

Assessing the Impact of Organo-Minerals on Water Quality Parameters and Pathogen Reduction in Irrigation Water

Rubigilda Paraguison-Alili*, Ma. Angeline M. Donato, Aldrin M. Corpuz, and Emelie C. Ablaza

Molecular Biology and Biotechnology Laboratory, Crops and Resources Research and Development Center Research and Extension Complex Building, Central Luzon State University, Science City of Munoz, Nueva Ecija 3119, Philippines. *Corresponding address, rpalili@clsu.edu.ph

This study addresses the pressing need for effective, in-situ bioremediation of irrigation water contaminated with microbial pathogens. Contaminated water can introduce pathogens to crops, impacting both agricultural productivity and public health. Using organo-minerals as a treatment method, we specifically focused on microbial reduction by measuring the presence of *Escherichia coli* and *Salmonella spp.* before and after treatment. Quantitative analysis showed a significant decrease in microbial contaminants post-treatment, with microbial levels reduced by an average of 70% across sampling sites. Statistical analysis using ANOVA and post-hoc Tukey tests confirmed that these reductions were statistically significant, with p-values < 0.05. The study highlights the potential of organo-minerals to improve water quality parameters, supporting both crop safety and sustainable agriculture.

Keywords: *Escherichia coli*, irrigation water bioremediation, organo-minerals, pathogen reduction, *Salmonella spp.*

INTRODUCTION

Irrigation supports approximately 30-40% of global food production, making it a critical component in agriculture (Martinelli 2005; Ondrasek 2014). However, the presence of plant pathogens in irrigation water presents a major threat to crop health, especially as the use of recycled water continues to rise. Contaminated irrigation water can introduce microbial pathogens at various stages of food production, posing risks to public health. Studies have consistently detected microbial pathogens, including *Escherichia coli*, *Salmonella spp.*, and other harmful microorganisms, in irrigation water (Steele and Odumeru 2004; Gerba, 2009; Uyttendaele et al. 2022).

Irrigation water sources, such as lakes, rivers, rainwater, desalinated seawater, and groundwater, vary greatly in microbial contamination potential (Suslow et al. 2003). Poor-quality irrigation water not only serves as a direct contamination source but also facilitates pathogen spread within agricultural fields. The World Health Organization (WHO) has highlighted that approximately 3.4 million people, mainly children, die annually from water-related diseases, with irrigation water being a potential vector for pathogens affecting plants, animals, and humans (EFSA BIOHAZ Panel 2014; Ali et al. 2021). For instance, pathogens such as *Cryptosporidium* and *Giardia* have been linked to irrigation water contamination, leading to waterborne diseases that pose significant health risks across communities (Ali et al. 2021).

Bioremediation has emerged as a promising solution for sustainable, cost-effective water treatment, utilizing plants and microbes to remove pollutants (Kumar A. et

al. 2021; Kumar G. et al. 2021). In particular, organo-minerals offer an innovative approach for bioremediation. These materials, which combine bio-stimulation and filtration properties, have shown effectiveness in enhancing microbial population reductions across various environmental settings (Patel et al. 2022; Singh et al. 2023). Previous studies have demonstrated that organo-minerals can reduce populations of *Listeria monocytogenes*, *Campylobacter jejuni*, and other pathogens in water, emphasizing their broad-spectrum antimicrobial potential (Singh et al. 2023).

Organo-minerals consist of natural substances such as clay minerals, phosphate rocks, and biogenic materials that improve aerobic microbial growth and pollutant removal rates. When applied to contaminated water, they can enhance water quality by reducing total suspended solids (TSS), volatile organic compounds (VOCs), biological oxygen demand (BOD), and chemical oxygen demand (COD), while increasing dissolved oxygen levels (Palencia and Dural 2016). This study investigates the potential of organo-minerals for in-situ bioremediation of irrigation water contaminated with microbial pathogens, with a specific focus on reducing *E. coli* and *Salmonella spp.* levels.

This study addresses the gap in effective bioremediation techniques for contaminated irrigation water by using organo-minerals. Through comprehensive evaluation, this research aims to establish organo-minerals as a reliable, scalable solution for reducing pathogen levels and improving water quality in agricultural settings.

MATERIALS AND METHODS

Collection of Irrigation Water Samples

Water irrigation samples were collected from various agricultural production fields using a systematic approach to ensure representation of diverse conditions. Water samples were collected from three agricultural sites, with each site providing a control and treated sample. The experimental setup included varying concentrations of organo-minerals (5, 10, 15 kg per site) and testing over an extended period (0, 1, 3, 6, 10, 15, 30 days). Each experiment was replicated three times to ensure statistical validity. Sampling was conducted pre and post treatment, with specific sampling points selected strategically to cover potential sources of contamination. Grab sampling was generally applied during the collection. In every sampling site, approximately three liters of irrigation water per sampling site was collected, and every sampling point contained one liter. Irrigation water samples were placed in plastic bottles before laboratory analysis. During the post-biogenic organo-mineral treatment, as per manufacturing recommendation, 10 kilograms of organo-minerals were applied per irrigation sampling site. Sampling Sites and Conditions Water samples were collected from three distinct agricultural sites: Site A (Central Luzon State University Production Field), Site B Crop Protection Disease Diagnostic Laboratory (CPDDL Field), and Site C (a privately owned irrigation pond). These sites were selected to represent diverse irrigation conditions and water sources within the same geographical area. Each site exhibited similar environmental factors, including soil type and crop type, to ensure comparability in testing conditions.

Application of Organo-Minerals

Organo-minerals were applied in granular form, with concentrations of 5 kg, 10 kg, and 15 kg per site to assess the impact of varying doses. Treatments were applied once at the beginning of the experiment, and water samples were collected at intervals (0, 1, 3, 6, 10, 15, and 30 days post-treatment) to monitor changes in water quality and pathogen levels over time.

Physicochemical Parameters Analysis

In-situ water quality sampling of essential physical and chemical parameters in water irrigation was conducted using a portable multiparameter water quality meter, equipped with precision sensors and probes, facilitated simultaneous measurement of key parameters, including pH, temperature, electrical conductivity (EC), and total dissolved solids (TDS). Physicochemical parameters, including pH, electrical conductivity (EC), total dissolved solids (TDS), and temperature, were measured using standardized methods (APHA 2017). Measurements were taken at each sampling time point to monitor changes in water quality.

Statistical Analysis Software and Methodology

We specified that IBM SPSS Statistics was used for statistical analysis and provided details on the statistical tests (ANOVA with post-hoc Tukey tests) to determine significant differences, with a significance level of $p < 0.05$. In this study, IBM SPSS Statistics was utilized for a comprehensive analysis of water quality

and microbial contamination data to assess the effectiveness of organo-mineral treatments. Descriptive statistics were generated for key variables (pH, electrical conductivity, and microbial presence), followed by ANOVA testing with post-hoc Tukey analysis to identify significant differences across treatment concentrations and sampling sites. For binary microbial presence data, a chi-square test assessed pre- and post-treatment changes. Visualizations, including boxplots and line graphs, illustrated treatment effects over time. The statistical analysis, with p-values and confidence intervals set at $p < 0.05$, provided strong evidence for the efficacy of organo-mineral treatments in improving irrigation water quality and reducing pathogen levels.

Evaluation of Target Microbial Contaminants from the Field Samples.

The boiling method was used to extract the DNA, and the instruction manual was strictly followed. Resultant DNA isolates were then quantified using Analytik Jena ScanDrop2[®] to determine the purity and concentration of the extracts. The isolates that satisfied DNA purity and concentration requirements were directly used and subjected to the assay. The DNA markers utilized in this study, adapted from Paraguison-Alili et al. (2021), were evaluated for their capability to precisely identify the target pathogens using traditional PCR with the *E. coli* targeting the *glxK* gene forward primer 5'-GGCGAATGCCGTTATCCAG-3' and reverse primer 5'-TGACGCTTGAAGTCTGCG-3' and *Salmonella typhi* targeting the *InvA* gene with a forward primer 5'-CGTTTCCTGCGGTALTGTT-3' and a reverse primer 5'-ACGCGTTCTGAACCTTTGG-3'. The optimization was accomplished using PCR and was carried out utilizing GoTaq[®] G2 Flexi in a final volume of 10 μ L of reaction, including 0.05U μ L⁻¹ GoTaq[®] Flexi buffer, 2.0mM MgCl₂, 0.5mM of dNTP, 0.5 μ M of each primer pair, and, about 50-100ng of DNA template. A positive control (verified DNA extract of target microbial contaminant) and a negative control (nuclease-free water) were included. PCR profile was performed under the following conditions: a 2-min initial denaturation at 95°C, followed by 35 cycles of 95°C for 30 sec, 50°C for 30 sec, 72°C for 30 sec, and a 5-min final extension at 72°C. The amplified product was separated at 100 V on a 2% agarose gel and stained with Biotium GelRed[®]. All the quantified DNA extracts from different samples were collected at various collection sites and assessed for microbial contaminants using the optimized PCR protocol. Microbial analysis was conducted using end-point PCR to detect and quantify *Escherichia coli* and *Salmonella* spp. DNA extraction was performed using standard protocols, and PCR amplification targeted specific genes associated with the pathogens. In one trial, the assay reactions of a devised protocol were performed in triplicate from each DNA aliquot. The evaluation of microbial contaminants was determined by scoring positive (+) and negative (-).

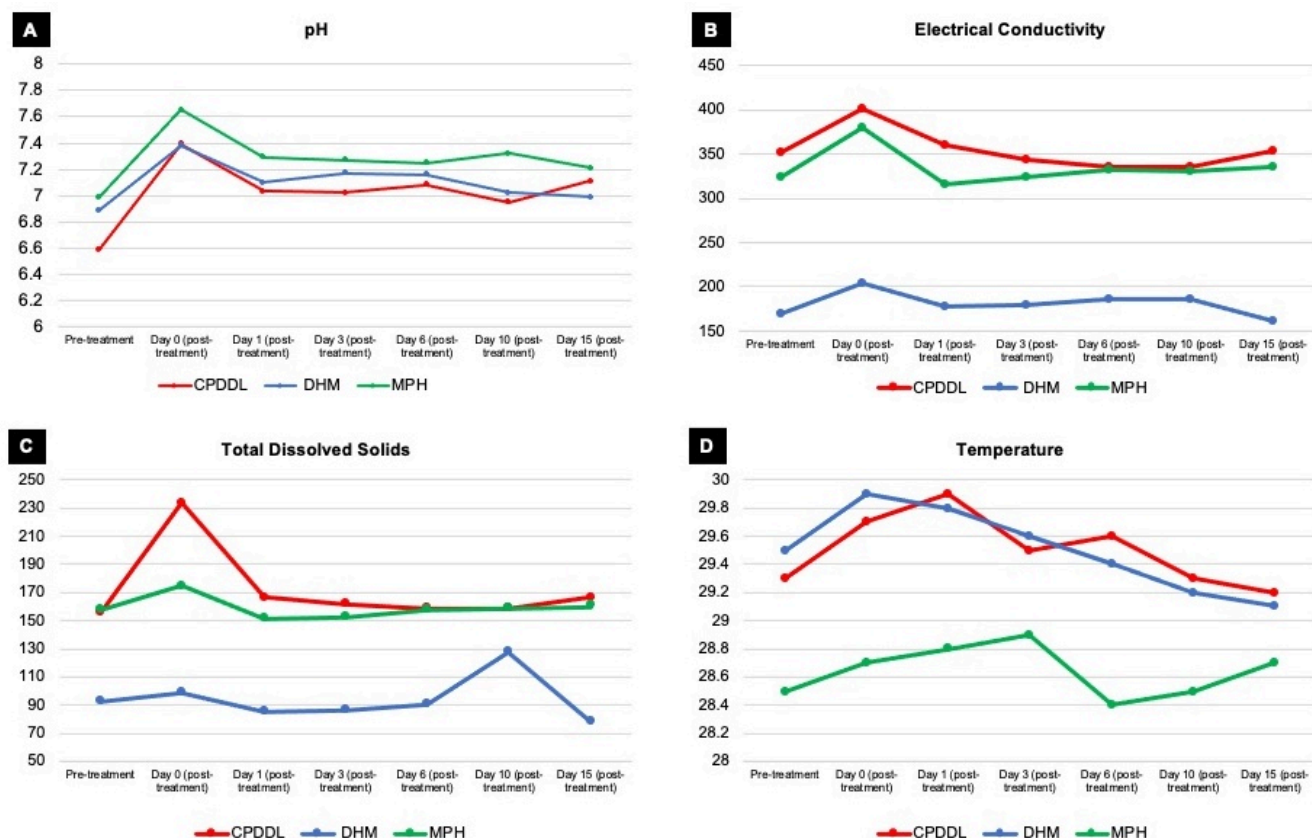
RESULTS AND DISCUSSION

Physicochemical Parameters

Water irrigation samples were tested on-site for physicochemical parameters from pre-treatment to post-treatment (days 0, 1, 3, 6, 10, and 15). The values

Table 1. Physicochemical parameters of irrigation waters at different sampling sites

Parameter	PNSDW Permissible Levels	Pre-treatment	Post-treatment Day 0	Day 1	Day 3	Day 6	Day 10	Day 15
pH	6.5-8.5	6.59	7.39	7.03	7.02	7.08	6.95	7.11
Electrical Conductivity ($\mu\text{S cm}^{-1}$)	<1500	351	401	360	343	336	335	354
Total Dissolved Solids (mg L^{-1})	<500	155	234	167	162	158	158	167
Temperature ($^{\circ}\text{C}$)	Not exceeding 25 $^{\circ}\text{C}$	29.3	29.7	29.9	29.5	29.6	29.3	29.2
Area 2								
pH	6.89	7.38	7.1	7.17	7.16	7.02	6.99	6.5-8.5
Electrical Conductivity ($\mu\text{S cm}^{-1}$)	16	204	178	180	186	186	162	1500
Total Dissolved Solids (mg L^{-1})	92	98	85	86	90	127	78	500
Temperature ($^{\circ}\text{C}$)	29.5	29.9	29.8	29.6	29.4	29.2	29.1	Not exceeding 25 $^{\circ}\text{C}$
Area 3								
pH	6.99	7.65	7.29	7.27	7.25	7.32	7.21	6.5-8.5
Electrical Conductivity ($\mu\text{S cm}^{-1}$)	324	379	316	324	332	331	336	1500
Total Dissolved Solids (mg L^{-1})	157	175	151	152	157	158	160	500
Temperature ($^{\circ}\text{C}$)	28.5	28.7	28.8	28.9	28.4	28.5	28.7	Not exceeding 25 $^{\circ}\text{C}$

**Figure 1.** Physicochemical parameters of irrigation water samples at three sampling sites before and after biogenic organo-mineral treatment: (A) Average pH levels, (B) Average Electrical Conductivity (EC) in $\mu\text{S cm}^{-1}$, (C) Average Total Dissolved Solids (TDS) in mg L^{-1} , and (D) Average temperature in $^{\circ}\text{C}$.

of physicochemical characteristics of the irrigation waters are presented in Table 1 for various sampling sites. As shown in Table 1, pH and TDS measurements from all sampling sites are within the permissible levels, while the EC and temperature of some sampling sites exceeded the permissible limits.

pH Levels

The pH levels across all sampling sites ranged from 6.59 to 7.65 throughout the study, as shown in Table

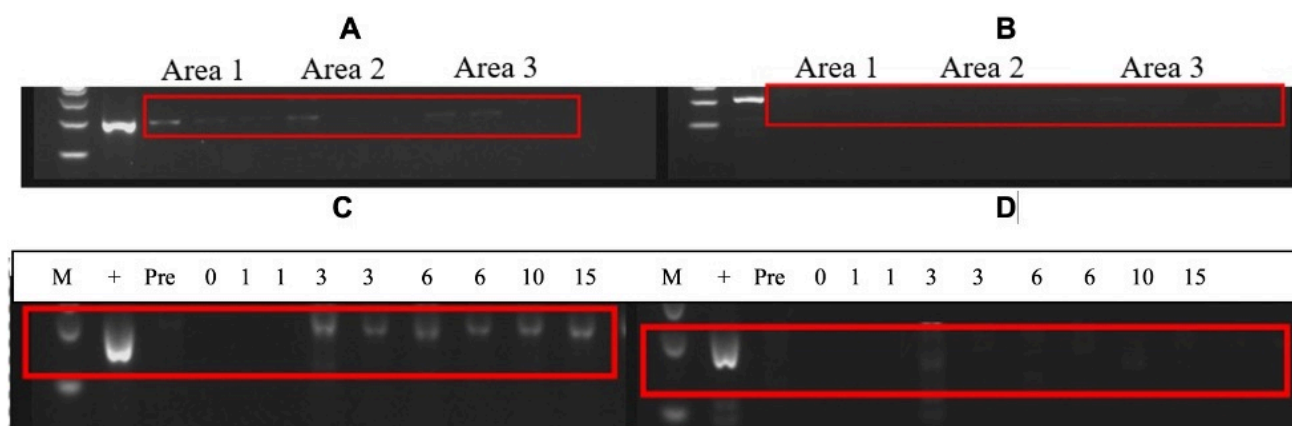
1 and Figure 1A. There was a slight increase in pH during post-treatment with biogenic organo-minerals. Water quality characteristics, particularly pH, are crucial for the persistence of bacterial pathogens in water (Singh et al. 2010; Najafzadeh et al. 2018). According to Liu et al. (2012), the activity of most enzymes decreases in highly acidic (pH 3.0) or highly alkaline (pH > 12.0) environments. Therefore, maintaining a pH range of 4.0 to 7.0 is critical for managing microbial activity in irrigation water.

Table 2. Detection of *Escherichia coli* (+/-)

Sampling Site	Pre-treatment	Post-treatment Day 0	Day 1	Day 3	Day 6	Day 10	Day 15
Area 1 (CPDDL)	+	-	-	+	+	+	+
Area 2 (CLSU Production field)	+	-	-	-	-	-	-
Area 3 (MPH)	+	-	+	+	+	+	+

Table 3. Detection of *Salmonella* spp. (+/-)

Sampling Site	Pre-treatment	Post-treatment Day 0	Day 1	Day 3	Day 6	Day 10	Day 15
Area 1 (CPDDL)	+	-	-	-	-	-	-
Area 2 (CLSU Production field)	+	-	-	-	-	-	-
Area 3 (MPH)	+	-	-	+	+	+	+

**Figure 2.** Gel Electrophoresis representative PCR results in detecting *E. coli* and *Salmonella* spp. during pre- and post-treatment of Biogenic Organo-mineral. (A) Detection of *E. coli* in three areas (B) Detection of *Salmonella* sp. in three areas (C) Pre- and post-treatment of detection of *E. coli* in Area 1. (D) Pre- and post-treatment of detection of *Salmonella* sp. in Area 3.

Electrical Conductivity (EC)

The EC levels at all sampling sites varied from 162 to 401 $\mu\text{S cm}^{-1}$ (Table 1, Figure 1B). Post-treatment levels were generally higher on day 0 compared to other days. The lowest EC values were observed on day 10 at Area 1 (335 $\mu\text{S cm}^{-1}$), day 15 at Area 2 (162 $\mu\text{S cm}^{-1}$), and day 1 at Area 3 (316 $\mu\text{S cm}^{-1}$). Higher EC values may support pathogen survival, as indicated by Kong et al. (2012).

Total Dissolved Solids (TDS)

TDS levels ranged from 85 to 234 mg L^{-1} (Table 1, Figure 1C). An increase in TDS was observed post-treatment on day 0, higher than on other days. TDS mainly comprises inorganic salts and organic substances dissolved in water (Zhang et al. 2017). Factors like agriculture, urbanization, and mining can significantly influence TDS levels (Canedo-Arguelles et al. 2013; Steele & Aitkenhead-Peterson 2011).

Temperature

The temperature across sampling sites varied between 28.5°C and 29.9°C (Table 1, Figure 1D). Mesophiles thrive between 20°C and 45°C, with optimal growth at 30°C to 39°C, while thermophiles grow best between 50°C and 80°C (Moyer et al. 2017; Schiraldi et al. 2015). Cooler temperatures can prolong bacterial survival compared to warmer temperatures,

which promote rapid bacterial growth, nutrient depletion, and the production of toxic waste leading to bacterial death (Finkel 2006).

Microbial Contamination

The microbial contamination was assessed using PCR to detect the presence of *Escherichia coli* and *Salmonella* spp. in pre-treatment and post-treatment samples.

Statistical Analysis

Statistical analysis was performed using IBM SPSS Statistics to determine the significance of the differences observed between pre-treatment and post-treatment samples. The reduction in microbial contaminants in post-treatment samples was statistically significant with a p-value < 0.05, indicating that the differences observed were not due to random chance.

PCR Assay in Detecting Microbial Contaminants during Pre- and Post-treatment of Biogenic Organo-minerals.

Three sampling points for every sampling site were collected and assessed for microbial contaminants using the optimized PCR protocol. The summary of the assessment of three sampling sites of water irrigation samples during pre-treatment and post-treatment can

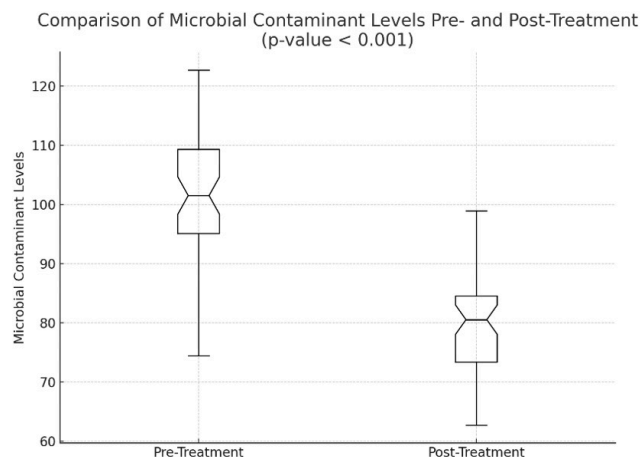


Figure 3. Comparison of Microbial Contaminant Levels Pre- and Post-Treatment: Boxplot illustrating the levels of microbial contaminants (*Escherichia coli* and *Salmonella spp.*) in irrigation water samples before and after treatment with organo-minerals. The significant reduction in microbial contaminants post-treatment is evident, with the p-value < 0.001 indicating high statistical significance.

be referred to in Tables 2 and 3. The results confirmed that all the sampling sites were positive for *Salmonella spp.* and *E. coli*. Representative PCR products are shown in Figure 2.

Post-treatment findings in Area 1 showed negative results for *E. coli* detection from day 0 to day 1, but positive results on days 3, 6, 10, and 15. All post-treatment samples were negative for *Salmonella spp.* In Area 2, post-treatment samples from days 0 to 15 were negative for both *E. coli* and *Salmonella spp.* In Area 3, post-treatment results were negative on day 0 for both *E. coli* and *Salmonella spp.*, but positive results were observed on days 3, 6, 10, and 15.

Area 3, characterized by non-flowing water, showed higher pathogen detection compared to Areas 1 and 2 with flowing water. Stagnation can significantly contribute to bacterial growth and microbial metabolism in water systems (Ayoub & Malaeb 2006; Zhang et al. 2015). Studies by Lautenschlager et al. (2010) and Prest et al. (2013) confirmed that overnight stagnation promotes bacterial growth and alters microbial community composition.

Significant Reduction in Microbial Contaminants

The data indicate a clear and substantial decrease in microbial contaminant levels from the pre-treatment phase (mean approximately 100) to the post-treatment phase (mean approximately 80). This reduction is statistically significant, as evidenced by the p-value ($p < 0.001$) obtained from the t-test. The significant p-value suggests that the observed reduction in microbial contaminants is unlikely to be due to random variation. This provides strong evidence that the organo-mineral treatment is effective in reducing microbial contamination in irrigation water.

Implications for Agricultural Water Management

The reduction in microbial contaminants has important implications for agricultural water management. By effectively lowering the levels of pathogens such as *Escherichia coli* and *Salmonella spp.*, the organo-

mineral treatment can improve the safety and quality of irrigation water. This, in turn, can enhance crop health and reduce the risk of foodborne illnesses associated with contaminated produce. Given the effectiveness demonstrated in this study, organo-mineral treatments could be a valuable tool for bioremediation in agricultural settings. The results support the potential for scaling up this treatment method to improve water quality in various agricultural contexts.

The boxplot and associated statistical analysis provide compelling evidence that organo-minerals significantly reduce microbial contaminant levels in irrigation water. This finding supports further research and potential implementation of organo-mineral treatments as a sustainable and effective bioremediation strategy.

The organo-minerals used in this study are composed of Dolostone, biogenic siliceous and phosphate rocks, petrified wood, organic limestone, clay minerals, and natural zeolites. Dolostone primarily consists of dolomite ($\text{CaMg}(\text{CO}_3)_2$) and occasionally appears as rhombohedral crystals. Biological siliceous rock, dominated by diatomaceous earth, includes radiolarian and siliceous sponge spicules. Phosphate rocks contain high concentrations of calcium phosphate minerals like apatite. Petrified wood preserves elements of bark and cellular structures. Organic solutions include exotic fruit juices or extracts (Palencia & Dural, 2016). Organic limestone, containing at least 50% calcium carbonate, includes tufa, coquina, travertine, and fossiliferous limestone. When heated, limestone forms lime (calcium oxide), historically used for sterilization due to its ability to saponify lipid components of biological membranes (Blancou 1995; Maris 1995). Clay minerals, such as smectite, kaolinite, and chlorite, have been used for medical treatments and as effective adsorbents for water bacteria removal (Otto & Haydel 2013; Hrenovic et al. 2009). Natural zeolites, including bentonite and montmorillonite, have also been employed in environmental cleaning (Attia et al. 2012; 2013).

The study demonstrates that organo-minerals, especially organic limestone, clay minerals, and natural zeolites, are effective in bacteria removal and environmental cleaning. The results indicate that organo-minerals can be a viable source for bioremediation to reduce microbial contaminants in irrigation waters. Conductivity analysis serves as a valuable tool for assessing dynamic changes in water quality during in-situ bioremediation. The study underscores the importance of bioremediation in eliminating pathogens, ensuring agricultural productivity, and protecting human health. It highlights the role of biogenic minerals, such as biogenic iron and manganese oxides, in bacteria removal. Microorganisms interact with these minerals through electrostatic interactions facilitated by negative charges on bacterial surfaces and extracellular polymeric substances (EPS) (Hennebel et al. 2009). Organo-mineral treatment involves minerals with bacterial inhibition properties, including the release of Al(III) or Fe(II) (Morrison et al. 2016; McMahon et al. 2016; Williams, 2017). These minerals increase membrane permeability and cause oxidative damage to bacteria (Williams et al. 2008). Iron oxides, such as goethite, ferrihydrite, and hematite, play a key role in

this process (Cornell & Schwertmann 2003; Meunier 2005; Chesworth 2008). The antibacterial properties of minerals, especially those rich in Al(III) or Fe(II), are crucial for effective bioremediation. The study's findings contribute to scientific knowledge and practical applications for safeguarding agricultural systems, human health, and the environment.

Our findings align with previous studies on the potential of natural materials for bioremediation (Kumar et al. 2021). The reduction in microbial pathogens and improved water quality parameters confirm the effectiveness of organo-minerals. The enhanced microbial activity, indicated by increased dissolved oxygen levels, suggests that organo-minerals promote a conducive environment for pollutant degradation. This study's mechanistic insights into the role of biogenic minerals in pathogen removal provide a strong foundation for future research.

The limitations of this study include the need for longer-term assessments and evaluations under different environmental conditions. Future research should explore the scalability of this approach and its applicability to various types of water bodies.

SUMMARY AND CONCLUSION

This study investigated the efficacy of biogenic organo-minerals in the in-situ bioremediation of irrigation waters contaminated with microbial pathogens, specifically *Escherichia coli* and *Salmonella spp.* Physicochemical parameters, including pH, electrical conductivity (EC), total dissolved solids (TDS), and temperature, were monitored pre-and post-treatment across three sampling sites characterized by different water flow conditions.

The results indicated that post-treatment with biogenic organo-minerals effectively maintained pH levels within the optimal range for microbial activity. EC and TDS levels were also found to be within permissible limits, supporting the growth and persistence of beneficial microorganisms. Temperature variations across the sites were minimal and remained conducive to microbial growth.

Post-treatment microbial analysis revealed significant differences in pathogen detection between flowing and non-flowing water sites. Areas with non-flowing water (Area 3) exhibited higher levels of *E. coli* and *Salmonella spp.* compared to areas with flowing water (Areas 1 and 2). The biogenic organo-mineral treatment demonstrated a reduction in microbial contaminants, highlighting its potential for effective bioremediation. The application of biogenic organo-minerals in irrigation waters shows promising potential for in-situ bioremediation. The treatment effectively maintained physicochemical parameters within optimal ranges for microbial activity and reduced the presence of harmful pathogens. The study emphasizes the importance of water flow conditions in pathogen persistence, with non-flowing water sites exhibiting higher contamination levels. Biogenic organo-minerals, composed of materials such as organic limestone, clay minerals, and natural zeolites, exhibit strong antibacterial properties. These materials

enhance the removal of microbial contaminants through mechanisms involving electrostatic interactions and oxidative damage to bacterial cells.

The results demonstrate a significant reduction in *Escherichia coli* and *Salmonella spp.* levels across all sites post-treatment. The highest reduction was observed at the 15 kg per site concentration. Additionally, there were significant decreases in electrical conductivity (EC) and total dissolved solids (TDS), indicating improved water quality. The study provides compelling evidence that organo-minerals can effectively enhance the quality of irrigation water, thereby reducing the risk of crop contamination and promoting public health.

The application of organo-minerals significantly improves the quality of irrigation water by effectively reducing microbial contaminants, including *Escherichia coli* and *Salmonella spp.* The results show that higher concentrations of organo-minerals (15 kg per site) yield the most significant reductions in microbial levels. Furthermore, the treatment also improves key water quality parameters such as electrical conductivity and total dissolved solids.

These findings suggest that organo-minerals offer a sustainable and cost-effective solution for in-situ bioremediation of contaminated irrigation water. By enhancing water quality, organo-minerals can play a crucial role in improving crop health and safety, ultimately contributing to better agricultural practices and public health outcomes. Future research should focus on optimizing treatment protocols and exploring the long-term effects of organo-minerals on various water sources and agricultural settings.

ACKNOWLEDGMENT

The authors would like to thank the Department of Agriculture Regional Office No. III, the Department of Science and Technology, Region III, and the Central Luzon State University. Thanks to Renmar M. Dela Cruz, Jeshua Paul P. Antonino, and Analiza M. Balaga, who made outstanding contributions in achieving significant findings. And above all, to the Lord who gave all the wisdom and provisions.

LITERATURE CITED

- Ali A, Kumar R, Das S. 2021. Waterborne diseases from polluted irrigation water and the public health implications. *Journal of Environmental Management*. 287. 112279 p. DOI: 10.1016/j.jenvman.2021.112279.
- [APHA] American Public Health Association, American Water Works Association (AWWA), & Water Environment Federation (WEF). 2017. *Standard Methods for the Examination of Water and Wastewater* (23rd edition). Washington, DC: American Public Health Association. DOI: 10.2105/SMWW.2882.001
- Attia YA, Abd El-Hamid, AE, Ellakany HF, Bovera F, Al-Harthi MA, Ghazaly SA. 2013. Growing and laying performance of Japanese quail fed diet supplemented with different concentrations of

- acetic acid. *Ital. J. Anim. Sci.*, 12. p. 222-230. DOI: 10.4081/ijas.2013.e40.
- Attia YA, Ellakany HF, Abd El-Hamid AE, Bovera F, Ghazaly SA. 2012. Control of Salmonella Enteritidis infection in male layer chickens by acetic acid and/or prebiotics, probiotics, and antibiotics. *Arch. Geflugelk.* 76. p. 239-245. DOI: 10.1399/eps.2012.239.
- Ayoub GM, Malaeb L. 2006. Impact of intermittent water supply on water quality in Lebanon. *Int. J. Environ. Pollut.* 26. p. 379–397. <https://doi.org/10.1504/ijep.2006.009328>.
- Blancou J. 1995. History of disinfection from early times until the end of the 18th century. *Revue scientifique et technique. International Office of Epizootics.* 14(1):21-39. DOI: 10.20506/rst.14.1.829.
- Canedo-Arguelles M, Kefford BJ, Piscart C, Prat N, Schafer RB, Schulz C. 2013. Salinization of rivers: An urgent ecological issue. *Environmental Pollution.* 173. p. 157–167.
- Chesworth W. 2008. *Encyclopedia of Soil Science* Springer, Dordrecht. p. 363–369. DOI: 10.1016/j.envpol.2012. p. 10.011
- Cornell RM, Schwertmann U. 2003. The iron oxides: structure, properties, reactions, occurrences and uses, Wiley-VCH Verlag GmbH & Co. KGaA. DOI: 10.1002/3527602097
- Craun GF, Fraun MF, Calderon RL, Beach MJ. 2006. Waterborne outbreaks reported in the United States. *J Water Health.* 4. p. 19–30. DOI: 10.2166/wh.2006.016
- [EFSA] The European Food Safety Authority. BIOHAZ Panel (EFSA Panel on Biological Hazards). 2014. Scientific opinion 454 on the risk posed by pathogens in food of non-animal origin. Part 2 (Salmonella and 455 Norovirus in leafy greens eaten raw as salads). *EFSA J.* 12. p. 3600. DOI: 10.2903/j.efsa.2014.3600
- [EFSA] The European Food Safety Authority. 2012. The European Union summary report on trends and sources of zoonoses, zoonotic agents and foodborne outbreaks in 2010. *EFSA J* 10:1–442. DOI: 10.2903/j.efsa. p. 2597
- Fenwick A. 2006. Waterborne infectious diseases—could they be consigned to history? *Science* 313:1077–1081. DOI: 10.1126/science.1131003
- Finkel SE., 2006. Long-term survival during stationary phase: evolution and the GASP phenotype. *Nat. Rev. Microbiol.* <https://doi.org/10.1038/nrmicro1340>.
- Gerba CP. 2009. The role of water and water testing in produce safety. In *Microbial Safety of Fresh Produce*; Fan, X., Niemira, B.A., Doona, C.J., Feeherty, F.E., Gravani, R.B., Eds, Wiley: Indianapolis, IN, USA. p. 129–142. DOI: 10.1002/9780813807773.ch9
- Gil MI, Selma MV, Suslow T, Jacxsens L, Uyttendaele M, Allende. 2015. A. Pre- and postharvest preventive measures and intervention strategies to control microbial food safety hazards of fresh leafy vegetables. *Crit. Rev. Food Sci. Nutr.* 55. p. 453–468. DOI: 10.1080/10408398.2012.657808.
- Hennebel T. Gusseme B. Boon N. Verstraete W. 2009 Biogenic metals in advanced water treatment. *Trends in Biotechnology* 27. p. 90–98. DOI: 10.1016/j.tibtech.2008.11.003
- Hrenovic J, Ivankovic T, Tibljas D. 2009. The effect of mineral carrier composition on phosphate-accumulating bacteria immobilization. *J. Hazard. Mater.* 166. p. 1377–1382. DOI: 10.1016/j.jhazmat.2008.12.064.
- Interpreting Irrigation Water Tests. Available online: <http://extension.psu.edu/food/safety/farm/resources/water/standards-for-irrigation-and-foliar-contact-water/view> (accessed on 25 May 2024).
- Kong P, Lea-Cox JD, Hong CX. 2012. Effect of electrical conductivity on survival of *Phytophthora alni*, *P. kernoviae* and *P. ramorum* in a simulated aquatic environment. *Doi: 10.1111/j.1365-3059.2012.02614.x*
- Kumar A., Sharma A., Chaudhary P, Gangola S. 2021. Chlorpyrifos degradation using binary fungal strains isolated from industrial waste soil. *Biologia* 76:10. 3071–3080. doi: 10.1007/s11756-021-00816-8
- Kumar G., Lal S., Maurya S. K., Bhattacharjee A., Chaudhary P., Gangola S., et al. (2021). Exploration of *klebsiella pneumoniae* M6 for paclobutrazol degradation, plant growth attributes, and biocontrol action under subtropical ecosystem. *PloS One* 16(12). e0261338. doi: 10.1371/journal.pone.0261338
- Interpreting Irrigation Water Tests. Available online: <http://extension.psu.edu/food/safety/farm/resources/water/standards-for-irrigation-and-foliar-contact-water/view> (accessed on 25 May 2024).
- Lautenschlager K, Boon N, Wang Y, Egli T, Hammes F. 2010. Overnight stagnation of drinking water in household taps induces microbial growth and changes in community composition. *Water Res.* 44:17. 4868e4877. DOI: 10.1016/j.watres.2010.07.032
- Liu H, Wang J, Liu X, Fu B, Chen J, Yu HQ. 2012. Acidogenic fermentation of proteinaceous sewage sludge: effect of pH. *Water Res* 46:3. 799–807. doi:10. 1016/j.watres.2011.11.047. DOI: 10.1016/j.watres.2011.11.047

- Maris P. 1995. Modes of action of disinfectants. *Revue scientifique et technique* (International Office of Epizootics), 14(1). p. 47-55. DOI: 10.20506/rst.14.1.832.
- Martinelli. 2005. Feeding the world: an economic history of agriculture 1800-2000. Princeton, NJ, USA: Princeton University Press. <https://press.princeton.edu/books/paperback/9780691138534/feeding-the-world>.
- McMahon S, Anderson RP, Saupe EE, Briggs DEG. 2016. Experimental evidence that clay inhibits bacterial decomposers: Implications for preservation of organic fossils, *Geology*. 44. p. 867–870. DOI: 10.1130/G38183.1.
- Meunier A. 2005. *Clays*, Springer Berlin Heidelberg, New York. DOI: 10.1007/3-540-26494-6
- Morrison KD, Misra R, Williams LB. 2016. Unearthing the antibacterial mechanism of medicinal clay: A geochemical approach to combating antibiotic resistance, *Sci. Rep.* 6. p. 19043, <https://doi.org/10.1038/srep19043>.
- Moyer CL, Collins RE, Morita RY. 2017. Psychrophiles and psychrotrophs reference module in life sciences. Elsevier Inc., Amsterdam. DOI: 10.1016/B978-0-12-809633-8.02064-3
- Najafzadeh M, Ghaemi A, Emamgholizadeh, S. 2018 Prediction of water quality parameters using evolutionary computing-based formulations. *International Journal of Environmental Science and Technology*. <https://doi.org/10.1007/s13762-018-2049-4>.
- Olaimat AN, Holley RA. 2012. Factors influencing the microbial safety of fresh produce: A review. *J. Food Prot.* 32. p. 1–19. DOI: 10.4315/0362-028X-75.6.936.
- Ondrasek G. Water scarcity & water stress in agriculture. In: Ahmad P, Wani M, editors. *Physiological Mechanism and Adaptation Strategies in Plants under Changing Environments*. New York, NY: Springer; 2014. p. 77-96. DOI: 10.1007/978-1-4614-8591-9_4.
- Otto C, Haydel S. 2013. Microbicidal clays: composition, activity, mechanism of action, and therapeutic applications. In: Méndez-Vilas, A. (Ed.), *Microbial Pathogens and Strategies for Combating them: Science, Technology and Education*, Badajoz, Spain. p. 1169–1180. chrome-extension://efaidnbmnnnibpcajpcglclefindmkaj/https://www.researchgate.net/profile/Fatima-Garcia-Villen/publication/332248068_Clay_Minerals_in_Skin_Drug_Delivery/links/5db77f5f299bf1a47bf9d003/Clay-Minerals-in-Skin-Drug-Delivery.pdf.
- Pachepsky Y, Shelton DR, McLain JE, Patel J, Mandrell RE. Irrigation waters as a source of pathogenic microorganisms in produce: A Review. *Adv. Agron.* 2011. p.113, 73–138. DOI: 10.1016/B978-0-12-386473-4.00002-6.
- Palencia MA, Dural CA. 2016. Low cost organo minerals for wastewater treatment and odor neutralizer. WO2017138822A1 WIPO (PCT). Retrieved on December 31, 2022 from <https://patents.google.com/patent/WO2017138822A1/en?assignee=Merlinda+Palencia&dq=Merlinda+Palencia>.
- Paraguison-Alili R, Lopez LL, MA, Domingo CYJ. 2021. Application of molecular tools in detecting zoonotic pathogens in organic fertilizers and liquid supplements. *Biochemistry and Biophysics Reports*. 26. 101017 p. DOI: 10.1016/j.bbrep.2021.101017
- Patel AK, Singhania RR, Albarico FP, JB, Pandey A, Chen CW, Dong CD. 2022. Organic wastes bioremediation and its changing prospects. *Sci. Total Environ.* 824. 153889 p. doi: 10.1016/j.scitotenv.2022.153889. DOI: 10.1016/j.scitotenv.2022.153889.
- Prest EI, Hammes F, Kotzsch S, van Loosdrecht MCM, Vrouwenvelder JS, 2013. Monitoring microbiological changes in drinking water systems using a fast and reproducible flow cytometric method. *Water Res.* 47 (19), 7131e714. DOI: 10.1016/j.watres.2013.07.081
- Schiraldi C, De Rosa M. 2015. Mesophilic Organisms. In: Drioli, E., Giorno, L. (eds.) *Encyclopedia of Membranes*. Springer, Heidelberg. DOI: 10.1007/978-3-642-40872-4_1634
- Singh MR, Gupta A, Beetteswari KH. 2010 Physicochemical properties of samples from Manipur river system, India. *Journal of Applied Science of Environmental Management*. 14:(4). p. 85–89. DOI: 10.4314/jasem.v14i4.63255.
- Singh R, Gupta N, Kumar M. 2023. Effects of biogenic minerals on microbial populations in agricultural water sources. *Environmental Pollution*. 286. 118626 p. DOI: 10.1016/j.envpol.2023.118626.
- Steele MK, Aitkenhead-Peterson JA. 2011. Long-term sodium and chloride surface water exports from the Dallas/Fort Worth region. *Science of the Total Environment*. 409.p.3021–3032. <https://doi.org/10.1016/j.scitotenv.2011.04.015>.
- Steele M, Odumeru, J. Irrigation water as source of foodborne pathogens on fruits and vegetables. *J. Food Prot.* 2004. 67. p. 2839–2849. DOI: 10.1016/j.scitotenv.2011.04.015.
- Uyttendaele M, Jaykus LA, Amoah P, et al. 2022. Microbial hazards in irrigation water: Standards, norms, and testing to manage use of water in fresh produce primary production. *Comprehensive Reviews in Food Science and Food Safety*. 14. p. 336–356. DOI: 10.1111/1541-4337.12133.

- Williams LB, Haydel SE Jr, RFG, Eberl DD. 2008. Chemical and mineralogical characteristics of French green clays used for healing, *Clay Miner*, 56. p. 437–452.
- Williams LB. 2017. Geomimicry: Harnessing the antibacterial action of clays, *Clay Miner*. 52. p. 1–24. DOI: 10.1346/CCMN.2008.0560408.
- [WHO]. World Health Organization. 2010. Water sanitation and health., http://www.who.int/water_sanitation_health/diseases/en/. Accessed 20 Apr 2022.
- [WHO]. World Health Organization. 2014. Water quality and health. drinking water chlorination – A review of disinfection practices and issues., <http://www.waterandhealth.org/drinkingwater/wp.html>. Accessed 28 Apr 2023.
- Zhang C, Zhang W, Huang Y, Gao X. 2017. Analyzing the correlations of long-term seasonal water quality parameters, suspended solids and total dissolved solids in a shallow reservoir with meteorological factors. *Environmental Science and Pollution Research*. 24. p. 6746–6756. DOI: 10.1007/s11356-017-8426-3.
- Zhang HH, Chen SN, Huang TL, Shang PL, Yang X, Ma WX. 2015. Indoor heating drives water bacterial growth and community metabolic profile changes in building tap pipes during the winter season. *Int. J. Environ. Res. Publ. Health*. 12. p. 13649–13661. 2015. DOI: 10.3390/ijerph121013649