Microbiological Quality Evaluation and Multivariate Analysis of Wastewater Treatment Efficiency in a Semi-Arid Urban Setting: The Case of Al-Muameera WWTP, Iraq

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Abstract
Microbial contamination continues to be a general challenge in discharged and reused wastewater, especially in semi-arid regions where treated effluent has become an alternative water sources. This work assesses the microbiological quality of sewage influent and effluent at Al-Muameera Wastewater Treatment Plant (WWTP), Hilla City, central Iraq as well as investigates the correlation between microbial indicators/parameters in raw and treated wastewater via multivariate statistics.Wastewater samples (influent/effluent) were taken in a six-month sampling campaign (November 2024–April 2025). Total coliform bacteria (TCB) and fecal coliform bacteria (FCB) were counted as indicators for microbial contamination, and the $\log_{10}$ transformation of bacterial counts was performed to normalize their frequency distribution. Multivariate statistical analyses (PCA, HCA and heatmap visualizations based on Pearson correlation of the integrated physicochemical and microbiological data) were conducted.The findings demonstrated an enormous amount of bacterial count in the influent wastewater, which was originated from raw domestic sewage. After treatment, bacterial numbers decreased significantly, although high concentrations of both TCB and FCB remained in the treated effluent. Results of Boxplot analysis clearly distinguished influent and effluent bacterial concentration, whereas PCA and cluster analysis successfully categorized samples according to treatment stage and showed strong correlations among bacterial indicators, organic matter, and suspended solids. Heatmap also indicated distinct correlations of the microbial parameters with specific physicochemical variables, particularly BOD_5, COD and TSS.Such results suggest that, although the conventional treatment in the Al-Muameera WWTP operation may represent a partial human microbial risk reduction, bacteria removal is not sufficient to warrant safe effluent disposal or unrestricted reuse. The combined use of microbiological indicators and multivariate statistical approach, developing a computer based method (software package) thus provides an effective tool to assess treatment efficacy and public health risks in semi-arid urban areas.
Keywords
Microbiological water quality; Total coliform; Fecal coliform; Multivariate analysis; PCA; Wastewater treatment plant; Semi-arid region, Iraq

1. Introduction

Microbiological pollution of water has been recognized as a serious environmental and public health challenge, particularly in developing countries that are undergoing urbanization such that the capacity to treat wastewater safely for discharge or reuse is lacking. Raw or poorly treated urban wastewater usually contains a high concentration of pathogenic and indicate microorganisms from human and animal faeces. Once in the environment, these microorganisms can pollute surface waters, soils and crops leading to a potential increase of water related diseases and imposing major challenges for sustainable water management (Qadir *et al.,* 2020; World Health Organization, 2019).

Reclaimed wastewater is playing increasingly significant roles in water resource protection and the provision of urban and agricultural water supplies, especially in semi-arid and arid regions. Nevertheless, the reuse of reclaimed water without an appropriate microbiological quality control could increase health risks, especially in areas with insufficient regulatory compliance and routine surveillance. Consequently, microbial indicators continue to serve as simple and affordable means to monitor the sanitary quality of wastewater and provide an indication of potential pathogen presence (Ashbolt *et al*., 2010).These include total coliform bacteria (TCB) and fecal coliform bacteria (FCB), which are the parameters most frequently employed to characterize microbial contamination of wastewater systems. Total coliforms as a marker for hygienic quality and system status and fecal coliforms, including thermotolerant coliforms, with more direct link to fecal pollution sources and potential risk to human health (Edberg *et al.,* 2000). Although more streamline methods for molecular detection are available, the coliform group of indicators is still widely used in developing countries because of their simplicity, low cost and already established regulatory significance.Conventional WWTPs predominantly based on primary sedimentation and secondary biological treatment in general are not designed for full pathogen removal, but to reduce organic matter and suspended solids. Although these systems can reduce number of microbes by sedimentation, predation and through bad environmental conditions (Murei & Momba, 2024; Shirdashtzadeh *et al.,* 2022), their efficiency in removing bacterial indicators is frequently low when there are no disinfection or tertiary treatment steps included (Bonetta *et al.*, 2022; Howard *et al.,* 2004). Consequently, the level of bacteria present in treated effluents can remain high enough to cause potential hazards in receiving ecosystems and public health.Microbial treatment efficiency cannot be assessed by only measuring the bacterial indicators, but their relationship with certain physico-chemical properties, nutrients and suspended load should also be investigated. These relationships are generally non-linear and complex, and thus univariate analysis is inadequate to reveal the underlying associations in WWTPs. Multivariate statistical methods are powerful tools to help elucidate such interactions by decreasing data dimensionality, identifying the overarching patterns and understanding associations among different variables (Williams, 2010; Ebrahimi *et al.*, 2021).

PCA, HCA, and the correlation-based heatmap visualization have been increasingly used in wastewater investigations for the examination of treatment efficiency, for classification of samples according to pollution patterns or as guidance on what controls microbial persistence. PCA extracts controlling gradients of water quality variation, while cluster analysis groups samples according to similarity and allows distinguishing between influent and effluent. Heatmaps combined with correlation analysis gives better intuitive explanations of relationships between microbial indicators and physicochemical variables in implementing integrated interpretation of treatment performance (Debassi *et al.*, 2025; Fang *et al.*, 2018).

The main barriers to wastewater treatment in Iraq include growth of the population, aging network, low sludge production and lack of adequate facilities. Numerous studies have reported the physicochemical enhancements obtained from conventional WWTPs, whereas systematic exploration of microbiological quality and its interaction with these factors is still limited. The literature is deficient in particular with respect to studies that use the multivariate statistical analysis combined with microbial parameters for assessing treatment efficiency and potential health risks under semi-arid climatic conditions.

The Al-Muameera wastewater treatment plant (WWTP) also known as the Pollution Control Plant is located in Hilla City, middle of Iraq, and represents a unique opportunity to fill this gap. The plant serves largely residential service area, has no polishing (i.e., disinfection or tertiary treatment), and is characteristic of many conventional-type WWTPs in the area. Evaluation of its microbiological performance and the relationships between bacterial indicators and physicochemical parameters are important for the analysis of risks that may exist, as well to find ways to improve treatment.

Therefore, the aims of this study are (i) to assess microbiological quality of influent and effluent wastewater of Al-Muameera WWTP in terms of total coliforms and fecal coliforms as major indicators; (ii) inspect correlation between microbial indicator group and spatial variation in key physicochemical parameters; and (iii) utilize multivariate statistical tools such as PCA, HCA and heatmap plot for evaluating treatment efficiencies with attention to potential environmental consequences highlighting eco-toxicity or health risks. The study was designed to provide data for evidence-based wastewater management and risk-informed reuse in semi-arid urban scenarios.

2. Materials and Methods

2.1. Study Area and Wastewater Treatment Plant.

This research was carried out at Al-Muameera Wastewater Treatment Plant (WWTP) situated in Hilla City and Babylon Province, central Iraq. The area has an arid climate and hot summer with mild winter that affect the wastewater quality and treatment process. The WWTP receives influent from a predominantly residential area and wastewater flow is mainly domestic with little or no industrial discharge.

The treatment plant used is a traditional one, including primary screening for preliminary treatment and removal of coarse material, primary settling to remove most suspended solids, and secondary biological treatment. The Tertiary treatment and disinfection units are absent in plant, which is similar to many other municipal WWTPs in semi-arid developing area.

2.2. Sampling Strategy

Wastewater monitoring took place during a six-month sampling period between November 2024 and April 2025. Sampling was performed every two months at two fixed points of the plant: influent (before treatment) and effluent (after secondary treatment). All samples were aseptically collected into sterile glass containers for microbiological examinations and transported to the laboratory in isothermal box near 4 °C. Microbiological analyses started within 6 h from sampling to avoid bacterial die-off or overgrowth. Physicochemical parameters utilized in the multivariate analyses were determined from concurrent monitoring conducted during those corresponding sampling events.

2.3. Microbiological Analyses

Microbiological quality of waste water was determined by TCB and FCB, the indicator organisms. The MPN method was carried out employing membrane filtration technique by the standard methods for the examination of water and wastewater.

Suitable sample volumes were filtered through sterile 0.45 μm filter membranes and then transferred onto selective culture plates. The total coliform bacteria were incubated at 37 °C for 24 h and the fecal coliform bacteria at 44.5 °C for also 24 hours. After incubation, typical colonies were counted and the values were reported as colony forming units/100 mL (CFU/100 mL).

We applied a log transformation to the bacterial counts in order to increase the robustness of statistical methods:

$\log_{10}(CFU/100 \text{ mL} + 1)$ were used in all the statistical and multivariate procedures.

2.4. Physicochemical Data Integration

Physicochemical variables such as temperature, pH, DO, TSS, BOD_5, COD, nutrients (NH_3-N, NO_2-N, NO_3-N), TN and total TP and ions in water were combined with microbial information to conduct multivariate analysis.

All physicochemical analyses were carried out according to the international standard methods, and data was taken during the same period of time as those for microbiological examinations to guarantee temporality for multivariate interpretation.

2.5 Multivariate Statistical Analysis

Using multivariate statistical analyses, the connections among microbiological parameters and physicochemical characteristics were investigated as well as treatment performance trends.

To eliminate the influence of eliminate the influence of differing units and magnitude independent, we standardized all variables before analysis (z-score normalization). PCA was used to reduce dimensionality of data and characterize the main gradients controlling variation in wastewater quality. Only components with eigenvalues greater than one were considered for interpreting the results.

Ward's clustering method and Euclidean distance were employed in hierarchical cluster analysis (HCA) to group samples based on similarity and source (influent vs. effluent). The dendrogram was used to view the results.

The correlations between microbiological and physicochemical variables were determined using the Pearson correlation coefficients. A correlation heatmap was applied to provide a graphical overview of positive and negative correlations between parameters.

Standard scientific statistical packages were used for the completion of all statistics and multivariate analyses.

2.6. Data Display and Quality Control

Results are reported applying data descriptive statistics, boxplots, multivariate ordination diagrams, heatmaps, dendrograms for clustering. Quality control procedures consisted mainly of blanks, duplicate analysis, and laboratory standardization protocols to guarantee data reliability and reproducibility.

2.7 Ethical Considerations

No experiments on animals or humans were conducted in this study. All samplings and analyses were performed in accordance with laboratory safety rules and environmental monitoring criterion. The research was used strictly for academic and environmental evaluation.

Results

3.1. Microbiological Characteristics of WWTP Influent and Effluent

The microbiological quality of wastewater from Al-Muameera WWTP Table 1 shows the quality of wastewater at intake and effluent in the AlMuameera WWTP before treatment and after treatment. Raw wastewater had very high levels of both TCB and FCB concentrations, which were characteristic of raw sewage. An extensive variation in the bacterial counts was found between sampling moments.

Some reduction was achieved in the bacterial counts of both indicators after treatment, although treated effluent still had high concentrations of bacteria. Average TCB levels decreased from 2.35×10^8 CFU/100 mL in influent to 1.76×10^8 CFU/100 mL in effluent samples. Moreover, the mean FCB densities decreased from 5.25×10^7 CFU/100 mL before treatment to 4.17×10^6 CFU/100 mL after treatment.

Table 1. Total and fecal coliform bacteria (CFU/100 mL) in influent and effluent wastewater released by Al-Muameera WWTP throughout the study period (n = 12).

Indicator	Influent (Mean ± SD; Min–Max)	Effluent (Mean ± SD; Min–Max)
Total coliform (TCB)	$2.35 \times 10^8 \pm 4.93 \times 10^8$ ($0 - 1.78 \times 10^9$)	$1.76 \times 10^8 \pm 4.48 \times 10^8$ ($0 - 1.59 \times 10^9$)
Fecal coliform (FCB)	$5.25 \times 10^7 \pm 3.67 \times 10^7$ ($1 \times 10^7 - 1.4 \times 10^8$)	$4.17 \times 10^6 \pm 6.69 \times 10^6$ ($0 - 2 \times 10^7$)

3.2 Fluctuation of Bacterial Indicators Pre-Treatment and Post-Treatment

The distribution of TCB and FCB concentrations for before and after treatment are presented in Figure 1 after logarithmic transformation ($\log_{10}(CFU/100 \text{ ml} + \text{density})$). The distinction between the influent and effluent samples is evident in the boxplots for bacterial indicators.

The range between the first and third quartile was higher, with lower median values for influent samples than effluent samples, demonstrating that there is greater fluctuation and more contamination before treatment. Even though median bacterial in effluent were reduced, there was some overlap between the influent and effluent distributions, particularly for TCB.
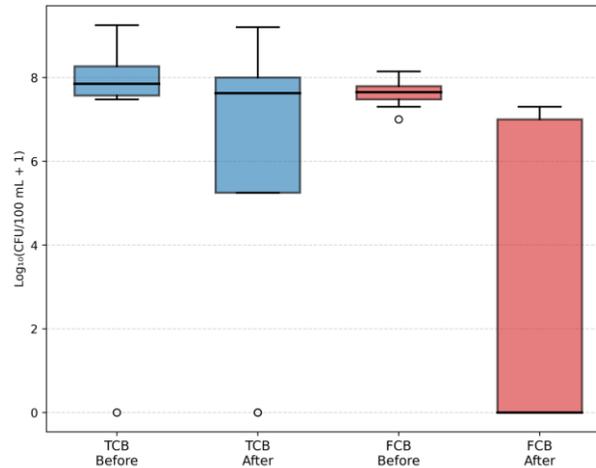
Figure 1. Boxplots of log₁₀-transformed TCB and FCB Before &after Treatment at the Al-Muameera WWTP.

3.4. Correlation analysis and heatmap display

The Pearson correlation between physicochemical and microbiological parameters is described in Figure 2 heatmap. The bacterial indicator was significantly associated with the organic pollution parameters, specifically BOD_5, COD and TSS.

There was a reverse correlation of bacterial indicators with dissolved oxygen representing the effect of more aerated conditions on lowered bacterial load. The heatmap reveals unique patterns of associations between variables and emphasizes a strong correlation between microbial contamination and organic matter.
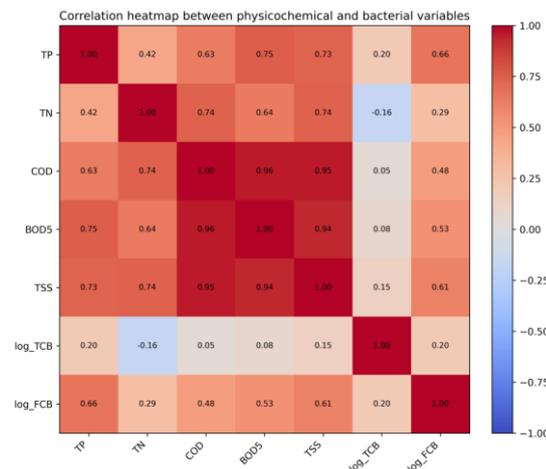

Figure 2. Heat map for Pearson correlation coefficients between physicochemical and microbiological parameters in the influent and effluent of wastewater at the Al-Muameera WWTP. Bacterial data were log₁₀-transformed before analyses.

3.5. Hierarchical Cluster Analysis

The gray rows represent dendrograms based on complete linkage hierarchical cluster analysis (HCA) according to combined physico-chemical and microbiological parameters. The dendrogram obtained is depicted in Figure 3.

The clustering is clearly seen for the original and preprocessed data, into inlet (influent) samples and outlet (effluent) samples. Based on this, the influent samples were found in their unique cluster. with Alternatively; we could see a higher bacterial load and organics levels as well in While the effluent samples ran separately (which shown an improvement water quality after treatment). Smaller sub-clusters within each group were evidences of temporal variability between sampling periods.
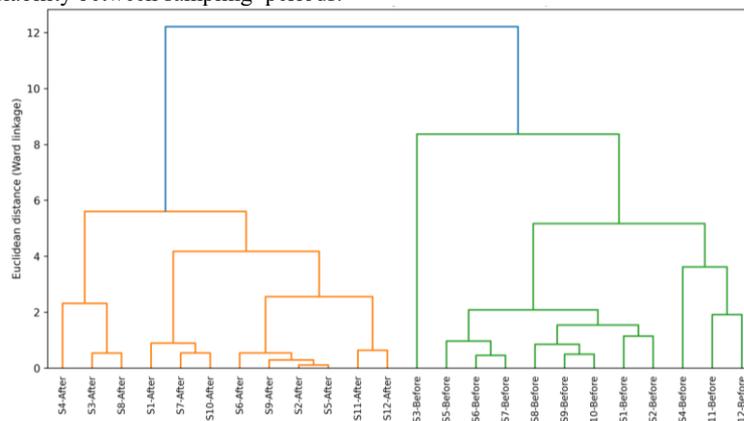

Figure 3. A dendrogram of the hierarchical cluster analysis (using ward's method) showing the patterns of similarity between influent and effluent wastewater samples from Al-Muameera WWTP according to standardized physicochemical and microbiological parameters.

Discussion

4.1. Environmental Role of Microbial Indicators in Treated Wastewater

TCB and FCB (microbial indicators of faecal pollution) is one of the most important bio-indicators that may be used to assess ecological integrity of receiving water bodies. High amounts of these indicators in the treated effluent indicate remaining organic load and ineffective treatment, that may have a negative impact on aquatic environment especially for semi-arid regions as having less dilution capability.

It shown, even after treatment at the Al-Muameera WWTPs which lead to a decrease in bacterial loads, significant levels of both FCB and TCB are found in treated effluent. Ecologically, such persistency suggests that present day treatment is failing to significantly reduce the overall microbial impact on the receiving environment. Similar results have been reported in arid and semi-arid regions where no improvement has been recorded with the treatment of sewage, as treated effluents emerge as source of microbial and organic load into river waters and canals (Edokpayi et al., 2017).Discharge of high bacterial load effluent catalyzes shifts in the microbial community structure in the environment waterbody, and stimulates recruitment of heterotrophic activity, thereby stimulating organic matter breakdown to lead to increased oxygen consumption. This can result in indirect potential effects on aquatic biodiversity, because those species which are pollution-tolerant and have weakened ecosystem resistance would be more favoured.

4.2. Characterization of patterns of bacterial reduction

From the boxplot it was apparent that the input and output water samples differed significantly in terms of bacterial content and partial removal had occurred during treatment. However, the level of matching between boxplot value distribution information and out distributions is far from perfect - particularly so for TCB- suggesting variation in treatment efficacy, and vulnerability to operational situations as well as ambient conditions.For environmental reasons, the variability is significant as episodic sudden releases of highly contaminated effluent can exert a much greater impact on receiving waters during periods of low flow. In semi-arid catchments, which generally have lower base flows, short-lived pulse inputs of microbially degraded material and organic matter can also disproportionately affect water quality and ecosystem function (Pelletier et al., 2020).

Results from conducted similar studies in Iran, Egypt and Jordan exhibited also approximate partial removals of coliforms from secondary-treated effluents as a local trend associated particularly with conventional treatment plants which do not apply disinfection or advanced polishing stages (Bouri et al., 2008; Tabatabaei et al., 2020).

4.3 Multivariate Interpretation of Environmental Factors Mediating Microbial Persistence

Multivariate statistics evidenced to be efficient as a method for the identification of environmental factors influencing microbial resistance in wastewater. PCA analysis indicated that principal components had clear correlations between bacterial indicators and organic physicochemical factors (BOD_5, COD) of organic origin as well as solid matters particulate form (TSS).

These correlations indicate that microbial persistence and transport generally occur in association with organic substrates and suspended particles, which can provide protection against environmental stressors and very likely contribute to bacterial survival as a component of treated effluent. This is a well-documented phenomenon in wastewater-impacted aquatic ecosystems because bacteria attached to particles become more resistant to environmental degradation and predation (Debassi *et al.*, 2025; Howard *et al.,* 2004).

The influent and effluent samples are distinguished in PCA space implying that treatment dramatically modifies the composition of wastewater, although the proximity of effluent samples to organic-related loadings emphasizes the lack of complete uncoupling microbial contamination from organic material under current operating conditions.

4.4. Environment explanation of the correlation patterns

Heatmap analysis also confirmed the close connection of microbial indicators with the organic pollution parameters. Significant positive relationships between coliform bacteria and BOD_5, COD and TSS imply a continuous supply of organic matter to the microbial populations.

In contrast, the negative relations of bacterial indicators and dissolved oxygen would have demonstrated the significance of aerobic conditions on disabling longevity survivability. In terms of the management of environmental aspects, this study shows that there would be more advantages by way of aeration optimization and organic loading reduction to improve effluent quality for lesser ecological impact.

Such correlation trends have been reported in polluted riverine waste stream systems where high bacterial densities are a function of associated biodegradable organic matter that subsequently reduces the dissolved oxygen availability and cause multiple stresses loading on an ecosystem (Yordanova et al., 2026, Orel *et al*., 2022).

4.5. Cluster analysis and ecological separation of treatment types

HCA discriminated between influent and effluent samples well, verifying the general benefit of cationic treatment on water quality. Such distinct separation indicates the good capacity of combining microbiological and physical-chemical parameters to act as an integrated indicator of treatment efficacy.

However, the fact that sub-clusters were observed within the effluent group indicates temporal changes in environmental conditions and treatment efficacy. Such variability becomes especially important in semi-arid locations, where variations in temperature, hydraulic loading and influent composition can affect treatment performance and impact downstream ecosystem.

4.6. Comparison to International Environmental Studies

Compared to international studies, the microbial reduction achieved at the Al-Muameera WWTP is in line with data on the performance for conventional WWTPs of developing and water-stressed countries. Published reports from South Asia, the Middle East, and North Africa all show that organic matter removal is sufficient, with microbial reduction incomplete without disinfection (Bonetta *et al*., 2022, Fang *et al*., 2024).In contrast, WWTPs with tertiary treatment (including disinfection) exhibit much lower microbial densities in effluent, i.e., they exert reduced ecological pressure on receiving waters. Nonetheless, the latter systems are more expensive to operate and require technical expertise which may reduce the appropriateness of system types in many semi-arid settlements around the world.

4.7. Environmental implications and treatment options

From environmental aspect point of view, these results suggested that the treated effluent from Al-Muameera WWTP might have cumulative effects of microbial and organic loads on the receiving water bodies, particularly during lower flow in both Qom and Thahr catching. Although effluent is considered as one of the main water sources in a water scarce catchment such as this one, its environmental use should be adequately managed to limit ecological deterioration.

Integration of microbial indicator data with multivariate statistical analysis is a useful tool to assess and identify environmental risk sources that can help inform water management. These types of responses are especially relevant in semi-arid environments that have narrow environmental thresholds and low levels of ecosystem resistance.

Conclusions and Environmental Recommendations

5.1 Conclusions

An integrated microbiological and multivariate analysis on wastewater treatment efficiency at Al-Muameera Waste Water Treatment Plant (WWTP), Central Iraq: An emphasis on semi-arid environmental perspective The results indicate that the conventional secondary treatment alone partially removes microbial contamination; but, large numbers of both total and fecal coliform bacteria remain in the treated effluent.

Partial least squares algorithms unambiguously separated influent from effluent wastewater, demonstrating the general efficiency of the treatment in changing wastewater features. However, the fact that robust associations between CWM and FBP parameters dominated by microbial indicators persist suggests strong relations of bacterial persistence with organic and particulate loads. This association emphasizes the lot of physicochemical factors as drivers for microbial communities, and also demonstrates that the traditional wastewater treatment methodologies are not efficacious to provide environmental protection.

Regarding water bodies, the fact that treated effluent's microbial indicators seem to persist for long periods of time may be an indication of possible ecological stress in receiving systems (mainly in those situated in semi-arid river basins with reduced dilution capacity). This pressure can induce change in microbial community structure, enhance oxygen demand and add to cumulative ecological stress with implication for resilience of ecosystems and sustainability of water quality.

In general, the findings are consistent with international evidence from semi-arid developing regions that show that conventional wastewater treatment adequately reduces organic pollution but may fall short of effectively managing microbial input to freshwaters in the absence of advanced treatment or disininction stages.

5.2. Environmental Recommendations

On the basis of results obtained in this study, the following recommendations from an environmental point of view are suggested for sustainable wastewater treatment and preservation of aquatic biota:

1. **Effluent Improvement by Environmental Protection:** Secondary treatment requirements, including low-cost disinfection or polishing units, to reduce microbial loads and decrease ecological effects on receiving waters during dry weather flows warrant consideration.

2. **Organic Matter and Particle Removal Optimization**: Organic matter and suspended solids can indirectly reduce microbial resistance by minimising substrates and protective niches for bacteria survival in treated effluent.

3. **Adoption of Integrated Environmental Monitoring**: Programs of routine monitoring should involve the use of microbiological parameters in combination with relevant physico-chemical factors to allow for an overall evaluation of effluent quality and environmental risk.

4. **Use of Multivariate Analysis in Assessment of Environmental Pollutants:** Multivariate statistical techniques, such as PCA and cluster analysis, must be included routinely for assessing wastewater treatment efficiency at a point in time and the environmental conditions exerting control on effluent quality.

5. **Adaptive Effluent Discharge Management:** Effluent outflow schemes can be remodeled in a seasonal pattern, according to river flow and ecological vulnerability, to alleviate environmental stress during sensitive periods.

6. **Integration of the risk environment into reuse planning:** Where considerations for wastewater reuse are being made, site-specific environmental risk assessments should be used in informing land application and focus on the protection of the ecosystem rather than water resource augmentation.

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