



Enhancing microbiological stability in municipal water distribution: A descriptive statistical analysis for public health assurance

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Abstract

The microbiological quality of municipal water is of prime importance for public health and represents a prominent concern for authorities to maintain and control the bioburden level within safe limits. An appropriately functioning network must ensure the biological stability of the water distribution system throughout the way of water from the processing and purification station until reaching the ultimate consumers. The study examines methodologies used to evaluate different segments from a local distribution system of city water by examining short-term results of the total microbial count obtained from several available points of use through a one-year period. Microbial variations observed in city's water system. Autumn has the highest bioburden. Locations exceeding 10,000 CFU/100 mL threshold warrant investigation. Non-uniform distribution across the system suggests potential influence of distance, pipe type, and biofilms. Early warning system established to monitor bioburden surges. Descriptive analysis showed that six lines from 19 examined sections from the distribution system had lower dispersion in the microbiological data between minimum and maximum results than the remaining parts of the distribution pipes. Moreover, multiple comparison test outputs returned significant differences in microbiological density datasets between high-count and low-count use points. Thus, surveying the layout design of the municipal water distribution infrastructure would be crucial to restate its impact on the sanitary distribution of water. In conclusion, this refined study provides a nuanced examination of microbiological stability in municipal water distribution, offering targeted insights for effective public health management. By implementing the present assessment, public health authorities can spot and identify defective areas in the distribution system and take necessary actions to prevent or mitigate potential risks of waterborne diseases.

Keywords: Bioburden, Municipal water, Correlation matrix, Coefficient of variation, Pareto chart

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1. Introduction

Water is essential for life, health, and well-being. However, not all water sources are equally safe and reliable. Municipal water, or tap water, is the main source of water for various industries and homes in many countries. It is delivered through underground pipes that connect the water treatment plants to the end-users. Municipal water undergoes rigorous treatment and processing to remove most of the impurities and contaminants that may pose health risks or affect the water quality. Municipal water is tap water that is delivered to

various industries and homes via underground pipes (Sensorex, 2020). This water is fully treated and processed before being delivered to these destinations, which means that the majority of impurities are removed before it can be potable or used in the shower (Netsol Water Solutions Pvt, 2022). Even though the Environmental Protection Agency (EPA) has established water quality standards that each municipality must meet, the quality of tap water can vary from community to community (Netsol Water Solutions Pvt, 2022; US EPA, 2013). Therefore, it is important to monitor and evaluate the quality of municipal water and to identify and address the potential challenges and risks that



may compromise its safety and suitability for different purposes. The goal of public health authorities is to assess the microbiological stability of municipal water distribution in urban regions, using various indicators and methods. The methodologies should provide valuable insights for effective public health management and water quality improvement (Apure, 2022).

Through a network of pipelines, reservoirs, and valves, municipal water distribution systems provide treated water to a variety of residences and businesses. This water's microbiological stability is crucial to guarantee its safety and purity for use and consumption by humans. The idea of giving customers drinking water with the same microbial quality at the tap as it produces at the water treatment facility is known as “microbiological stability” (Sensorex, 2020). However, during distribution, unchecked bacterial and other microbe development may happen, posing a risk to hygienic conditions as well as aesthetic and functional issues (Netsol Water Solutions Pvt, 2022). Microbial proliferation, for instance, can lead to the emergence of opportunistic diseases, like Legionella, which can endanger the health of susceptible people (US EPA, 2013). Additionally, microbial development can change the water's color, taste, odor, and turbidity.

In general, drinking municipal water must be relatively safe. For a municipality to provide water to residents and businesses, the water must first be treated and tested (CDC, 2022). If it does not meet local or national standards, the water must be treated again until a certain number of contaminants have been removed (World Health Organization, 2008). As previously stated, some municipalities are more effective than others at treating tap water, which means that certainty about the quality of municipal water cannot be assured from the final consumer point of view (CDC, 2020). The concept of biological stability of drinking water refers to providing consumers with drinking water of the same microbial quality as that produced at the water treatment facility (Park et al., 2016; Prest et al., 2016). Uncontrolled growth of bacteria, on the other hand, can occur during distribution in water mains and premise plumbing, resulting in hygienic (e.g., development of opportunistic pathogens), aesthetic (e.g., deterioration of taste, odor, color), or operational (e.g., fouling or bio-corrosion of pipes) issues (Hayward et al., 2022; Ibekwe & Murinda, 2019). Diverse microorganisms compete for limited available nutrients for growth in drinking water (Hibbing et al., 2010).

Water is an essential resource, and ensuring its safety is a top priority for municipalities around the world (Eissa, 2017). One critical aspect of this is assessing microbiological stability, which involves analyzing the presence of microorganisms in water distribution systems (Cabral, 2010). Descriptive statistical analysis plays a crucial role in this process, as it helps to identify trends and patterns in the data (Eissa, 2015). By using statistical tools

such as mean, standard deviation, and correlation analysis, experts can gain valuable insights into the health of these systems and make informed decisions about how to maintain and improve their performance (Eissa, 2016a). In this article, we will explore the importance of descriptive statistical analysis in assessing microbiological stability in municipal water distribution systems, and how this approach can help to ensure the safety and well-being of communities around the world. The importance of descriptive statistical analysis in assessing microbiological stability in municipal water distribution systems will be explored in this article, and how this approach can help to ensure the safety and well-being of communities around the world. However, it is also acknowledged that the validity and reliability of this method depend on the sample size and frequency of testing, which may be constrained by practical and financial factors in the initial phases of surveillance.

The safety and quality of drinking water are of utmost importance, and as such, it is crucial to ensure that municipal water distribution systems are microbiologically stable (Eissa, 2018). One way to assess this is through the current in-depth analysis, which allows us to better understand the trends and patterns in microbial data. By examining key factors such as microbial load and variation in the count, potential sources of defects should be identified and proactive measures to prevent them.

The microbial quality of water is critical for the validity of human use, especially since humanity is approaching an advanced stage of water scarcity. Due to the challenges encountered with city water use and its safety microbiologically, an effective examination technique, including quantitative and descriptive methodologies must be adopted by the public health authority to assess the microbial quality of the municipal distribution system. A focus on a specific topic concerning biological stability shall be discussed herein via bioburden density in tap water to measure the magnitude of the stability of this inspection property at the preliminary stages of monitoring with a limited number of samples available. This study aims to assess the microbiological stability of municipal water distribution systems in a selected city, using descriptive statistical analysis of microbial data. This approach allows us to better understand the trends and patterns in microbial data and to identify key factors that influence the microbial load and variation in the water. By examining these factors, we can detect potential sources of defects and propose proactive measures to prevent them.

2. Material and Methods

Municipal water samples were collected monthly for one year when available or accessible.

2.1. Location and study subject

A specific area embracing a tape water distribution network was selected for investigation of the total microbial aerobic count. The examined piping system is

contained within a single district included within the Giza governorate. The study included 19 sampling points of use after two storage tank units from the processed city water. The selection of sampling points and criteria considered several factors. First, the availability and accessibility of data on the water distribution network and water quality parameters within the district were crucial. Additionally, the representativeness and diversity of water consumption patterns and water demand allocation methods within the district were considered. The research objectives and proposed model also played a role, with the relevance and applicability of the chosen district being a key factor.

Spatial distribution and coverage of sampling points were crucial. Points were chosen to represent different zones and branches of the network, ensuring a comprehensive overview. Finally, the functional and operational characteristics of the points of use themselves were taken into account. This included factors like the type of water use (domestic, commercial, etc.), water consumption frequency and duration, and the presence of storage tanks or other devices that could influence water quality. Only points with reliable water quality measurements, such as total microbial aerobic count and turbidity, were included.

2.2. Type of microbiological analysis

The microbiological quality of city water samples was assessed by measuring the bioburden density in 100 mL of water as Colony Forming Unit (CFU). The samples were collected monthly from the available points of use throughout the year 2022. The Total Viable Aerobic Count (TVAC) was determined by filtering the samples through 0.45 μm sterile membrane filters and incubating them on appropriate culture media (Eissa et al., 2022). The neutralization was performed by adding sodium thiosulfate to the sample containers, as recommended by the ISO 19458:2006 standard on the sampling bottles (Eissa & Rashed, 2022; Eissa et al., 2022).

2.3. Statistical analysis and software platforms

A variety of statistical techniques were employed to analyze the microbiological data, including Pareto analysis, outlier detection, and non-parametric tests like the Wilcoxon signed-rank test (for comparing medians of paired samples) and the Kruskal-Wallis test (for comparing medians of multiple groups). The Spearman correlation matrix was also used to assess relationships between microbial levels at different use points. These techniques helped identify significant differences in bioburden levels across seasons, locations within the water network, and even specific use points.

3. Results

3.1. Water microbial density and time factor

The analysis of microbial counts in the city's water system revealed significant seasonal variations. Figure 1 utilizes

the Pareto principle to highlight the most impactful seasons and months on the overall bioburden. When combined, spring and autumn bioburden levels contribute over 80% of the total microbial count. Further breakdown by month shows an even greater disparity. Two fall months, September and November, account for more than half (54%) of the total bioburden, while two spring months, April and May, contribute only 23.5%. Interestingly, even summer's August month falls within the top contributors, accounting for an additional 7.5%, bringing the combined impact of these three seasons to over 85% of the total microbial count.

3.2. Microbiological variations and extremities over the study term for the analyzed point-of-use

The procedure of sample preparation, collection and processing was consistent with previous similar research that was conducted on the same line (Eissa & Rashed, 2022). This procedure was chosen because it is a standard and reliable method for detecting and enumerating the total microbial population in water samples (Eissa & Rashed, 2022; Eissa et al., 2022). It is also important to neutralize the residual disinfectant in water samples before analysis, as it can inhibit the growth of microorganisms and affect the accuracy of the results. Aberrant Samples Detection for the water use ports labeled SK1, ST2, RNT, LER, PCW, MIQ, G02, G04, G07, G3A, G3B, G18, G19, G33, G52, G68, G71, G75 were statistically tested as could be seen in Figure 2. The number of outliers detected was 1, 1, 1, 2, 2, 2, 2, 3, 1, 2, 2, 2, 3, 2, 1, 0, 0, 1, respectively. Thus, the excursion rates (%) were 8.3%, 9.1%, 8.3%, 16.7%, 18.2%, 16.7%, 16.7%, 25.0%, 8.3%, 16.7%, 16.7%, 16.7%, 25.0%, 16.7%, 8.3%, 0.0%, 0.0%, 8.3%, respectively. This finding was summarized in Figure 2 with two extreme rates (maximum 25.0% and minimum 00.0%) of the excursion rates shown in addition to the statistics computed in the clustered column diagrams.

The Coefficient of Variation (CV) is a statistical measure that relates the standard deviation of a set of values to its mean. It is often expressed as a percentage and is used herein to compare the degree of variability between distinct sets of microbiological counts in a water line (Figure 3). For estimated values, the CV can be used to assess the precision of the. A lower CV indicates a more precise estimation of water quality (G68, G07, G71 and G03), while a higher CV suggests greater uncertainty or variability around the estimate (ST2, G18, G02 and PCW). By expressing the coefficient of variation as a percentage, it provides a standardized measure of dispersion that can be used to compare data sets across different domains. A fluctuation in the coefficient of variation is a useful statistical tool that can help investigators and analysts to assess the degree of variability and precision in the water distribution system datasets.

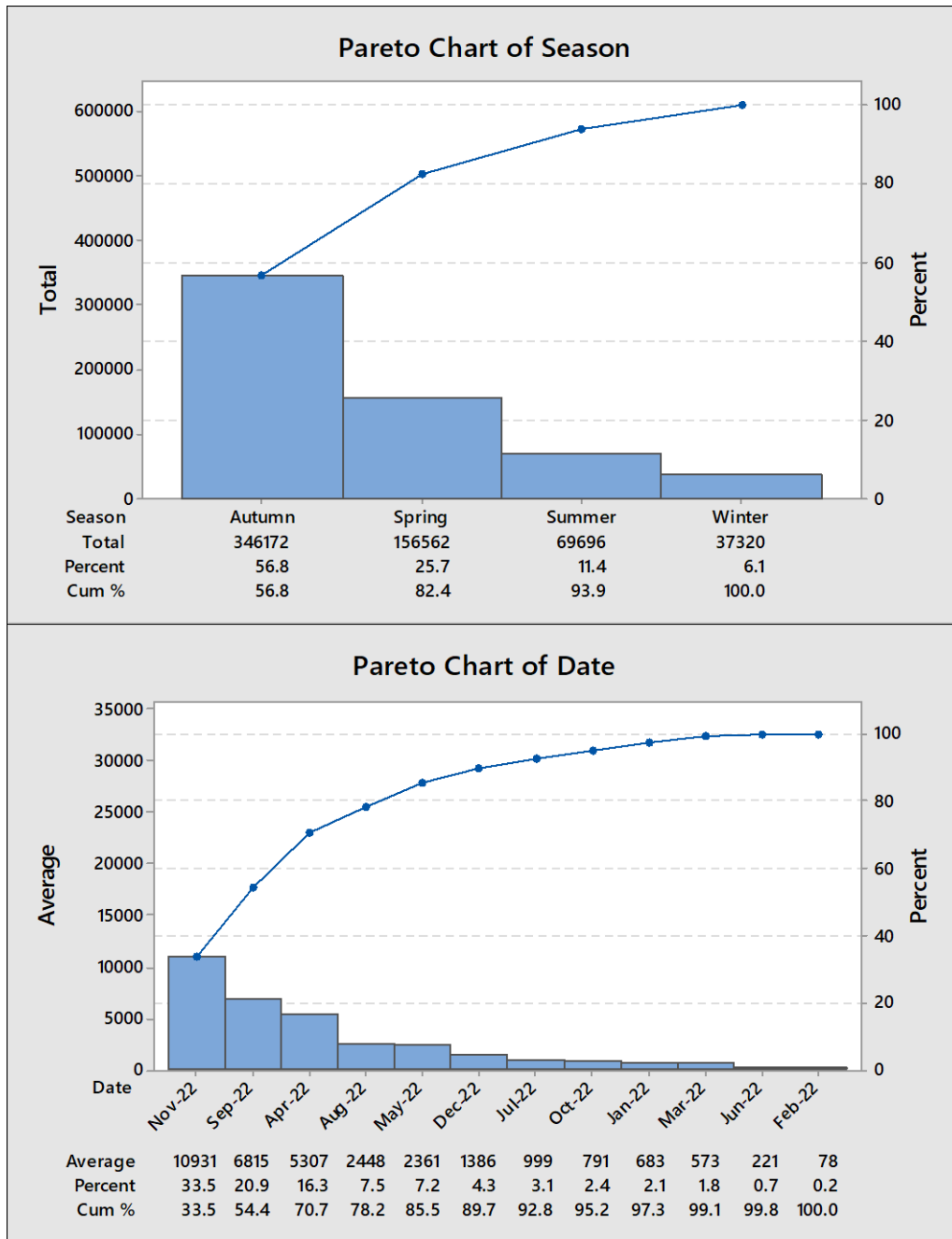


Figure 1. Pareto analysis showing the periods embracing major bioburden level in city water distribution system

The first diagram presents two Pareto charts that illustrate the bioburden levels in a city’s water distribution system across various seasons and dates. Bioburden refers to the quantity of microorganisms present in a sample. Figure 1 depict the total or average bioburden levels for each category, along with the cumulative percentage of the total bioburden. The categories are organized in descending order of bioburden levels, from left to right. These charts are useful in identifying the most significant factors that contribute to the bioburden levels in the water system. The upper chart displays the total bioburden levels for each season. Autumn records the highest bioburden level, followed by spring, summer, and winter. This suggests that the bioburden levels fluctuate with the seasonal changes in

temperature, rainfall, and water demand. The lower chart reveals the average bioburden levels for specific months. It is observed that some dates have significantly higher bioburden levels than others, which could be attributed to factors such as water quality, maintenance, or contamination events.

The second diagram demonstrates the results of outlier detection of microbial count in the examined distribution lines of municipal water. The upper graph uses vertical bars to represent microbial counts at different use points labeled from SK1 to G75. The blue bars indicate outliers, while the red bars indicate non-outliers. The y-axis is labeled as “Frequency” ranging from 0 to 12. The lower graph is another bar plot representing the distribution of

microbial counts at different use points. Figure 2 shows the interquartile range (IQR), lower limits, and upper limits using blue, red, and green lines respectively. The y-axis is also labeled as “Statistical Limits” but has a different scale $\times 10,000$. This diagram illustrates that the microbial count varies significantly across the use points, and that some use points have extremely high or low values that deviate from the normal range.

Figure 3 depicts the Coefficient of Variation (CV) for bioburden samples estimated from different locations from the distribution lines of city water under examination. The CV values are represented on a bar graph, with each bar

corresponding to a specific location or sample type. The CV is a measure of the relative variability of a data set, expressed as a percentage. It is calculated by dividing the standard deviation by the mean and multiplying by 100. A higher CV indicates a higher degree of dispersion or heterogeneity in the data, while a lower CV indicates a higher degree of consistency or homogeneity in the data. The graph shows that the CV values vary widely across the different locations and sample types. This suggests that the bioburden levels in the city water are not uniform and may depend on factors such as the distance from the source, the type of pipe, the presence of biofilms, and the sampling method.

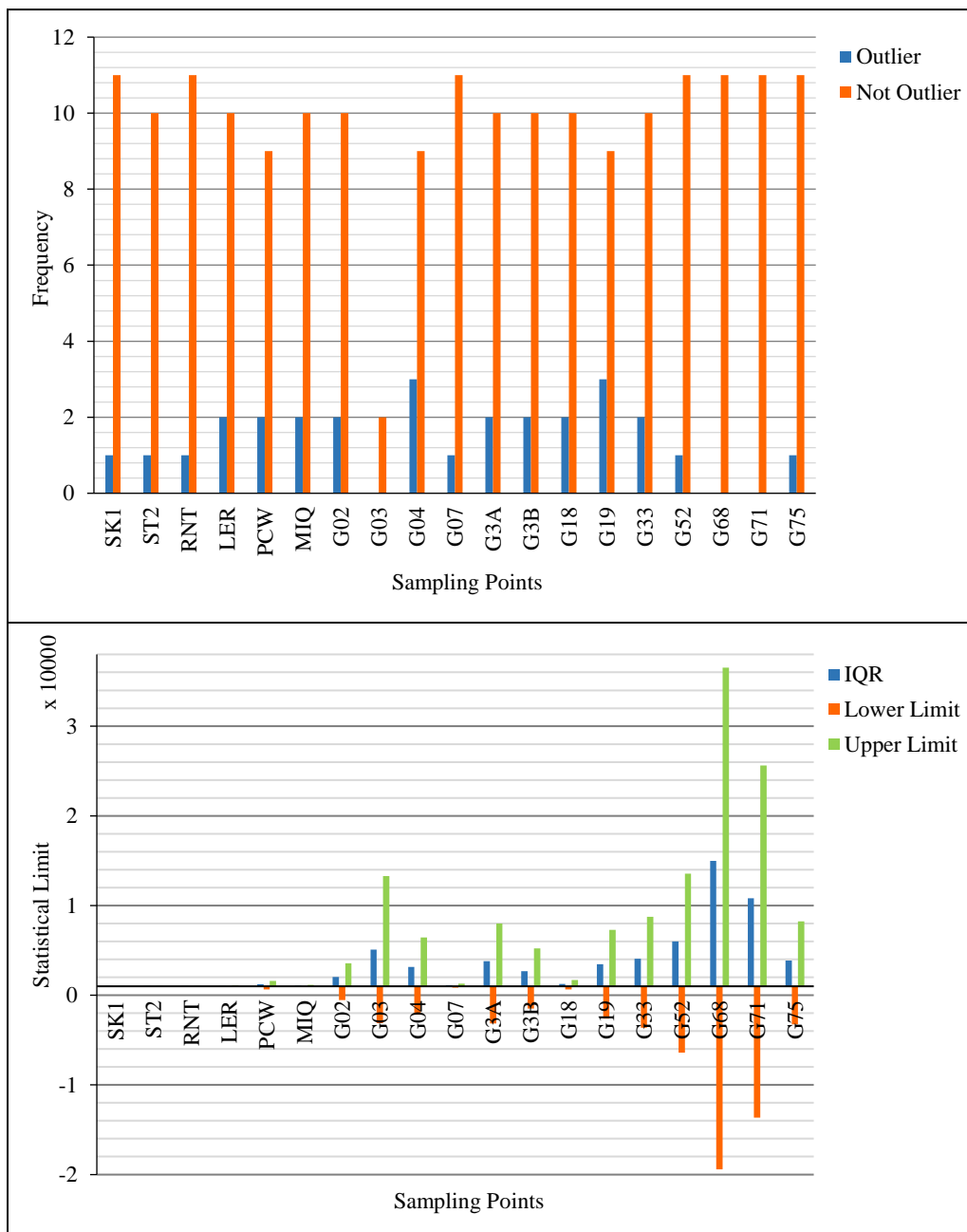


Figure 2. Outlier detection of microbial count in the examined distribution lines of municipal water

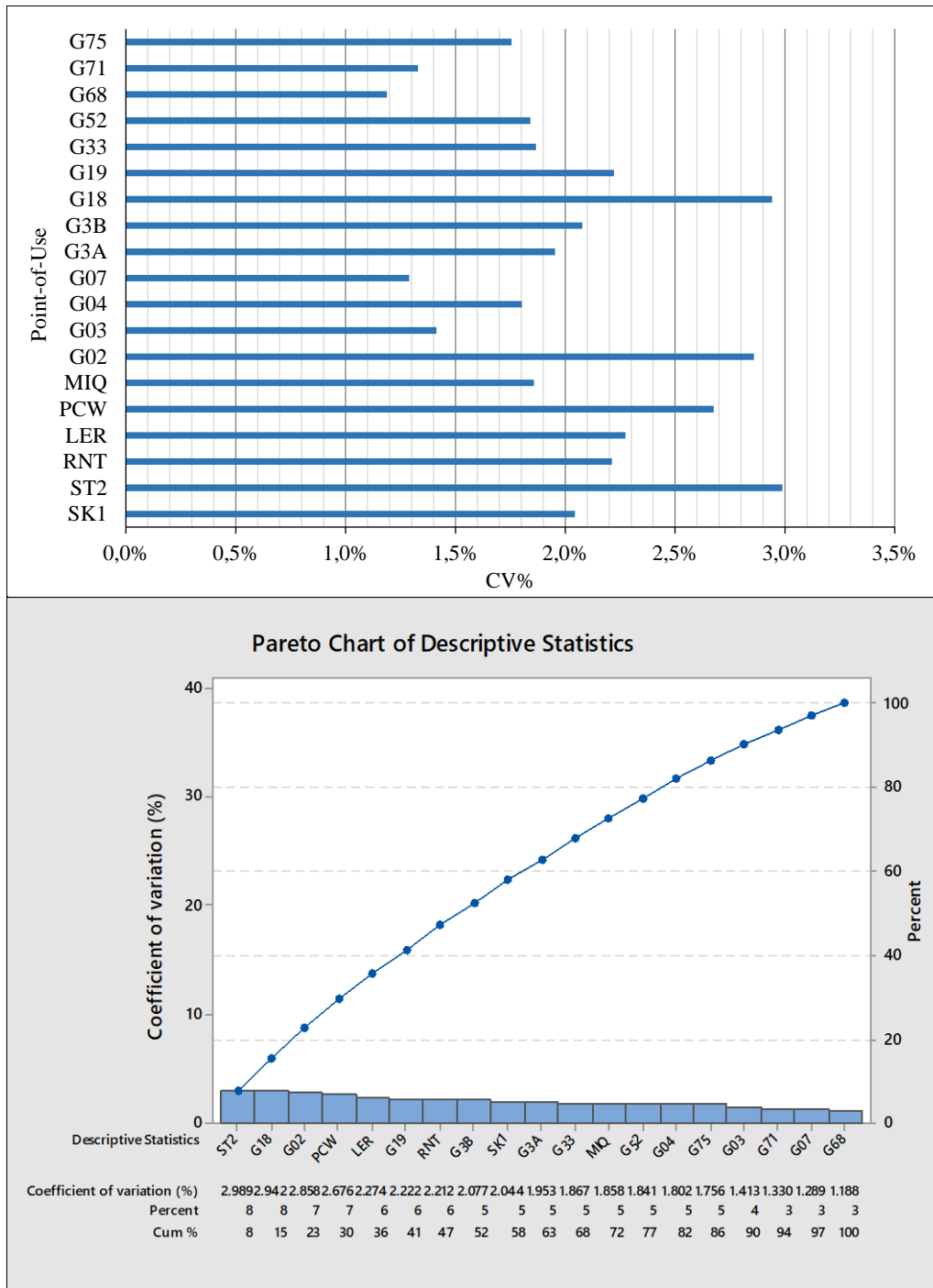


Figure 3. Coefficient of variation (CV) for the bioburden samples estimated from different locations from the distribution lines of city water under examination

3.3. Establishment of early warning alarm using cut-off threshold

All Setting a warning limit for the microbiological count of city water distribution lines would be a desirable measure. This cut-off limit was set arbitrarily far below the action threshold of 50,000 CFU/100 mL value namely 10,000 CFU/100 mL. The non-parametric *t*-test analog test was used to identify the lines with a higher potential of bioburden density. With this respect distribution segments that showed actual medians that are statistically not

significantly different from the reference value at $\alpha=0.05$ were the main source of the microbial density aberrancy i.e., use ports G68, G71 and G3A with minority in G19 and G33. This is reflected in the discrepancy values and indicated by the two-tailed *p*-value as could be seen in Figure 4. The remaining points of use were far below this alarming limit and hence are statistically different from it. This study established an early warning alarm for the microbiological count of city water distribution lines using a cut-off threshold of 10,000 CFU/100 mL. This threshold was chosen to be much lower than the action threshold of

50,000 CFU/100 mL to ensure timely detection and prevention of microbial contamination. By applying a non-parametric test, the study identified the distribution segments that had a higher potential of bioburden density and were not significantly different from the reference value. These segments were G68, G71, and G3A, with some contribution from G19 and G33, as shown in Figure 4. These segments should be monitored closely and subjected to appropriate interventions to reduce the bioburden levels and improve the water quality. The remaining points of use were well below the alarming limit and were significantly different from the reference value, indicating a satisfactory microbial stability.

Figure 4 shows the results of a non-parametric t -test analog that compares the median microbial counts of different water lines with a reference value of 10,000 CFU/100 mL, which is the cut-off threshold for the early warning alarm. The p -values indicate the probability of obtaining a result equal to or more extreme than the observed one, assuming that the null hypothesis is true. The null hypothesis is that the median microbial count of each water line is equal to the reference value. The lower the p -value, the more evidence there is to reject the null hypothesis and conclude that the water line has a significantly different median microbial count from the reference value. The figure illustrates that some water lines have major or minor microbial counts that are statistically referenced to the alarming threshold value, meaning that they have significantly higher or lower median microbial counts than the reference value. These water lines are G68, G71, G3A, G19, and G33, as shown by their low p -values and high discrepancies. These water lines should be monitored closely and subjected to appropriate interventions to reduce the bioburden levels and improve the water quality. The remaining water lines have median microbial counts that are not significantly different from the reference value, indicating a satisfactory microbial stability.

3.4. Descriptive statistical distribution of microbiological data

The descriptive statistical analysis of the microbial count per 100 mL of water for different sampling locations is shown in Figure 5 in descending order of magnitude. The datasets demonstrated non-Gaussian distribution with strong skewness to the right side. The significance of demonstrating a non-Gaussian distribution for microbial count data is that it indicates that the data are not symmetric and have a long tail on the right side, meaning that there are some extreme values or outliers that are much higher than the average. This implies that the data are over-dispersed and have more variability than expected from a normal distribution. This affects the interpretation of the data because it means that the mean and standard deviation are not sufficient to describe the central tendency and

dispersion of the data and those parametric statistical tests that assume normality may not be valid or reliable. Therefore, alternative methods such as non-parametric tests, transformation, or modeling with appropriate distributions (such as Poisson, negative binomial, or zero-inflated models) may be needed to analyze the data and draw meaningful conclusions.

This distribution is important in the context of water quality assessment because it reflects the heterogeneity and complexity of the microbial communities in different water sources and environments. Microbial count data are influenced by many factors, such as sampling methods, sequencing technologies, environmental conditions, and biological interactions. These factors can cause variation in the abundance and diversity of microbes across samples and locations, resulting in skewed and over-dispersed data. Understanding the distribution of the data can help to identify the sources and patterns of microbial contamination, assess the risks and impacts of waterborne diseases, and evaluate the effectiveness of water treatment and management strategies. Thus, most of the sampling ports showed results that did not pass the normality test (at a significance level α 0.05). The stability of the bioburden level could be demonstrated by points RNT, SK1, ST2, MIQ, LER and G07. However, rising level of variability could be explored in G03, PCW, G75, G04, G18, G3B, G71, G52, G68, G33, G3A, G02 and G19, respectively. The rising level of variability in the sampling ports indicates that the microbiological counts are not consistent and may fluctuate over time or space. Some factors that may contribute to this variability are:

- *Water quality and treatment:* The source water quality, the type and dosage of disinfectants and the effectiveness of filtration and chlorination may affect the microbial diversity and abundance in the water supply systems.
- *Water distribution system:* The age, material, and design of the pipes, the presence of biofilms, the water pressure and flow rate, and the occurrence of leaks or breaks may influence the microbial community composition and dynamics in the water distribution system.
- *Building water supply system:* The configuration, maintenance, and usage of the building water supply system, such as the faucets, showers, heaters, and coolers, may impact the microbial growth and survival in the building water supply system.
- *Environmental factors:* The temperature, seasonality, light irradiation, pH, nutrients, organics, and antibiotics may affect the microbial activity and diversity in the water supply system.

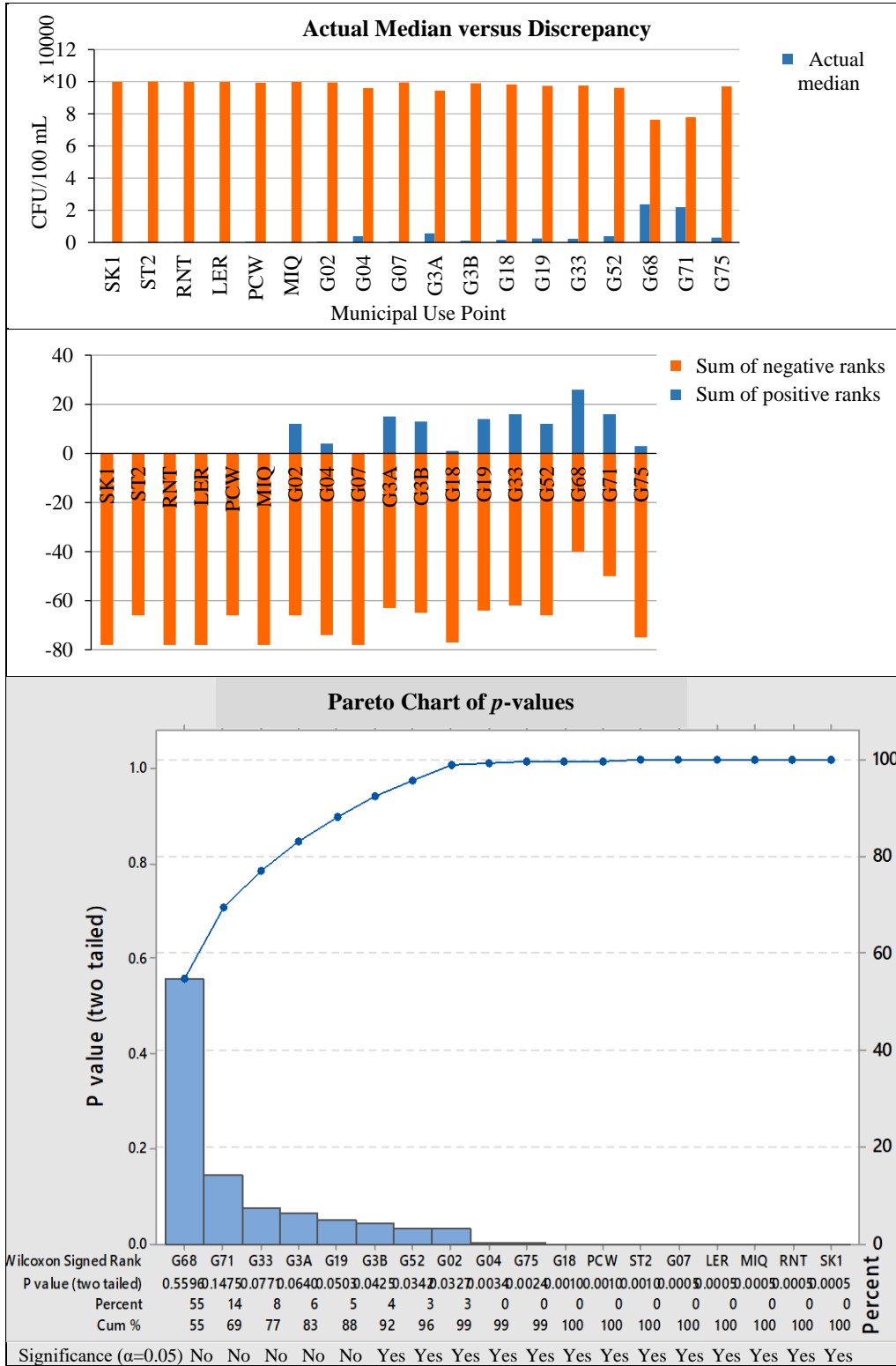


Figure 4. The output of the non-parametric *t*-test analog shows lines with major and minor microbial counts that are statistically referenced to the alarming threshold value. The top part of the figure displays a bar chart comparing the actual median and discrepancy at various municipal use points. The bars represent different values, with red indicating discrepancies and blue representing the actual median. The discrepancy is the difference between the actual median and the reference value. The middle part shows the sum of negative ranks for each municipal use point. The negative ranks are the ranks of the observations that are below the reference value. The bottom part, labeled as “Pareto Chart of *p*-Values”, illustrates the *p*-values associated with each municipal use point in a descending order.

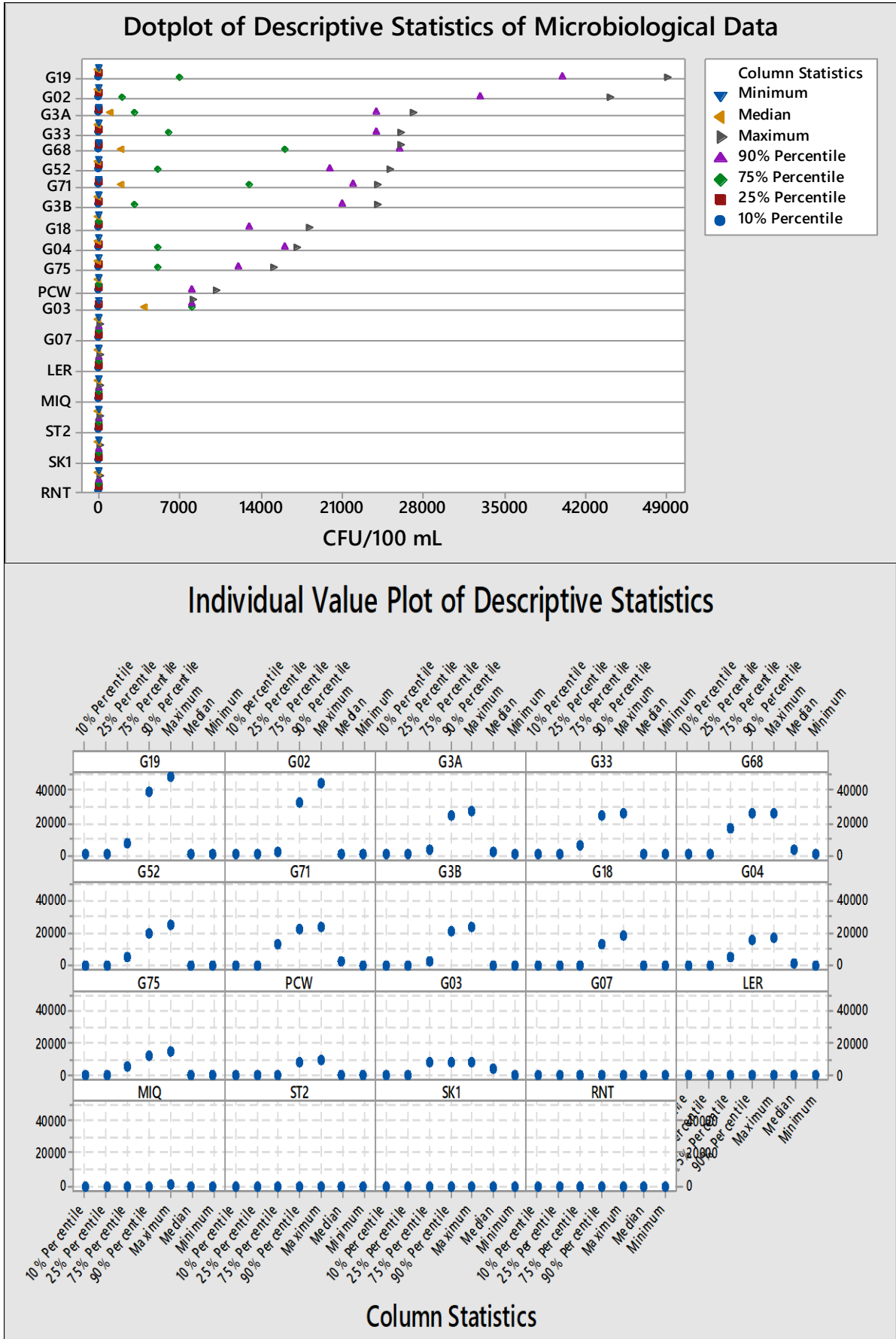


Figure 5. Column statistics showing data spreading of microbiological count from city water from different point-of-use

The two ways for data visualization mentioned in Figure 5 could be described as the following:

- *Dot plot of descriptive statistics*: This plot provides a visual representation of the spread and central tendency of microbiological data from different points-of-use. Each point-of-use is represented by different colored dots that indicate various percentiles, median, and minimum/maximum values. This visualization aids in quickly identifying the distribution and variability of microbial counts across different water lines, helping to assess their microbial stability.
- *Individual value plot*: This plot displays individual data points for each point-of-use, offering a detailed view of the microbiological count at each specific location. It allows for an in-depth analysis to identify any outliers or specific points with unusually high or low microbial counts, contributing to a comprehensive understanding of the water lines' microbial stability.

3.5. Pairwise comparison between microbiological record data from points of use under investigation

A water sample from one site, specifically the coast near UPV hatchery, was taken to the Microbiology Laboratory of IA-UPV for the total plate count (TPC) of bacteria present. Only one site was processed instead of two due to time constraints and the availability of materials in the laboratory. Five-fold dilutions were prepared with sterile saline solution. An aliquot (100 μ L) of each sample dilution was spread onto plates with prepared media using an L-rod. The plates were then inverted, placed inside transparent plastic cellophane, and stored in the laboratory at room temperature for 72 hours. Only the first, third, and fifth dilutions were used in this study. Two replicates per sample were prepared and executed. All plates were monitored every 24 hours, and the number of colonies present was counted. For the calculation of bacteria colonies using the TPC method, the following procedure was employed.

Table 1 shows the main finding of the statistical test highlighting the presence of detectable differences in the bioburden quality between different lines of the distribution system. This finding is in line with the previous outcome of the column statistics that described the nature of data dispersion. Detailed examination of the sources of significant variability could be projected from Figures 6 and 7 which drew the mean rank difference for significant and non-significant pairwise comparisons, respectively.

Figure 6 shows the mean rank difference for various pairs of municipal water distribution segments, indicating the relative performance or condition of these segments in terms of microbiological density. Significant differences are marked with "Yes" in the Significance column and indicate that the segments have different levels of microbial contamination. Non-significant differences are not marked and indicate that the segments have similar levels of

microbial contamination, with no clear advantage for either segment. Figure 7 shows the individual value plot of the mean difference in rank for the microbiological count of different water distribution system segments. The x-axis represents the Dunn's multiple comparisons test, which is a statistical method to compare the means of multiple groups after a non-parametric analysis of variance (ANOVA). The y-axis represents the mean difference in rank, which is the difference between the average ranks of two groups in a non-parametric test. The blue dots represent the individual value points of the mean difference in rank for each pair of water distribution system segments. The horizontal line at $y=0$ indicates no difference in rank between the two groups being compared.

The dot plot reveals variations in microbiological density across different segments of a municipal water distribution system. Some segments have consistently higher ranks (worse conditions) than other segments. This suggests that these segments may have different sources, pathways, or factors affecting their microbial quality. A few use points were the reason behind this significance since they contributed to unusually high microbial density in water samples. This could be illustrated in Figure 8 where the count of the main effectors in the municipal distribution system is small in relation to the other stable and low bioburden water lines. With reference to Figure 5, the points of discrepancy could be identified between the two distinct groups. Figure 9 shows two important criteria:

- The ranking of the microbiological sampling output results for each distribution port demonstrates the range, spreading and scatter of the data points.
- Histogram showing the similarity in the microbial count distribution shape and spreading that could be observed with the most points.

Table 1. The output of non-parametric multiple comparisons Kruskal-Wallis test for municipal water distribution system

Table Analyzed [‡]	Results of the distribution line data
<u>Kruskal-Wallis test</u>	
<i>p</i> -value	< 0.0001
Exact or approximate <i>p</i> -value?	Approximate [€]
<i>p</i> -value summary	****
Do the medians vary signif. ($p < 0.05$)	Yes [§]
Number of groups	19
Kruskal-Wallis statistic	80.72
<u>Data summary</u>	
Number of treatments (columns)	19
Number of values (total)	214

[‡]Reported at 95% Confidence Interval (CI)

[€]Asymptotic *p*-value

[§]If the *p*-value is small, the hypothesis that the difference is due to random sampling can be rejected, and one can instead conclude that the populations have different distributions.

**** A tiny *p*-value where $p \leq 0.0001$

Histogram of Bioburden: The upper graph shows the frequency of microbial counts across different distribution ports. Each bar represents the number of samples that fall within a certain range of microbial counts. The histogram helps to visualize the distribution and variation of microbial counts across different ports, and to compare the relative levels of contamination among them. **Kruskal-Wallis Test Ranks:** The lower graph shows the ranks of the microbiological data for each examined use point, based on the Kruskal-Wallis test. This is a non-parametric statistical test that compares the medians of multiple groups of data. The test assigns a rank to each data point, from the lowest to the highest value, and then calculates the average rank for each group. The lower the average rank, the lower the median of the group. The graph plots the average ranks for each distribution port, using different symbols. The graph helps to identify which ports have significantly higher or lower microbial counts than others, and to test the null hypothesis that all ports have the same median.

3.6. Non-parametric correlation matrix for the points of use of municipal water system

The apparent shape of the distribution in the histograms of Figure 8 showed truncated spreading of the microbial count data. Generally, the datasets do not follow a normal distribution. Hence, surveying the correlation between use points under examination was conducted using a non-parametric Spearman matrix. Table 2 shows the detailed output of the calculated two-tailed correlation coefficient (r) and the corresponding p -values with the strength of the association indicated and marked with visual color for ease of scanning and spotting of the significant correlation. The lowest negative but strong correlation was found between G07 and G3B. The correlation data of these points appeared to be highly associated. Section G3B appeared to be highly determined by G02 and PCW. On the other hand, SK1 and ST2 fields appeared to be highly correlated. Similarly, G02 with G33, MIQ with G07, G52 with G68, RNT with G18, ST2 with G52, MIQ with G3B and G19, G52 and G18 with LER and G68 with G71 correlation data were found to be highly linked together. A very strong positive correlation existed between G52 and ST2 in the bioburden data and was sufficiently strong with SK1. A strong correlation appeared between G71 and G68 but they do not share this strength level with other points.

4. Discussion

Water is one of the most essential resources on the planet, and its quality is crucial for human health and well-being (Bruyninckx, 2018). Municipal water distribution systems play a vital role in ensuring that people have access to safe and clean drinking water (Dinka, 2018). However, these systems can be vulnerable to microbiological contamination, which can pose serious health risks to the public (Vacs Renwick et al., 2019). That's why it's crucial to conduct regular assessments of the microbiological stability of water distribution systems (Liu et al., 2018). Descriptive statistical analysis is an important tool that can help experts to interpret and analyze the data collected from these assessments (Noiva

et al., 2016). In the following sections, we'll explore the importance of descriptive statistical analysis in assessing the microbiological stability of municipal water distribution systems and how it can be used to identify potential risks and improve the quality of drinking water. The biological stability of municipal water is of prime importance for public health use (Pruden, 2014). Thus, safety must be ensured for human use (WHO, 2011). One of the important criteria that should be monitored and evaluated is the microbiological content of the water. The bioburden level should be ensured to be within the permissible limit from the departure from the station until reaching the consumers (Health Canada, 2021). The microbiological efficacy of the distribution system could be examined through the magnitude of the bioburden density and the stability of the microbial enumeration over a specific time and from one segment to another.

4.1. Water microbial density and time factor

The provided information makes a compelling case for the seasonal variation in microbial count and its implications for public health. The use of the Pareto principle in Figure 1 highlights the significant impact of certain seasons and months on bioburden, indicating the need for targeted interventions. The reference to Edam and Abdelgalil (2022) underscores the empirical basis of the findings, adding credibility to the assertion that autumn contributes significantly to the total bioburden. Moreover, the combination of autumn and spring bioburden accounting for over 80% of the total microbial count emphasizes the concentrated impact of specific seasons on water quality.

Edam and Abdelgalil (2022) is a recent study that investigated the seasonal variation of microbial community, potential opportunistic pathogens and antibiotic resistance genes in a novel underground reservoir of an island. The study found that the microbial community and function showed significant seasonal changes, with autumn having the highest diversity and abundance of microbes. The article summarized the current knowledge on the microbial ecology of water and its relation to public health. The researchers discussed the factors that influence the microbial composition and dynamics of water, such as temperature, nutrient availability, hydrological conditions, and human activities. The paper also highlighted the potential risks of waterborne pathogens and the need for effective monitoring and management of water quality.

The detailed breakdown by month further strengthens the argument, demonstrating the disproportionate contribution of certain months to the overall bioburden. The reference to Figueras and Borrego (2010) supports the significance of seasonal impact on water quality, emphasizing the need for tailored sanitization and disinfection strategies to manage bioburden levels effectively. In conclusion, the evidence presented suggests that seasonal variations have a substantial impact on the biological quality of city water, necessitating careful consideration and targeted action by public health authorities to maintain control over water quality and safeguard public health.

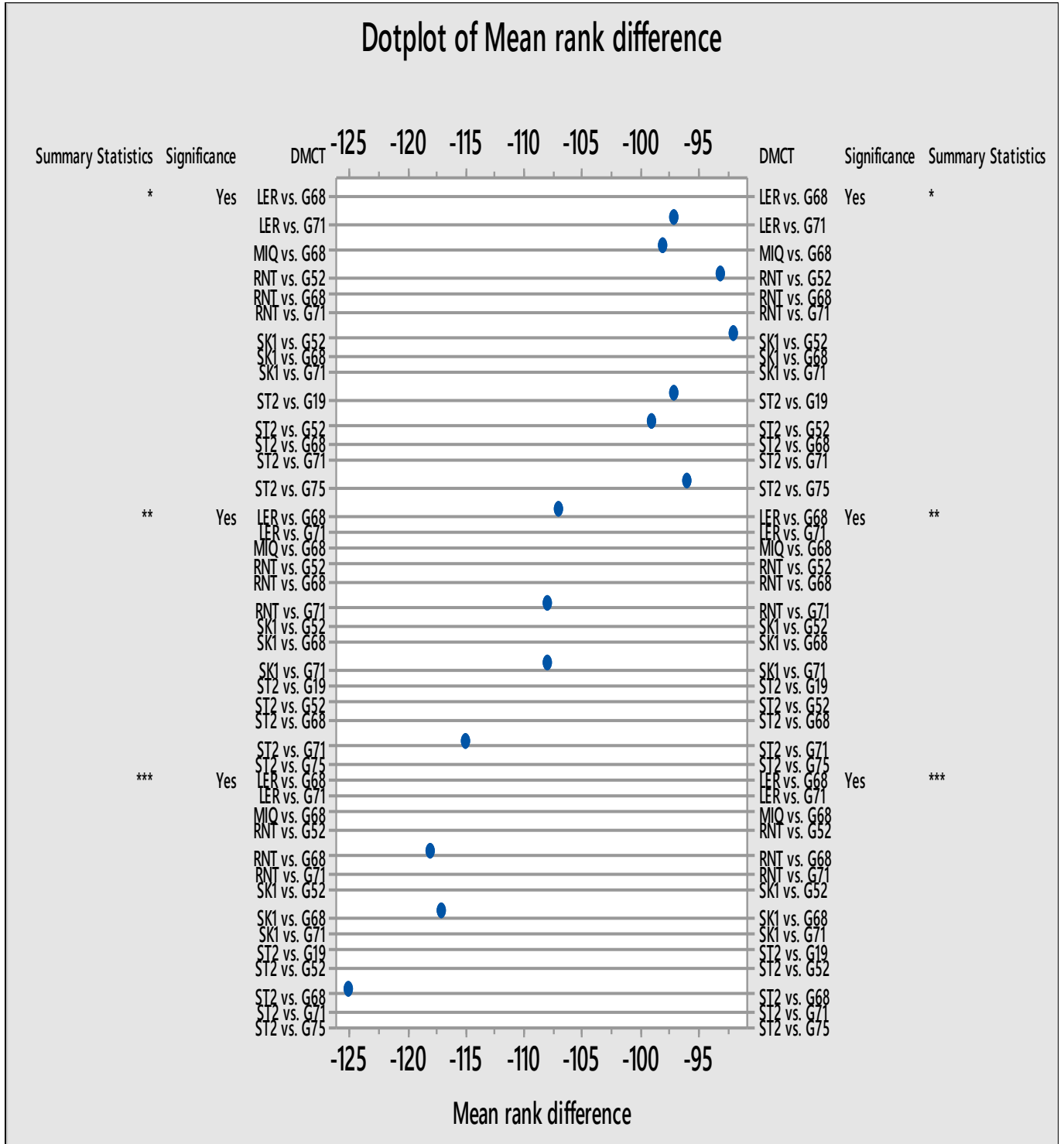


Figure 6. The outcome of the mean rank difference from the statistically significantly different segments of the water distribution system in the microbiological density

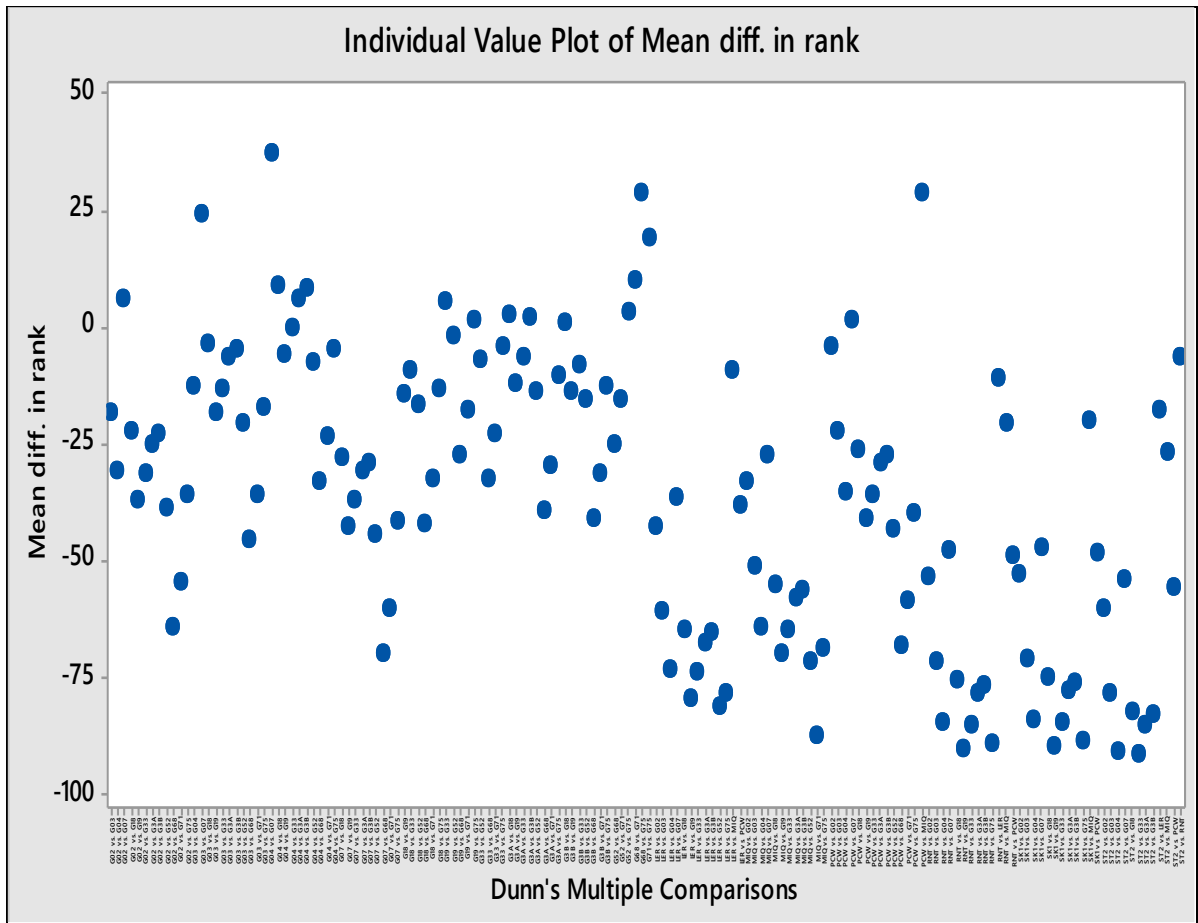


Figure 7. The outcome of the mean rank difference computed for the statistically not different water distribution system segments in the microbiological count

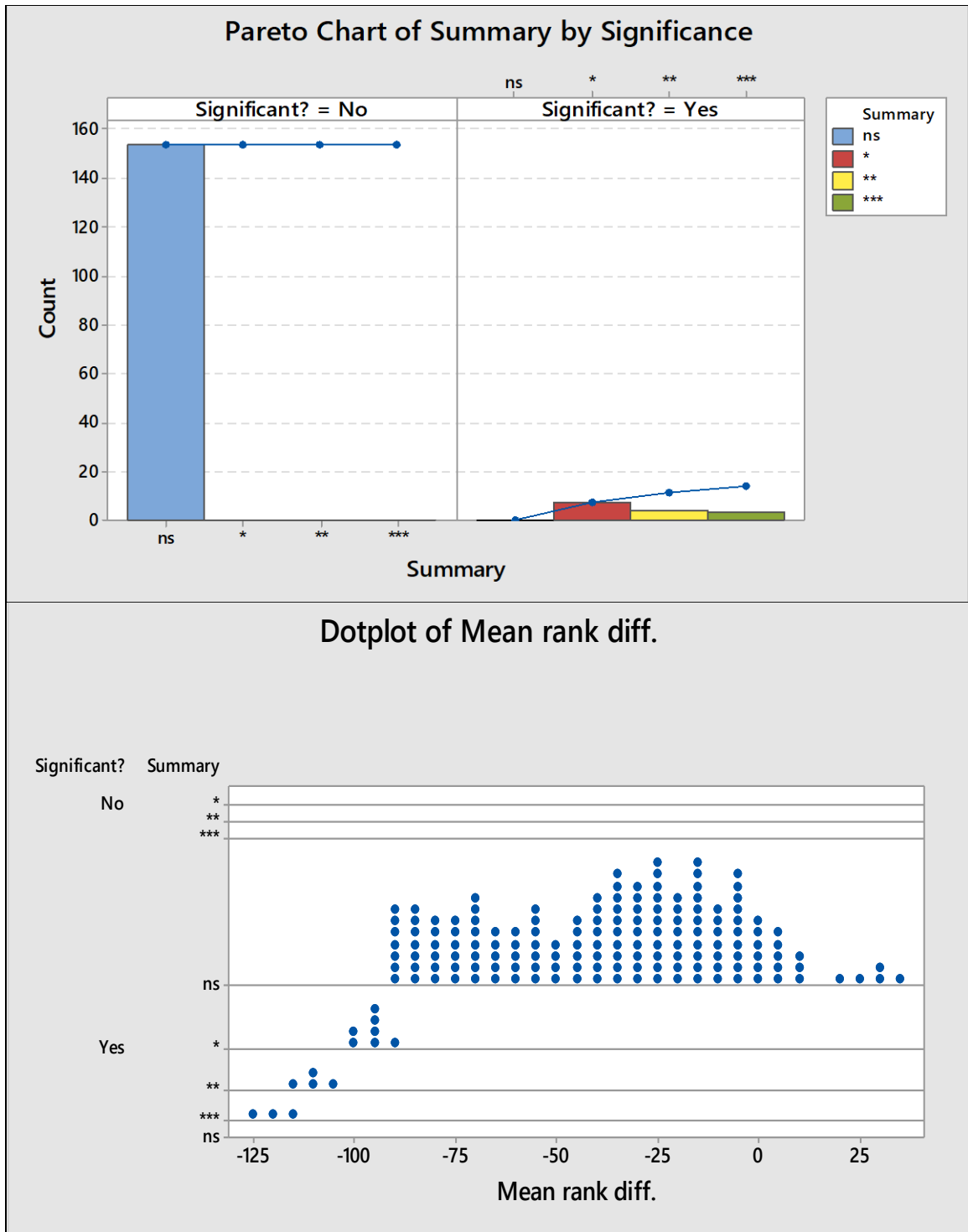


Figure 8. Mean rank analysis of the non-parametric Kruskal-Wallis test summing up the multiple comparison results (ns: not significant ($p > 0.05$), $*p \leq 0.05$, $**p \leq 0.01$) and $***p \leq 0.001$)

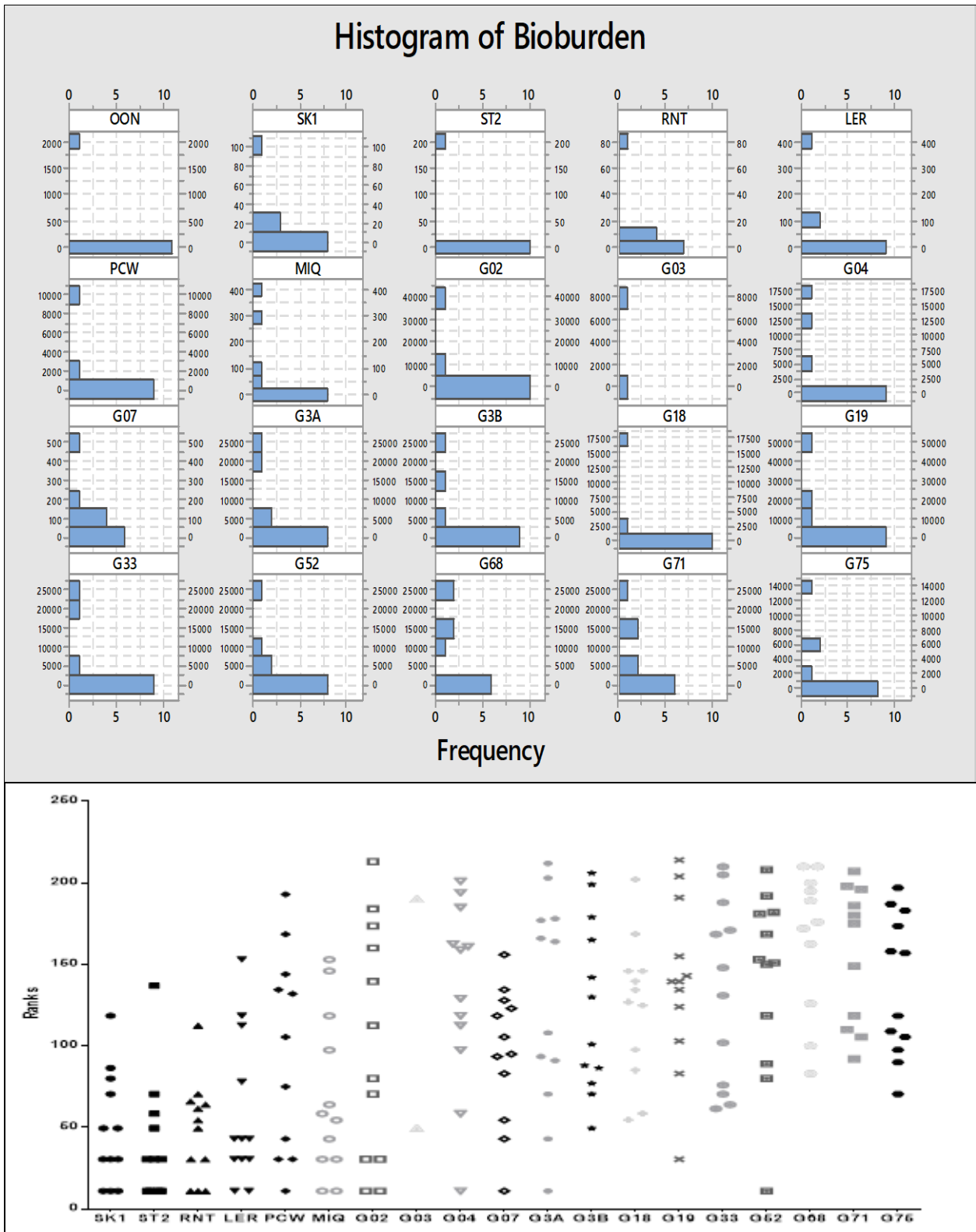


Figure 9. Histogram for distribution visualization (upper graph) and Kruskal-Wallis test showing the ranks of microbiological data for each examined use point (lower graph)

Table 2. Non-parametric Spearman correlation matrix showing two-tailed correlation coefficient (r) and p-values

Correlation Matrix	SK1	ST2	RNT	LER	PCW	MIQ	G02	G04	G07	G3A	G3B	G18	G19	G33	G52	G68	G71	G75
SK1		0.79	0.34	0.77	-0.33	0.59	0.31	0.24	-0.29	0.41	0.60	-0.50	0.03	0.22	0.80	0.57	0.31	0.29
ST2	0.79		0.31	0.65	-0.53	0.51	0.12	0.34	-0.33	0.23	0.47	-0.61	0.40	-0.07	0.87	0.30	0.10	0.13
RNT	0.34	0.31		0.56	0.25	-0.06	-0.22	0.23	0.17	-0.04	-0.04	-0.47	-0.19	-0.35	0.37	0.25	0.11	-0.07
LER	0.77	0.65	0.56		-0.46	0.36	0.09	0.27	0.01	0.17	0.13	-0.46	0.04	0.12	0.57	0.35	0.23	0.00
PCW	-0.33	-0.53	0.25	-0.46		-0.35	0.09	0.18	0.31	-0.14	-0.17	0.20	-0.47	-0.13	-0.41	-0.16	0.02	-0.15
MIQ	0.59	0.51	-0.06	0.36	-0.35		0.66	0.14	-0.44	0.39	0.72	0.20	0.47	0.60	0.52	0.20	-0.19	0.54
G02	0.31	0.12	-0.22	0.09	0.09	0.66		0.32	-0.24	0.18	0.48	0.33	0.32	0.67	0.17	0.03	0.12	0.15
G04	0.24	0.34	0.23	0.27	0.18	0.14	0.32		0.09	0.34	0.09	0.02	0.05	0.36	0.39	0.06	0.35	-0.16
G07	-0.29	-0.33	0.17	0.01	0.31	-0.44	-0.24	0.09		-0.43	-0.73	0.15	-0.39	-0.41	-0.57	-0.36	-0.16	-0.13
G3A	0.41	0.23	-0.04	0.17	-0.14	0.39	0.18	0.34	-0.43		0.72	0.20	-0.11	0.70	0.48	0.76	0.49	0.31
G3B	0.60	0.47	-0.04	0.13	-0.17	0.72	0.48	0.09	-0.73	0.72		-0.02	0.17	0.56	0.69	0.71	0.36	0.53
G18	-0.50	-0.61	-0.47	-0.46	0.20	0.20	0.33	0.02	0.15	0.20	-0.02		0.08	0.57	-0.57	-0.21	-0.30	0.14
G19	0.03	0.40	-0.19	0.04	-0.47	0.47	0.32	0.05	-0.39	-0.11	0.17	0.08		0.20	0.19	-0.36	-0.50	-0.29
G33	0.22	-0.07	-0.35	0.12	-0.13	0.60	0.67	0.36	-0.41	0.70	0.56	0.57	0.20		0.17	0.31	0.25	0.15
G52	0.80	0.87	0.37	0.57	-0.41	0.52	0.17	0.39	-0.57	0.48	0.69	-0.57	0.19	0.17		0.55	0.36	0.28
G68	0.57	0.30	0.25	0.35	-0.16	0.20	0.03	0.06	-0.36	0.76	0.71	-0.21	-0.36	0.31	0.55		0.74	0.45
G71	0.31	0.10	0.11	0.23	0.02	-0.19	0.12	0.35	-0.16	0.49	0.36	-0.30	-0.50	0.25	0.36	0.74		0.14
G75	0.29	0.13	-0.07	0.00	-0.15	0.54	0.15	-0.16	-0.13	0.31	0.53	0.14	-0.29	0.15	0.28	0.45	0.14	

Correlation coefficient (r)	Lower	Upper	Lower	Upper
Very weak	0.00	0.19	0.00	-0.19
Weak	0.20	0.39	-0.20	-0.39
Moderate	0.40	0.59	-0.40	-0.59
Strong	0.60	0.79	-0.60	-0.79
Very Strong	0.80	1.00	-0.80	-1.00

p*	SK1	ST2	RNT	LER	PCW	MIQ	G02	G04	G07	G3A	G3B	G18	G19	G33	G52	G68	G71	G75
SK1		0.6	27.1	0.5	28.9	4.8	31.6	45.3	33.1	18.3	4.3	8.3	91.7	49.6	0.3	7.1	36.2	36.1
ST2	0.6		34.6	3.6	9.0	11.3	72.6	29.9	27.2	48.3	15.0	3.9	22.2	76.2	0.1	36.2	76.2	69.8
RNT	27.1	34.6		6.2	45.8	81.2	45.6	47.6	60.4	88.0	87.2	11.0	53.3	25.4	23.5	44.7	74.6	81.1
LER	0.5	3.6	6.2		13.0	25.0	77.7	39.3	97.8	60.2	69.0	12.2	91.4	71.6	5.7	29.1	48.9	96.9
PCW	28.9	9.0	45.8	13.0		27.1	78.2	60.0	35.9	66.7	60.8	55.9	13.7	69.8	20.5	62.5	95.3	63.8
MIQ	4.8	11.3	81.2	25.0	27.1		2.2	66.2	14.7	21.0	1.1	52.0	12.8	4.4	8.9	55.7	56.0	7.5
G02	31.6	72.6	45.6	77.7	78.2	2.2		30.4	44.5	57.5	11.3	28.8	30.7	2.0	59.8	92.2	71.7	64.6
G04	45.3	29.9	47.6	39.3	60.0	66.2	30.4		78.3	27.6	78.3	95.1	89.1	25.6	21.0	85.4	28.6	61.9
G07	33.1	27.2	60.4	97.8	35.9	14.7	44.5	78.3		16.9	0.9	64.6	21.1	18.4	5.6	26.8	63.4	68.3
G3A	18.3	48.3	88.0	60.2	66.7	21.0	57.5	27.6	16.9		1.1	52.3	72.8	1.4	12.1	0.9	12.9	33.1
G3B	4.3	15.0	87.2	69.0	60.8	1.1	11.3	78.3	0.9	1.1		95.1	59.9	6.3	1.6	1.7	27.3	7.9
G18	8.3	3.9	11.0	12.2	55.9	52.0	28.8	95.1	64.6	52.3	95.1		79.7	5.6	5.4	51.2	35.9	65.5
G19	91.7	22.2	53.3	91.4	13.7	12.8	30.7	89.1	21.1	72.8	59.9	79.7		53.8	55.3	26.3	12.2	35.0
G33	49.6	76.2	25.4	71.6	69.8	4.4	2.0	25.6	18.4	1.4	6.3	5.6	53.8		58.8	35.1	45.1	63.5
G52	0.3	0.1	23.5	5.7	20.5	8.9	59.8	21.0	5.6	12.1	1.6	5.4	55.3	58.8		8.3	27.3	37.9
G68	7.1	36.2	44.7	29.1	62.5	55.7	92.2	85.4	26.8	0.9	1.7	51.2	26.3	35.1	8.3		1.2	16.9
G71	36.2	76.2	74.6	48.9	95.3	56.0	71.7	28.6	63.4	12.9	27.3	35.9	12.2	45.1	27.3	1.2		69.4
G75	36.1	69.8	81.1	96.9	63.8	7.5	64.6	61.9	68.3	33.1	7.9	65.5	35.0	63.5	37.9	16.9	69.4	

* Expressed as %

p-value	p-value %	Evidence for rejecting H ₀
More than 0.1	> 10%	Very weak to none
Between 0.1 - 0.05	5% 10%	Weak
Between 0.05 - 0.01	5% 1%	Strong
Less than 0.01	< 1%	Very strong

4.2. Microbiological fluctuations and outliers over study period for the examined point-of-use

The results in the corresponding result section provided highlights the significance of microbiological fluctuations and outliers at various water use ports, as well as the use of the Coefficient of Variation (CV) to assess the variability and precision of microbiological counts in a water line. The reference to Insee (2016) adds credibility to the explanation of the CV as a statistical measure that relates the standard deviation of a set of values to its mean. Furthermore, the reference to Hayes (2022) supports the assertion that the CV is used to compare the degree of variability between distinct sets of microbiological counts, reinforcing the importance of this statistical measure in the context of water quality assessment.

Additionally, the reference to Jelliffe et al. (2015) underscores the utility of the CV in assessing the precision of estimates, further emphasizing its relevance in evaluating water quality. The explanation of how a lower CV indicates a more precise estimation of water quality at certain ports, while a higher CV suggests greater uncertainty or variability, provides a clear understanding of the implications of the CV in this context. The discussion of fluctuation in the coefficient of variation as a useful statistical tool for assessing variability and precision in water distribution system datasets is supported by the references and adds depth to the analysis. Finally, the information provided offers a comprehensive understanding of the significance of microbiological fluctuations and outliers at water use ports, as well as the use of the CV as a statistical measure to assess variability and precision in water quality assessment.

4.3. Descriptive statistical distribution of the data related to microbiology

The establishment of an early warning alarm using a cut-off threshold for microbiological counts in city water distribution lines is a critical measure for safeguarding public health. The reference to Eissa et al. (2015) supports the rationale for setting a warning limit for microbiological counts below the action threshold, emphasizing the importance of this early warning system in monitoring water quality. Furthermore, the use of the non-parametric *t*-test analog test to identify lines with a higher potential of bioburden density, as mentioned in the context of GraphPad Software, LLC (2023), adds credibility to the methodology used in this study. This statistical approach is crucial for identifying segments of the distribution system that exhibit aberrant microbial density, providing a basis for establishing the early warning alarm.

The discussion of distribution segments that showed actual medians statistically not significantly different from the reference value at $\alpha=0.05$, such as use ports G68, G71, and G3A, as well as the indication of discrepancy values and two-tailed *p*-values, as shown in Figure 4, demonstrates the rigorous statistical analysis employed in identifying the

sources of microbial density aberrancy. The reference to the remaining points of use being far below the alarming limit and statistically different from it further reinforces the effectiveness of the early warning alarm system in distinguishing between acceptable and aberrant microbiological counts. In summary, the information provided offers a comprehensive understanding of the rationale and statistical methodology behind the establishment of an early warning alarm using a cut-off threshold for microbiological counts in city water distribution lines.

4.4. Comparing microbiological record data from under investigation points of use pairwise

The significance of variations between different municipal water distribution segments can be investigated through non-parametric multiple comparisons using the Kruskal-Wallis test, as indicated by Eissa (2016b). This statistical approach is crucial in the medical and public health context for understanding and addressing variations in bioburden quality across different lines of the distribution system. The reference to Table 1, which highlights the main findings of the statistical test, provides empirical evidence of detectable differences in bioburden quality between different lines of the distribution system, underscoring the importance of investigating these variations.

Furthermore, the detailed examination of the sources of significant variability projected from Figures 6 and 7, which depict the mean rank difference for significant and non-significant pairwise comparisons, respectively, offers a comprehensive understanding of the nature and extent of the variations in bioburden quality. The identification of a few use points as the reason behind the significance of these variations, due to their contribution to unusually high microbial density in water samples, is a critical insight that can inform targeted interventions to address the sources of contamination. The main effectors contributing to unusually high microbial density in water samples are the factors marked with significance levels (*, **, ***), which indicate that there are statistically significant differences in the mean rank of the bacterial counts among the different use points. These factors could include contamination sources, environmental conditions, or human activities affecting water quality. For example, some of the possible factors are: type of water source, implemented treatment, location of the site, population growth, lack of protection, agriculture, urbanization/sanitation, and flooding threats.

These factors affect the overall water quality by introducing or promoting the growth of pathogenic or opportunistic microorganisms, such as *Escherichia coli*, *Legionella*, *Pseudomonas*, etc., which can pose health risks to humans and animals. Moreover, these factors can also alter the physicochemical properties of water, such as pH, turbidity, dissolved oxygen, etc., which can affect the taste, odor, color, and clarity of water.

The reference to Figure 8, which illustrates the count of the main effectors in the municipal distribution system in relation to other stable and low bioburden water lines, further supports the identification of key points of discrepancy and their impact on overall bioburden quality. Additionally, the reference to Figure 5, which identifies points of discrepancy between two distinct groups, and Figure 9, which provides criteria for ranking the microbiological sampling output results and demonstrates the range, spreading, and scatter of the data points, offers valuable insights into the distribution and shape of microbial count data. In summary, the information provided offers a comprehensive understanding of the significance of variations in bioburden quality in municipal water distribution segments and the importance of employing non-parametric statistical tests and visualizations to investigate and address these variations from a medical and public health perspective.

4.5. Non-parametric correlation matrix for the municipal water system's points of use

The significance of variations between different municipal water distribution segments could be investigated through non-parametric multiple comparisons Kruskal-Wallis test (Eissa, 2016b). The apparent shape of the distribution in the histograms of Figure 8, showing truncated spreading of the microbial count data, is a crucial observation from a public health and medical perspective. This observation suggests that the datasets do not follow a normal distribution, highlighting the non-normal nature of the microbial count data. The surveying of the correlation between use points under examination using a non-parametric Spearman matrix, as conducted by Eissa and Rashed (2022), is a significant approach in understanding the relationships and associations within the microbial count data. The detailed output presented in Table 2, which includes the calculated two-tailed correlation coefficient (r) and the corresponding p -values, provides valuable insights into the strength of the associations and correlations among the use points.

The identification of the lowest negative but strong correlation between G07 and G3B, along with the visual color marking for ease of scanning and spotting of the significant correlation, underscores the importance of understanding the interrelationships between different use points in the distribution system. The specific correlations identified, such as the strong associations between G02 and PCW, SK1 and ST2, G52 and G68, and others, offer valuable insights into the interconnectedness of the use points and their impact on the bioburden data. The presence of very strong positive correlations between G52 and ST2, as well as strong correlations between G71 and G68, further highlights the complex interdependencies within the bioburden data, providing critical information for public health and medical interventions. In summary, the findings from the non-parametric Spearman matrix analysis provide valuable insights into the correlations and

associations between use points in the distribution system, offering a comprehensive understanding of the relationships within the microbial count data from a public health and medical perspective.

The significance of variations between different municipal water distribution segments can be investigated through non-parametric multiple tests. This statistical approach is crucial in the medical and public health context for understanding and addressing variations in microbial quality across different lines of the distribution system. The presence of very strong positive correlations between few points within the municipal network system, highlight the complex interdependencies within the bioburden data and provide critical information for public health and medical interventions. The identification of a few use points as the reason behind the significance of these variations, due to their contribution to unusually high microbial density in water samples, is a critical insight that can inform targeted interventions to address the sources of contamination. Finally, the non-parametric statistical tests reveal significant variations in bioburden quality between different use points and lines of the distribution system, which have important implications for water quality monitoring and public health protection strategy.

5. Conclusion

The biological stability of the municipal distribution systems is an important aspect to attain and maintain. Monitoring and controlling the microbiological quality throughout the distribution lines is vital until reaching the final consumers. Only six points from the 19 water-use ports showed more than 65% of the total bioburden recovered from this study with one line alone demonstrating an average of 16%. The study is limited by the amount of data available at the early stages of the infrastructure examination. More results are required to be gathered to construct control charts that might show the behavior of the inspection characteristics under investigation. However, the municipal system showed at the initial examination that control measures must be put in place to improve the microbiological stability despite the fact of presence of low microbial count within some sections in the system yet the fluctuations and variabilities were considerably high. Correlating obtained results with layout infrastructure should be addressed in other separate investigations using engineering design to correct for structure-related issues. Future research could build upon the findings of this study by expanding the scope and frequency of data collection, as well as exploring the factors that influence the biological stability of the municipal distribution systems.

Future research can refine our understanding of bioburden variations in water distribution systems. Collecting more samples from diverse water use points over extended periods would allow for more accurate control charts and trend identification. Examining the connections between biological instability and water source, treatment, pipe

materials, and flow rates could shed light on root causes. Finally, experimenting with interventions like treatment modifications, pipe cleaning, or biostatic agents would help determine the most effective strategies for reducing bioburden and ensuring consistent water quality.

Maintaining the biological stability of municipal water distribution systems is crucial for public health. Our study highlights that only six out of 19 water-use ports contribute significantly to the total bioburden, warranting immediate control measures. While data limitations exist, this work lays the groundwork for future investigations, emphasizing the need for control charts and correlation studies with infrastructure design. The inconclusive correlation matrix suggests potential design-related defects, prompting further exploration using logarithmic transformations. Recommendations include investigating the impact of source feed water and advocating for regular monitoring programs nationwide. The presented statistical analysis by public health authorities remains pivotal for ongoing assessments and enhancements in water safety protocols.

Conflict of interest

The author declares that there is no conflict of interest.

Ethical Approval

For this type of study, formal consent is not required.

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