SCIENTIA PLENA
www.scientiaplena.org.br
VOL. 21, NUM. 02 2025
doi: 10.14808/sci.plena.2025.026201

The action of the compound 17β-estradiol in Fleischmann® yeast

A ação do composto17β-estradiol na levedura Fleischmann®

D. T. Sarabia[1]; M. S. Mascarenhas[1]*; M. Batistote[1]

[1]*Universidade Estadual de Mato Grosso do Sul, 79804-970, Dourados-MS, Brasil*

**maria_mascarenhas@outlook.com*
(Recebido em 20 de novembro de 2024; aceito em 06 de fevereiro de 2025)

Water is an essential resource for the survival of living organisms and the balance of the planet; however, its quality has been compromised by the presence of potentially toxic elements (PTEs), which can cause damage to aquatic ecosystems and human health. Thus, this study aimed to analyze the chemical characteristics of different types of estrogens and evaluate the toxic action of the compound 17β-estradiol on Fleischmann® yeast. A solution of 17β-estradiol, with purity greater than 99%, was prepared in ethanol and diluted to reach a concentration of 100 ng.L^{-1}. The yeast was cultured in a liquid medium containing water, 2% glucose, and 17β-estradiol, with exposure times of 30, 60, 90, 120, and 150 minutes. Colony growth was assessed on Petri dishes containing solid Sabouraud medium, while cell mortality rate and budding were analyzed by cell counting using a Neubauer chamber and methylene blue staining. The chemical characteristics of 17β-estradiol, such as low volatility, low water solubility, and high hydrophobicity, contribute to its persistence in the environment. Exposure of the yeast to the compound resulted in gradual inhibition of colony growth, with greater impact after 150 minutes. The study demonstrated that even low concentrations of 17β-estradiol caused toxic effects, increasing cell mortality and reducing budding after 90 minutes of exposure, indicating potential risks for other organisms and humans. The yeast stood out as an efficient bioindicator.
Keywords: *Saccharomyces cerevisiae*, estrogens, emerging compounds.

A água é um recurso essencial para a sobrevivência dos organismos vivos e o equilíbrio do planeta, porém sua qualidade tem sido comprometida pela presença de elementos potencialmente tóxicos (PTEs), que podem causar danos aos ecossistemas aquáticos e à saúde humana. Assim, este estudo visou analisar as características químicas dos diferentes tipos de estrogênios e avaliar a ação tóxica do composto 17β-estradiol na levedura Fleischmann®. Uma solução de 17β-estradiol, com pureza superior a 99%, foi preparada em etanol e diluída até atingir uma concentração de 100 ng.L^{-1}. A levedura foi cultivadas em meio líquido contendo água, 2% de glicose e 17β-estradiol, permanecendo nos tempo de exposição de 30, 60, 90, 120 e 150 min. O crescimento das colônias foi avaliado em placas de Petri contendo meio sólido Sabouraud, enquanto a taxa de mortalidade e o brotamento das células foram analisados por contagem celular utilizando uma câmara de Neubauer e corante azul de metileno. As características químicas do 17β-estradiol, como baixa volatilidade, baixa solubilidade em água e alta hidrofobicidade, contribuem para sua persistência no ambiente. A exposição da levedura ao composto resultou em inibição gradual do crescimento das colônias, com maior impacto após 150 min. O estudo demonstrou que mesmo baixas concentrações de 17β-estradiol causaram efeitos tóxicos, aumentando a mortalidade celular e reduzindo o brotamento após 90 min. de exposição, indicando riscos potenciais para outros organismos e humanos. A levedura se destacou como bioindicador eficiente.
Palavras-chaves: *Saccharomyces cerevisiae*, estrogênios, compostos emergentes.

1. INTRODUCTION

Water is an essential natural resource for the survival of living organisms and for maintaining the balance of the planet. However, the quality of this vital resource has been compromised due to various human activities [1]. The increasing presence of chemical products, such as medicines, cosmetics, cleaning products and anticorrosive, has been identified in bodies of water [2]. It has resulted in the degradation of environments. These compounds are widely recognized as emerging pollutants, substances that may pose environmental risks due to their ability to react with other substances present in the environment, generating new compounds whose effects are still

uncertain but have the potential to cause significant harm to the ecosystem. Such elements are considered "emerging" when they arise from new sources, alternative routes of human exposure, or new treatment approaches. [3].

These emerging contaminants are also recognized as Potentially Toxic Elements (PTEs). This type of contamination emerges as a deep threat to the environment, making it imperative to implement effective detection and monitoring strategies [4]. The presence of contaminants in water resources not only threatens the balance of aquatic ecosystems, but also endangers living organisms, given the ability of these chemical substances to cause harm [5]. In this context, the detection and monitoring of these elements in water emerge as a fundamental foundation for preserving the environment and promoting the population's quality of life. Since the presence of these contaminants throughout the hydrological cycle, which includes groundwater, surface water courses and effluents from wastewater treatment plants, raises concerns about the negative impact on terrestrial and aquatic life and human health [1].

Emerging contaminants such as pesticides, personal care products, X-ray contrast media, endocrine disruptors and pharmaceuticals have been extensively reported in wastewater, groundwater and surface water in recent years [5]. These substances, whether natural or synthetic, are associated with significant changes in the natural processes of several species, especially fish [6]. In the group of emerging contaminants, pharmaceutical and personal care products (PFCPs) have been increasingly mentioned in the scientific community. Analgesics, antiseptics, antibiotics and other compounds in this category are among the most investigated. Due to the common polar functional groups in PFCPs, the identification and removal of substances becomes a challenging task [7].

The source of emerging contaminants influences the characteristics of these substances. The transport and transformation of these contaminants depend on physical-chemical characteristics and environmental parameters, such as water solubility, temperature, polarity, volatility, organic matter content, pH, precipitation, altitude and latitude [8]. These factors are crucial in determining the life expectancy of an emerging contaminant in the environment. There are diverse sources of emerging contaminants, both in terms of number and nature. Point sources of pollution and diffuse sources are two main types [9]. When present in bodies of water and beyond permitted limits, these compounds can cause toxic effects to living organisms, altering morphological, biochemical and genetic aspects, which has driven research to minimize the effects of these pollutants in the environment or even to promote its removal.

The application of the use of microorganisms as an efficient, ecological and economical tool in the detection and removal of emerging pollutants has been systematically used in recent years. However, more studies are needed about the use of microbial community with the potential to degrade potentially toxic elements [10]. However, some studies report that different groups of microorganisms used alone or in combination can be used both to promote the accumulation and to degrade numerous PTEs [6].

In this sense, the yeast *Saccharomyces cerevisiae* can be an innovative tool to evaluate the toxicity of emerging compounds, as it has characteristics that make it effective. Firstly, this microorganism has an extraordinary ability to survive, multiply and even adapt to adverse conditions, which can allow a rapid response to environmental stimuli [11]. Furthermore, it has a high degree of genetic correlation with eukaryotic organisms, including humans [12]. Such attributes are important to be considered for their use as a bioindicator to evaluate the genotoxic and mutagenic potential of potentially toxic elements (PTEs), which may be present in various ecosystems [13]. Another advantage of *S. cerevisiae* is its ease of cultivation, growth and manipulation in the laboratory, as it is a non-pathogenic organism, which allows experiments to be carried out under controlled conditions. This experimental capacity makes it possible to evaluate the yeast's response to different samples, facilitating the identification of potential contaminants and the quantification of the effects caused [14].

This microorganism is capable of selectively accumulating potentially toxic elements, concentrating them, allowing a direct analysis of the effect of exposure time and minimum inhibitory concentration in a timely manner [10]. This characteristic can provide important information about the presence and concentration of toxic elements in the environment. Understanding the response of *S. cerevisiae* to contaminants in natural habitats can be an

important tool for implementing strategies to preserve environmental quality, promoting public health and the conservation of ecosystems.

In fact, environmental degradation, perpetrated by different organisms that cohabit the environment, manifests characteristics that provide a dynamic response to xenobiotic substances, such responses being correlated with changes in their physiology, morphology and adaptations, due to the capacity to accumulate such substances [15]. In this sense, the use of microorganisms provides a faster and more efficient response to the toxicity of compounds and their effects at the cellular and molecular level [16]. Among other aspects, they highlight the reduced cost, quick analysis time and reduction of waste generated during analysis as advantageous [17].

The use of microorganisms as bioindicators has stood out as a promising approach, allowing the assessment of the presence and impact that these contaminants can cause ecosystems. These are living organisms capable of responding, in a measurable way, to environmental changes resulting from the presence of pollutants. They reflect the interaction between the environment and organisms, providing valuable information about the quality of ecosystems. Among potential bioindicators, *S. cerevisiae*, a unicellular yeast, has been widely used in microbiological research and offers significant advantages for contaminant detection. Therefore, the study aims to analyze the chemical characteristics of estrogen types, as well as evaluate the toxic action of the compound 17β-estradiol on Fleischmann® yeast.

2. MATERIAL AND METHODS

2.1 Study development location and Microorganism used

The studies were developed at Centro de Estudos em Recursos Naturais – CERNA, in Universidade Estadual do Mato Grosso do Sul, Dourados/MS. In this study the *S. cerevisiae* Fleischmann® lineage was used.

2.2 Evaluation of estrogen types

The research was carried out through an exploratory/descriptive bibliographic review of chemical characteristics of estrogen types. Furthermore, exploratory/descriptive research contributes to the understanding and contextualization of the researched data and focuses on themes in order of importance regarding their content [18].

2.3 Preparation of 17β-estradiol solution

To prepare the stock solution, the compound 17β-estradiol with a purity level of (>99%) was solubilized in ethyl alcohol PA and vortexed. Successive dilutions were performed to a concentration of 100 ng. L^{-1}.

2.4 Preparation of cultivation conditions

For cell growth, yeasts were grown in 10 mL test tubes containing 0.05 g of lyophilized yeast, 1.0 g of glucose, 9.0 mL of sterile distilled water and vortexed. After sample homogenization, 1.0 mL of the compound 17β-estradiol at a concentration of 100 ng. L^{-1} was added to the reaction medium. The tubes were incubated at a temperature of 30 °C at 250 rpm, for times of (30, 60, 90, 120 and 150) minutes, methodology adapted from Sarabia et al. (2019) [19]. Aliquots were collected for analysis of colony growth, assessment of mortality rate and budding.

2.5 Colony growth

To evaluate colony growth in response to 17β-estradiol, previously cultured cells were used. Petri dishes containing Sabouraud solid medium were sterilized in an autoclave for 20 min and

0,5 µL of the culture solution was dripped onto the plate. The plates were incubated at 30 °C for 48 hours, or until colonies appeared. The analysis performed visually and qualitatively, according to the methodology adapted by Mueller et al. (2020) [20].

2.6 Assessment of mortality and sprouting rate

To evaluate the mortality rate and the number of shoots, previously cultured cells were used. Aliquots of 10 µL of the sample were removed and added to an Eppendorf containing 90 µL of methylene blue dye and shaken. The samples were counted in a Neubauer chamber with the aid of an optical microscope [21].

2.7 Statistical analysis

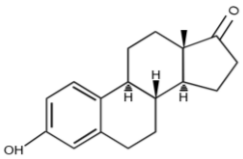
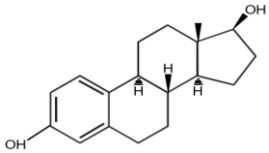
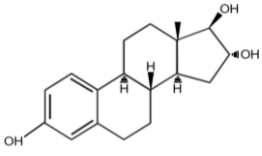
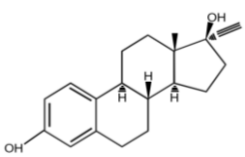
The data obtained were analyzed with Microsoft Excel 16 software and the graphs were plotted in GraphPad Prism version 9.5.1 showing mean and standard deviation.

3. RESULTS AND DISCUSSION

Hormones can be classified as natural or synthetic hormones, which vary according to their structural and molecular form. Chemical characteristics such as the amount of carbon and hydroxyl make a difference in relation to the type of estrogen, (Table 1). The physical-chemical characteristics and environmental conditions are fundamental parameters in the degradability of these compounds and in the lifetime of these molecules presents in ecosystems.

Estrogens are presented as estrone (E1), 17β-estradiol (E2), estriol (E3) and 17α-ethynylestradiol (EE2) and act as possible endocrine disruptors, being natural or synthetic [22]. Its fate in aquatic ecosystems has been a cause for great concern. The detection of 17β-estradiol in surface waters is a global concern. Long-term exposure to this compound, even in very small concentrations, has caused changes in aquatic organisms [23].

Table 1. Classification and chemical characteristics of estrogen types.

Estrogen	Structure Formula	Molecular Formula	Classification
Estrone (E1)		$C_{18}H_{22}O_2$	Natural
17β-estradiol (E2)		$C_{18}H_{24}O_2$	Natural
Estriol (E3)		$C_{18}H_{24}O_3$	Natural
17α-ethynylestradiol (EE2)		$C_{20}H_{24}O_2$	Synthetic

Source: Adapted from Li et al. (2020) [24].

According to Li et al. (2020) [24], estrogens are organic compounds with low volatility, low solubility in water, weak acids and hydrophobicity; this makes them easily degraded in aquatic organisms and remain in sediments for a long time. However, due to the large amounts of chemicals found in the environment, extra attention must be paid to endocrine disruptors, as they are compounds that can trigger harmful effects, both for the environment and for human health hermaphroditism. Among these we have natural hormones such as estrone, 17*β*-estradiol and estriol that can alter hormone levels, increase the risk of cardiovascular diseases, prostate cancer, breast cancer, induce reproductive disorders, malformations, infertility and hermaphroditism [25].

According to Wilkinson et al. (2022) [7], the main sources of pollution come from hospital waste, sewage treatment plants, septic tanks and pharmaceutical production sites. Furthermore, these compounds can be disposed of in the ecosystem through sewage treatment plants (STPs), which are not always carried out effectively and can result in the release of effluents containing high concentrations of chemical products [26].

However, another important point to be discussed is the concentration of these compounds in the environment, since the levels found from below the detection limits to hundreds of nanograms per liter. According to studies carried out by Alnashiri (2021) [27], presented several compounds in different concentrations, as they ranged from 0.1 to 219.800 ng. L^{-1}, these include compounds such as heavy metals, pesticides, perfluorinated acids, benzotriazoles, hormones, alkylphenols, pharmaceuticals and personal care products.

In the evaluation of colony growth in response to the action of the compound 17*β*-estradiol at the times analyzed, it was observed that even at a low concentration the yeast showed inhibition in colony development. The reduction in colony growth occurred gradually in relation to the time of exposure to the compound, so the greatest inhibition occurred at 150 minutes (Figure 1). Possibly the longer duration of action of 17*β*-estradiol against yeast triggered a more severe response in inhibiting cell growth.

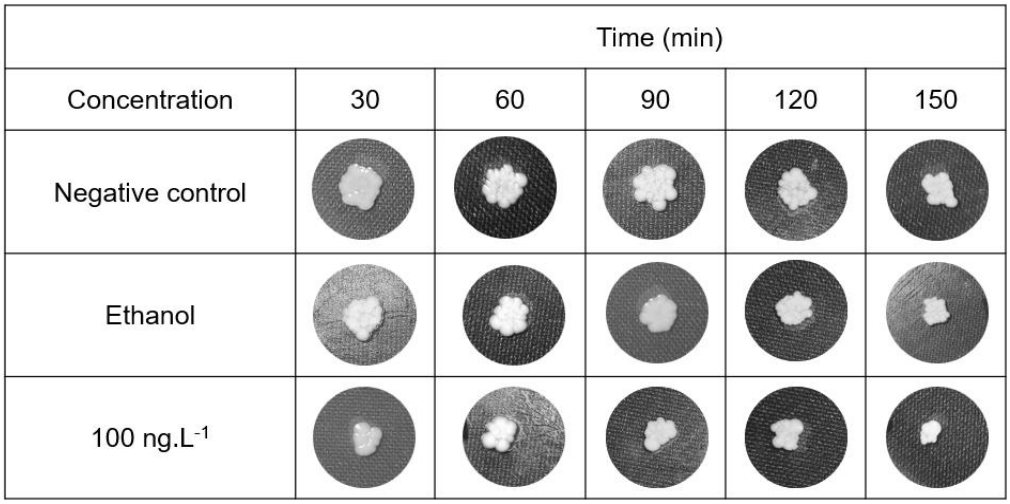


Figure 1. Evaluation of Fleischmann® yeast colony on the action of 17β-estradiol at a concentration of 100 ng.L^{-1} at different exposure times.

There are several test organisms used as estrogen detection agents in water, such as fish, microcrustaceans, molluscs, fungi such as yeast, bacteria and others [4]. A study carried out using zebrafish, modified in the expression of a fluorescent protein, found that in the presence of 17β-estradiol at a concentration of 100 ng.L^{-1}, there was induction of specific expression of the protein in different tissues and with different response patterns in relation to the compound [28]. Studies developed using the pesticide 2,4-dichlorophenoxyacetic acid, in evaluating the dose-response relationship against the yeast *S. cerevisiae*, showed cytotoxic and genotoxic changes [10]. A widespread test in the literature is the Yeast Estrogen Screen (YES assay), a biological assay that uses yeast to detect the presence and measure the activity of estrogenic

compounds in environmental samples, foods or chemical products. This test was used by da Cunha et al. (2019) [29], to detect endocrine disruptors with estrogenic activity in surface waters of Santa Maria Madalena (RJ) and as a result observed that there was estrogenic activity, with concentrations of 23 ng.L^{-1} in the dry season and 10.4 ng.L^{-1} in the rainy season, indicating high and medium ecological risk, respectively.

In the analysis of the yeast cell mortality rate in relation to the compound 17β-estradiol at the analyzed times, a gradual increase in the lethality of the Fleischmann® yeast can be observed. It is noted that in the 90-minute period there is a certain resistance of the yeast to survival in relation to the action of the compound, however in the longer periods of exposure to the compound there was grater lethality to the cells. Regarding budding, a gradual decrease in the number of buds is observed, mainly at 90 minutes, at the tie of the second generation of yeast. It can be observed that the number of sprouts in the mortality rate occur more markedly in the longer periods of action of the compound, which may have possibly induced the Fleischmann® yeast to a toxicity response in relation to the action of the compound (Figures 2A e 2B).

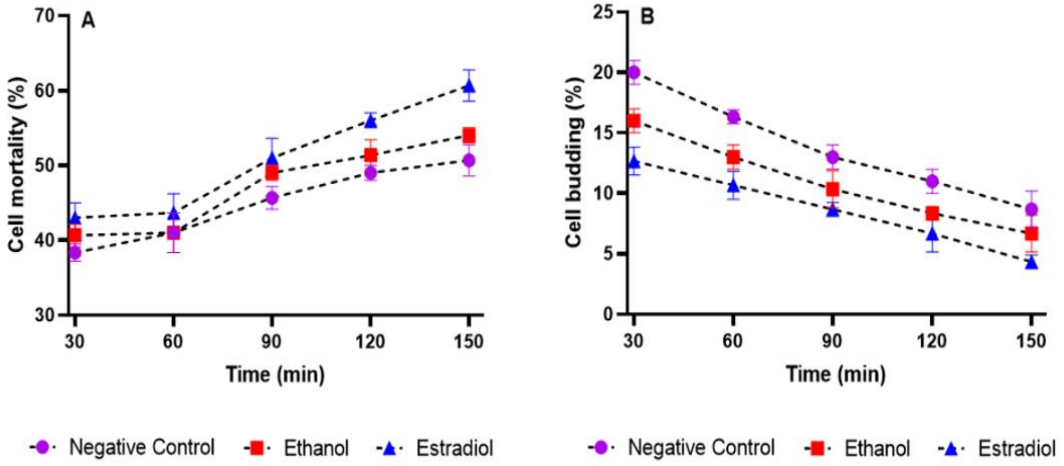


Figure 2. Evaluation of the mortality rate (A) and budding yeast cell (B), Fleischmann®, on the action of 17β-estradiol, at different exposure times.

Saccharomyces cerevisiae was used as a test organism to evaluate the toxicity of chemicals to verify changes in gametogenesis, in other words, in cell division and correlate the effects in humans due to the high dree of homology. 17β-estradiol was listed in this study as one of the chemicals that is toxic to yeast, and when mixed with other compounds can enhance its effects. Studies developed by Wang et al. (2024) [30], using fish as a biomarker of gene expressions in the response of hepatocytes to the action of the natural hormones such as estrone, 17β-estradiol and estriol, the data showed several gene responses. Literature reports show that mussels cultured with 17β-estradiol suffered hormonal imbalances compromising food safety.

Furthermore, there are several test organisms used as bioindicators in toxicology studies. In this study, we can observe that Fleischmann® yeast presented a sensitivity response to toxicity to the compound 17β-estradiol in relation to colony growth, mortality rate and budding, mainly in low concentrations and in longer exposure times. Such cellular characteristics are important to be considered in test organisms. However, additional studies should be carried out to better understand the yeast's response mechanisms to the toxic action of the compound, which could become a valuable tool for use in environmental analyses

4. CONCLUSION

The study showed the importance of investigating emerging compounds such as estrogens present in the environment which can interfere with organisms. The physical-chemical

characteristics of the types of estrogens are important, as these compounds can be natural and synthetic in nature; the structural and molecular formulas are fundamental, as these molecules interfere in the degradation and interaction in ecosystems.

In the evaluation of the growth of Fleischmann® yeast colonies, a more pronounced inhibition of growth occurred in the longer periods of exposure to 17β-estradiol. In the evaluation of the mortality and budding rate, the 90-minute period provided greater cell development, however, in the times of greater exposure to the compound, there was a marked loss of yeast cell vitality under the conditions analyzed. The exposure time in relation to the dose of the compound triggered an effective response of the yeast against the toxic action of 17β-estradiol.

5. ACKNOWLEDGMENTS

The Universidade Estadual de Mato Grosso Do Sul (UEMS); Programa de Pós-Graduação em Recursos Naturais (PGRN); Fundação de Apoio ao Desenvolvimento do Ensino, Ciência e Tecnologia do Estado de Mato Grosso do Sul (FUNDECT); Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq); Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) – Código 001.

6. BIBLIOGRAPHICAL REFERENCES

1. Mishra RK. Fresh water availability and its global challenge. BJMAS. 2023 May;4(3):1-78. doi: 10.37745/bjmas.2022.0208
2. Vargas-Berrones K, Bernal-Jácome L, de León-Martínez LD, Flores-Ramírez R. Emerging pollutants (EPs) in Latin América: A critical review of under-studied EPs, case of study-Nonylphenol. Sci Total Environ. 2020 Jul;726:138493. doi: 10.1016/j.scitotenv.2020.138493
3. Selak A, Reberski JL, Klobučar G, Grčić I. Ecotoxicological aspects related to the occurrence of emerging contaminants in the Dinaric karst aquifer of Jadro and Žrnovnica springs. Sci Total Environ. 2022 Jun;825:153827. doi: 10.1016/j.scitotenv.2022.153827
4. Pourret O, Hursthouse A. It's time to replace the term "heavy metals" with "potentially toxic elements" when reporting environmental research. Int. J. Environ. Res. Public Health. 2019 Nov;16(22):4446. doi: 10.3390/ijerph16224446
5. Anifowoshe AT, Roy D, Dutta S, Nongthomba U. Evaluation of cytogenotoxic potential and embryotoxicity of KRS-Cauvery River water in zebrafish (*Danio rerio*). Ecotoxicol Environ Saf. 2022 Mar;233:113320. doi: 10.1016/j.ecoenv.2022.113320
6. Aolin H, Qin L, Zhu S, Hu X, Yin D. Combined effects of pH and dissolved organic matter on the availability of pharmaceuticals and personal care products in aqueous environment. Sci Total Environ. 2024 Jun;929:172637. doi: 10.1016/j.scitotenv.2024.172637
7. Letinski DJ, Parkerton TF, Redman AD, Connelly MJ, Peterson B. Water solubility of selected C9–C18 alkanes using a slow-stir technique: comparison to structure–property models. Chemosphere. 2016 May;150:416-23. doi: 10.1016/j.chemosphere.2015.12.038
8. Puri M, Gandhi K, Kumar MS. Emerging environmental contaminants: A global perspective on policies and regulations. J Environ Manage. 2023 Apr;332:117344. doi: 10.1016/j.jenvman.2023.117344
9. Kumar A, Alam A, Tripathi D, Rani M, Khatoon H, Pandey S, et al. Protein adaptations in extremophiles: an insight into extremophilic connection of mycobacterial proteome. Seminars Cell Developm Biol. 2018 Dec;84:147-57. doi: 10.1016/j.semcdb.2018.01.003
10. Sarabia DT, Mueller LP, Santos MSM, Batistote M. Physiological response to the action of the agrotoxic 2,4-Dichlorophenoxiacetic acid in *Saccharomyces cerevisiae*. RSD. 2021;10(11):e408101119912. doi: 10.33448/rsd-v10i11.19912
11. Hori Y, Engel C, Kobayashi T. Regulation of ribosomal RNA gene copy number, transcription and nucleolus organization in eukaryotes. Nat Rev Mol Cell Biol. 2023 Jun;24(6):414-29. doi: 10.1038/s41580-022-00573-9
12. Brtnicky M, Datta R, Holatko J, Bielska L, Gusiatin ZM, Kucerik J, et al. A critical review of the possible adverse effects of biochar in the soil environment. Sci. Total Environ. 2021 Nov 20;796:148756. doi: 10.1016/j.scitotenv.2021.148756
13. Echeverri-Jaramillo G, Jaramillo-Colorado B, Junca H, Consuegra-Mayor C. Towards the development of microbial ecotoxicology testing using chlorpyrifos contaminated sediments and marine yeast isolates as a model. Microorganisms. 2022 Oct;10(10):2019. doi: 10.3390/microorganisms10102019

14. Daskalova AV, Tomova AA, Kujumdzieva AV, Velkova LG, Dolashka PA, Petrova VY. Menadione and hydrogen peroxide trigger specific alterations in RNA polymerases profiles in quiescent *Saccharomyces cerevisiae* cells. Biotechnol. Biotechnol. Equip. 2021 Jan;35(1):1190-9. doi: 10.1080/13102818.2021.1941255
15. Li X, Zhao B, Luo L, Zhou Y, Lai D, Luan T. In vitro immunotoxicity detection for environmental pollutants: Current techniques and future perspectives. TrAC. 2023 Jan;158:116901. doi: 10.1016/j.trac.2022.116901
16. Chowdhury S, Dubey VK, Choudhury S, Das A, Jeengar D, Sujatha B, et al. Insects as bioindicator: A hidden gem for environmental monitoring. Front Environ Sci. 2023 Mar;11:1146052. doi: 10.3389/fenvs.2023.1146052
17. Zhang M, Sun Q, Chen P, Wei X, Wang B. How microorganisms tell the truth of potentially toxic elements pollution in environment. J Hazard Mater. 2022 Jun;431:128456. doi: 10.1016/j.jhazmat.2022.128456
18. Sagova-Mareckova M, Boenigk J, Bouchez A, Cermakova K, Chonova T, Cordier T, et al. Expanding ecological assessment by integrating microorganisms into routine freshwater biomonitoring. Water Res. 2021 Mar;191:116767. doi: 10.1016/j.watres.2020.116767
19. Sarabia DT, Mueller LP, Santos MSM, Batistote M. Ação de toxicidade dos agrotóxicos atrazina e ácido 2, 4-Diclorofenoxiacético na levedura Fleischmann®. Rev Ed Amb Ação. 2019 Jun;18(68).
20. Mueller LP, Santos MD, Cardoso CA, Batistote M. The effects of thermal and ethanolic stress in industrial strains of *Saccharomyces cerevisiae*. RSD. 2020 Oct;9(10):e6819109091. doi: 10.33448/rsd-v9i10.9091
21. Lee SS, Robinson FM, Wang HY. Rapid determination of yeast viability Biotechnol Bioeng Symp. 1981;11:641-9.
22. Gong J, Lin C, Xiong X, Chen D, Chen Y, Zhou Y, et al. Occurrence, distribution, and potential risks of environmental corticosteroids in surface waters from the Pearl River Delta, South China. Environ Pollut. 2019 Aug;251:102-9. doi: 10.1016/j.envpol.2019.04.110
23. Song J, Nagae M, Takao Y, Soyano K. Field survey of environmental estrogen pollution in the coastal area of Tokyo Bay and Nagasaki City using the Japanese common goby *Acanthogobius flavimanus*. Environ Pollut. 2020 Mar;258:113673. doi: 10.1016/j.envpol.2019.113673
24. Li C, Wei Y, Zhang S, Tan W. Advanced methods to analyze steroid estrogens in environmental samples. Environ. Chem. Lett. 2020 May;18:543-59. doi: 10.1007/s10311-019-00961-2
25. Ciślak M, Kruszelnicka I, Zembrzuska J, Ginter-Kramarczyk D. Estrogen pollution of the European aquatic environment: a critical review. Water Res. 2023 Feb;229:119413. doi: 10.1016/j.watres.2022.119413
26. Patel M, Kumar R, Kishor K, Mlsna T, Pittman Jr CU, Mohan D. Pharmaceuticals of emerging concern in aquatic systems: chemistry, occurrence, effects, and removal methods. Chem Rev. 2019;119(6):3510-673. doi: 10.1021/acs.chemrev.8b00299
27. Alnashiri HM. Assessment of physicochemical parameters and heavy metal concentration in the effuents of sewage treatment plants in Jazan Region, Saudi Arabia. J King Saud Univ Sci. 2021 Dec;33(8):101600. doi: 10.1016/j.jksus.2021.101600
28. Risjani Y, Darmawan A, Renitasari DP, Ayuknita AL, Rahma F, Effendi S, et al. Histopathological aberration and 17-β-estradiol imbalance in green mussel *Perna viridis* population cultured in Java Sea, Indonesia. Egypt J Aquat Res. 2023 Jun;49(2):197-203. doi: 10.1016/j.ejar.2022.07.002
29. da Cunha DL, Muylaert S, do Nascimento MTL, da Silva GGM, da Fonseca EM, Bila DM, et al. Atividade estrogênica de desreguladores endócrinos em águas superficiais do município de Santa Maria Madalena, Sudeste do Brasil. Eng Sanit Ambient. 2021Jan;26(1):21-8. doi: 10.1590/s1413-415220180133
30. Wang Y, Lv J, Xie Z, Huai N, Zhang K, Zhou Y, et al Gene expression profiles of *Chinese medaka* (*Oryzias sinensis*) primary hepatocytes in response to estrone (E1), 17β-estradiol (E2) and estriol (E3). J Environ Sci. 2025 Jun;152:368-75. doi: 10.1016/j.jes.2024.03.047