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## The Use of Non-Parametric Bootstrap in Computing of Microbial Risk Assessment from Lettuce Consumption Irrigated with Contaminated Water by Sanitary Sewage in Infulene Valley

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Abstract: The Metropolitan area of Maputo (Mozambique Capital City) is located in semi-arid zone (800 mm annual rainfall) with 1101170 million inhabitants. On the west side, there are the flatlands of Infulene where the Mulauze River flows towards to the Indian Ocean, receiving at this site, the storm water contaminated with sanitary sewage from Maputo, transported through a concrete open channel. In Infulene, local communities grow salads crops such as tomato, onion, garlic, lettuce, and cabbage, which are then commercialized and consumed in several markets in Maputo City. Lettuce is the most daily consumed salad crop in different meals, generally in fast-foods, breakfasts, lunches, and dinners. However, the risk of infection by several pathogens due to the consumption of lettuce, using the Quantitative Microbial Risk Assessment (QMRA) tools, is still unknown since there are few studies or publications concerning to this matter in Mozambique. This work is aimed at determining the annual risk arising from the consumption of lettuce grown in Infulene valley, in Maputo, using QMRA tools. The exposure model was constructed upon the volume of contaminated water remaining in the lettuce leaves, the empirical relations between the number of pathogens and the indicator of microorganisms (E. coli), the consumption of lettuce (g) and reduction of pathogens (days). The reference pathogens were Vibrio cholerae, Cryptosporidium, norovirus, and Ascaris. The water quality samples (E. coli) were collected in the storm water channel from January 2016 to December 2018, comprising 65 samples, and the urban lettuce consumption data were collected through inquiry in Maputo Metropolis covering 350 persons. A nonparametric bootstrap was performed involving 10,000 iterations over the collected dataset, namely, water quality (E. coli) and lettuce consumption. The dose-response models were: Exponential for Cryptosporidium, Kummer Confluent hypergeomtric function (1F1) for Vibrio and Ascaris Gaussian hypergeometric function (2F1-(a,b;c;z) for norovirus. The annual infection risk estimates were performed using R 3.6.0 (CoreTeam) software by Monte Carlo (Latin hypercubes), a sampling technique involving 10,000 iterations. The annual infection risks values expressed by Median and the 95th percentile, per person per year (pppy) arising from the consumption of lettuce are as follows: Vibrio cholerae (1.00, 1.00), Cryptosporidium (3.91x10<sup>-3</sup>, 9.72x  $10^{-3}$ ), nororvirus (5.22x $10^{-1}$ , 9.99x $10^{-1}$ ) and Ascaris (2.59x $10^{-1}$ , 9.65x $10^{-1}$ ). Thus, the consumption of the lettuce would result in greater risks than the tolerable levels ( $< 10^{-3}$  pppy or  $10^{-6}$  DALY) for all pathogens, and the Vibrio cholerae is the most virulent pathogens, according to the hit-single models followed by the Ascaris lumbricoides and norovirus. The sensitivity analysis carried out in this work pointed out that in the whole QMRA, the most important input variable was the reduction of pathogens (Spearman rank value was 0.69) between harvest and consumption followed by water quality (Spearman rank value was 0.69). The decision-makers (Mozambique Government) must strengthen the prevention measures related to pathogens reduction in lettuce (i.e., washing) and engage in wastewater treatment engineering.

Keywords: annual infections risk, lettuce, non-parametric bootstrapping, quantitative microbial risk assessment tools

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