOPUS ID: 57667083200; WoS Researcher ID: C-7725-2011
Mohd. SHANNAWAZ, SCOPUS ID: 56349680200; WoS Researcher ID: AAY-3219-2020
Farah NIAZI, SCOPUS ID: 57223949830; WoS Researcher ID: AAF-9702-2020
Bhavna KUMAR, SCOPUS ID: 57209177097; WoS Researcher ID: AAY-3219-2020
Aanchal Anant AWASTHI, SCOPUS ID: 5721717429

Date of receipt of the manuscript: 21/06/2024
Date of acceptance for publication: 30/09/2024

OH&RM ONE HEALTH & RISK MANAGEMENT

VOL. 5, ISSUE 4
2024

BIOLOGICAL SCIENCES

OPEN ACCESS

THE IMPACT OF MICROALGAE CHLORELLA VULGARIS AND SCENEDESMUS QUADRICAUDA ON THE GROWTH PARAMETERS OF THE CILIATE PARAMECIUM CAUDATUM

Elena ROSCOV, Ion TODERAS, Laurentia UNGUREANU, Daria TUMANOVA

Institute of Zoology, Moldova State University, Republic of Moldova

Corresponding author: Elena Roscov, e-mail: elena.roscov@sti.usm.md

DOI: 10.38045/ohrm.2024.4.04 CZU: 582.263:593.171.4

Keywords: *microalgae, Chlorophyta, Ch. vulgaris, S. quadricauda, P. caudatum, population size, reproduction rate.*

Introduction. *An important aspect of research in the field of microbial ecology is understanding the interactions between organisms, particularly the influence they have on one another within aquatic ecosystems. Green microalgae (phylum Chlorophyta) represent a diverse group of photosynthetic organisms, playing a significant role in ecological cycles. Investigations have focused on evaluating the impact of two microalgae species, Chlorella vulgaris and Scenedesmus quadricauda, on the growth parameters of the natural population of Paramecium caudatum, making it a suitable indicator for assessing the influence of these organisms on the food chain.*

Material and methods. *The research was based on the work of Kokova, V. (1982), and Likhachev, S.V. (2020). The productivity of the ciliates was determined by their division rate, according to Zaika V.E. (1983) and Spinei L. (2009). Experiments were conducted over intervals of 24-144 hours, using microalgae solution concentrations at 0.1, 0.5, 1, and 10 mg/L.*

Results. *For Chlorella vulgaris the lower doses generated a significant stimulatory effect on P. caudatum, resulting in notable increases in both number and reproduction rate.*
In contrast, for Scenedesmus quadricauda, lower doses also had a pronounced stimulatory effect, while higher doses yielded varied results, including both increases and decreases in parameters.

Conclusions. *Both strains of microalgae demonstrate stimulatory potential on the natural population of P. caudatum, particularly at lower doses.*

Cuvinte-cheie: *microalge, Chlorella vulgaris, Scenedesmus quadricauda, Paramecium caudatum, efectivul numeric, rata de reproducere.*

IMPACTUL MICROALGELOR CHLORELLA VULGARIS ŞI SCENEDESMUS QUADRICAUDA ASUPRA PARAMETRILOR DE CREŞTERE A CILIATEI PARAMECIUM CAUDATUM

Introducere. *Un aspect important al cercetărilor în domeniul ecologiei microorganismelor constă în înţelegerea interacţiunilor dintre organisme, în special influenţa pe care o au acestea asupra ecosistemului acvatic. Microalgele verzi (Filumul Chlorophyta) reprezintă un grup divers de organisme fotosintetice, care au un rol semnificativ în ciclurile ecologice. Astfel, investigaţiile au fost orientate spre evaluarea impactului a două specii de microalge, Chlorella vulgaris şi Scenedesmus quadricauda, asupra parametrilor de creştere a populaţiei naturale de Paramecium caudatum.*

Material şi metode. *Ca suport metodologic au servit cercetările propuse de cercetătorii Kokova V. (1982) şi Likhachev S.V. (2020). Productivitatea infuzoriilor a fost determinată după viteza dividerii lor, conform cercetătorilor Zaika V. E. (1983) şi Spinei L. (2009). Experimentele s-au desfăşurat în intervale de timp de 24 - 144 ore, utilizând concentraţiile soluţiilor de microalge de 0,1; 0,5; 1 şi 10 mg/l.*

Rezultate. *Pentru Chlorella vulgaris s-a constatat că dozele mai mici au generat un efect stimulativ semnificativ asupra P. caudatum, cu creşteri notabile ale numărului şi ratei de reproducere. În cazul Scenedesmus quadricauda, dozele mai mici au avut un efect stimulator pronunţat, în timp ce dozele mari au dus la rezultate diferenţiate, inclusiv creşteri şi scăderi ale parametrilor.*

Concluzii. *Concluziile indică faptul că ambele microalge au un potenţial stimulator asupra populaţiei naturale P. caudatum, în special dozele mai mici.*

INTRODUCTION

Green algae (Chlorophyta) belong to the group of photosynthetic organisms known chlorophyta, which are green in color due to the presence of chlorophyll. With their ability to harness the solar energy and convert it into food, green algae play a crucial role in the planet's energy cycle. Important species in this class are *Chlorella vulgaris Beijerinck 1890* and *Scenedesmus quadricauda Chodat 1926*, which are studied for their photosynthetic properties, high nutrient content, bioactive compounds and potential applications in various fields (1, 2, 3).

A significant aspect of research in microbial ecology is understanding the interactions between organisms, particularly their influence on others within aquatic ecosystems. *Paramecium caudatum Ehrenberg, 1833*, a widely distributed ciliate protist, serves as a valuable model organism for such studies due to its well-documented biological characteristics and sensitivity to environmental changes (4).

Thus, the general development mechanisms of the entire ecosystem can be uncovered, and the interactions between populations can be analysed (5). In addition to investigating industrial cultivation possibilities for autotrophic microalgae, considerable attention is also given to establishing methods for producing large biomass quantities of heterotrophic invertebrate organisms (6).

The purpose of this work was to investigate the interactions between green microalgae from the phylum Chlorophyta, specifically or particularly *Chlorella vulgaris* and *Scenedesmus quadricauda* species, and their effect on the population of *Paramecium caudatum* in a controlled culture media (nutrient medium). Assessing the impact of these green algae on the behavior, reproduction, and other biological aspects of the ciliate will contribute to a deeper understanding of aquatic resource dynamics and the potential benefits or risks associated with the presence of these microorganisms in aquatic ecosystems.

This study provides a specific example of the interactions between certain microorganisms, contributing to a broader understanding of ecological relationships (3).

MATERIAL AND METHODS

The study focused on the ciliate *Paramecium caudatum*, from which separate clonal specimens were obtained for subsequent research through binary fission. *Saccharomyces cerevisiae* yeast served as their food source. Mass cultures and individual lines were used in experiments, obtained by isolating a single specimen from a culture in full development. Cultivation was conducted in micro-aquariums, following classical methods proposed by K. Kokova, V. (1982) (7); Likhachev, S.V., (2020) (8) with daily counting of cells. An optimal culture medium was created for the paramecia, including chemical composition and environmental conditions. During the cultivation of protozoa, significant attention was paid to the studies of trophic conditions, as well as the qualitative composition of their food and nutritional intensity. From each individual, a line of descendants was established, and parameters such as growth rates, lifespan, reproduction, and natural population size were monitored according to Zaika V.E (1983) (9); Spinei L. (2009) (10). These parameters were determined by counting the number of divisions per day for each line over a period of six days and data analysis.

The strains of green microalgae from the phylum Chlorophyta were obtained from Laurentia Ungureanu, a corresponding member of the ASM (Academy of Science of Moldova), research professor, and doctor habilitatus in biological sciences, as well as from Daria Tumanova, PhD in biological sciences, in the laboratory of Hydrobiology and Ecotoxicology at the Institute of Zoology. The strains were patented and deposited in the Non-pathogenic Microorganism National Collection at the Institute of Microbiology and Biotechnology, Technical University of Moldova. The microalgae were cultivated in standardized media under controlled conditions of temperature, light, and nutrients, following the methods described by Wasser S.P. et al. (1989) (11). The algal biomass was separated from the growing medium by filtration and centrifugation.

Test groups were divided based on the species of microalgae (*Chlorella vulgaris*, *Scenedesmus quadricauda*) and their concentrations, subjected to a time interval of 24 to 144 hours. Concentrations of microalgal solutions of 0.1 mg/L, 0.5 mg/L, 1 mg/L, and 10 mg/L were prepared. Control groups were also included to assess the accuracy of the procedures and confirm the results.

Quantitative analysis was performed in 10 replicates at a temperature of 25°C, over 24-144

hours using the binocular microscope MBC-9 with ocular magnification of 14x.

Appropriate statistical methods were employed to assess the significance of differences between groups (Statistica 7.0, Excel 2007, Biostat). The t-Student test (significance test) was used to test for a statistically significant difference between the values obtained in the experimental and control groups. Interpretation of P values for the significance test indicates the level of statistical significance, thus *P<0.05 (statistical correlation is significant (S, 95% confidence)), **P<0.01 (statistical correlation is significant (S, 99% confidence)), ***P<0.001 (statistical correlation is highly significant (HS, 99.9% confidence)) and P>0.05 (statistical correlation is insignificant, IS).

RESULTS

The study investigated the sensitivity of the ciliate *Paramecium caudatum* to cultures of *Chlorophyta* microalgae, represented by the species *Chlorella vulgaris* and *Scenedesmus quadricauda*, as well as to microalgal culture liquids. The focus was on evaluating the response of the test organism to various concentrations (0.1, 0.5, 1, and 10 mg/L) and different time intervals (24, 48, 72, 96, 120, and 144 hours).

Evaluating the impact of different concentrations of *Chlorella vulgaris* over a 24-hour interval revealed statistically significant effects at concentrations of 0.1 and 10 mg/L. Specifically, the concentration of 0.1 mg/L showed a significant negative effect on population dynamics, resulting in a decrease of 43.33%, while the concentration of 10 mg/L resulted in a more pronounced decrease of 60.00% (fig. 1).

The reproduction rate of *Cw* at concentrations of 0.1 and 10 mg/L exhibited significant reductions of 31.84% and 50.84%, respectively. Medium concentrations of 0.5 and 1 mg/L also had a negative impact, but the effects were weaker, suggesting only a slight, insignificant reduction in *Nt* and *Cw* (tab. 1, fig. 1).

Table 1. Experimental results for the tested cultures with the microalgae *Chlorella vulgaris* as food for the ciliates *Paramecium caudatum.*

Experimental groups	N	Chlorella vulgaris							
		Sample size (Nt) M±ES	Difference compared to the control			Repro duction rate (Cw) M±ES	Difference compared to the control		
			d	%	t_d		d	%	t_d
24 h									
Control	*10*	*6.00±0.67*				*1.79±0.11*			
0.1 mg/L	*10*	*3.40±0.55*	*-2.60*	*43.33*	*3.00***	*1.22±0.18*	*-0.57*	*31.84*	*2.70***
0.5 mg/L	*10*	*4.90±0.53*	*-1.10*	*18.33*	*1.29*	*1.59±0.12*	*-0.20*	*11.17*	*1.23*
1 mg/L	*9*	*5.89±1.10*	*-0.11*	*1.83*	*0.09*	*1.77±0.17*	*-0.02*	*1.12*	*0.10*
10 mg/L	*10*	*2.40±0.17*	*-3.60*	*60.00*	*5.21****	*0.88±0.07*	*-0.91*	*50.84*	*6.98****
72 h									
Control	*9*	*109.00±18.69*				*1.56±0.05*			
0.1 mg/L	*10*	*157.60±35.38*	*+48.60*	*44.59*	*1.21*	*1.69±0.09*	*+0.13*	*8.33*	*1.26*
0.5 mg/L	*10*	*178.40±18.80*	*+69.40*	*63.67*	*2.62***	*1.73±0.03*	*+0.17*	*10.90*	*2.92***
1 mg/L	*10*	*187.40±30.00*	*+78.40*	*71.93*	*2.22**	*1.74±0.05*	*+0.18*	*11.54*	*2.55**
10 mg/L	*10*	*136.00±24.71*	*+27.00*	*24.77*	*0.87*	*1.64±0.08*	*+0.09*	*5.77*	*0.95*
120 h									
Control	*10*	*199.40±27.14*				*1.06±0.04*			
0.1 mg/L	*10*	*354.90±80.94*	*+155.50*	*77.98*	*1.82*	*1.17±0.06*	*+0.07*	*6.60*	*0.97*
0.5 mg/L	*10*	*437.30±101.26*	*+237.90*	*119.31*	*2.27**	*1.22±0.06*	*+0.11*	*10.38*	*1.53*
1 mg/L	*10*	*301.30±28.72*	*+101.90*	*51.10*	*2.58***	*1.14±0.02*	*+0.09*	*8.49*	*2.01**
10 mg/L	*9*	*223.33±44.06*	*+23.93*	*12.00*	*0.46*	*1.08±0.05*	*0.00*	*0.00*	*0.00*
144 h									
Control	*10*	*160.30±15.78*				*0.85±0.02*			
0.1 mg/L	*10*	*343.90±45.68*	*+183.60*	*114.54*	*3.80****	*0.97±0.03*	*+0.12*	*14.12*	*3.33****
0.5 mg/L	*10*	*460.50±32.87*	*+300.20*	*187.27*	*8.23****	*1.02±0.01*	*+0.17*	*20.00*	*7.60****
1 mg/L	*10*	*285.60±42.72*	*+125.30*	*78.17*	*2.75***	*0.94±0.05*	*+0.09*	*10.59*	*1.67*
10 mg/L	*9*	*222.78±36.18*	*+62.48*	*30.98*	*1.58*	*0.90±0.05*	*+0.05*	*5.88*	*0.93*

*Note: * - P<0.05 (S); ** - P<0.01 (S); *** - P<0.001 (HS); P>0.05 (NS)*

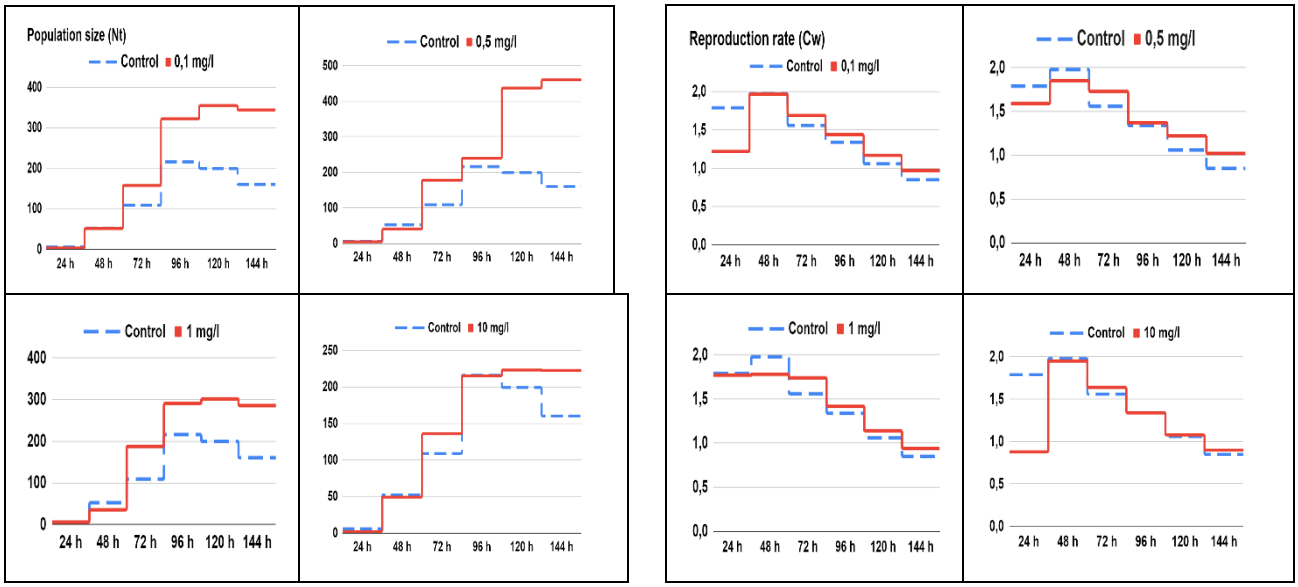


Figure 1. The dynamics of the population size (Nt) and reproduction rate (Cw) of *Paramecium* after administration of 0.1 mg/L, 0.5 mg/L, 1 mg/l, and 10 mg/L of *Chlorella vulgaris* medium over 24…144 hours (ĩ(hours)).

After 144 h incubation, it was observed that at a concentration of 0.1 mg/L, Nt increased significantly by +183.60 with an increase of 114.54% (t_d=3.80; P<0.001), while at 0.5 mg/L, it increased by +300.20, with a substantial increase of 187.27% (t_d=8.23; P<0.001). The statistical correlation is highly significant (HS, 99.9% confidence). Concentrations higher than 1 and 10 mg/L showed positive effects but were statistically insignificant (tab. 1).

Thus, the experimental results demonstrated that the tested concentrations of *Chlorella vulgaris* on the ciliate *P. caudatum* positively influence the abundance (Nt) and reproduction rate (Cw), with these effects increasing at higher concentrations, especially over longer time intervals.

To further investigate the effects of the analyzed test, the solution in which the microalga Chlorella vulgaris was cultivated was introduced into the nutrient medium of the paramecia. It was demonstrated, that after 24 h of incubation, concentrations lower than 0.1, 0.5, and 1 mg/L had significant negative effects, leading to a decrease in Nt and Cw. The deviation is at the 5% probability level when P value <0.05 (S). The concentration of 1 mg/L had the greatest negative impact, with a significant and considerable reduction in Nt, indicated by a difference of -2.40 and a significant percentage decrease of -40.00%, and in Cw indicated by a difference of -0.40 and a significant percentage decrease of -22.35%. The deviation is negatively significant (P<0.001) at a 0.1% probability level. The concentration of 10 mg/L has a nonsignificant positive impact of +0.30 (5.00%), suggesting a possible stimulating effect (tab. 2, fig. 2).

After 72 hours of incubation, the concentration of 0.1 mg/L continues to have a negative influence, but with values closer to the control values, with a difference of only - 2.22 (2.04%). As the concentrations increased, the effects became positive, remaining nonsignificant at 0.5 and 1 mg/L, and significant at 10 mg/L. At the concentration of 10 mg/L, Nt increases by 93.68% (t_d=4.90; P<0.001) and Cw by 14.10% (t_d=4.31; P<0.001) compared to the control. The deviation is statistically significant when the probability (P) is 0.1%. The values decreased after 120 hours, then increased again after 144 hours.

At 144 hours of incubation, the concentration of 0.1 mg/L improved both the population size and reproduction rate, approaching the control level. Concentrations of 0.5, 1, and 10 mg/L generated a significant increase compared to previous intervals, surpassing the control in the case of 1 mg/L concentration by 73.30% and 10 mg/L by 52.42%.

The results demonstrated that the cultural media of the microalgae *Chlorella vulgaris* at higher concentrations of 1 and 10 mg/L had a more pronounced effect, positively influencing popu-

Table 2. Experimental results of testing the liquid culture of the microalgae *Chlorella vulgaris* as food for *Paramecium caudatum* ciliates

Experimental groups	N	Liquid culture of the microalgae *Chlorella vulgaris*							
		Population size (Nt) M±ES	Difference compared to the control			Reproduction rate (Cw)M±ES	Difference compared to the control		
			d	%	t_d		d	%	t_d
24 h									
Control	10	6.00±0.67				1.79±0.11			
0.1 mg/L	9	3.89±0.57	-2.11	35.16	2.40*	1.36±0.14	-0.43	24.02	2.42*
0.5 mg/L	8	4.00±0.40	-2.00	33.33	2.56*	1.39±0.11	-0.40	22.35	2.57*
1 mg/L	10	3.60±0.39	-2.40	40.00	3.10**	1.28±0.11	-0.51	28.49	3.28***
10 mg/L	10	6.30±1.64	+0.30	5.00	0.17	1.84±0.26	+0.05	2.79	0.18
72 h									
Control	9	109.00±18.69				1.56±0.05			
0.1 mg/L	9	106.78±13.14	-2.22	2.04	0.10	1.56±0.04	0.00	0.00	0.00
0.5 mg/L	10	120.30±33.65	+11.30	10.37	0.29	1.60±0.10	+0.04	2.56	0.36
1 mg/L	10	142.30±24.59	+33.30	30.55	1.08	1.65±0.06	+0.09	5.77	1.15
10 mg/L	9	211.11±9.23	+102.11	93.68	4.90***	1.78±0.01	+0.22	14.10	4.31***
120 h									
Control	10	199.40±27.14				1.06±0.04			
0.1 mg/L	10	160.10±33.02	-39.30	19.71	0.92	1.02±0.05	-0.04	3.77	0.62
0.5 mg/L	10	208.10±39.40	+8.70	4.36	0.18	1.07±0.05	+0.01	0.94	0.16
1 mg/L	9	147.56±30.85	-51.84	26.00	1.26	1.00±0.08	-0.06	5.66	0.67
10 mg/L	10	169.10±24.88	-30.30	15.20	0.82	1.03±0.03	-0.03	2.83	0.60
144 h									
Control	10	160.30±15.78				0.85±0.02			
0.1 mg/L	10	183.70±27.18	+23.40	14.60	0.74	0.87±0.04	+0.02	2.35	0.45
0.5 mg/L	9	188.56±37.49	+28.26	17.63	0.69	0.87±0.03	+0.02	2.35	0.55
1 mg/L	10	277.80±33.81	+117.50	73.30	3.15**	0.94±0.03	+0.09	10.59	2.50*
10 mg/L	9	244.33±42.26	+84.03	52.42	1.86*	0.92±0.05	+0.07	8.24	1.30

Note: * - P<0.05 (S); ** - P<0.01 (S); *** - P<0.001 (HS); P>0.05 (NS)

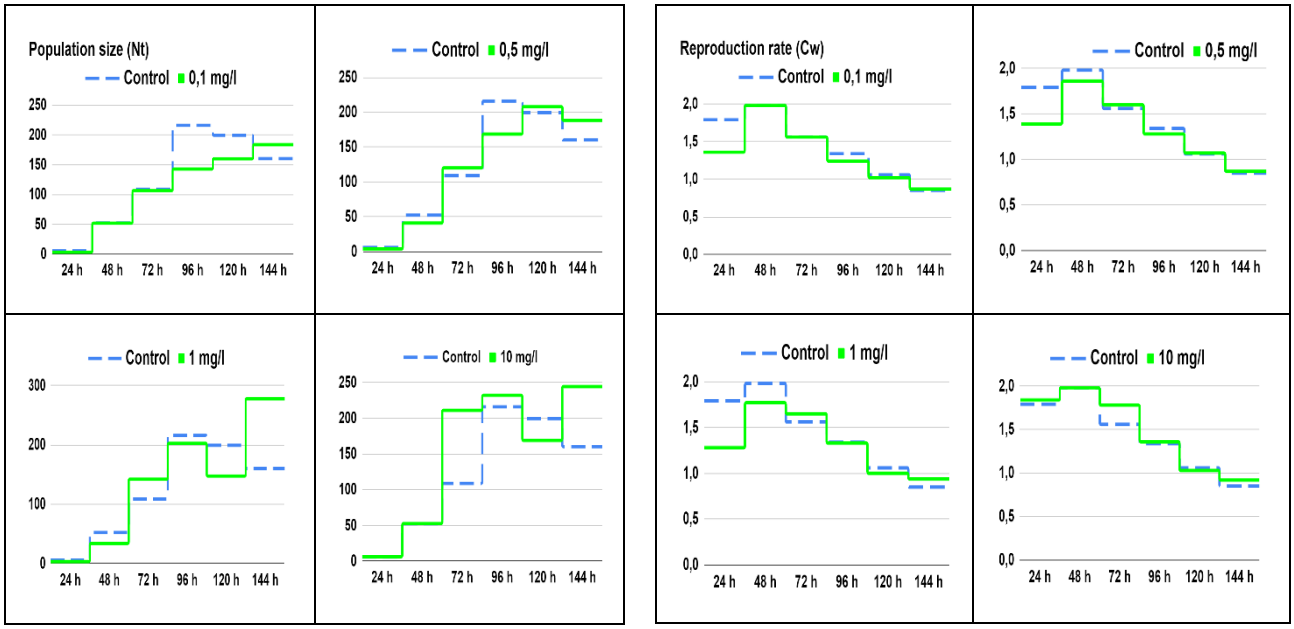


Figure 2. The dynamics of population size (Nt) and reproduction rate (Cw) of *Paramecium* after administration of 0.1 mg/L, 0.5 mg/L, 1 mg/L, and 10 mg/L of the media with microalgae *Chlorella vulgaris* over 24…144 hours (ĩ(hours)).

lation size and reproduction rate at 72, 96, 120, and 144 hours of incubation. In contrast, at 24 and 48 hours, the effects were less consistent than at lower concentrations (0.1 and 0.5 mg/L). Therefore, with the increase in incubation time, the effects of the concentrations became positive.

At 144 hours the concentration of 1 mg/L indicated a significant increase (Nt-td = 3.15, P<0.01 (S); Cw-td = 2.50, P<0.05 (S)), suggesting a stimulatory effect at this concentration and duration. The results at 10 mg/L were more varied, demonstrating a complex influence on the *Paramecium* culture.

The next stage involved adding the microalgae *Scenedesmus quadricauda* to the nutrient medium of *Paramecium*.

After 24 hours of incubation of the experimental samples, it was observed that at 0.1 mg/L, the population size (Nt) of *Paramecium* increased by 14.29%, and the reproduction rate (Cw) increased by 11.20%, indicating a positive influence on reproduction. At 1 mg/L, on the contrary, we observed a highly significant decrease in Nt by 42.86% (t_d=3.54; P<0.001), and Cw decreased by 44.80% (t_d=3.15; P<0.01), suggesting a significant negative influence on reproduction. At 10 mg/L, *Nt* decreased by 46.29% (td=3.54; P<0.001), and *Cw* decreased by 49.60% (td=3.48; P<0.001), with the negative effects becoming more pronounced at this higher concentration. The statistical correlation is highly significant (HS, 99.9% confidence) (tab. 3).

Table 3. Experimental results of testing the culture containing microalgae *Scenedesmus quadricauda* as food for the ciliates *Paramecium caudatum.*

Experimental groups	N	*Scenedesmus quadricauda*							
		Population size (Nt) M±ES	Difference compared to the control			Reproduction rate (Cw)M±ES	Difference compared to the control		
			d	%	t_d		d	%	t_d
24 h									
Control	10	3.50±0.39				1.25±0.11			
0.1 mg/L	9	4.00±0.64	+0.50	14.29	0.67	1.39±0.02	+0.14	11.20	1.25
0.5 mg/L	10	3.10±0.29	-0.40	11.43	0.82	1.13±0.10	-0.12	9.60	0.81
1 mg/L	9	2.00±0.25	-1.50	42.86	3.54***	0.69±0.14	-0.56	44.80	3.15**
10 mg/L	8	1.88±0.24	-1.62	46.29	3.54***	0.63±0.14	-0.62	49.60	3.48***
72 h									
Control	9	90.00±33.64				1.50±0.12			
0.1 mg/L	10	101.00±24.27	+11.00	12.22	0.27	1.54±0.09	+0.04	2.67	0.27
0.5 mg/L	10	71.40±22.96	-18.60	20.67	0.46	1.42±0.13	-0.08	5.33	0.45
1 mg/L	8	51.57±13.34	-38.43	42.70	1.06	1.31±0.09	-0.19	12.67	1.27
10 mg/L	10	49.60±18.09	-40.40	44.89	1.06	1.30±0.12	-0.20	13.33	1.18
120 h									
Control	9	211.11±52.79				1.07±0.06			
0.1 mg/L	10	194.50±35.91	-16.61	7.87	0.26	1.05±0.05	-0.02	1.87	0.26
0.5 mg/L	10	267.00±46.50	+55.89	26.47	0.79	1.12±0.07	+0.05	4.67	0.54
1 mg/L	10	152.33±27.41	-58.78	27.84	0.99	1.01±0.04	-0.06	5.61	0.83
10 mg/L	8	114.50±31.48	-96.61	45.76	1.57	0.95±0.05	-0.12	11.21	1.54
144 h									
Control	9	262.67±23.76				0.93±0.02			
0.1 mg/L	10	203.50±29.23	-59.17	22.53	1.57	0.89±0.04	-0.04	4.30	0.10
0.5 mg/L	10	367.10±34.24	+104.43	39.76	2.51*	0.98±0.02	+0.05	5.38	1.77
1 mg/L	9	261.00±46.70	-1.67	0.63	0.03	0.93±0.03	0.00	0.00	0.00
10 mg/L	9	139.67±36.44	-123.00	46.83	2.83**	0.82±0.05	-0.11	11.83	2.04*

Note: * - P<0.05 (S); ** - P<0.01 (S); *** - P<0.001 (HS); P>0.05 (NS)

After 72 hours of incubation, the low concentration of 0.1 mg/L indicated positive values for Nt and Cw, although these effects were statistically insignificant (tab. 3). Meanwhile, the higher concentrations of 0.5, 1, and 10 mg/L led to a decrease in the studied parameters. The negative effects were less pronounced compared to 48 hours (fig. 3). This suggests a negative influence

of the *Scenedesmus* culture, but a statistically insignificant on the population size and reproduction rate at higher doses.

The concentration of 0.5 mg/L of *Scenedesmus* exposed for 120 h resulted in a non-significant increase in Nt (26.47%) and Cw (4.67%). As time progressed, at 144 h, the concentration of 0.5 mg/L significantly increased the mean Nt value by 39.76% (t_d=2.51; P<0.05) and contributed non-significantly to an increase in Cw by 5.38% (t_d=1.77; P>0.05) compared to the control (tab. 3, fig. 3). This indicates a significant positive effect. From 24 to 96 h, these values were lower, which can be explained by the adaptation period of the ciliates to the addition of *Scenedesmus quadricauda*, after which these indices increased.

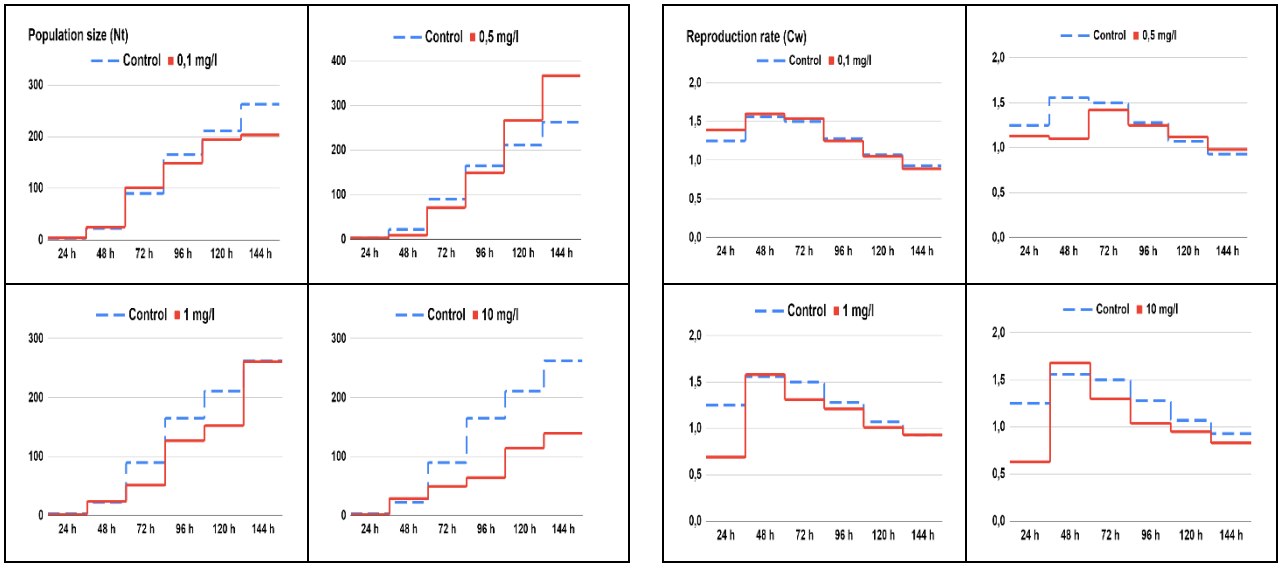


Figure 3. The dynamics of the population size (Nt) and reproduction rate (Cw) of *Paramecium* after exposure to 0.1 mg/L, 0.5 mg/L, 1 mg/L, and 10 mg/L of *Scenedesmus quadricauda* culture for 24...144 hours.

In the *Paramecium* culture medium, liquid cultures of *Scenedesmus quadricauda* were added at concentrations of 0.1, 0.5, 1, and 10 mg/L. The experimental results showed that after 24 hours of incubation, the concentrations that negatively affected *Paramecium* were 0.1 and 10 mg/L (tab. 4, fig. 4). The *Nt* values decreased by 24.86% (0.1 mg/L) and 26.86% (10 mg/L), and Cw values decreased by 22.40% (0.1 mg/L) and 24.80% (10 mg/L) compared to control values. The significance threshold is P>0.05, so the values were insignificant (IS). The negative effect was more pronounced at the higher dose. At 1 mg/L, Nt increases by 3.71%, and Cw increases by 3.20%. While this indicates a positive effect, it is minimal and nearly equivalent to the control values.

In Table 4, it was observed that after 72 hours of incubation, at a concentration of 0.1 mg/L, Nt decreased by 6.55%, and Cw by 1.33%, demonstrating a negative but minor effect. The concentration of 0.5 mg/L had a positive effect on *Paramecium*, but insignificant, Nt increased by 66.44% (t_d=1.16; P>0.05), and Cw increased by 11.33%. At a concentration of 1 mg/L, Nt significantly decreased by 67.22% (t_d=1.16; P>0.05), and Cw significantly decreased by 24.67% (t_d=2.66; P>0.01(S)). The deviation is significant since t_d (t-Student significance test) is greater than 2.58 with a 99% confidence interval. At 10 mg/L, Nt decreased by 43.09%, and Cw decreased by 12.67%. However, the negative effect was less pronounced compared to 1 mg/L.

After 120 hours, at 0.1 mg/L, Nt increased by 10.37%, and Cw increased by 1.87%. This is a positive but modest effect, stimulating the numerical growth of the *P. caudatum* culture and maintaining a relatively constant reproduction rate. At 0.5 mg/L, there was a significant inhibitory effect, resulting in a decrease in Nt by 21.89% and Cw by 4.67%. The 1 mg/L dose had a more pronounced inhibitory effect, causing a reduction in both parameters, Nt by 24.73%, and Cw by 5.61%. The negative effect is more pronounced compared to 24 hours. Conversely, the

10 mg/L dose had a significant stimulatory effect, contrary to expectations, leading to an increase in both the number and reproduction rate, Nt by 28.46%, and Cw by 4.67%. This effect was positive but statistically insignificant, with a significance threshold of P>0.05 (IS).

After 144 hours the concentration of 0.1 mg/L continued to have a positive but insignificant effect, maintaining a numerical increase and a relatively constant reproduction rate, Nt increased by 9.77%, and Cw increased by 1.08%. Higher concentrations of 0.5 mg/L, 1 mg/L, and 10 mg/L had negative effects on the *Paramecium* (fig. 4). For example, the concentration of 0.5 mg/L had an extremely pronounced inhibitory effect, leading to a decrease in both the number and reproduction rate. Doses of 1 mg/L and 10 mg/L had a more or less moderate, but still inhibitory effect, resulting in a significant reduction in both parameters.

Thus, *Scenedesmus quadricauda* and its culture medium, tested as food for paramecia, significantly influence reproduction, with both positive and negative effects depending on the administered dose and exposure time. Lower concentrations such as 0.1 mg/L and 0.5 mg/L, generally had a stimulative effect on reproduction, while higher concentrations of 1 mg/L and 10 mg/L had varied effects, expressed through increases and decreases in parameters, suggesting a more complex effect.

The research allowed us to assess the degree of ecological plasticity of the natural population of *P. caudatum* in relation to environmental factors, which will serve as a scientific basis for the development of safe monitoring and conservation measures for the species.

Table 4. Experimental results of testing the liquid culture of the microalgae *Scenedesmus quadricauda* as food for *Paramecium caudatum* ciliates.

Experimental groups	N	Liquid culture of the microalgae *Scenedesmus quadricauda*							
		Population size (Nt) M±ES	Difference compared to the control			Reproduction rate (Cw) M±ES	Difference compared to the control		
			d	%	t_d		d	%	t_d
24 h									
Control	10	3.50±0.39				1.25±0.11			
0.1 mg/L	8	2.63±0.45	-0.87	24.86	1.46	0.97±0.18	-0.28	22.40	1.33
0.5 mg/L	10	2.90±0.37	-0.60	17.14	1.12	1.06±0.15	-0.19	15.20	1.02
1 mg/L	8	3.63±0.45	+0.13	3.71	0.22	1.29±0.14	+0.04	3.20	0.22
10 mg/L	9	2.56±0.36	-0.94	26.86	1.77	0.94±0.15	-0.31	24.80	1.67
72 h									
Control	9	90.00±31.73				1.50±0.12			
0.1 mg/L	10	84.10±20.40	-5.90	6.55	0.16	1.48±0.11	-0.02	1.33	0.12
0.5 mg/L	10	149.80±40.55	+59.80	66.44	1.16	1.67±0.09	+0.17	11.33	1.13
1 mg/L	10	29.50±7.54	-60.50	67.22	1.86	1.13±0.07	-0.37	24.67	2.66**
10 mg/L	9	51.22±6.61	-38.78	43.09	1.20	1.31±0.04	-0.19	12.67	1.50
120 h									
Control	9	211.11±52.79				1.07±0.06			
0.1 mg/L	9	233.00±37.80	+21.89	10.37	0.34	1.09±0.03	+0.02	1.87	0.30
0.5 mg/L	10	164.90±31.58	-46.21	21.89	0.75	1.02±0.04	-0.05	4.67	0.69
1 mg/L	10	158.90±33.66	-52.21	24.73	0.83	1.01±0.04	-0.06	5.61	0.83
10 mg/L	10	271.20±47.03	+60.09	28.46	0.85	1.12±0.03	+0.05	4.67	0.75
144 h									
Control	9	262.67±23.76				0.93±0.02			
0.1 mg/L	9	288.33±60.47	+25.66	9.77	0.39	0.94±0.07	+0.01	1.08	0.14
0.5 mg/L	8	174.88±49.72	-87.79	33.42	1.59	0.86±0.06	-0.07	7.53	1.11
1 mg/L	9	240.11±35.87	-22.56	8.59	0.52	0.91±0.03	-0.02	2.15	0.55
10 mg/L	8	215.00±41.60	-47.67	18.15	1.00	0.90±0.03	-0.03	3.23	0.83

Note: * - P<0.05 (S); ** - P<0.01 (S); *** - P<0.001 (HS); P>0.05 (NS)

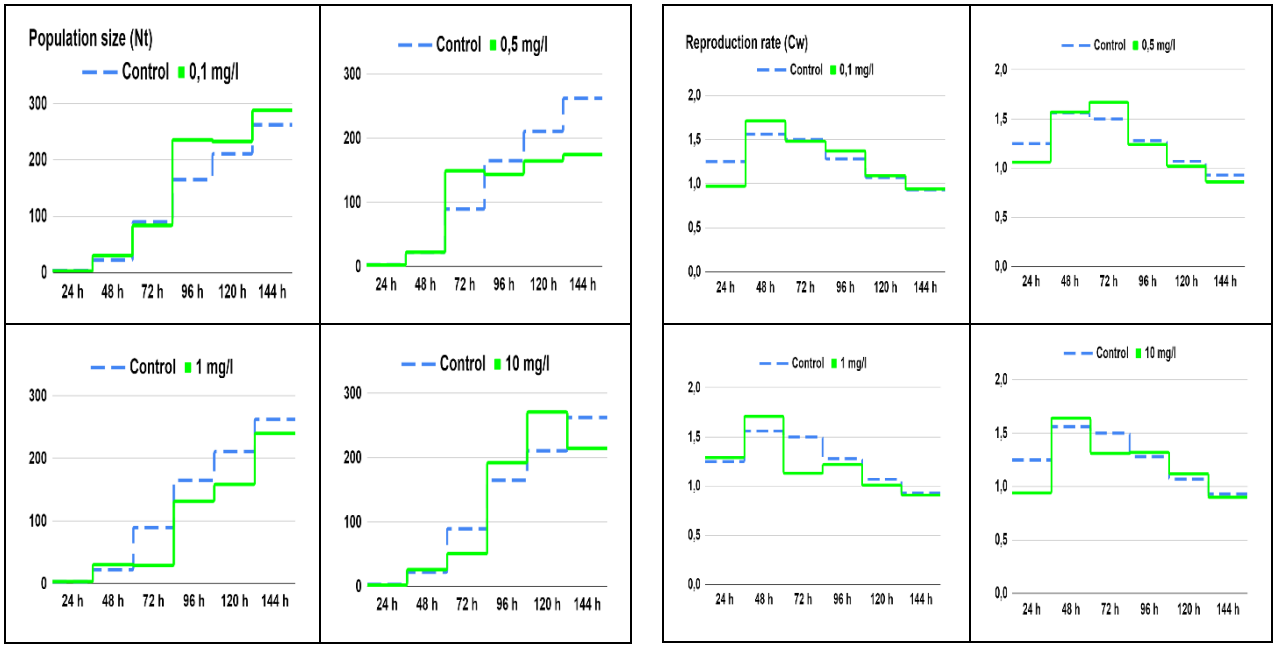


Figure 4. The dynamics of the population size (Nt) and reproduction rate (Cw) of *Paramecium* after exposure to 0.1 mg/L, 0.5 mg/L, 1 mg/L, and 10 mg/L o f *Scenedesmus quadricauda* culture medium for 24...144 hours.

DISCUSSIONS

Microalgae of the phylum *Chlorophyta* play a crucial role in the planet's energy cycle through their ability to harness solar energy and convert it into food. Of the notable species, *Chlorella vulgaris* and *Scenedesmus quadricauda* serve as the foundation of many aquatic ecosystems. Through photosynthesis, they produce oxygen and convert carbon dioxide into organic material, playing a key role in the global carbon cycle. This process sustains algae and generates the primary food source for various aquatic organisms. Thus, these investigations not only contribute to the development of our knowledge of ecological mechanisms, but also emphasize the importance of the conservation and proper management of these fragile environments.

Some studies have developed feeding protocols for bee families at the end of winter, a period of limited foraging, using nutrient paste enriched with biologically active supplements derived from the biomasses of aquatic monocellular microalgae, including *Scenedesmus quadricauda*, *Scenedesmus apiculatus*, and *Oocystis borgei*. The research has shown that feeding bee families enriched with biologically active supplements from the biomass of aquatic microalgae contributes to an increase in the prolificacy of brood – by 7.8-10.3%, in the quantity of brood capped – by 7.7-9.3%, in the bee family's power – by 7, 1-9.3%, family disease resistance – by 1.8-3.8%, bro brood viability in the brood nest – by 1.2-1.7%, amount of accumulated brood in the brood nest – by 15.527.6%, amount of wax grown on combs – by 13.3-36.7% and amount of accumulated honey in the brood nest – by 28.0-38.9% (2).

Additional studies on the use of *Chlorella vulgaris* and *Scenedesmus quadricauda* microalgae have exerted a positive effect on the germination of sugar seed by increasing the efficiency and regularity of this critical process for *Beta vulgaris* seeds. The best results, in germination indices as well as root morphological traits, were reached by using *C. vulgaris* extract at medium concentrations of 1 mg Corg/L and 2 mg Corg/L, while the *S. quadricauda* extract, the concentration effects on germination indices were less evident and differences among concentrations were not significant. Only one concentration of 1 mg Corg/L, had a positive effect (3).

In comparison to previous research, we examined the effects of these two microalgae on the unicellular organism *Paramecium caudatum*, particularly their influence on interactions within an aquatic ecosystem.

Our experimental results indicated that *Chlorella vulgaris* and its medium generally had a significant stimulatory effect on *P. caudatum* during the time intervals of 72 to 144 hours. It is important to note that interpretation may vary depending on the specific context of the experiment and the tested components. Data analysis shows that responses to the tested components are complex and depend on multiple factors, including concentration, time interval, and complex interactions between them.

The microalga *Chlorella vulgaris* showed a more pronounced positive effect compared to its liquid media at time intervals of 120 and 144 hours, overall having a significant stimulating effect on *P. caudatum*.

Lower concentrations (0.1 and 0.5 mg/L) had more pronounced effects, generating significant increases of parameters (Nt and Cw). Higher concentrations (1 and 10 mg/L) also had a stimulatory effect, but with variability in intensity, including an unexpected stimulation at the dose of 10 mg/L. The results demonstrate that *Chlorella vulgaris* may serve as a potential food source for *P. caudatum*.

Microalgae *Scenedesmus quadricauda* and its culture medium, tested as food for paramecia, significantly influence reproduction, with both positive and negative effects depending on the administered dose and exposure time. Lower concentrations, such as 0.1 mg/L and 0.5 mg/L, generally had a stimulative effect on reproduction. Higher doses of 1 mg/L and 10 mg/L had varied effects, expressed through increases and decreases in parameters, suggesting a more complex effect. The exposure time (hours) of 24h, 48h, 72h, 96h, 120h, and 144h plays a crucial role on *P. caudatum* response to their presence.

Both microalgae, *Chlorella vulgaris* and *Scenedesmus quadricauda*, generally exhibited stimulative effects on *Paramecium caudatum* compared to their culture media.

Lower doses of both microalgae tended to be more effective in stimulating the growth and reproduction of *P. caudatum.*

Higher doses may have varied effects, suggesting the need for careful control of concentrations used in laboratory practices or potential practical applications.

Research on the interactions between microalgae in the phylum *Chlorophyta*, such as *Chlorella vulgaris* and *Scenedesmus quadricauda* and organisms like *Paramecium caudatum*, can provide the necessary understanding in restoring water quality in water pools. Furthermore, exploring the use of these microalgae as a nutritional supplement can bring significant benefits to agriculture and environmental conservation.

CONCLUSIONS

1. In conclusion, lower concentrations of tested *Chlorella vulgaris* culture (except for the 10 mg/L concentration in certain cases) have a positive effect on the natural population of *P. caudatum*, especially over longer time intervals.
2. The tested concentration of 0.5 mg/L *Chlorella vulgaris* in *P. caudatum* food significantly influence the population size and reproduction rate of the ciliate, with these effects increasing over the incubation period, by 119.31% at 120 h and 187.27% at 144 h compared to the control.
3. Higher concentrations (1 and 10 mg/L) of *Chlorella vulgaris* culture medium in *P. caudatum* food positively influence the population size and reproduction rate at 72, 96, 120, and 144 h of incubation, while the effects at 24 and 48 h are less consistent. The concentration with significant stimulating effect was 1 mg/L, generating increases in numerical abundance (by 73.30%) and reproduction rate (by 10.59%) compared to the control.
4. The effects of *Scenedesmus quadricauda* and its culture medium, tested as food for *P. caudatum*, were differentiated based on dose and time interval.
5. Lower doses (0.1 and 0.5 mg/L) generally produced a significant stimulatory effect, resulting in notable increases in both population size and reproduction rate. In contrast, higher doses (1 and 10 mg/L) exhibited varied effects, including both increases and decreases in parameters, indicating a more complex interaction.

CONFLICT OF INTERESTS

The authors declare no conflict of interest.

ACKNOWLEDGMENT

The cost for this study were covered by Subprogram 010701: "Evaluation of the structure and functioning of animal world and aquatic ecosystems under the influence of biotic and abiotic factors in the context of ensuring ecological security and the well-being of the population" within the Institute of Zoology, MSU.

REFERENCES

1. Baglieri A, Sidella S, Barone V, Fragalà F, Silkina A, Nègre M, Gennari M. Cultivating *Chlorella vulgaris* and *Scenedesmus quadricauda* microalgae to degrade inorganic compounds and pesticides in water. *Environmental Science and Pollution Research (ESPR).* 2016;23:18165-18174. doi:10.1007/s11356-016-6996-3
2. Cebotari V, Toderaş I, Buzu I, Ungureanu L. Măsuri de ajustare a apiculturii convenţionale la cea organică în Republica Moldova [Measures of converting the conventional beekeeping into the organic beekeeping in the Republic of Moldova.]. *Buletinul Academiei de Ştiinţe a Moldovei. Ştiinţele vieţii*. 2020;3(342):7-28. Available at: https://ibn.idsi.md/ro/vizualizare_articol/130424 (Accessed 06.09.2024).
3. Puglisi I, Barone V**,** Fragalà F, Stevanato P, Baglieri A, Vitale A. Effect of Microalgal Extracts from *Chlorella vulgaris* and *Scenedesmus quadricauda* on Germination of *Beta vulgaris* Seeds. *Plants.* 2020;*9*(6):675. doi:10.3390/plants9060675
4. Chesnokova S, Chugay N. Biologicheskie metody otsenki kachestva ob"ektov okruzhayushchey sredy: ucheb. posobie. [Biological methods for assessing the quality of environmental objects]. ladimir: Izd-vo Vladim. gos. un-ta. 2008. Available at: https://dspace.www1.vlsu.ru/handle/123456789/5105 (Accessed 06.09.2024).
5. Pisman T, Galayda Ya, Shirobokova I. Experimental and mathematical modeling of the consumer's influence on productivity of algae in a model aquatic ecosystem. *Advances in Space Research*. 2005;35(9):521-1527. doi:10.1016/j.asr.2004.12.048
6. Sadchikov A. P. Kul'tivirovaniie vodnykh i nazemnykh bespozvonochnykh: printsipy i metody: ucheb. Posobie. [Cultivation of aquatic and terrestrial invertebrates: principles and methods: textbook.]. MAKS Press. 2009. Available at: https://books.google.it/books?id=0M99kgAACAAJ (Accessed 05.09.2024).
7. Kokova V. Nepreryvnoie kul'tivirovanie bespozvonochnykh. [Continuous cultivation of invertebrates.]. Novosibirsk. «Nauka» Sibirskoie otdeleniie. 1982. Available at: https://shorturl.at/rZS3d (Accessed 05.09.2024).
8. Likhachev S, Pimenova Ye, Zhakova S. Biotestirovanie v ekologicheskom monitoringe: uchebno-metodicheskoie posobiie. [Biotesting in environmental monitoring: educational manual.]. Ministerstvo sel'skogo khoziaistva Rossiiskoi Federatsii, federal'noie gosudarstvennoie biudzhetnoie obrazovatel'noie uchrezhdeniie visshego obrazovaniia «Permskii gosudarstvennyi agrarno-tekhnologicheskii universitet imeni akademika D.N. Prianishnikova». Perm': IPTS «Prokrost». 2020;64–71. Available at: https://shorturl.at/VoBdD (Accessed 04.09.2024).
9. Zaika V. Sravnitel'naya produktivnost' gidrobiontov. [Comparative productivity of aquatic organisms.]. Kiiev: Nauk. Dumka, 1983. https://files.znu.edu.ua/files/Bibliobooks/Domni/0000918.pdf (Accessed 04.09.2024).
10. Spinei L, Lozan O, Badan V. Biostatistica [Biostatistics]. Univ. de Stat de Medicină şi Farmacie „Nicolae Testemiţanu". 2009;186. Available at: https://management.usmf.md/sites/default/files/inline-files/Biostatistica.pdf (Accessed 04.09.2024).
11. Wasser S, Kondrat'yeva N, Masyuk N. i dr. Vodorosli. [Algae.]. Spravochnik. Kiiev: Nauk. Dumka, 1989. Available at: https://shorturl.at/eTAlK (Accessed 04.09.2024).

Date of receipt of the manuscript: 07/06/2024
Date of acceptance for publication: 30/09/2024

OH&RM ONE HEALTH & RISK MANAGEMENT

VOL. 5, ISSUE 4
2024

MEDICAL SCIENCES

OPEN ACCESS

ETHICS INSTITUTIONALIZATION IN HEALTHCARE FACILITIES IN THE REPUBLIC OF MOLDOVA

Rodica GRAMMA

Nicolae Testemițanu State University of Medicine and Pharmacy, Republic of Moldova

Corresponding author: Rodica Gramma, e-mail: rodica.gramma@usmf.md

DOI: 10.38045/ohrm.2024.4.05

CZU: 582.263:593.171.4

Keywords: *ethics management, code of ethics, ethics leadership, ethics training, ethics audit.*

Introduction. *The process of institutionalizing ethics is composed of the implementation of tools such as: codes, ethical leadership, training, ethics committees, ethics audits, ethics consultants. The article presents an analysis of the situation on the role of these ethics' tools on the activity of the healthcare facilities of the Republic of Moldova.*

Material and methods*. The research was carried out on 2 target groups: 1. a cross-sectional study on a sample of 1070 employees of 120 hospital and primary care institutions and 2. a sample of 134 members of ethics / bioethics committees. Two questionnaires were developed and transposed into Google forms to be completed anonymously. Comparative evaluation was performed using the 95%CI. The standard Chi-Squared Test (α = 0.05) for Independence without Yates' Correction was considered for 3x3 and 7x5 contingency tables.*

Results. *In the country's medical institutions, the level of institutionalization of ethics was low, and the application of ethics management tools varied significantly. Only 13% (95% CI: 11.0, 15.0) of respondents reported the existence of an institutional ethics code, while 30.4% (95% CI: 27.6, 33.1) admitted they were unaware of such a code within their institution. Furthermore, only 25.5% (95% CI: 22.9, 28.1) of respondents indicated that institutional values were promoted by all employees. Just 36.2% (95% CI: 30.4, 36.1) felt they had the necessary knowledge to make decisions in ethical dilemmas.*

Conclusions. *The detected gaps lead us to the conclusion that the continuous integration of ethics into institutional activities must become essential for the managers of medical institutions in the country. This is an important condition for ensuring the quality of services. Implementing ethics programs should be an indispensable part of developing institutional strategies.*

Cuvinte cheie: *managementul eticii, codul de etică, conducerea etică, instruirea în etică, auditul etic.*

INSTITUȚIONALIZAREA ETICII PROFESIONALE ÎN INSTITUȚIILE MEDICALE DIN REPUBLICA MOLDOVA

Introducere. *Procesul de instituționalizare a eticii constă în implementarea unor instrumente precum: codul de etică, leadershipul etic, instruirea eficientă, comitetele de etică, auditul pentru etică și, consultanți eticieni. Articolul prezintă o analiză a situației cu privire la rolul acestor instrumente de etică în activitatea instituțiilor de sănătate din Republica Moldova.*

Material și metode. *Cercetarea a fost realizată pe 2 grupuri țintă: un studiu transversal pe un eșantion de 1070 de angajați din 120 de instituții spitalicești și de asistență primară, și un eșantion de 134 de membri ai comitetelor de etică/bioetică, pentru acestea fiind elaborate două chestionare anonime în Ggoogle forms.*

Rezultate. *În instituțiile medicale din țară se atestă un nivel precar de instituționalizare a eticii, iar instrumentele de management etic au fost aplicate foarte diferit. Doar 13% (CI95% 11,0, 15,0) dintre respondenți au indicat prezența unui cod instituțional, iar 30,4% (CI95% 27,6, 33,1) recunosc că nu au știut despre așa cod în instituția lor. Doar 25,5% (CI95% 22,9, 28,1) dintre respondenți consideră că valorile instituționale au fost promovate de către toți angajații, și doar 36,2% (IC95% 30,4, 36,1) dintre cei chestionați posedă cunoștințele necesare pentru a lua decizii în situații de dileme etice.*

Concluzii. *Lacunele depistate ne sugerează că, integrarea continuă a eticii în activitatea instituției ar trebui să fie prioritară pentru managerii instituțiilor medicale din țară. Aceasta este o condiție importantă în asigurarea calității serviciilor, iar implementarea programelor de etică ar trebui să devină o parte indispensabilă a strategiilor de dezvoltare instituțională.*

INTRODUCTION

In the recent specialized literature, the *Management of Ethics* has been increasingly discussed, as a new distinct field within organizational management. This represents the management of all elements related to the moral aspect of an organization, be it a commercial firm, hospital or university. Ethics management deals with the development of those management tools that contribute to the ethical development of an organization, determine the desirable situation and decide on the measures to be taken to achieve it, consistent with other forms of management (1, 2, 3).

Organizations are recommended to adopt an *ethics program* with distributed responsibilities. The process of governing and managing an organization's ethical performance through an *ethics program* is based on four pillars, namely: a) institutionalization of ethics; b) ethical risk assessment; c) development of ethical standards; and d) ethical performance reporting and disclosure. Thus, the environment in which organizations exist is an important determinant of ethical behaviour. Institutionalizing ethics aims to integrate ethical standards into an organization's strategies and operations and build an organizational culture (4).

There is a strong relationship between the work climate formed in an institution and the behaviour of employees, which is strongly influenced by organizational practices. Through institutionalized norms, policies and procedures, an organization alerts and empowers its employees to decide what is correct behaviour and thus creates an ethical culture (5). The managers should decide regarding the ways of influence to be applied – either through building the correct perception of ethical behaviour or through tools to promote ethical behaviour among their employees (6).

To organize the ethics of an institution, it is necessary to create an "ethical infrastructure" (7). Thus, the activities of organizing ethics in the institution can be carried out by means of a series of instruments, such as: the elaboration of institutional codes of ethics; ensuring ethical leadership, i.e. behaviours of managers worthy of following; the activity of institutional ethics committees; ethics training within the organization; ethics audits; appointing the person responsible (ethics consultant) for ethical issues and promoting an ethical climate and culture (1, 2, 7).

The *aim* of the research was to determine the role and impact of these ethical tools on the activities of healthcare facilities in the Republic of Moldova.

MATERIAL AND METHODS

A cross-sectional study was carried out, which included a sample of 1070 participants (±3%; expected frequency – 5 0%). The study was carried out in the period of 2022-2023.

The questionnaire was transposed into a *Google forms* and distributed through human resources departments, professional associations and social networks. The invitation to participate in the survey was sent to120 hospital and primary care institutions from the Republic of Moldova.

The questionnaire contained 57 questions to identify problems related to the ethical dilemmas faced by the employees, the ways and possibilities to solve them, the existing ethical tools, as well as the level of respect for the patient's rights in providing medical services. The five-level Likert scale (for ordinal variables), single-choice or multiple-choice matrices (for dichotomous or nominative variables in accordance with the question) were applied. At the same time, for some questions, the respondents were given the possibility of their own answers, by including the open answer option and being asked to formulate suggestions for improving the ethics management of their institution.

Another questionnaire in a similar format was created for the members of ethics / bioethics committees of medical institutions and was completed by 134 respondents. The questionnaire was composed of 25 questions with closed and open answer options, the purpose of which was to evaluate the opinions of members with reference to the role and importance of committees and about their membership activity.

No personal data were recorded, the completion of the questionnaires was done anonymously.

Statistical analysis: Descriptive statistics for discrete data were performed by estimating relative frequencies, completed with 95% confidence in-

tervals (95% CI). Comparative evaluation was performed using the 95% CI. The standard Chi-Squared Test ($\alpha = 0.05$) for Independence without Yates' Correction was considered for 3x3 and 7x5 contingency tables. Visualization was performed using barplots and heatmaps. The programs used for the statistical analysis were SPSS 26 and Google Collab.

RESULTS

The research found that in the medical institutions of the country there was a low level of ethics institutionalization, and the tools of ethics management were used very different, from case to case.

Codes of ethics

More than half of the respondents – 56.6% (95% CI 53.7, 59.6) indicated that in their institutions, there was no separate code, and thus the Code of Ethics of the Medical Worker and the Pharmacist was automatically applied (approved by Government Decision no. 192/2017). Practically, one third of the respondents – 30.4% (95% CI 27.6, 33.1) did not know about the code of ethics in their own institution. Only a small number – 13% (95% CI 11.0, 15.0) indicated the presence of an institutional code. Every third employee in republican hospitals – 37.9% (95% CI 32.8, 41.9), and among those in municipal hospitals – 36.5% (95% CI 30.0, 43.5), admitted that they did not know about the code of ethics governing the institution where they worked. The rate of those who did not know about the code of ethics was likely higher, possibly due to reporting bias.

One of the essential functions of the code should be the unanimous endorsement of a set of values that are commonly agreed upon by all staff. We noted that only a fourth of the study respondents – 25.5% (95% CI: 22.9, 28.1) considered that the institutional values were promoted by all employees. 40.5% (95% CI: 37.5, 43.4) of respondents believed that only some employees in their institution knew the values, and 34% (95% CI: 31.2, 36.9) could not provide any opinion. We could assume that refraining from answering was a way to avoid a negative response, which could affect the image of the collective in which they operated.

More than half of the respondents working in the private sector – 57.1% (95% CI: 37.2, 75.5) considered that in their institutions all employees knew the values declared at the institutional level, while among employees from public district hospitals, this value was only 20.4% (95% CI: 16.8, 24.4). A possible explanation could be the fact that private institutions tend to have much more insistent policies to maintain a high degree of satisfaction of the beneficiary (the paying customer).

The respondents were asked about the way in which they were informed about the ethical principles and values that underlie the institution's activity. Also, we found the association between this parameter and the hospital type. It is worrying the fact that there were employees who indicated that no one from the institution informed them about this subject (fig. 1).

An ethical leadership

The managerial staff, for the most part, was appreciated with a high score, which was an advantage for the institutions. However, the number of those who rated the behaviour of their managers with a low score should not be ignored. The assessment of the managers' behaviour was proposed through a five-level Likert scale from 1 to 5, where 1 meant 'strongly disagree' and 5 meant 'strongly agree.' 15.2% of physicians appreciated the behaviour of their manager with only 1 or 2 points out of 5 (1 point – 9.1% (n=63, 95%CI 7.1, 11.5), 2 points – 6.1% (n=42, 95%CI 4.4, 8.1)). A critical opinion was also identified towards the vice directors – 18.8% of doctors gave a very low score to their behaviour, namely 8.7% (n=60, 95%CI 6.7, 11.1) – 1 point, and 10.1% (n=70, 95%CI 8.0, 12.6) – 2 points. Additionally, 13.6% of doctors rated the behaviour of the immediate heads of the subdivisions in which they worked as unsatisfactory (6.4% (n=44, 95%CI 4.7, 8.5) – 1 point, and 7.2% (n=50, 95%CI 5.4, 9.4) – 2 points).

The appreciation of the behaviour of the representatives of the institution's administration has improved with the increase of the respondents' work experience (fig. 2). This trend can be explained by the loyalty that has developed in employees over time and the fact that the group of respondents with a long work experience can include more representatives of different levels of management in the institution.

Among the negative assessments, the many respondents mentioned the presence of favouritism, lack of transparency and inappropriate influences in managerial decisions.

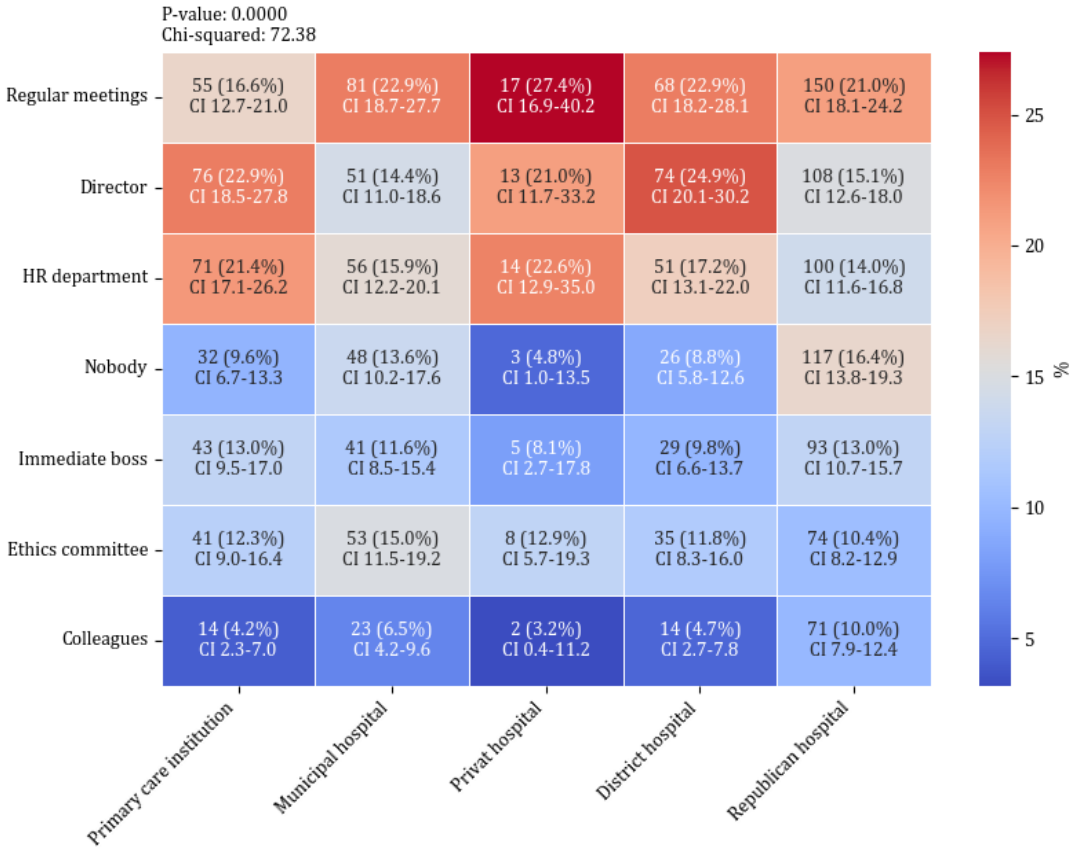


Figure 1. The source of information about the institution's values, by institution type, absolute figures, %.

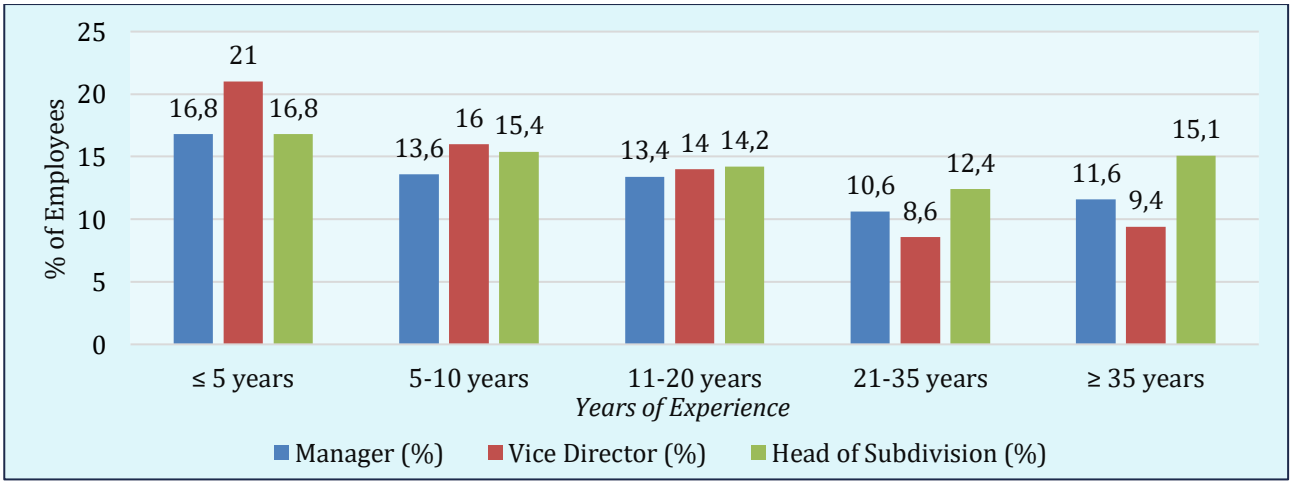


Figure 2. Negative assessment (1-2 points from 5) of managers' behaviour, by work experience, %.

Ethics/bioethics committees

The emergence of ethics/bioethics committees in medical institutions is conditioned by the mandatory criteria of the accreditation process. The presence and activity of these committees has been verified through the evaluation and accreditation procedure of the institution for a period of 5 years (8, 9). Absolutely in all the institutions included in the study (100%) there are such committees.

Only 44.1% (95%CI 41.1, 47.1) of the respondents rated the ethics committee as a very relevant and useful structure. The number of those who were unaware of the committee's activity was alarmingly high at 18.9% (95%CI 16.5, 21.2), to which could be added those who could

not assess its relevance – 20.8% (95%CI 18.4, 23.3). One in six respondents, 16.2% (95%CI 14.0, 18.4), stated that this structure in the institution was irrelevant and formal (fig.3).

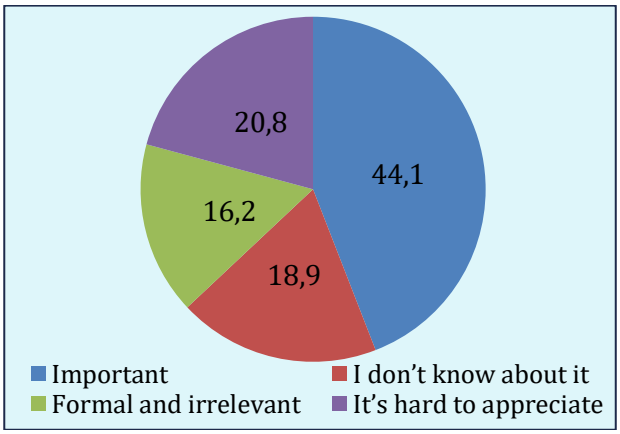


Figure 3. Usefulness of the institutional ethics/bioethics committee, employees' opinion, %.

Some of the members of ethics/bioethics committees themselves were skeptical of the necessity of the committees they were part of. Practically, every fifth member included in the study (18.9%) could not appreciate the usefulness of these committees or considered them formal structures just for filling the accreditation procedure.

Regarding the transparency of the work of the ethics/bioethics committee, only half – 55.4% (95%CI 52.4, 58.4) – of the employees confirmed that its decisions were known within the institution. It is alarming that 18.6% (95%CI 16.3, 20.9) stated that their colleagues did not know about the committee's activities. To this group could be added those who hesitated to give an answer – 26% (95%CI 23.4, 28.6), probably because they were not familiar with any procedures that would guarantee transparency and communication in this field.

Ethical training of employees

The ability of an organization to reach its full potential remains dependent on the knowledge, skills, and values of each employee. The research determined that there were significant reservations regarding the ability of employees to recognize ethical dilemmas and conduct ethical analyses. Only a little more than a third of the respondents, 38.5% (95%CI: 35.6, 41.4), considered that they had a collective with sufficient capacity for ethical analysis. At the same time, one in three respondents—33.7% (95%CI: 30.9, 36.6)—believed that employees in their institution needed additional training and negatively evaluated their ability to recognize and solve moral dilemmas.

Half of the respondents from the private sector—50% (95% CI: 30.6, 69.4)—believed that their collective had a high capacity for ethical analysis. In contrast, only 33.9% (95% CI: 29.6, 38.5) of the respondents from republican hospitals provided the same answer, followed by those from primary care institutions—38.8% (95% CI: 31.9, 46.0)—and from municipal hospitals—41.8% (95% CI: 35.0, 48.8).

Just over a third of employees—36.2% (95% CI: 30.4, 36.1)—considered that they possessed the necessary knowledge to make decisions in ethical dilemmas. Additionally, 43.2% of respondents considered that they had insufficient knowledge and would like additional training (95% CI: 36.8, 42.7). The group of those who considered that they needed extensive training—10.1% (95% CI: 7.5, 11.0)—was equal to those who could not assess their knowledge—10.3% (95% CI: 7.7, 11.2).

Doctors were more critical in assessing their own knowledge regarding ethical evaluation compared to nurses (fig. 4).

The interest focused on discovering where employees received the knowledge to deal with ethical issues. It was of great concern that the number of doctors who considered having received no training in ethics throughout university was 30.8% (95%CI 28.1, 33.6), and during residency studies was 50.2% (95%CI 47.2, 53.2), where the foundation for ethical analysis should be laid. One in five doctors – 21.2% (95%CI 18.8, 23.7) – had no such training at work, while 29.1% (95%CI 26.3, 31.8) denied having received such training in continuing education. Additionally, some (13.7%) believed that the training received was useless.

Ethics audit

The ethical audit, as well as the presence of ethical consultants, is not a practice applied in medical institutions in Moldova. However, the results of the carried out research were the basis for the development of a Grid for Ethics Audit, intended for evaluation of medical institutions on three dimensions: (a) the place of ethics in the policies,

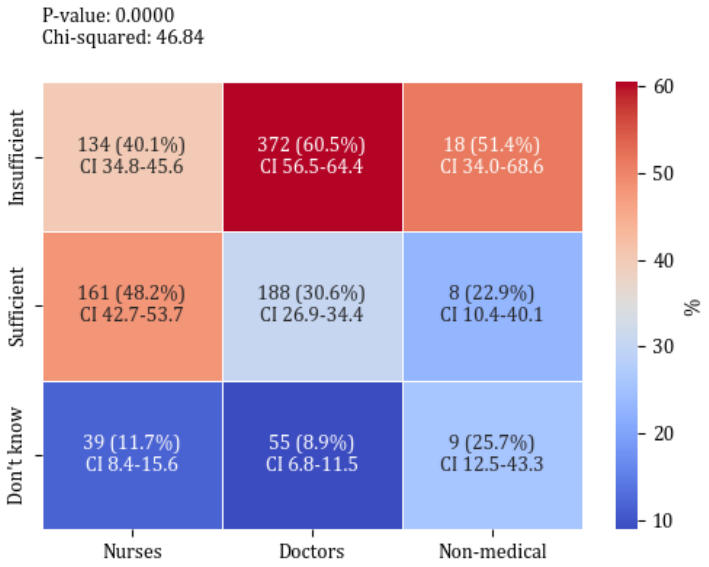


Figure 4. Appreciation of own level of knowledge in the field of ethics, by profession, %.

programs and strategic activities; (b) promotion of ethical values and human rights principles; (c) institutional environment (ethical climate) and employees` satisfaction.

The grid was applied by the author in two hospitals. An extensive assessment was made, which identified certain gaps and non-conformities regarding ethical environment and the existing risk of human rights violations, both from the perspective of the patient and of the employee. As a common conclusion it was determined that ethics is included in the institutional policies only in a general form, without any dedicated activities or concrete programs. The institutions have not developed sufficient procedures and tools that would ensure the appropriate conditions for respecting the patient's rights, especially regarding informing procedure and informed consent collecting, accepting the patient's refusal, communicating with non-native patients, etc. Based on the performed audit, the institutions received a set of solid recommendations for improving the situation.

The grid was published (10), being presented to managers from the healthcare system who can use it for self-evaluation/internal audit of their institutions at a certain frequency.

DISCUSSIONS

The research identified a series of gaps in the organization of the ethical dimension in healthcare facilities of the Republic of Moldova, both hospital and primary care institutions. Tools proposed in the ethics management theories are insufficiently implemented. The role of ethics codes, ethics committees and of the periodic ethical training at the workplace is insufficiently explored. At the level of medical institutions, there are also identified some gaps in the promotion of values-based leadership and ethical model of managers. Ethical counselling is very little applied in daily clinical activity. There are no consultants dedicated to the ethical assistance of employees in medical institutions. The ethical audit is not a procedure implemented in the evaluation practice of the medical institutions of the Republic of Moldova.

The described managerial tools have an extremely important role in organizing ethical environments in medical institutions, especially to ensure a high quality of the services provided and to increase the satisfaction of both the providers and the beneficiaries of medical services (1, 11).

Firstly, the codes of ethics are valuable tools for improving the ethical climate of an organization. During the last years, many works are dedicated to the structure and content of ethical codes, their importance for organizations being emphasized (12). Managers cannot continuously and directly communicate to employees the ethical behaviour that is expected of them. For this, a more structured and constant form of communication is needed. Therefore, a code of ethics is required as the primary means of guiding em-

ployees to behave ethically. Managers should be interested in transforming the code of ethics from a formal text printed on paper into a living document woven into the organizational culture (13). Mureşan V. (7) identifies several important aspects in defining a code of ethics, considering that this document clearly demarcates the field of morals in the life of an organization. The code will promote the desirable values and virtues of the organization, which must always be educated among employees so that their moral actions are habitually voluntary, regardless of the circumstances. Thus, a code will impose a minimum number of expectations that apply to all employees, providing clarity regarding their responsibilities (1).

Secondly, a manager will always be seen as a model and example of behaviour for the organization they lead, and the promotion of an ethical leadership is an effective tool for the institutionalization of ethical behaviours (14). The leaders' behaviour and personality are very important in building trust of employees (15). The behaviour and image of the manager will be replicated by employees, at different levels. It is hard to imagine that we can find an ethical climate in an or ganization where the manager has integrity problems, is opportunistic, suspected of acts of corruption and bribery, has an arrogant behaviour and does not have self-control over his emotions, allowing his aggressive impulses to take over in communicating with employees (16).

Thirdly, institutional ethics committees are considered important tools in the management of the medical institution. The activity of these structures should be organized on three dimensions: continuous education of the staff, ethical consultation and policy development (17). These structures should provide essential support to healthcare managers in ethically complicated decision-making situations, when conflicts and contradictions of values are evident (18). The formal presence of committees in the structure of medical institutions is only a loss for managers who should benefit to the maximum from the support of these multidisciplinary teams, which can offer them useful visions and approaches.

Fourthly, to ensure a high quality of services, the management of an institution will have to ensure the high degree of ethical competence of its employees as well as the continuous professional development that it provides for its staff. On-the-job training is considered one of the main tools of the process of institutionalizing ethics. Such trainings should be organized with periodicity, succeeding from the problems identified during the daily practical activity (19). However, even if the effectiveness and the positive impact of ethical training on organizational outcomes is demonstrated, in practice, ethics training efforts are often met with resistance and scepticism by employees. That is why it is very important to draw special attention to the format and application content of these trainings, in order not to turn them into formal meetings without practical utility (20).

Fifthly, the ethics audit is approached as a proactive tool of ethics management for the development of integrity in organizations (21). This is an assessment to determine whether it is necessary to make any changes in the organization's environment and to strengthen its ethical policies (2). An ethics audit can help executives assess how well an organization has met its legal and ethical obligations, uncover or prevent ethical risks, and strategically plan social responsibility activities (22).

Some authors recommend replacing the word *audit* with *ethical assessment* or *ethical inventory*, considering that the assessment of the level of compliance of an organization with the context of assumed values is much broader and deeper, compared to the verification of the correspondence of some numbers, as is the financial audit, which seems to be narrower than the ethical one (23).

An ethics audit should cover three broad areas: (a) the organization's values; (b) governance; and (c) legal compliance (2). The requested purpose of the audit will determine the choice of matters that are included in the audit examination (1).

At the same time, the research had some limitations, as it did not allow to specifically identify the ethical problems of each institution, as well as its causes. This can be developed, subsequently, by applying the audit grids at the institutional level, in the organizational environments of the medical institutions in the country, which will allow the identification of gaps and non-conformities, as well as the identification of solutions for the institutionalization of ethics to be able to ensure compliance with moral values and the principles of fundamental human rights.

CONCLUSIONS

1. The activities of organizing ethics in the institution can be carried out by means of a series of instruments, such as: the elaboration of institutional codes of ethics; ensuring ethical leadership; the activity of institutional ethics committees; ethics training within the organization; ethics audits; appointing the person responsible for ethical issues and promotion of ethical climate and culture.
2. For an adequate process of institutionalizing ethics, it is important to implement all tools, because each of them has its own goals and purposes that add value to this complex process.
3. The organization and maintenance of an ethical environment and culture, implementation of ethics programs, should be the priorities of a manager interested in the quality of the services provided in their institution, as well as in building an organization with a high degree of morality.
4. The implementation of ethics management programs should become an indispensable part of the strategies and development plans of medical institutions, through which employees should adopt and conform to common values, which would determine ethical decision-making behavior.

CONFLICT OF INTERESTS

There is no conflict of interest regarding the material presented in the paper.

ACKNOWLEDGMENT

The article presents the results of the study carried out as part of the postdoctoral research project with the title "Management of ethics in healthcare facilities for the respect of human rights in the provision of health services" financed by the National Agency for Research and Development of the Republic of Moldova, 2022-2023.

ETHICAL APPROVAL

The study was approved by the Research Ethics Committee of the State University of Medicine and Pharmacy "Nicolae Testemițanu" (Decision no. 1 of 16.02.2022).

REFERENCES

1. Kaptein M. Ethics Management. Auditing and Developing the Ethical Content of Organizations. Springer, 1998. doi:10.1007/978-94-011-4978-5
2. Menzel DC. Ethics Management for Public and Nonprofit Managers. Leading and Building Organizations of Integrity. Third edition. London: Taylor&Francis, 2017. Available from: https://books.google.md/books/about/Ethics_Management_for_Public_Administrat.html?id=dc2-TlTW6BMC&redir_esc=y (Accessed 26.09.2024)
3. Martínez C, Skeet AG, Sasia PM. Managing organizational ethics: How ethics becomes pervasive within organizations. *Bus Horiz*. 2021; 64(1):83-92. doi:10.1016/j.bushor.2020.09.008
4. Kaptein M. The Effectiveness of Ethics Programs: The Role of Scope, Composition, and Sequence. *J Bus Ethics.* 2015;132(2):415-431. doi:10.1007/s10551-014-2296-3
5. Resende MM, Porto JB, Gracia FJ, Tomás I. Unethical behavior at work: the effects of ethical culture and implicit and explicit moral identity. *Ethics&Behavior.* 2023;34(6):438-457. doi:10.1080/10508422.2023.2243632
6. Teresi M, Pietroni DD, Barattucci M, Giannella VA, Pagliaro S. Ethical Climate(s), Organizational Identification, and Employees' Behavior. *Front Psychol.* 2019;10:1356. doi:10.3389/fpsyg.2019.01356
7. Constantinescu M, Muresan V. *Instituționalizarea eticii: mecanisme și instrumente* [Institutionalizing ethics: mechanisms and tools]. București: Editura Universității din București, 2013. Available from: https://philpapers.org/archive/CONIEM.pdf (Accessed 26.09.2024).
8. Parliament of the Republic of Moldova. Law no. 552/2001 regarding health assessment and accreditation. Published in: Monitorul Oficial Republic of Moldova, 2001; 155-157: 1234. Available from: https://www.legis.md/cautare/getResults?doc_id=112510&lang=ro (Accessed 25.09.2024).
9. Ministry of Health of the Republic of Moldova. Regulation on the health assessment and accreditation procedure. Approved by MoH Order no. 582/2024 Available from: https://www.legis.md/cautare/getResults?doc_id=144052&lang=ro (Accessed 25.09.2024).
10. Gramma R. *Valori, etică și drepturi în managementul instituțiilor medicale* [Values, Ethics and Rights in the Management of Healthcare Facilities]. Chișinău: T-Par, 2024.
11. Bokolia SK. Managerial Ethics. *JETIR.* 2019; 6(5): 29-37. Available afrom: https://www.jetir.org/papers/JETIRCC06006.pdf (Accessed 24.09.2024).

12. Babri M, Davidson B, Helin S. An Updated Inquiry into the Study of Corporate Codes of Ethics: 2005–2016. *J Bus Ethics*. 2021;168:71-108. doi:10.1007/s10551-019-04192-x
13. Collings-Hughes D, Townsend R, Williams B. Professional codes of conduct: A scoping review. *Nursing Ethics*. 2022;29(1):19-34. doi:10.1177/09697330211008634
14. Zydziunaite V. Leadership Values and Values Based Leadership: What is the Main Focus? *Applied Research In Health And Social Sciences Interface And Interaction*. 2018;15(1):43-58. doi:10.2478/arhss-2018-0005
15. Malik M, Mahmood F, Sarwar N, Obaid A, Memon MA, Khaskheli A. Ethical leadership: Exploring bottom-line mentality and trust perceptions of employees on middle-level managers. *Curr Psychol*. 2022;27:1-16. doi:10.1007/s12144-022-02925-2
16. Zhang Y, Zhou F, Mao J. Ethical Leadership and Follower Moral Actions: Investigating an Emotional Linkage. *Front Psychol*. 2018;9:1881. doi:10.3389/fpsyg.2018.01881
17. Crico C, Sanchini V, Casali PG, Pravettoni G. Evaluating the effectiveness of clinical ethics committees: a systematic review. *Med Health Care Philos*. 2021;24(1):135-151. doi:10.1007/s11019-020-09986-9
18. Raoofi S, Arefi S, Khodayari Zarnaq R, Azimi Nayebi B, Mousavi MSS. Challenges of hospital ethics committees: a phenomenological study. *J Med Ethics Hist Med.* 2021;14:26. doi:10.18502/jmehm.v14i26.8282
19. Caldwell JL, Ortiz AY, Fluegge ER, Brummett MJ. The Effectiveness of Ethics Training Strategies: Experiential Learning for the Win. Intern *J Bus and Manag Research*. 2020;8:124-131. doi:10.37391/IJBMR.080407
20. Andersson H, Svensson A, Frank C, Rantala A, Holmberg M, Bremer A. Ethics education to support ethical competence learning in healthcare: an integrative systematic review. *BMC Med Ethics*. 2022;23(1):29. doi:10.1186/s12910-022-00766-z
21. Ojasoo M. CSR reporting, stakeholder engagement and preventing hypocrisy through ethics audit. *Journal of Global Entrepreneurship Research.* 2016;6(1):14. doi:10.1186/s40497-016-0056-9
22. Hofmann PB. To Minimize Risk, Ethics Audits Are as Essential as Financial Audits. *J Healthc Manag.* 2019;64(2):74-78. doi:10.1097/JHM-D-19-00030
23. Beste T. *The Corporate Ethics Audit as a New Tool for Management by Values*. Saarbrucken: VDM Verlag Dr.Muller GmbH&Co, 2011.

Rodica GRAMMA, SCOPUS ID: 54683803100; WoS Researcher ID: AAD-4691-2022

Date of receipt of the manuscript: 19/05/2024
Date of acceptance for publication: 10/11/2024

REQUIREMENTS FOR AUTHORS

Rules of drafting
The manuscript (written in English and French) should be in accordance with the guidelines published in: *Uniform Requirements for Manuscripts Submitted to Biomedical Journal (1994) Lancet 1996, 348, V2; 1-4* (www.icmje.org). The manuscripts should be written in font Cambria, size 11 points, spaced at 1.0, fully justified alignment, fields 2 cm on all sides. All pages must be numbered consecutively (in the right bottom corner) and continuously. Abbreviations should be explained at first occurrence in the text and should not be excessively used. The manuscripts must not exceed the number of words (without the title, affiliation, abstract and references): review articles – 4,500 words; research articles – 3,000 words; expert opinions – 2,500 words; case presentation – 1,700 words; experimental and clinical notes – 1,300 words; book reviews and presentations – 2,000 words; teaching articles – 4,000 words. The volume of tables and figures should not exceed ⅓ from the volume of the manuscript. The journal reserves the right to make any other formatting changes. Rejected manuscripts are not returned.

All manuscripts submitted for publication should be accompanied by two abstracts: in the language of origin of the article and English.

Title and authors
The title should be as short as possible (maximum – 120 signs with spaces), relevant for the manuscript content. The names of the authors should be written in full: name, surname (*e.g.:* Jon JONES). Affiliation should include: Department/Unit/Chair, University/Hospital, City, Country of each author. Beneath the affiliation, the author`s details and contact information – e-mail address (*e.g.:* corresponding author: Jon Jones, e-mail: jon.jones@gmail.com).

The structure of the manuscript
The manuscript should comprise the following subheadings (capitalized):

- **SUMMARY**
- **INTRODUCTION** (will reflect the topicality and the general presentation of the problem studied, purpose and hypothesis of the study)
- **MATERIAL AND METHODS**
- **RESULTS**
- **DISCUSSIONS**
- **CONCLUSIONS**
- **CONFLICT OF INTERESTS**
- **ACKNOWLEDGEMENT** (optional)
- **ETHICAL APPROVAL** (specify the presence or absence of a positive opinion from the ethics committee: no, date, institution ad informed consent)
- **REFERENCES**

The **summary** should contain 1,600 signs with spaces:

- **Introduction**
- **Material and methods**
- **Results**
- **Conclusions**
- **Key words:** 3-5 words

The summary should not include tables, charts, and bibliographic notes; information not included in the article.

Figures. The text included in figures should be written in font Cambria, 10 point. Each figure should be accompanied by a heading and legend. They should be numbered with Arabic numerals and placed in parentheses (*e.g.:* fig. 1). Both the title (*e.g.* Figure 1) and legend are centred, below the figure.

Tables. The text included in tables should be written in font Cambria, 10 point. Each table should be accompanied by a heading. Tables should be inserted into the text and adjusted to the width of the page. The tables are numbered in Arabic numerals and mentioned in body text in parentheses (*e.g.* tab. 1). The title of the table is centred on the top of the table (*e.g.* Table 1).

References are numbered in the order they appear in the paper. The reference sources are cited at the end of the article by using AMA style and will include only the references cited within the text (the reference is numbered within round parentheses). The in-text citations that appear more than once are numbered si-milarly as in the first citation. The number of references should not exceed 50 sources. The scientific authors are responsible for the accuracy of their writings. The reference list should include only those references that have been consulted by the authors of the manuscript. The elements of the reference sources are written exactly in accordance with the requirements.

For more information see: http://journal.ohrm.bba.md/index.php/journal-ohrm-bba-md/editing_guidelines

CERINȚE PENTRU AUTORI

Reguli de tehnoredactare

Pregătirea manuscrisului (elaborat în limbile engleză și franceză) va fi în conformitate cu instrucțiunile publicate în: *Uniform Requirements for Manuscripts Submitted to Biomedical Journals (1994) Lancet 1996, 348, V2; 1-4* (www.icmje.org). Manuscrisele trebuie să fie cu font Cambria, dimensiune 11 puncte, spațiat la interval 1,0, aliniere justificată, câmpurile 2 cm pe toate laturile. Toate paginile trebuie să fie numerotate consecutiv (în colțul de jos, în partea dreaptă) și să includă nume-rotarea continuă a paginilor. Abrevierile trebuie să fie explicate la prima apariție în text și nu trebuie utilizate excesiv. Manuscrisele nu trebuie să depășească (fără a număra titlul, afilierea, rezumatul și refe-rințele): pentru articole de sinteză/referate – 4500 de cuvinte; pentru articole de cercetare – 3000 de cuvinte; pentru opinii ale experților – 2500 de cuvinte; prezentare de caz și imagini din practica clinică/laborator – 1700 de cuvinte; note experimentale și clinice – 1300 de cuvinte; recenzii și prezentări de carte – 2000 de cuvinte; articole didactice – 4000 de cuvinte. Volumul tabelelor și figurilor nu trebuie să depășească ⅓ din volumul manuscrisului. Revista își rezervă dreptul de a face orice alte modificări de formatare. Manuscrisele respinse nu sunt returnate.

Toate manuscrisele transmise spre publicare trebuie să fie însoțite de două rezumate: în limba de origine al articolului și în limba engleză.

Titlul și autorii

Titlul ar trebui să fie cât mai scurt posibil (maximum - 120 de semne cu spații), elocvent pentru conținutul manuscrisului. Numele autorilor vor fi scrise deplin: prenume, nume de familie (*ex:* Ion RUSU). Afilierea va include: Secția/Departamentul/Catedra, Universitatea/Spitalul, Orașul, Țara pentru fiecare autor. Se vor menționa obligatoriu, mai jos, datele autorului corespondent și informațiile de contact – adresa de e-mail (*ex:* autor corespondent: Ion Rusu, e-mail: ion.rusu@gmail.com).

Structura manuscrisului

Manuscrisul va cuprinde următoarele subtitluri (scrise cu majuscule):

- **REZUMAT** (vezi cerințele mai jos)
- **INTRODUCERE** (se va reflecta actualitatea și prezentarea generală a problemei studiate, scopul și ipoteza studiului)
- **MATERIAL ȘI METODE**
- **REZULTATE**
- **DISCUȚII**
- **CONCLUZII**
- **CONFLICT DE INTERESE**
- **MULȚUMIRI ȘI FINANȚARE** (optional)
- **APROBAREA ETICĂ** (se va specifica prezența sau lipsa avizului pozitiv de la comitetul de etică: nr, data, instituția și acordul informat)
- **REFERINȚE**

Rezumatul va conține până la 1600 de semne cu spații și va cuprinde:

- **Introducere**
- **Material și metode**
- **Rezultate**
- **Concluzii**
- **Cuvinte cheie**: 3-5 cuvinte

În rezumat nu vor fi incluse tabele, grafice și note bibliografice; informații care nu sunt prezentate în studiu.

Figuri. Textul inclus în figuri trebuie să fie scris cu font Cambria, dimensiune 10 puncte. Fiecare figură trebuie să fie însoțită de titlu și legendă. Ele vor fi numerotate cu cifre arabe și vor fi menționate în text în paranteze (*ex:* fig. 1). Titlul (*ex:* Figura 1) și legenda figurii trebuie să fie scrisă centrat, sub figură.

Tabele. Textul inclus în tabele trebuie să fie scris cu font Cambria, dimensiune 10 puncte. Fiecare tabel trebuie să fie însoțită de titlu. Tabelele vor fi inserate în text, fără a depăși lățimea unei pagini. Ele vor fi numerotate cu cifre arabe și vor fi menționate în text în paranteze (*ex:* tab. 1). Titlul tabelului va fi poziționat deasupra tabelului centrat (*ex:* Tabelul 1).

Referințele trebuie să fie numerotate în ordinea apariției în text. Citarea sursei de referință va fi conform stilului *AMA*, plasată la sfârșitul articolului și va include doar referințele citate în text (menționând numărul de referință în paranteză rotundă). Dacă aceeași referință este citată de mai multe ori, ea va fi trecută în text cu același număr ca la prima citare. Numărul total de referințe nu va depăși 50 de surse. Acuratețea datelor ține de responsabilitatea autorului.

Pentru mai multe informații consultații: http://journal.ohrm.bba.md/index.php/journal-ohrm-bba-md/editing_guidelines

EXIGENCES POUR LES AUTEURS

Normes de rédaction
La préparation des manuscrits (rédigés en anglais et français) sera conforme aux instructions publiées dans *Uniform Requirements for Manuscripts Submitted to Biomedical Journals (1994) Lancet 1996, 348, V2 ; 1-4* (www.icmje.org). Les manuscrits doivent être en police Cambria, taille 11 points, espacés à l'intervalle 1,0, alignement justifié, champs 2 cm de tous les côtés. Toutes les pages doivent être numérotées consécutivement (dans le coin inférieur droit) et inclure une numérotation continue des pages. Les abréviations doivent être expliquées lors de la première apparition dans le texte et ne doivent pas être utilisées de manière excessive. Les manuscrits ne doivent pas dépasser (sans mentionner le titre, l'affiliation, le résumé et la bibliographie) le vo-lume suivant: pour articles de synthèse/rapports – 4500 mots; pour les articles de recherche – 3000 mots; pour les opinions d'experts – 2500 mots; présentation de cas et photos de la pratique clinique/de laboratoire – 1700 mots; notes expérimentales et cliniques – 1300 mots; commentaires et présentations de livres – 2000 mots; articles pédagogiques – 4000 mots. Le volume des tableaux et des figures ne doit pas dépasser ⅓ du volume du manuscrit. La revue se réserve le droit d'apporter toute autre modification de formatage. Les manuscrits rejetés ne sont pas retournés.

Tous les manuscrits à publier doivent être accompagnés par deux résumés: dans la langue originale et en anglais.

Titre et auteurs
Le titre doit être le plus court que possible (maximum – 120 signes avec espaces), éloquent pour le contenu du manuscrit. Les noms des auteurs seront écrits complets: prénom, nom (*ex:* Albert LEBRUN). Quant à l'affiliation, on devra indiquer: Section/ Département/Chaire, Université/Hôpital, Ville, Pays – pour chaque auteur. Les données de l'auteur correspondant et les coordonnées – adresse e-mail (*ex:* auteur correspondant: Albert Lebrun, e-mail: albert.lebrun@gmail.com*)* seront obligatoires ci-dessous.

Structure du manuscrit
Le manuscrit comprendra les sous-titres suivants (avec lettres majuscules):

- **RÉSUMÉ** (voir les exigences ci-dessous)
- **INTRODUCTION** (reflétera l'actualité et la présentation générale du problème étudié, le but et l'hypothèse de l'étude)
- **METHODES**
- **RESULTATS**
- **DISCUSSIONS**
- **CONCLUSIONS**
- **CONFLIT D'INTERETS**
- **REMERCIEMENTS ET FINANCEMENT**
- **APPROBATION ÉTHIQUE** (préciser la présence ou l'absence d'avis favorable du comité d'éthique: no, date, institution et consentement éclairé)
- **REFERENCES**

Le **résumé** contiendra 1600 signes avec espaces:

- **Introduction**
- **Méthodes**
- **Résultats**
- **Conclusions**
- **Mots clés**: 3-5mots.

Le résumé ne comprendra pas des tableaux, graphiques et des notes bibliographiques; des informations non présentées dans l'étude.

Figures. Le texte inclus dans les figures doit être écrit avec police Cambria, taille 10 points. Chaque figure doit être accompagné par un titre et une légende. Ceux-ci seront numérotés avec des chiffres arabes et mentionnés dans le texte entre parenthèses (*ex:* fig. 1). Le titre (*ex:* Figure 1) et la légende de la figure doivent être centrés, au-dessous de la figure.

Tableaux. Le texte inclus dans les tableaux doit être écrit avec police Cambria, taille 10 points. Chaque tableau doit être accompagné par un titre. Les tableaux seront numérotés avec des chiffres arabes, mentionnés dans le texte entre parenthèses (*ex:* tab. 1), et seront insérés dans le texte, sans dépasser la largeur d'une page. Le titre du tableau sera placé au-dessus du tableau, centré (*ex:* Tableau 1).

Les **références** doivent être numérotées dans l'ordre où elles apparaissent dans le texte. La citation de la source de référence sera de style *AMA*, placée à la fin de l'article et n'inclura que des références citées dans le texte (mentionnant le numéro de référence entre parenthèses rondes). Si la même référence est citée plusieurs fois, elle sera transmise dans le texte avec le même numéro que celui de la première citation. Le nombre total de références ne dépassera pas 50 sources. La responsabilité pour l'exactitude des données est à la charge de l'auteur. Il faut indiquer dans le manuscrit seulement les références vraiment consultées par les auteurs. Les composants des sources de référence doivent être rédigés strictement selon les exigences.

Pour plus d'informations, voir: http://journal.ohrm.bba.md/index.php/journal-ohrm-bba-md/editing_guidelines

ТРЕБОВАНИЯ ДЛЯ АВТОРОВ

Правила составления

Подготовка рукописи (разработанной на английском и французском языках) будет осуществляться в соответствии с инструкциями, опубликованными в: *Uniform Requirements for Manuscripts Submitted to Biomedical Journals (1994) Lancet 1996, 348, V2; 1-4* (www.icmje.org). Авторы должны использовать шрифт Cambria, размер 11 точек, с интервалом 1,0, выравнивание по ширине, поля 2 см со всех сторон. Все страницы должны быть пронуме-рованы последовательно (в правом нижнем углу) и включать непрерывную нумерацию страниц. Сокращения должны быть объяснены при первом появлении в тексте и не должны использоваться чрезмерно. Объем рукописей не должен превышать (без названия, принадлеж-ности, резюме и литературы): для обзорных статей/рефератов – 4500 слов; для научных ста-тей – 3000 слов; для экспертных заключений – 2500 слов; для презентации случаев из клини-ческой/лабораторной практики – 1700 слов; для экспериментальных и клинических заметок – 1300 слов; для рецензий и презентаций книг – 2000 слов; для учебных статей – 4000 слов. Объем таблиц и рисунков не должен превышать ⅓ от объема рукописи. Журнал оставляет за собой право вносить любые другие изменения форматирования. Отклоненные рукописи не возвращаются.

Все рукописи, представленные для публикации, должны сопровождаться двумя резюме: на языке оригинала статьи и на английском языке.

Название и авторы

Название должно быть как можно короче (максимум – 120 знаков с пробелами), но дос-таточно информативным для содержания рукописи. Фамилии авторов будут написаны полностью: имя, фамилия (*например:* Иван ИВАНОВ). Принадлежность будет включать: Отделение/ Департамент/Кафедра, Университет /Больница, Город, Страна для каждого автора. Данные соответствующего автора и контактная инфор-мация – адрес электронной почты (*например:* контактная информация: Иван Иванов. e-mail: ivan.ivanov@gmail.com) будут обязательно ниже.

Структура Рукописи

Рукопись будет включать в себя следующие подзаголовки (они должны быть заглавными):

- **РЕЗЮМЕ** (см. требования ниже)
- **ВВЕДЕНИЕ** (будет отражать актуаль-ность и общее представление изучаемой проблемы, цель и гипотезу исследования)
- **МАТЕРИАЛЫ И МЕТОДЫ**
- **РЕЗУЛЬТАТЫ**
- **ДИСКУССИИ**
- **ВЫВОДЫ**
- **КОНФЛИКТ ИНТЕРЕСОВ**
- **БЛАГОДАРНОСТИ И ФИНАНСИРОВАНИЕ**
- **ЭТИЧЕСКОЕ ОДОБРЕНИЕ** (указать наличие или отсутствие одобрения со стороны комитета по этике: №, дата, учреждение и информированное согласие)
- **ЛИТЕРАТУРА**

Резюме должно содержать 1600 знаков с пробелами и будет включать в себя следующие подзаголовки:

- **Введение**
- **Материалы и методы**
- **Результаты**
- **Выводы**
- **Ключевые слова:** 3-5 слов

Резюме не должно включать таблицы, диаграммы и библиографические заметки, инфор-мацию, не представленную в исследовании.

Рисунки (графики, диаграммы). Текст, включенный в рисунки, должен быть написан в Cambria, размер 10 пунктов. Каждый рисунок должен сопровождаться заголовком и описанием. Название (*например:* Рисунок 1) и описание рисунка должны быть вписаны по центру, в низу рисунка. Они должны быть пронумерованы арабскими цифрами и указаны в тексте в скобках (*например:* рис. 1).

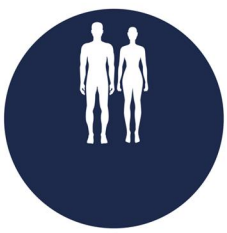
Таблицы. Текст, включенный в таблицы, должен быть написан в Cambria, размер 10 пунктов. Каждая таблица должна сопровождаться заголовком. Они должны вставляться в текст, не превышая ширину страницы. Должны быть пронумерованы арабскими цифрами и указаны в тексте в скобках (*например:* таб. 1). Название таблицы должно располагаться над таблицей в центре (*например:* Таблица 1).

Литература. Источники должны быть пронумерованы в порядке их появления в тексте. Ссылки на источники должны быть в стиле *AMA*, помещены в конце статьи и включать только источники, цитируемые в тексте (упоминание номера источника в круглых скобках). Если один и тот же источник цитируется несколько раз, он будет передан в тексте с тем же номером, что и первый раз. Общее количество источников не должно превышать 50. Ответственность за точ-ность данных лежит на авторе. Будут цитиро-ваться только те источники, с которыми ознакомились авторы рукописи. Компоненты справочных источников должны быть написаны строго в соответствии с требованиями.

Дополнительная информация на: http://journal.ohrm.bba.md/index.php/journal-ohrm-bba-md/editing_guidelines

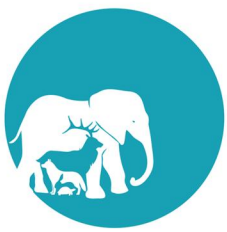
The *One Health* concept

Human health



The WHO defined health in 1946 as "a state of complete physical, mental and social well-being and not merely the absence of disease or infirmity", with the later addition of "the capacity to lead a socially and economically productive life".

Animal health



The OIE defines animal welfare in 2008: an animal is in good condition if it is healthy, enjoys comfort, is well fed, is safe, is able to display its innate (natural) behavior and does not suffer from unpleasant conditions such as pain, fear and stress.

Plant and environmental health

Environmental health refers to those aspects of human health that include the quality of life determined by physical, biological, socio-economic and psycho-social factors in the environment. The interrelationships of people with the environment concern medicine, when an ecological system is in a state of equilibrium, the health of the population prevails.

Globally, the *One Health* concept is a worldwide strategy to expand interdisciplinary collaborations and communications in all aspects related to the health care of humans, domestic animals or wildlife, which can no longer be approached separately, but only jointly.

One Health addresses not only human and animal disease concerns, but also issues related to lifestyle, diet, exercise, the impact of different types of human-animal relationships, and environmental exposures that can affect both populations. In order to achieve the expected effects, it is also necessary to educate the population to make them aware of the risk factors and benefits of prevention, as well as communication and understanding between patients and healthcare providers.


Scopus®
Academic Resource Index ResearchBib
WORLD of JOURNALS
DOAJ DIRECTORY OF OPEN ACCESS JOURNALS
CORE
We are Crossref Member
Instrumentul Bibliometric Național www.ibn.idsi.md
НАУЧНАЯ ЭЛЕКТРОННАЯ БИБЛИОТЕКА eLIBRARY.RU
INTERNET ARCHIVE
OCLC WorldCat®
OpenAIRE
CYBERLENINKA
ROAD
BŞM
Google Scholar
zenodo