# Biological treatment of domestic wastewater by *Escherichia coli*

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*Received 27 Aug. 2024, Accepted 1 Oct. 2024, published 30 Dec. 2024.*

***DOI: 10.52113/2/11.02.2024/62-75***

**ABSTRAC:** *Escherichia coli* may be utilized to decrease some of the contaminants in wastewater from the Al Muthanna wastewater treatment plant's final sedimentation basins, according to the results of the current research. Using chromogenic urinary tract infection (UTI) agar to create dark blue colonies, bacteria were isolated from the intended area and identified. *Escherichia coli* was used in the experiment, which was carried out in a lab setting, and it was put to contaminated water that had previously been autoclave-sterilized. Following the incubation of sterile contaminated water and bacteria in the incubator under optimal circumstances, various physical and chemical variables were measured to indicate its potential to eliminate certain pollutants. The findings indicated that the pH had a minor tendency toward alkalinity and that the salinity, nitrates, electrical conductivity, and total alkalinity were all low. On the other hand, the bacteria were very effective in lowering phosphate, calcium, magnesium, and overall hardness. After treatment, the readings of ammonia raised. The results demonstrated how well bacteria work to lower copper and zinc levels in treated water. During the course of the treatment, while the bacteria showed no efficacy in lowering iron levels.

**Keywords:** Biological treatment, wastewater, *E.coli*, heavy metals.

## 1. Introduction

Water is a vital resource that is necessary for human survival as well as the general well-being and success of all species on Earth [1]. Water contamination has been a problem for humans throughout time as a result of both fast population increase and technological improvement. Furthermore, it is a frequent practice in developing nations like Ethiopia, Kenya, Nigeria, and India to discharge effluent into water bodies without first applying the required wastewater treatment [2]. The ecosystem and public health may be severely harmed by the presence of many contaminants in wastewater, including microorganisms, organic and inorganic impurities, and heavy metals [3]. Trash and debris disposal into natural water ponds may have detrimental effects on aquatic ecosystems, endangering both human health and natural habitats [4]. Thus, before being released into the environment, wastewater has to be properly cleaned and treated. Water pollution can no longer be completely removed by traditional wastewater treatment techniques. As a result, the treated water may still contain trace levels of pollutants [5]. Pollutants are hazardous materials that may harm the environment and interfere with a variety of plant cellular processes [6]. Alternative wastewater treatment techniques are

needed because of the detrimental impact that contaminants have on aquatic habitats and human life [7], [8]. Traditional environmental cleaning techniques that include physical and chemical processes deteriorate the environment and may lead to secondary contamination. Consequently, one alternative to these conventional techniques is biological treatment. Water, soil, sludge, and waste streams will all be cleaned up via bioremediation [9]. Microbial biotechnology is a discipline that is surprisingly expanding and changing, offering a variety of solutions for handling environmental problems.

This work aimed to study the ability of *E.coli* to treat pollutants in domestic waste water.

## 2. Materials and Methodology

### 2.1 Description of the Wastewater Treatment plant

The Central Wastewater Treatment Plant is located in the Al-Muthanna Governorate – the Al-Rumaytha district in the Al-Daboush area, about (25 km) away from the city center (Samawa). The stages of treatment include several steps. It begins with the mechanical or physical treatment stage, where heavy water (wastewater) enters from the main conveying pipe in pump station 2 in Rumaytha to the main entry station (inlet). It contains a clip to hold stones, cans, pieces of cloth and nylon. Then to the basins for removing sand and fat, and then to the narrowing basins to narrow and accelerate the movement of water. The water enters the aeration ponds, where the primary treatment is carried out by aerobic bacteria to remove phosphorus, nitrogen and nitrates. In the second part of the aeration ponds, the water is treated by aerobic bacteria that analyze heavy water, and then to the sedimentation ponds to rid the water of suspended matter. Then to the chlorination ponds and finally to the final station that pumps the treated water to the drain (information from the station management).

### 2.2. Microbial Isolation and Sample Collection

as soon as samples are taken from the necessary location (sedimentation ponds). In order to determine whether common bacteria were present in wastewater samples, water samples were collected in 1000 ml glass bottles that had been thoroughly cleaned, rinsed, and sterilized. The samples were then inoculated onto UTI chromogenic agar, or MacConkey agar. A 1 ml waste water sample was transferred to 9 ml of normal saline, and 300 µl was taken to be grown on the previously described agar. The sample was then incubated at 37 °C for 48 hours. The medium's chromogenic guide will be used to diagnose the bacteria that have grown. [10] *E. Coli* colonies seem dark blue.

### 2.3. Treatment mechanism using bacterial isolates

*E. coli* was isolated and used in the treatment procedure. An autoclave was used to sterilize the wastewater sample from the site, and 10

milliliters of each bacterial isolate's culture were then added to 200 milliliters of wastewater in 500 milliliter plastic bottles. The treatment was then incubated at 30 degrees Celsius for a week. As per [11], the experiment was carried out. Alkalinity (T.K), phosphates ($PO_4^-$), nitrates ($NO_3^-$), nitrite ($NO_2^-$), ammonia (NH3), total dissolved solids (TDS), salinity, hydrogen ion concentration (pH), total hardness (TH), calcium ($Ca^{+2}$), magnesium ($Mg^{+2}$), and heavy metals (Cu, Zn,Fe). were measured using the techniques indicated in the following table:


**Fig 1: Map of study site.**

Table 1: Physiochemical parameters and methods were used in the current study.

| NO | Parameters | Acronyms | Unit | Methods |
|---|---|---|---|---|
| 1 | Water Temperature | WT | C˚ | Mercury Thermometer (0-100C)[12] |
| 2 | Electrical conductivity | EC | πs/cm | multi-meter Sm801[13] |
| 3 | Salinity | - | ‰ | By the Conductivity Equation Salinity‰ =EC x 640 x $10^{-6}$;[14] |
| 4 | Total dissolved solids | TDS | mg/l | gravimetrically according to[15] |

| | | | | |
|---|---|---|---|---|
| 5 | Total suspended solid | TSS | mg/l | gravimetrically according to[15] |
| 6 | Hydrogen ion concentration | pH | - | multi-meter Sm801[13] |
| 7 | Total hardness | TH | mg $CaCO_3$/l | EDTA-2Na titration using EBT as an indicator;[15] |
| 8 | Calcium | $Ca^{+2}$ | mg $CaCO_3$/l | EDTA-2Na titration using hydrogen peroxide as an indicator;[15] |
| 9 | Magnesium | $Mg^{+2}$ | mg/l | Calcium hardness (as mg $CaCO_3$/L) minus overall hardness (as mg Mg/L)*0.243;[15] |
| 10 | Total Alkalinity | TA | mg/l | As $CaCO_3$ by titration method[15] |
| 11 | Phosphates | $PO_4$ | µl/g | Molybdate ascorbic acid method;[16] |
| 12 | Nitrates | $NO_3$ | µl/g | Cadmium reduction method; [17] |
| 13 | Nitrite | $NO_2$ | µl/g | Colorimetric methods;[17] |
| 14 | Ammonia | NH4 | µl/g | Handbook of common methods in Limnology,[18] |
| 15 | Copper | Cu | µl/g | by flame atomic absorption spectrophotometer;[15] |
| 16 | Iron | Fe | µl/g | by flame atomic absorption spectrophotometer; [15] |
| 17 | Zinc | Zn | µl/g | by flame atomic absorption spectrophotometer;[15] |

### 2.4. Statistical Analysis

SPSS was used to examine the data.V.12. Sequential factorial trials were used for statistical analysis, and manually generated Least Significant Differences (LSD) were used. P value < 0.05 was taken into account statistically noteworthy.

## 3. Results and Discussion

*E. Coli* typically develops in a temperature range of 23 to 40 °C, however the present study's data suggest that it mutates and divides more effectively around 30 °C [19].

The statistical analysis shown in Figure 2 (a) verified that the temperature values of water treated by bacteria differed significantly ($p \leq 0.05$).

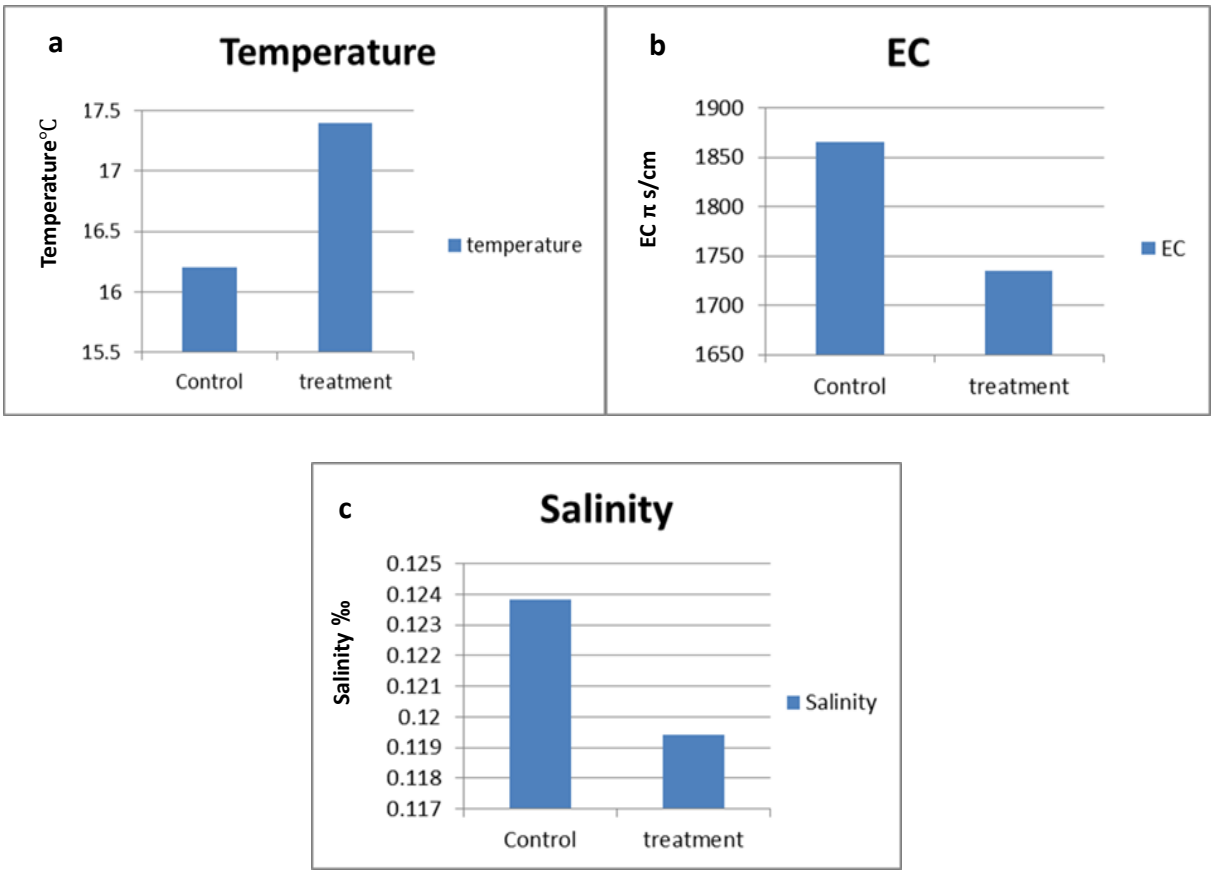


**Figure 2:** Some physical parameters for domestic water during treatment period by *E.coli* (a:temperature, b: Electrical conductivity and c:Salinity.

After being treated with *E. coli*, the electrical conductivity value in the present investigation dropped from 1999µs/cm to 1915µs/cm. Figure 2 (b), 4.20 percent of the EC was removed. This might be because the bacteria are raising pH or dissolved inorganic carbon by catalyzing carbonate deposition via a variety of metabolic pathways, including photosynthesis, urinalysis, ammonia, denitrification, sulfate reduction, anaerobic sulfide oxidation, and methane oxidation. [20], [21], [22], and [23]. Moreover, metal ions may be absorbed by cell walls that contain negatively charged functional groups, such as carboxyl and

amine phosphates [24],[25]. Because bacteria have genes that allow them to react to high salinity or stressful environments, and because they also physically absorb salts via certain metabolic pathways, the higher the salinity, the faster the bacteria grow [26]. 4.20 percent of the salinity was removed. The statistical analysis revealed that there was no significant change ($p \leq 0.05$) in the electrical conductivity and salinity values of the water treated by bacteria, as shown in Figure 2 (c). nucleation sites for $CaCO_3$ precipitation [30]. The statistical study revealed that the total Hardness values in the water treated by bacteria did not change significantly ($p \leq 0.05$).

After being treated with *E. coli*, the pH values in the study (figure 3) (a) slightly rose in an alkaline direction. This might be attributed to the microbial breakdown of proteins and amino acids in the wastewater into ammonia, which raises the pH of the sample. The presence of microorganisms that break down organic compounds is also indicated by the change in pH of wastewater [27]. The findings of the statistical study showed that the pH values in the water treated by bacteria did not change significantly ($p \leq 0.05$).

After treating the contaminated water with *E. coli*, the overall hardness values fell from 1100 mg $CaCO_3$/L to 1040 mg $CaCO_3$/L, with a removal percentage of 5.45%, as shown in Figure 3(b).This might be because the negatively charged surfaces of microorganisms attach cations, particularly $Ca^{2+}$, to their surfaces, scavenging them from aquatic environments. As a result, microbes are perfect locations for the nucleation of crystals [28], [29].$CaCO_3$ precipitation therefore depends on the proper supply and concentration of calcium. In order to promote further calcite formation, bacterial cells provide an alkaline environment and serve as

Following *E. coli* treatment, calcium levels dropped from 240 mg $CaCO_3$/l to 165 mg $CaCO_3$/L. Figure 3(c) shows the percentage of removal (31.25%). In bacterial cells, calcium has a "general reset" function and aids in the structure of the alleged bacterial "skeleton" [31].Prokaryotic cell growth is significantly influenced by $Ca^{2+}$, which is also involved in a variety of bacterial functions such as gene expression, chemotaxis, transport, cell differentiation, and pathogenicity. Wall-less *E. coli* that grows in a L shape needs calcium for growth; without it, the cells stop dividing, expand, form huge vacuoles, and finally decompose [30]. The results of the statistical study showed that the amounts of calcium in the water treated by bacteria differed significantly ($p \leq 0.05$).

Additionally, magnesium concentrations dropped from 208 mg $CaCO_3$/L to 188 mg $CaCO_3$/L after *E. Coli* therapy. Figure (3) (d) shows the percentage of removal (10.05%). It is required for the action of several enzymes

and for preserving the ribosome's structural integrity in live cells. The ion is necessary to keep the bacterial cell's permeability barrier intact. Spheroplast stability caused by magnesium ions also points to a possible role for them in maintaining the integrity of cell membranes. The presence of magnesium is known to be necessary for ribosome stability in vitro. Similarly, massive turnover (degradation and synthesis) of ribosomes occurs in live bacteria depriving them of magnesium, which breaks down the ribosomal protein. While ribosomal RNA may still be deposited in acids, it is not recycled when new ribosomes are simultaneously formed. [32]. The results of the statistical study showed that the amounts of magnesium in the water treated by bacteria differed significantly ($p \leq 0.05$).

Figure 3(e) shows that the total alkalinity dropped from 820 mg/L to 340 mg/L and that the elimination percentage was 58.54% while treating wastewater with *E. coli*. This is because carbonate precipitation is either enhanced or inhibited by extracellular polymerase (EPS), which plays a significant role in microbial calcification [24, 29]. Bacteria that are heterotrophic or autotrophic may both create EPS [33]. Divalent cations like $Ca^{2+}$ and $Mg^{2+}$ may be trapped in considerable quantities by EPS that include a variety of acidic residues and sugars [34]. EPS contains metallic bonds, including carboxyl, phosphate, amine, and hydroxyl groups [24],[35]. EPS adheres to these negatively charged groups in order to extract free cations from the solution. As a result, EPS inhibits calcium carbonate saturation and stops carbonate precipitation [36]. The results of the statistical analysis demonstrated that the total alkalinity levels in the bacterially treated water differed significantly ($p \leq 0.05$).

Following treatment, phosphate readings dropped as shown in figure 4(a), with an elimination percentage of 80.76 percent. Currently, wastewater treatment facilities biologically remove phosphorus by allowing microorganisms like bacteria to absorb dissolved orthophosphate, polyphosphate, and organophosphate. An acknowledged and less costly alternative for chemical phosphate removal in wastewater treatment is biological phosphate removal. Microorganisms utilize phosphorus for a variety of purposes, including cell maintenance, nucleic acid synthesis, the phospholipids that make up cell membranes, and internal chemical energy transfer processes involving ATP molecules. Additionally, some phosphorus is retained by cells for later use. The creation of net living biomass determines how much phosphate is eliminated. A different biotechnological and environmentally friendly approach to the environment and its inhabitants is biological phosphate removal (EBPR) [37]. The results of the statistical analysis showed that the levels of phosphate in the water treated by bacteria differed significantly ($p \leq 0.05$).

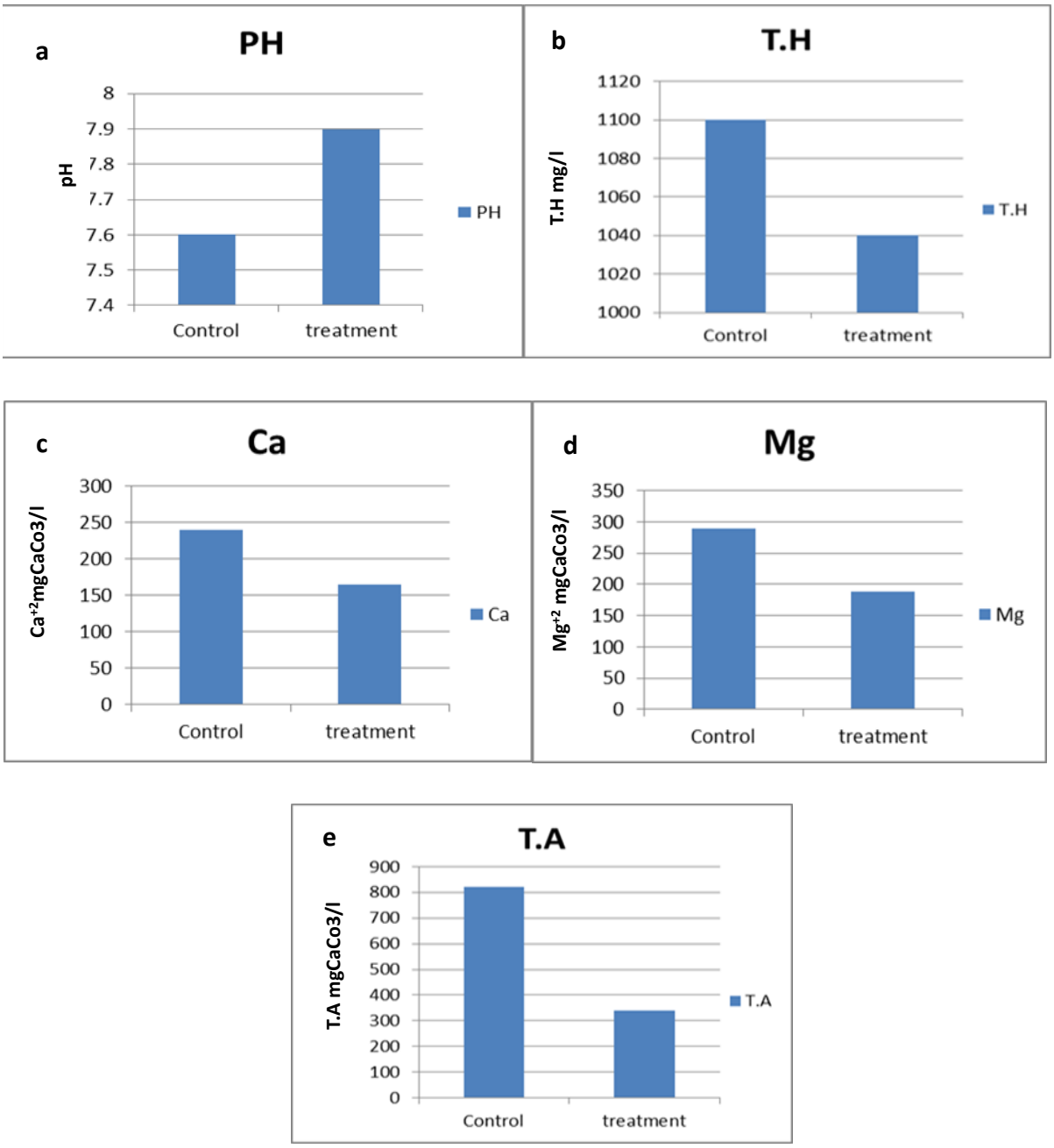


**Figure 3:** some chemical parameters for domestic water during treatment period by *E.coli* (a: pH, b: Total Hardness, c: Calcium, d: magnesium and e: total alkalinity).

The nitrate readings reduced following treatment, as shown in Figure 4(b), and the elimination percentage was 50.00%. Regarding bacteria Nitrate is an important component of *E. coli's* physiology and is the second most appropriate electron acceptor after oxygen. For respiration, *E. Coli* may use a variety of electron acceptors, including nitrite, nitrate, and oxygen. Nitrate is the second most energy-dense substance after oxygen. Recent research has shown that the availability of terminal electron acceptors and

the production of biofilms in *E. coli* are strain-dependent. For a variety of processes, including growth, assimilation, redox balance (homeostasis), and peripheral electron acceptor (respiration), *E. Coli* may reduce nitrate [38]. The results of the statistical analysis showed that the levels of nitrate in the water treated by bacteria differed significantly ($p \leq 0.05$).

According to the present findings, the bacteria were effective in lowering the nitrite concentrations during the course of the treatment. The elimination rate was 26.45% in Figure 4 (c). This might have to do with the fact that nitrite is a byproduct of the two-step biological process known as nitrification, which converts ammonium to nitrate. Ammonia-oxidizing bacteria convert ammonia to nitrite in the first and last stage, the bacteria that oxidize nitrite to nitrate finish the process. De-nitrification may take place if the effluent is later exposed to anaerobic conditions, which means the process by which facultative heterotrophic bacteria convert nitrate to nitrogen gas biologically [39]. The results of the statistical analysis showed that the levels of nitrite in the water treated by bacteria differed significantly ($p \leq 0.05$).

Ammonia levels rose from 2.26 mg/l to 4.5 mg/l during bacterial treatment. The consumption of proteins or amino acids found in contaminated water is the scientific explanation, which causes ammonia to be released into the water [40]. Figure 4(d). The results of the statistical analysis showed that the values of ammonia water treated by bacteria differed significantly ($p \leq 0.05$).

According to the available data, the bacteria were effective in lowering copper concentrations from 0.427 mg/l to 0.125 mg/l during the course of the therapy. According to Figure 5(a), 70.73% of the copper was removed. This was in line with what the study [41] found. This is because copper is a crucial micronutrient that plays a role in several physiological functions. It is a necessary cofactor in redox enzymes because it can move electrons between the copper ($Cu^{+}$) and copper ($Cu^{2+}$) states via a redox reaction. Enzymes such as cytochrome c oxidase, peptidylglycine alpha-amides monooxygenase, and superoxide dismutase SOD are essential for respiration, peptide processing, and defense against oxidative stress [42, 43]. Copper transporter 1 (Ctr1) brings copper ions ($Cu^{+}$) into cells, where they are then distributed across the cytosolic, mitochondrial, and Golgi pathways for use [44]. The statistical results demonstrated that the levels of copper in the bacterially treated water differed significantly ($p \leq 0.05$). The iron percentage was >0.35, according to [45], however the present study's findings were >0.001. This difference helps to explain why there are low or no iron concentrations and why bacteria do not benefit from them.

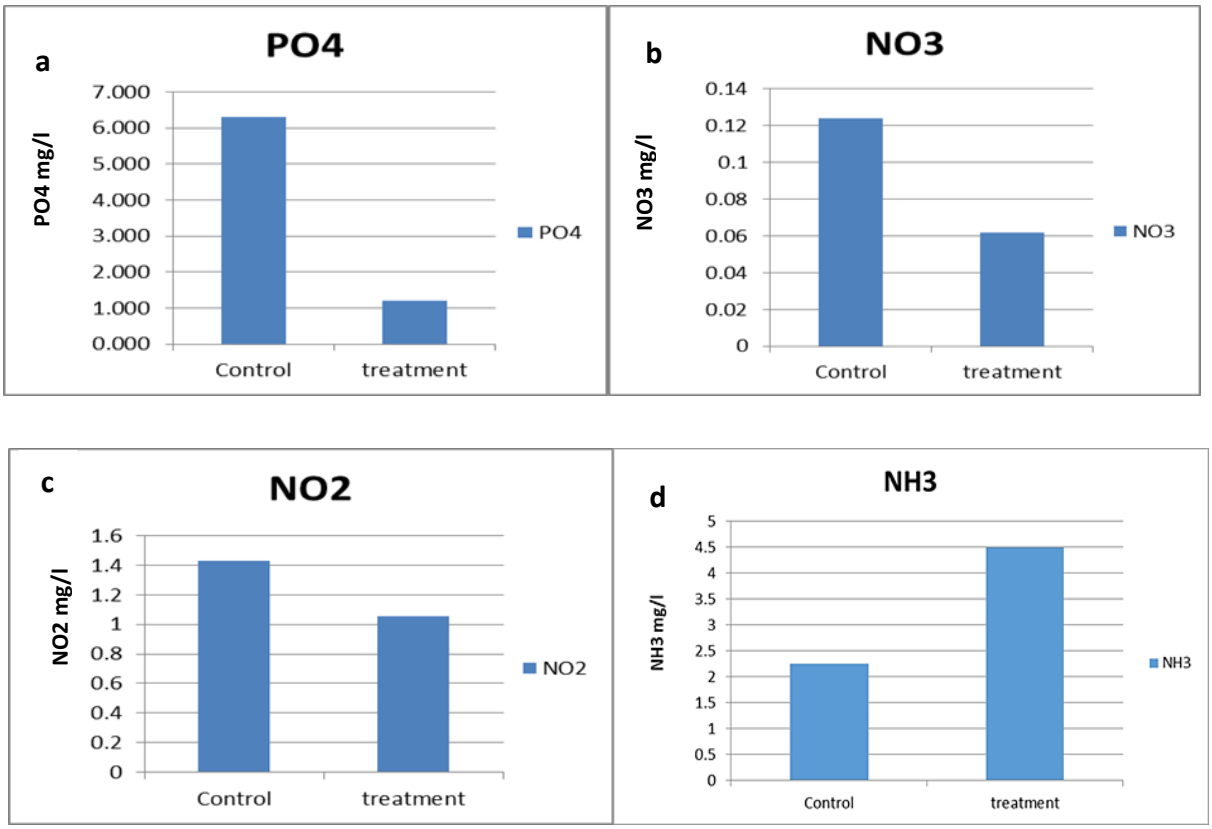


**Figure 4:** Some nutrients for domestic water during the treatment period by *E.coli* (a: phosphate, b: Nitrate, c: Nitrite and d: Ammonia).

The proportion of iron removed from contaminated water was 0%. Figure 5 (b) shows that the iron levels in the water treated by bacteria did not change significantly ($p \leq 0.05$) according to the statistical analysis findings.

With a removal percentage of 99.90%, the biological treatment of zinc with bacteria has a great reduction effectiveness. This could be because bacteria need zinc as a necessary element. It performs a variety of tasks, including acting as a catalyst for other molecules or proteins and being crucial for cell development and transcription. apoptosis, senescence, mitosis, and reaction to oxidative stress [46]. The statistical results shown in Figure 5(c) demonstrated a significant difference ($p \leq 0.05$) in the zinc levels in the bacterially treated water.

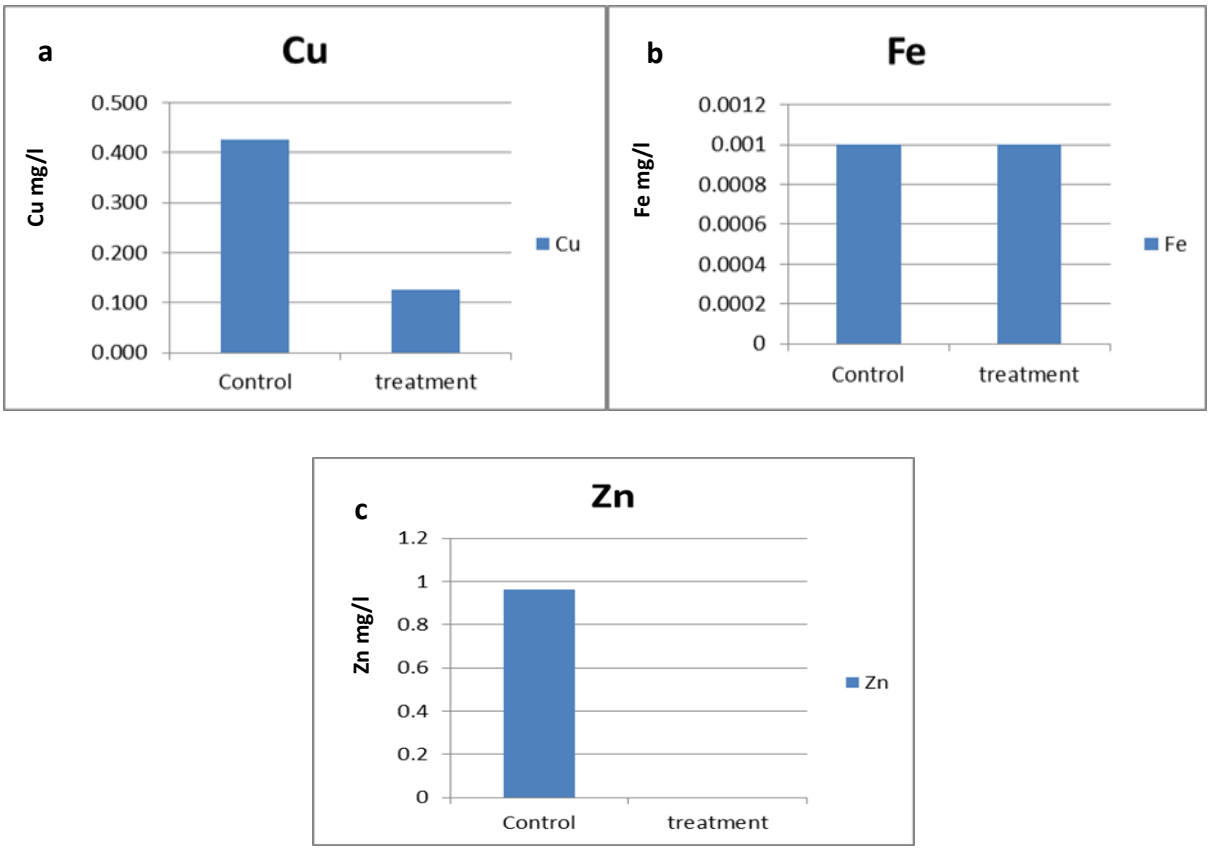


**Figure 1 5:** Some heavy metals for domestic water during treatment period by *E.coli* (a: Copper, b:Iron and c: Zinc).

## Conclusion

High effectiveness of *Escherichia coli* in removing pollutants from contaminated wastewater was observed. The results showed that the pH tended slightly towards alkaline side, and the values of electrical conductivity, salinity, nitrate , nitrite and total hardness were low, while showed high efficiency in reducing total alkalinity, calcium, magnesium, phosphate, zinc and copper. As for ammonia, its values increased after treatment, while it did not show any efficiency in reducing iron throughout the treatment period. Significant differences were found for each of (calcium, magnesium, phosphate, ammonia, zinc, copper, total alkalinity, nitrite, nitrate), and no significant differences were found for each (salinity, electrical conductivity, pH, total copper) after treating by bacteria.

## References

[1]. Iredell, S., 2020, Water Pollution and Cancer: An Updated Review, Science Insights, 43(4), 1079-1086.

[2]. Chai S, Tan G, Munawaroh H, 2021, Multifaceted roles of microalgae in the application of wastewater biotreatment: a review, Environmental Pollution, 269, 116236.

[3]. Zhou Q, Yang N, Li Y, 2020, Total concentrations and sources of heavy metal pollution in global river and lake water bodies from1972 to 2017, Global Ecology and Conservation, 22, e00925.

[4]. Zahra M, Maryam N., 2021, Phytoremediation of wastewater using aquatic plants, A review, Journal of Applied Research in Water and Wastewater, 8, 50-58.

[5]. Mohiyaden H, Sidek M, Hayder A, 2016, Conventional methods and emerging technologies for urban river water purification plant: a short review ,Journal of Engineering and Applied Sciences, 11, 2547–2556.

[6]. Cichy B, Kużdżał E, Krztoń H. Phosphorus recovery from acidic wastewater by hydroxyapatite precipitation, 2019, Journal of Environmental Management, 232, 421-427.

[7]. Kumar N, Bauddh K, Kumar S, Extractability and phytotoxicity of heavy metals present in petro chemical industry sludge,2013, Clean Technologies and Environmental Policy Journal. 15, 1033–1039.

[8]. Anand, S., Bharti, S., Dviwedi, N., Macrophytes for the reclamation of degraded waterbodies with potential for bioenergy production, 2017, SpringerSingapore, 333–351.

[9]. Kaur T, Devi R, Kour D., 2021, Plant growth promoting soil microbiomes and their potential implications for agricultural and environmental sustainability, Biologia, 76, 2687-2709.

[10]. Mulamattathil G, Bezuidenhout C and Ateba N., 2014, Isolation of Environmental Bacteria from Surface and Drinking Water in Mafikeng,South Africa,and Characterization Using Their Antibiotic Resistance Profiles, Journal of Pathogens,11, 371208.

[11]. Phong T, Duyen T and Diep N. Isolation and characterization of lipid-degrading bacteria in wastewater of food processing plants and restaurants in Can Tho City, Vietnam,2014, American Journal of Life Sciences, 2(6), 382.

[12]. Fresenius W and Quentin E. Water analysis: A practical guide to physico-chemical, chemical, and microbiological water examination and quality assurance.1988. (No. 628.161 W3).

[13]. Estefan G, Sommer R and Ryan J. Methods of soil, plant, and water analysis, 2013, A manual for the West Asia and North Africa region. Monitoring, Evaluation & Learning Repository, 3, 65-119.

[14]. Mackereth H, Heron J and Talliny F., 1978, Water analysis: Some revised method for limnologists, Sci. publ. fresh water Biological. Association, London , Journal of Water Resource and Protection, 36,1-120.

[15]. APHA (American public Health Association). (2017) ‘standard methods for the

examination of water and wastewater. 23rd', Washington DC, USA.

[16]. APHA, American Public Health Association (2003). Standard methods for the examination of water and wastewater. 20th ed. Washington DC, USA.

[17]. Parson, T.R.; Maite, y. and laui, C.M. Amannual of chemical and biological methods for sea water analysis pergamon press oxford,1984, 173

[18]. Lind T. Handbook of common methods in Limnology, 1979, 2nd ed.199

[19]. Pradeep K , Albert L., 2013, Pressure and Temperature Dependence of Growth and Morphology of Escherichia coli: Experiments and Stochastic Model, Biophysical Juornal, 105(3), 783–793.

[20]. Whiffin S, Van P, Harkes P., Microbial carbonate precipitation as a soil improvement technique,2007,Geomicrobiology Journal, 24(5), 417-423.

[21]. Fujita Y, Taylor L, Gresham L, 2008, Stimulation of microbial urea hydrolysis in groundwater to enhance calcite precipitation, Environmental science & technology, 42(8), 3025-3032.

[22]. De Belie, N., & De Muynck, W. Crack repair in concrete using biodeposition,2008, In Concrete repair, rehabilitation and retrofitting II (pp. 309-310). CRC Press10, 107

[23]. Pereira L, Silva N, Gomes C, 2006, Diarrhea-associated biofilm formed by enteroaggregative Escherichia coli and aggregative Citrobacter freundii, a consortium mediated by putative F pili.. Molecular Microbiology , 60(5), 1136–1151

[24]. Vickers, N. J. Animal communication: when i'm calling you, will you answer too? ,2017, Current biology, 27(14), R713-R715.

[25]. Fein B. 2006, Thermodynamic modeling of metal adsorption onto bacterial cell walls: current challenges, Advances in agronomy, 90, 179-202.

[26]. Stephen D, Meredith K, Leigh-Anne H., 2021, Freshwater salinization increases survival of Escherichia coli and risk of bacterial impairment, 191, 116812.

[27]. Sonune A and Garode M., 2015, Bioremediation potential of bacterial isolates for municipal wastewater treatment, Current world environment, 10(2), 619-625.

[28]. Stocks F, Galinat K, 1999, Microbiological precipitation of $CaCO_3$, Soil Biology and Biochemistry, 31(11), 1563-1571.

[29]. Ramachandran K, Ramakrishnan Vand Bang S. 2001, Remediation of concrete using micro-organisms. ACI Materials, Journal-American Concrete Institute, 98(1), 3-9.

[30]. Norris V, Grant S, Freestone P., 1996, Calcium signalling in bacteria, Journal of Molecular biology, 178, 3677–3682.

[31]. Onoda T, Enokizono J, Kaya H., 2000, Effects of calcium and calcium chelators on growth and morphology of Escherichia coli L-form NC-7, Journal of Bacteriology, 182(5), 1419-22.

[32]. Joan E, Lusk R , Eugene P., 1968, Magnesium and the Growth of Escherichia coZi,

The Journal of Biological chemistry, 243, 2618-2624.

[33]. Roberto D, Claudio S, Raffaella P and Massimo V., 2001, Exopolysaccharide-producing cyanobacteria and their possible exploitation: A review. Journal of Applied Phycology, 13: 293–299.

[34]. Kremer B, Kazmierczak J and Stal J. 2008, Calcium carbonate precipitation in cyanobacterial mats from sandy tidal flats of the North Sea, Geobiology, 6(1), 46-56.

[35]. Dittrich M and Sibler S. Calcium carbonate precipitation by cyanobacterial polysaccharides. 2010. Geological Society , London , Special Publications, 336 ( 1 ), 51-63 .

[36]. Christophe D, Pamela R, Olivier B, et al, 2009, Processes of carbonate precipitation in modern microbial mats. Ear th-Science Reviews, 96, 141-162.

[37]. Krishnaswamy, U.; Muthuchamy, M.; Perumalsamy, L. 2011, Biological removal of phosphate from synthetic wastewater using bacterial consortium, Juornal of Biotechnology, 9(1), 37-49

[38]. Alberto J, Mikael R, Keira M., 2020, Nitrate Metabolism Modulates Biosynthesis of Biofilm Components in Uropathogenic Escherichia coli and Acts as a Fitness Factor During Experimental Urinary Tract Infection, Frontiers in Microbiology, 11, 26.

[39]. Dongke Y., 2012, Evaluation of effluent organic nitrogen and its impacts on receiving water bodies, Civil and Environmental Engineering, 22, 140.

[40]. Yosuke M, Hisanari Y, Yohei T, 2017, Ammonia production from amino acid-based biomass-like sources by engineered Escherichia coli, 7, 83.

[41]. Khosravi, A., Javdan, M., Yazdanpanah, G., Malakootian, M., 2020, Removal of heavy metals by Escherichia coli (E. coli) biofilm placed on zeolite from aqueous solutions (case study: the wastewater of Kerman Bahonar Copper Complex), Applied Water Science, 10, 167.

[42]. Medeiros D, Jennings D. Role of copper in mitochondrial biogenesis via interaction with ATP synthase and cytochrome c oxidase, 2002, Bioenerg Biomembr, 34 (5), 389–395.

[43]. Steiger D, Fetchko M, Vardanyan A, Atanesyan L, Steine K, Turski M., 2010,The Drosophila copper transporter Ctr1C functions in male fertility, Journal of Biological Chemistry, 285 (22), 17089–17097.

[44]. Festa R, Thiele D. Copper: an essential metal in biology, 2011, Journal of Biological Chemistry, 21(21), 877–R883.

[45]. Bayan S., 2021, Adopting the Water Quality Index to assess the validity of groundwater in Al-Zubair city southern Iraq for drinking and human consumption, Ecology Environment and Conservation, 27(1), 73-79.

[46]. Guomei Q, Pengpeng X, Siqi L., 2020, Uptake system ZnuACB is essential for maintaining pathogenic phenotype of F4ac+ enterotoxigenic E. coli (ETEC) under a zinc restricted environment, 51(1), 12.