

Antimicrobial Activity of Vetiver (*Vetiveria zizanioides*) Against *Escherichia coli* and its Impact on Zooplankton Communities in the Karuvannur River

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Abstract: Rapid population growth in developing regions has intensified housing density, often without adequate sewerage infrastructure. Consequently, untreated waste frequently enters surface water systems, leading to significant microbial contamination and posing a global threat to freshwater resources. This study evaluated the antimicrobial activity and water purification potential of *Vetiveria zizanioides* as a phytoremediation medium for wastewater treatment. Over a one-month period, water samples from the Karuvannur River were analyzed for *Escherichia coli* abundance and zooplankton density before and after vetiver treatment. Results revealed a substantial reduction in *E. coli* concentrations coupled with an increase in zooplankton populations, suggesting improved water quality and a potential inverse relationship between pathogenic bacteria and aquatic microfauna. The observed antimicrobial effects are attributed to the bioactive properties of vetiver, including antioxidant activity, free radical scavenging, and phytoremediation mechanisms such as phytostabilization, phytofiltration, and phytoextraction. Owing to its low cost, adaptability, and environmental compatibility, *V. zizanioides* demonstrates significant potential as a sustainable phytoremediation agent for restoring polluted freshwater systems and enhancing ecological balance.

Keywords: *Vetiveriazizanioides*, antimicrobial activity, *Escherichia coli*, zooplankton, phytoremediation, water quality, Karuvannur River

Introduction

Water serves as an essential requirement for every living organism and holds a vital position in preserving community health. Nevertheless, water supplies, especially in countryside regions, face growing threats from contamination, resulting in increased occurrences of water-related illnesses like cholera, typhoid, and dysentery. Although various drinking water sources exist, the bacterial safety and cleanliness of these supplies continue to present major challenges. Based on World Health Organization (WHO) data, roughly 80% of water contamination in developing nations, including

India, stems from household waste. Reports indicate that 70% of India's water sources suffer from severe pollution, with water-linked diseases responsible for 75% of health problems and 80% of deaths among children. The spread of harmful bacteria through the fecal-oral pathway, commonly enabled by polluted water, represents a primary factor in this health crisis. Fecal coliform bacteria, especially *Escherichia coli* (*E. coli*), function as essential bacterial markers for water quality assessment. While these microorganisms may not be harmful by themselves, their existence suggests potential contamination and the likelihood of disease-causing germs being present. *E. coli*, a flexible anaerobic, gram-negative rod-shaped bacterium, normally lives in the digestive systems of people and animals. Finding it in water indicates a breakdown in the separation between waste and water supplies.

In terms of structure, *E. coli* appears as cylindrical, moving bacteria (through surrounding flagella), that don't form spores, with certain types having protective layers and attachment structures. They multiply easily on common growing materials, creating large, damp, pale grayish-white clusters. The bacterial outer wall includes an intricate lipopolysaccharide framework, supporting cell wall strength and disease-causing ability. The external protective covering helps avoid immune detection, improving their survival in both host organisms and water environments. Remarkably, *E. coli* exists beyond polluted water sources; scientists have found them in clean aquatic settings, plant surface communities, and underground water in isolated locations. This broad presence results from their capacity to endure conditions with limited nutrients, needing only basic requirements like simple sugars and nitrogen compounds. In raw or poorly processed drinking water supplies, particularly in developing areas, *E. coli* levels can vary from thousands to tens of thousands in each milliliter.

Water flow from farming and city areas serves as a main pathway for bringing *E. coli* into rivers and waterways, causing substantial water quality decline (USEPA, 2002). This contamination affects not only human wellness but also disturbs water-based ecosystems. Within various water-dwelling creatures, plankton groups—including phytoplankton (plant-type) and zooplankton (animal-type)—work as responsive biological indicators of water condition. Zooplankton specifically holds a crucial position in aquatic food networks, connecting primary producers (phytoplankton) to upper food chain levels like fish. Their numbers, variety, and community structure respond significantly to environmental shifts, including bacterial contamination. The existence and growth of *E. coli* in water systems has proven to negatively impact zooplankton groups. A reverse relationship frequently appears, where higher *E. coli* levels match with reduced zooplankton numbers. This negative connection emphasizes the environmental consequences of bacterial pollution and demonstrates the significance of combining plankton observation with bacterial evaluation when assessing freshwater condition.

Vetiveria zizanioides, widely recognized as vetiver grass, represents a perennial plant

species within the Poaceae (Gramineae) family. Originating from India and Africa, this remarkable grass has earned worldwide acclaim for its numerous environmental benefits. While historically employed for protecting soil and conserving water, vetiver grass has become increasingly valued over recent years for its ecological flexibility and capacity to restore damaged environments. This grass demonstrates exceptional resilience when facing challenging environmental circumstances, thriving in poor lateritic soils, flooded areas, and regions experiencing frequent droughts. The plant's extensive, thread-like root structure—reaching depths of 2–3 meters—serves a vital function in securing soil, preventing erosion, and maintaining moisture levels. This comprehensive root system additionally enhances the plant's effectiveness in filtering processes and nutrient uptake, positioning it as an ideal choice for securing slopes, restoring mining sites, creating protective barriers against wind and water, and providing animal feed.

Among the most notable recent uses of vetiver is its role in cleaning water systems. Multiple research efforts have confirmed its capacity to function as an organic filter, successfully lowering levels of nitrates, phosphates, toxic metals, and harmful microorganisms such as *Escherichia coli*—a key marker of sewage pollution. This quality makes vetiver especially beneficial in economically disadvantaged or countryside areas where untreated contaminated water flows into natural waterways. Beyond its environmental functions, vetiver holds commercial significance within the perfume and beauty sectors. The plant's fragrant roots produce essential oils containing high levels of sesquiterpenes, which serve extensively as stabilizing agents in perfume creation and in manufacturing soaps, beauty products, and folk remedies. Given its distinctive biological features, including substantial biomass generation and resistance to extreme acidity, salt content, alkalinity, and toxic metal levels, vetiver has become an effective, environmentally responsible, and affordable option for environmental cleanup. Its flexible nature—functioning well in both wet and dry conditions—allows it to flourish and operate successfully across diverse environmental settings. As a result, vetiver grass is gaining increasing acceptance as an environmentally conscious method for restoring polluted locations and enhancing water purity.

Materials and methods

Study site and sample collection

This research focused on assessing the antibacterial properties of *Vetiveria zizanioides* (commonly known as vetiver grass) while examining how *Escherichia coli* (*E. coli*) levels correlate with zooplankton variety. Water specimens were gathered from the Karuvannur River in Kerala, India's Thrissur District. The river begins in the Pumalai hills, which are situated within the Chimmony Wildlife Sanctuary—a region that forms part of the environmentally important Western Ghats. Stretching roughly 48 kilometers in total, the Karuvannur River receives significant flow from the

Kurumali River, one of its primary tributaries.

Collected surface water specimens from designated locations throughout the river system. The samples were promptly transferred to laboratory facilities while maintaining sterile protocols for subsequent microbiological and biological testing. The study involved measuring *E. coli* concentrations to determine the extent of bacterial contamination, alongside analysing zooplankton communities to gauge aquatic ecosystem health. Furthermore, water specimens underwent laboratory treatment with vetiver grass to evaluate its effectiveness in decreasing *E. coli* numbers and to observe any related changes in zooplankton populations.

Zooplankton Sampling and Enumeration

Zooplankton populations are known to exhibit spatial patchiness in aquatic ecosystems, which poses challenges for both sampling and quantitative interpretation. To address this, a standard plankton net with a mesh size of 30 µm was employed to concentrate samples, particularly in areas where zooplankton density was low or where a larger biomass was required for analysis.

Collected water samples were homogenized by thorough shaking to ensure uniform distribution of organisms. A subsample of 1 mL was extracted using a wide-mouthed dropper and transferred into a Sedgwick-Rafter counting chamber, which has a holding capacity of 1 mL. The chamber is divided into 25 large grids, each further subdivided into 25 smaller grids, facilitating systematic enumeration.

Zooplankton individuals present in each large grid were carefully observed and counted under a compound microscope. The total number of organisms per large grid was recorded, and the overall abundance was extrapolated to estimate the number of zooplankton per litre of the concentrated sample.

Bacteriological Analysis of Water Samples: Presumptive Test for *Escherichia coli*

The presence of *Escherichia coli*, a fecal coliform bacterium commonly found in the intestinal tracts of humans and other warm-blooded animals, serves as a key indicator of fecal contamination in water. To evaluate the sanitary quality of water samples, a three-tiered bacteriological analysis was conducted, comprising the presumptive test, confirmed test, and completed test.

Presumptive Test Procedure

The presumptive test is based on the ability of coliform bacteria to ferment lactose, resulting in the production of acid and gas. Lactose broth supplemented with Bromothymol Blue (BTB) was used as the culture medium. BTB functions as a pH indicator, changing colour in response to acid production during lactose fermentation. Aliquots of the water sample were inoculated into a series of test tubes containing the lactose broth. These tubes were incubated at 35–37°C for 24–48 hours. Gas production

was monitored using Durham tubes placed inverted within the broth tubes. The appearance of acid (colour change) and gas within the Durham tubes was considered a positive result for coliform presence.

The Most Probable Number (MPN) method was employed to estimate the concentration of coliform organisms in the water samples. This statistical approach is based on the number of positive tubes at different dilution levels and provides an estimation of the microbial load, correlating with the extent of fecal contamination.

Materials Required

The following materials and reagents were used to conduct the presumptive test for the detection of coliform bacteria in water samples:

1. Water samples (freshly collected from the study site)
2. Single-strength lactose broth (SSLB)
3. Double-strength lactose broth (DSLb)
4. Bromothymol blue (BTB) – pH indicator
5. Durham's tubes (for gas detection)
6. Routine microbiological laboratory setup (including autoclave, incubator, laminar flow hood, sterile pipettes, test tubes, and glassware)

Preparation of Culture Media

Single-Strength Lactose Broth (SSLB)	
Component	Quantity per 100 mL
Peptone	1.0 g
Sodium chloride (NaCl)	0.5 g
Lactose	1.0 g
Distilled water	100 mL
pH	Adjusted to 7.2–7.4

Double-Strength Lactose Broth (DSLb)	
Component	Quantity per 100 mL
Peptone	2.0 g
Sodium chloride (NaCl)	1.0 g
Lactose	2.0 g
Distilled water	100 mL
pH	Adjusted to 7.2–7.4

Both media were prepared according to standard microbiological protocols. The pH was adjusted using 1N NaOH or 1N HCl as required. The media were sterilized by autoclaving at 121°C and 15 psi for 15 minutes.

Presumptive Coliform Test (MPN Method)

Indicator Preparation

A 0.2% Bromothymol blue (BTB) solution was prepared in 0.02N NaOH and diluted to volume with distilled water. BTB (1.25 mL) was added per 100 mL of lactose broth. The dye appears bluish-green at pH 7.4 and turns yellow at pH 6.0, indicating acid production.

Media and Distribution

Single Strength (SSLB) and Double Strength (DSL) lactose broths were prepared, adjusted to pH 7.2–7.4, and supplemented with BTB. Durham's tubes were inserted into all test tubes. Media were distributed as follows:

- DSL: 10 mL in 3 tubes
- SSL: 1 mL in 3 tubes
- SSL: 9.9 mL in 3 tubes

Control tubes were included for each set.

Sample Inoculation and Incubation

Water samples were collected aseptically. Inoculation was done as follows:

- DSL (10 mL): 10 mL sample
- SSL (1 mL): 9 mL sample
- SSL (9.9 mL): 0.1 mL sample

Tubes were incubated at 37°C for 24 hours.

Vetiver Treatment

In this study, four plastic tubs, each containing 10 litres of collected water, were used to assess the efficacy of vetiver (*Vetiveria zizanioides*) in water quality improvement. A quantity of 50 grams of fresh vetiver roots was added to each tub.

After two weeks of treatment, water samples from each tub were analysed for *Escherichia coli* (*E. coli*) counts and zooplankton abundance. The remaining water was left undisturbed for an additional two weeks (total of one month), after which the same parameters were re-evaluated.

This experiment was repeated monthly for a duration of six months. The average values of *E. coli* and zooplankton counts were calculated to assess the treatment efficacy over time.

Result

The current study aimed to investigate the antimicrobial potential of Vetiver grass (*Chrysopogon zizanioides*) and to explore the correlation between *E. coli* reduction and changes in zooplankton abundance. Water samples were collected from the Karuvannur River, a known freshwater source possibly impacted by anthropogenic activities. The study evaluates the bioremediation capacity of vetiver by assessing:

- Reduction in faecal coliforms (*E. coli*), a major microbial contaminant.
- Changes in zooplankton abundance, used as a bioindicator of water quality.

Initial analysis of water sample (Pre-treatment Phase)

Table 1: Abundance of *E.coli* and zooplankton in untreated water sample

Organism	Pre-treated Water (org/L)
<i>E. coli</i>	13,000org/L
Zooplankton	11,000org/L

The initial analysis of river water showed a high load of faecal *E. coli* (13,000 org/L), indicating substantial microbial contamination—most likely from sewage discharge or agricultural runoff. Simultaneously, the zooplankton population was relatively low (11,000 org/L), reflecting poor water quality, as many zooplankton species are sensitive to organic and bacterial pollution. This inverse relationship aligns with previous studies where high *E. coli* levels negatively impact zooplankton diversity and density.

Effect of Vetiver Treatment over Time

Table 2: Abundance of *E.coli* and zooplankton in vetiver-treated water samples

Organism	After 2 Weeks (org/L)	After 1 Month (org/L)
<i>E. coli</i>	700org/L	300 org/L
Zooplankton	18,000org/L	24,000org/L

E. coli Reduction

There was a marked decline in *E. coli* population from 13,000 to 700 org/L after 2 weeks and further down to 300 org/L after 1 month. This ~97.7% reduction over one month suggests potent antibacterial activity of vetiver roots. The observed effect can be attributed to:

- Tannins and phenolic compounds found in vetiver roots, known to disrupt bacterial cell walls and inhibit microbial growth.
- Rhizofiltration and rhizodegradation, where plant roots absorb and promote

the breakdown of organic contaminants and pathogens.

This supports previous literature that vetiver roots exhibit strong antimicrobial properties, making them a cost-effective, natural alternative for water purification.

Zooplankton Increase

In contrast to *E. coli*, the zooplankton population increased significantly, from 11,000 to 18,000 org/L after 2 weeks, and further to 24,000 org/L after 1 month. This 118% increase over a month indicates that the aquatic environment became more favourable for zooplankton due to improved water quality. Zooplankton are highly sensitive to pollutants and are commonly used as bioindicators of ecological health. Their proliferation in the treated water suggests that:

- Reduction in bacterial load allowed zooplankton to thrive.
- Improved oxygen levels and reduced organic waste enhanced habitat conditions.
- Absence of chemical stressors (as vetiver is a natural treatment) promoted natural recolonization.

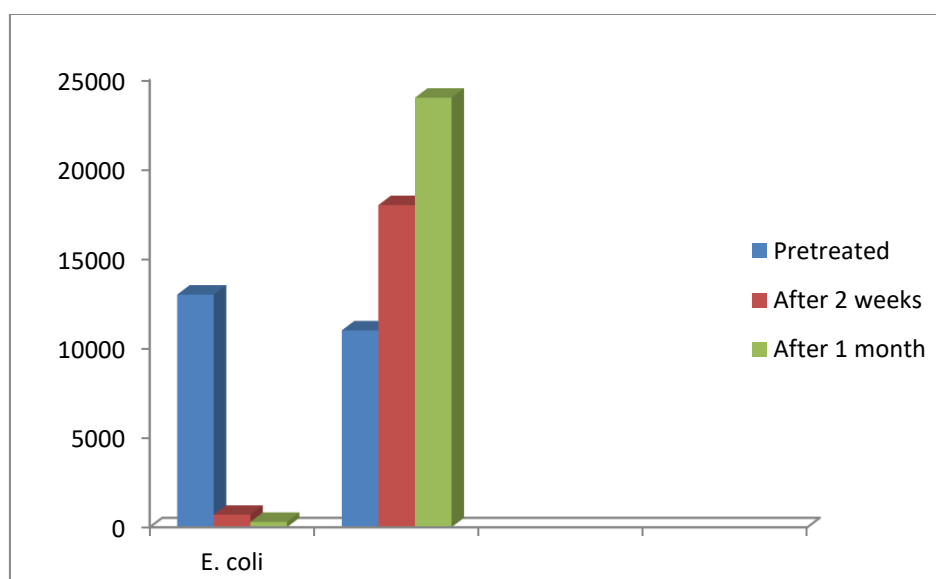


Figure I. Comparative Analysis of Zooplankton and *E. coli* Populations Following Vetiver Treatment

Figure I illustrates the temporal changes in *Escherichia coli* (*E. coli*) and zooplankton abundance in water samples before and after treatment with Vetiver grass (*Chrysopogon zizanioides*). The bar graph compares three sampling points: pre-treatment, after two weeks, and after one month of exposure to vetiver roots. The results demonstrate a significant reduction in *E. coli* counts from 13,000 org/L in the pre-treated sample to 700 org/L after two weeks, and further to 300 org/L after one month of vetiver treatment. This consistent decrease suggests that vetiver possesses

strong antibacterial activity, potentially attributed to phytochemicals such as alkaloids, tannins, and saponins that are known to disrupt bacterial growth and biofilms. Conversely, the zooplankton population exhibited a progressive increase, rising from 11,000 org/L in the untreated sample to 18,000 org/L after two weeks, and reaching 24,000 org/L by the end of one month. This rise in zooplankton density indicates a recovery of aquatic microfauna, as water quality improved due to the reduced microbial pollution.

The data clearly establish a negative correlation between *E. coli* and zooplankton abundance, where the suppression of faecal bacterial contamination through vetiver bioremediation facilitated a favourable habitat for zooplankton growth. This finding supports the use of zooplankton as bioindicators of water quality and highlights the ecological benefit of phytoremediation using vetiver.

Discussion

The findings of Gerrard Mark Aaron (2008) highlight the significant potential of Vetiver grass (*Vetiveria zizanioides*) as an effective and low-cost biological agent for wastewater purification. The study demonstrated that Vetiver grass can act as a primary purifier by removing contaminants and reducing pollutant loads, particularly in regions lacking access to advanced wastewater treatment infrastructure. Its dense root system, rapid growth, and high tolerance to pollutants make it especially suitable for phytoremediation applications. Moreover, the economic feasibility of using Vetiver supports its application in rural and low-income settings, offering a sustainable solution to freshwater pollution and sanitation challenges. These results underscore the relevance of nature-based solutions in environmental management and support further research into large-scale implementation of Vetiver-based wastewater treatment systems. Chaudhry et al. (1999) provides insight into the natural remediation potential of microbial communities, plants, and mycorrhizal fungi in iron- and zinc-contaminated filter cake waste. The successful colonization of such degraded substrates highlights the synergistic role of plant-microbe-mycorrhizae associations in stabilizing and detoxifying heavy metal-contaminated environments. The findings underscore the ecological importance of harnessing native or tolerant species and beneficial microbes for land restoration efforts. Devprakash and Srinivasan (2011) demonstrated that both alcoholic and aqueous extracts of *Vetiveria zizanioides* exhibit significant antimicrobial activity. This highlights the plant's potential as a natural source of bioactive compounds effective against pathogenic microorganisms. The presence of phytochemicals such as alkaloids, flavonoids, and essential oils likely contributes to its antimicrobial properties. These findings support the traditional use of vetiver in herbal medicine and suggest its utility in modern applications for water purification and microbial control. Incorporating Vetiver-based treatments may offer an eco-friendly and sustainable alternative to chemical antimicrobials in both environmental and clinical settings. Gerrard Mark Aaron (2008) highlighted vetiver

grass as an effective, low-cost natural purifier of wastewater. Its deep roots and pollutant tolerance make it ideal for improving water quality, especially in areas lacking conventional treatment systems. Grimshaw and Helfer (1995) demonstrated the effectiveness of Vetiver grass in soil and water conservation, land rehabilitation, and embankment stabilization. Its strong root system makes it ideal for preventing erosion and managing degraded lands sustainably. Mathivanan et al. (2007) reported that plankton populations in the Cauvery River serve as effective indicators of water pollution. Variations in plankton density and diversity reflect the ecological impact of contaminants, highlighting their usefulness in water quality monitoring. Nantachit et al. (2010) confirmed the antimicrobial potential of alkaloids extracted from *Vetiveria zizanioides* roots. The study supports Vetiver's role as a natural antimicrobial agent, highlighting its application in health and environmental sanitation. Sangeetha and Stella (2012) reported that vetiver extracts possess strong antimicrobial activity against various pathogenic microorganisms. This reinforces the plant's potential for use in natural antimicrobial formulations and eco-friendly sanitation practices.

Truong and Baker (1998) emphasized the effectiveness of the Vetiver Grass System in environmental protection. Its use in erosion control, water purification, and land rehabilitation demonstrates Vetiver's broad ecological benefits and practical application in sustainable land management. Zheng et al. (1997) conducted a preliminary study showing that Vetiver grass can effectively purify eutrophic water by reducing excess nutrients. This highlights its potential role in controlling nutrient pollution and improving water quality in aquatic ecosystems. Xia et al. (2002) demonstrated that Vetiver grass effectively absorbs and purifies contaminants from garbage leachate. Its strong uptake capacity and adaptability make it suitable for treating solid waste effluents and enhancing environmental sanitation.

Conclusion

The present study investigated the antimicrobial efficacy and water purification potential of *Vetiveria zizanioides* in the Karuvannur River, with particular focus on its effects on *Escherichia coli* populations and zooplankton abundance. The results demonstrated a notable decline in *E. coli* concentrations accompanied by an increase in zooplankton density in vetiver-treated samples, indicating improved water quality and a possible inverse relationship between pathogenic bacteria and aquatic microfauna. The observed effects can be attributed to the bioactive properties of vetiver, including its antioxidant activity, free radical scavenging ability, and phytoremediation mechanisms such as phytostabilization, phytofiltration, and phytoextraction. These attributes underscore its capacity to inhibit microbial contaminants while promoting ecological balance in aquatic systems. Given its cost-effectiveness, ease of cultivation, and environmental compatibility, *V. zizanioides* presents a viable phytoremediation agent for the restoration of polluted freshwater bodies. The findings support the integration of vetiver-based systems into sustainable

water management and ecological rehabilitation strategies.

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