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Microbial Menaces and their Biodiversity Present in Crops Irrigated with Polluted Yamuna Water in Peri-urban Agriculture area of Delhi and National Capital regions, India

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Crops can become contaminated with microorganisms capable of causing human diseases while still on the plant in fields or orchards, or during harvesting. The objective of this study was to assess the contamination level of microbial load, yeasts, molds, Coliform and *Escherichia coli* on their surface and biodiversity indexes of microorganisms. The bacterial counts were found to be higher than the Yeast and Moulds counts. The range of bacterial population is 8.72-5.04 log cfu/g and for the fungal is 6.53 - 2.30 log cfu/g. Organisms isolated from vegetables samples included *Saccharomyces*, *Aspergillus*, *Rhizopus*, *Staphylococcus*, *Klebsiella*, *Streptococcus*, *Bacillus*, *Escherichia*, *Pseudomonas*, *Salmonella* and *Diplococcus* with various percentages of occurrences. Streptococcus and Aspergillus are the most frequently isolated genera in all studied crops. Staphylococcus (86.36%) and Pseudomonas (77.27%) present in the studied crops. It's the main cause of unwanted diseases in Delhi and NCR. The biodiversity indexes i.e. Simpson (0.20) and Shannon (3.31) of microorganism show the high species richness and diversified microbial community. The microorganisms isolated are opportunistic and mostly pathogenic in nature. The microbial load also very high which gives the signal of health alertness before using these contaminated vegetables.

Keywords: Aspergillus, Diplococcus, Microbial load, Streptococcus, Vegetables, Simpson Index.

The consumption of contaminated raw vegetables may lead to food poisoning due to presence of harmful and pathogenic microorganism[1] The outbreaks of food poisoning cases have therefore highlighted the importance of microbial quality assessment of food commodities. Raw vegetables are known potential for a wide range of microorganisms, including human pathogens[2]. Pathogens commonly detected in fresh vegetables are *E. coli*, coliform bacteria, *Staphylococcus* sp., *Salmonella* sp *Campylobacter spp., Shigella spp., Clostridium botulinum, Listeria monocytogenes, Clostridium perfringens*, some *Bacillus spp., and Streptococcus*[3] The increasing numbers of pathogens in vegetables are due to the widespread use of human and animal faecal waste water and Industrial waste water in agricultural practices[4]. It is not surprising that enteric pathogens can contaminate agricultural produce and cause outbreaks of illness due to the following contaminated consumption[5].Spores of *Clostridium botulinum, Clostridium perfringens*, and *Bacillus cereus* can also be isolated from fresh vegetables and *Listeria monocytogenes* can be found in decaying vegetables[6].These microorganisms are likely to contaminate raw fruits and vegetables through contact with faeces, sewage, untreated irrigation water or surface water[7] Factors contribute

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to real increases in diseases associated with fruits and vegetables due to use of wastewater, increased application of improperly composted manures to soils in which fruits and vegetables are grown, extended time between harvesting and consumption, and changing food consumption patterns[8] The presence of a pathogen on produce is of less consequence if the rind, skin or peel is to be removed before consumption. Potato, Onion, Bottle gourd, Bitter gourd and all other vegetables having the peel, fall in this category[9] However, Microorganisms that have become trapped on the inner leaves of certain vegetables can be particularly difficult to remove by routine cleansing practices[10]. Application of improperly composted manure or water containing raw sewage to fields, or the use of water contaminated with faeces during processing of fruits and vegetables must be avoided[11]. The microbial quality of vegetables grown with wastewater is highly alarming. Several studies throughout the world have demonstrated a very close relation between the consumption of fruits and vegetables irrigated with raw wastewater and many food borne diseases like gastroenteritis, cholera, chemical toxicity etc[12]

Due to increasing consumption of vegetables as a result of awareness of its health benefits and report showing impacts of vegetables irrigated by using untreated wastewater on health were available[13]. The need to assess the microbial quality of irrigated vegetables in Delhi, India is eminent in order to propose alternatives to prevent consumers from disease outbreaks. Therefore, the present study was undertaken to examine the quality of Crops for microbial contamination, irrigated from waste water from Delhi and peri urban area.

MATERIALS AND METHOD

Study area

The study was carried out in Delhi –NCR peri urban area. The samples of crops were collected from the 15 different sites of Yamuna pusta region. i.e. Palla, Christian ashram, Jagatpur, Sonia Vihar, Wazirabad, Shastri park, Indraprastha, Okhla, Noida, Basantpur, Nehru vihar, Daryia nalla, Punjabi bagh, Keshopura and Nilothi. Crops grown in these areas are gone for the sale in local market. [Fig. 1].

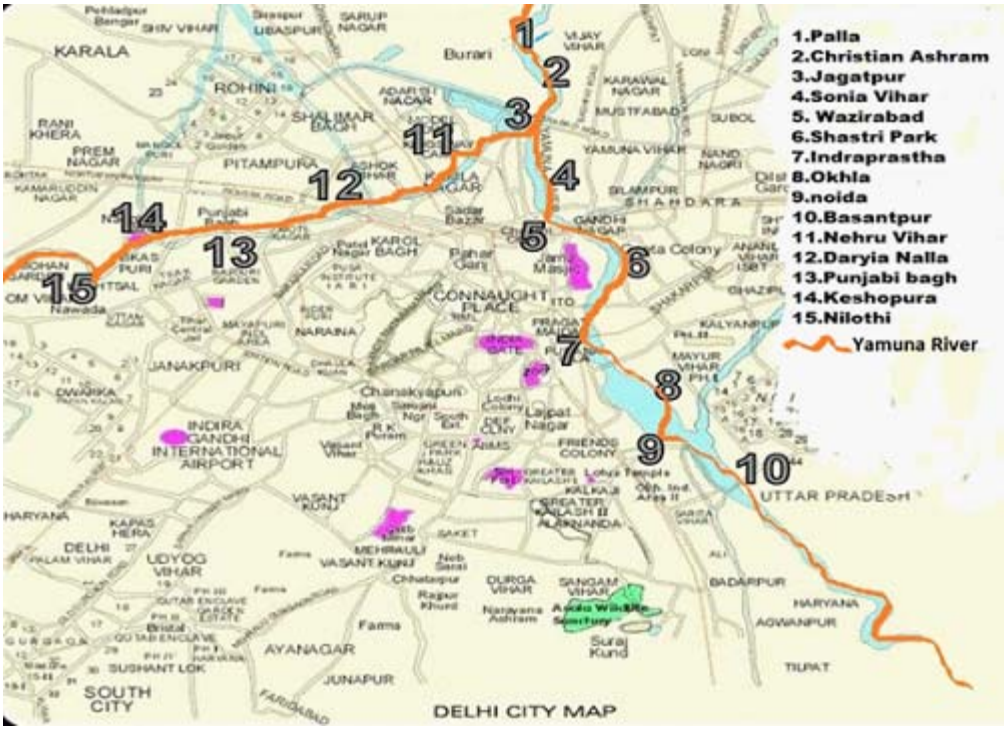

Fig. 1. Map of 15 sampling sites of Delhi-NCR peri urban area

Sample collection and processing

Four samples of each crop from 15 Different sites were collected as shown in Fig. 1. Random sampling procedure was adopted to collect the sample materials. The vegetables samples were collected in sterile polythene zip bags to laboratory for storage in refrigerator.

Microbial analysis of crops

Twenty five gram [25 g] of each crop sample were weighed and blended in 100 mL of sterile saline solution for 2 minutes under sterile conditions. The blender was carefully disinfected to prevent any cross contamination. The homogenates were collected in sterile bottles and stored at -20°C until needed. Aliquots [1 mL] of each homogenate were serially diluted in sterile saline solution. The diluents of buffered peptone water were then inoculated on to the respective media. Yeast and Mould count in crops was determined by the procedure described by IS 5403: 1999[14] Coliforms bacteria in crops were determined by the procedure described by IS 5401[II]: 2002[15] Total plate count in crops was determined by the procedure described by IS 5402:2002[16] *E. coli* in crops was determined by the procedure described by IS 5887[I]: 1976,[17]Salmonella, Streptococcus and Staphylococcus by IS 5887: 1976, [18]Vibrio by IS5887:1976,[18]Pseudomonas by IS 13428:2005.[19]Gram reaction and biochemical tests such as catalyse, oxidase, urease, citrate, methyl red test, Voges Proskauer, indole, sugar fermentation were performed to identify other bacteria [20]while fungal isolates were determined based on their cultural and microscopic morphology[21]

RESULTS AND DISCUSSION

Total twenty two different varieties of crops studied for the Microbial quality analysis. After studying all the crop samples, the bacterial count as found more than the fungal count in studied crops [Table 1]. The microbial study of crops also shows that the different crops have different concentration of microbial load on it which makes the crops, specific for the contamination and also not suitable to eat in regards to human.

The organisms isolated from the 22 different varieties of samples are *Saccharomyces*, *Aspergillus*, *Rhizopus*, *Staphylococcus*, *Klebsiella*, *Streptococcus*, *Bacillus*, *Escherichia*, *Pseudomonas*, *Salmonella* and *Diplococcus*. The percentage of occurrence of Aspergillus and Streptococcus (100), Staphylococcus(86.6), Pseudomonas(77.27), E.coli(59.09),Salmonella(36.36), Bacillus(36.6), Saccharomyces(31.81), Rhizopus(27.27), Klebsiella(22.72) and Diplococcus(4.54) in studied crops. The bacterial and fungal count of microorganism ranges from 8.72-5.08 log cfu/g and 6.53-2.3 log cfu/g respectively [Table 1].

On studying, the percentage of occurrence of microorganism in the crops, Streptococcus and Aspergillus are the organism present in all crops. While diplococcus is rarest

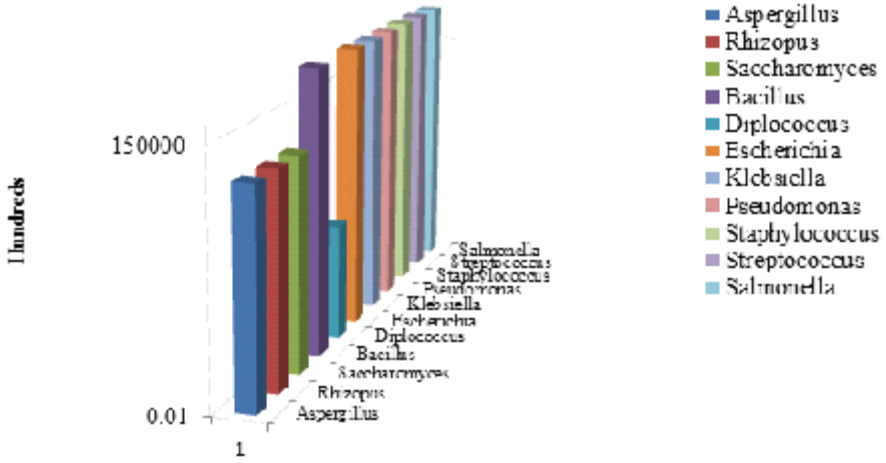

Fig. 2. Microbial population in crops

microorganism in the studied crops. Most of the microorganisms are pathogenic, which cause serious diseases to human who feed on these contaminated crops . *Pseudomonas* infects the pulmonary tract, urinary tract, burns, wounds, and also causes other blood infections[22]. *Aspergillus* causes otomycosis and aspergillosis in human[23]. *Bacillus* causes anthrax and food poisoning[24].

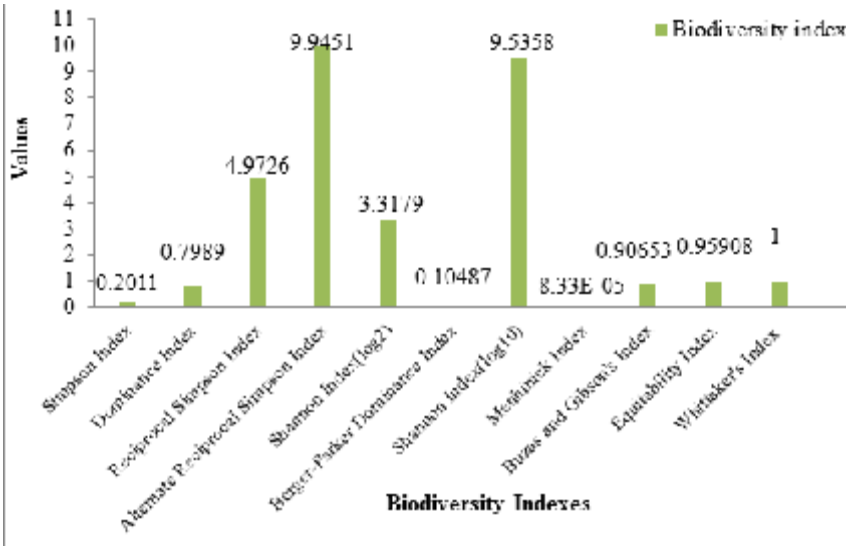

Fig. 3. Biodiversity Indexes

Table 1. Microbial load [fungus and bacteria] on crops

S.No.	Crops	Fungal Count [log cfu/g]±SD	Bacterial Count [log cfu/g] ±SD
1	Cucumber	4 ± 0.44	6.4 ± 0.45
2	Rice	2.3 ± 0.09	5.07 ± 1
3	Bitter gourd	3.3 ± 0.18	7.6 ± 0.31
4	Lady finger	4.4 ± 0.21	7.8 ± 0.39
5	Radish	4.1 ± 0.06	7.5 ± 0.12
6	Ridge gourd	5.3 ± 0.08	7.6 ± 0.39
7	Red Spinach	4.6 ± 0.04	7.8 ± 0.35
8	Mustard	4.4 ± 0.11	7.8 ± 0.24
9	Wheat	2.7 ± 0.15	5.04 ± 0.07
10	Spinach	5.7 ± 0.99	7.9 ± 0.10
11	Carrot	4.1 ± 0.22	7.5 ± 0.30
12	Armenian Cucumber	4 ± 0.04	7.5 ± 0.23
13	Pumpkin	3.3 ± 0.32	7.3 ± 0.15
14	Bottle gourd	5.4 ± 0.17	7.7 ± 0.17
15	Sorgham	4 ± 0.03	7.3 ± 0.06
16	Beans	3.3 ± 0.03	7.3 ± 0.06
17	Eddoe	6.5 ± 0.29	7.7 ± 0.06
18	Cabbage	4.6 ± 0.25	7.3 ± 0.22
19	Pigweed	4.08 ± 0.08	7.5 ± 0.40
20	Brinjal	3.3 ± 0.19	7.2 ± 0.02
21	Cauliflower	4.5 ± 0.07	8.7 ± 0.19
22	Tomato	4.3 ± 0.12	6.6 ± 0.33

Recommended limits in crops (WHO-ICMSF) : log 3 CFU/ g

Diplococcus causes Pneumonia[25] *Escherichia* causes urinary tract infection with diarrhoea and dysentery condition[26]. *Klebsiella* causes pneumonia,[27]urinary tract infections, septicemia, meningitis, diarrhea and soft tissue infections[28]. S*taphylococcus* causes a wide variety of diseases in humans and animals through either toxin production or penetration. Staphylococcal toxins are a common cause of food poisoning, as they can be produced by bacteria growing in improperly stored food items[29]. The most common sialadenitis is caused by staphylococci, as bacterial infections. *Streptococcus* causes the diseases that may be caused include streptococcal toxic shock syndrome, necrotizing fasciitis, pneumonia, bacteremia, acute rheumatic fever and acute glomerulonephritis [30]. *Salmonella* causes typhoid fever, paratyphoid fever, and food poisoning.

On other hand, the biodiversity study i.e. Alpha, Beta and Gamma diversity studied in the population [Tables 2 and 3]. The Simpson index comes around 0.20 and the approximation Simpson comes 0.10, the study reveals that the value of Simpson lean towards zero denotes the more diversified community[31]. Shannon index comes 3.31 which denote that the studied samples are highly diversified. All other values studied in alpha, beta and gamma diversity, support the diversification [Fig. 3].

The microbial quality of crops was investigated. *Rhizopus*, *Aspergillus*, *Saccharomyces* were fungi isolated from crops. Bacteria isolated included *Bacillus*, *Escherichia*, *Klebsiella*, *Staphylococcus*, *Streptococcus*, *Salmonella*, *Diplococcus* and *Pseudomonas* with various percentages of occurrences. Earlier, Similar organisms were isolated from fruits or crops[32]. Fungi are known to cause spoilage of crops. These microorganisms could contaminate the crops through the use of manure in the farm, spores from the atmosphere, wastewater use in irrigation and washing the crops[33]. Food-borne pathogens commonly detected in fresh crops which include *Salmonella*, *Shigella*, *E. coli*, *Streptococcus* and coliform bacteria[34]. The bacterial and fungal count of microorganism ranges from 8.72-5.08 log cfu/g and 6.53-2.3 log cfu/g, respectively. The presence of these microorganisms in crops creates a public health concern. The Alpha, Beta and Gamma diversity of the studied microorganism shows the excellent Simpson and Shannon index values. The value of these indexes is towards zero which proves that the biodiversity level of microorganism is diversified in studied crops. The high species diversification suggest that a greater number of successful species[35] and a more stable ecosystem, more ecological niches are available and the

Table 2. Alpha Biodiversity Indexes

Alpha Diversity	
Simpson Index	0.20
Simpson Index Approximation	0.10
Dominance Index	0.79
Dominance Index Approximation	0.89
Reciprocal Simpson Index	4.97
Alternate Reciprocal Simpson Index	9.94
Shannon Index	3.31
Berger-Parker Dominance Index	0.10
Shannon Index	2.29
Inverted Berger-Parker Dominance Index	9.53
Shannon Index	-0.998
Margalef Richness Index	0.424
Menhinick Index	8.33E-5
Rényi Entropy/Hill Numbers [r=0,1,2,"]	11,9.971, 9.94,H"-"
Buzas and Gibson's Index	0.906
Gini Coeffificient	6.098
Equitability Index	0.959
ln[] of Hill Numbers [0,1,2,"]:	2.39,2.299, 2.297,H"-"

Table 3. Beta Biodiversity Indexes

Beta Biodiversity	
Absolute beta Value [[S_0-c]-[S_1-c]...]:	10
Whittaker's Index [S/alpha]:	1
Sørensen's similarity index:	1
Alternate Whittaker's Index [S/alpha-1]:	0
Sørensen's similarity index [%]:	100%
Jaccard Index:	-1
Routledge beta-R Index:	3.667
Jaccard Index [%]:	-100%
Mountford Index:	-0.22
Number of Common Species:	11
Mountford Index [%]:	-22.22%
Bray Curtis dissimilarity	0
Gamma Biodiversity	
Absolute gamma [S_0+S_1...-c]:	0

environment is less likely to be hostile, complex food webs and environmental change is less likely to be damaging to the ecosystem as a whole.

In the present paper, the authors assessed the microbial load of twenty two different vegetable samples from fifteen sites of Delhi peri urban area. Contamination was found in all the studied crops. Cauliflowers, Lady Finger, Mustard, Red Spinach were the highly contaminated crops. Along with this, the value of Simpson and Shannon index is towards zero which proves that the biodiversity level of microorganism is diversified in studied crops. Beside soil microbes, other sources of their contamination are improper handling and waste water used for irrigation. Wastewater uses in agriculture will increase the microbial load in the crops[36]. Vegetables may be contaminated with pathogenic microorganisms during growing in the field or during harvesting, post harvesting, handling, processing and distribution. Therefore, vegetables may act as a reservoir for many microorganisms from which they will be colonized inside these vegetables and infect susceptible host. Sanitary measures should be adopted while handling crops to limit the level of microbial contamination

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