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Review

Tracking Microbes from Irrigation Water to Crops: The Potential of Metagenomics and Meta-Transcriptomics

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Abstract

Irrigated crops may harbor microbes from irrigation water that may be deleterious and pose risks to consuming humans and animals. To provide good and quality food void of contamination with harmful pathogens and reduce foodborne diseases, there exists the need to continuously monitor and trace the transition of microbes from irrigation water to crops. Traditional methods of culturing microbes provided the basic knowledge of the presence of microbes and their transition from irrigation water to the crop however, they are low throughput and cumbersome. The advent of new technologies that can provide high-throughput data have made monitoring of microbes in irrigation water easier generating multitudes of data for both culturable and fastidious microbes. Metagenomics and meta-transcriptomics techniques are promising as they boost generate massive data with less effort than the case of traditional methods. Their application in tracing microbes from irrigation water to crops showed that the crops could harbor harmful microbes present in irrigation water. However, further studies are required to improve databases, particularly for viruses and protozoa, standardizing metagenomics, and meta-transcriptomics data analysis protocols.

Keywords: water, irrigation, contamination, metagenomics, microbial communities, meta-transcriptomics, microbial communities.

Резюме

Напояваните култури могат да съдържат микроби от водата за напояване, които да бъдат вредни и да представляват риск за консумацията на хора и животни. За да се осигури добра и качествена храна без замърсяване с вредни патогени и да се намалят болестите, пренасяни с храни, е необходимо непрекъснато наблюдение и проследяване на преминаването на микробите от водата за напояване към културите. Традиционните методи за култивиране на микроби предоставят основни познания за наличието им и техния преход от водата за напояване към реколтата, но те са бавни и с ниска производителност. Появата на нови технологии, които могат да осигурят данни с висока производителност, направиха мониторинга на микробите във водата за напояване по-лесен, генерирайки множество данни както за култивирани, така и за взискателни микроби. Метагеномните и мета-транскриптомните техники са обещаващи, тъй като те стимулират генерирането на масивни данни с по-малко усилия, отколкото в случая с традиционните методи. Тяхното приложение при проследяване на микроби от водата за напояване до културите показа, че културите могат да съдържат вредни микроби, присъстващи във водата за напояване. Необходими са обаче допълнителни проучвания за подобряване на базите данни, особено за вируси и протозои, стандартизиране на протоколи за метагеномни и мета-транскриптомни анализи на данни.

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Introduction

Intense farming using irrigation is a reliable mode of food production that bypasses drought, and unreliable and erratic patterns of rainfall. The proportion of crops produced under irrigation will likely increase to meet the increased demand for food, which results from population growth and urbanization. In some farming areas, poor quality water such as wastewater may be used knowingly or unknowingly for irrigation. Due to the high nutrient content of wastewater, its application on agricultural fields improves the availability of nitrogen and phosphorus to crops (Hernández-Chover *et al*., 2024). However, the contamination of the soil and the plant with harmful microorganisms as well as other contaminants from the wastewater is a major pressing challenge of the application of wastewater (Sugurbekova *et al*., 2023). If overhead irrigation is used, aboveground fruits and vegetables may bear the contaminants which may at times survive until consumed. World Health Organization (WHO) (Mara and Kramer, 2008) recommended that wastewater should be treated before its application as irrigation water to minimize the load of various contaminants and pathogenic microbes. Despite the recommendation from WHO, many countries still irrigate crops with untreated wastewater (Herman *et al*., 2015).

Among the many challenges associated with irrigation with wastewater or other raw water sources is the introduction of enteric pathogens into the soil that can be absorbed by the crops or the splashing of these pathogens on the crop itself. The enteric pathogens that are found in fresh produce are the major causes of several foodborne diseases (Herman *et al*., 2015). Enteric pathogens introduced into the soil through irrigation with wastewater can survive for long periods in the rhizosphere of crop plants (Hamoud *et al*., 2023). Upon their establishment in the rhizosphere, the pathogens can penetrate the root of the crops via the subjacent rhizodermis layer and the inner root cortex (Detert and Schmidt, 2023). The pathogens that remain in the rhizosphere during the transportation from the soil to the root may influence the plant-associated microbes by competing for nutrients (Chepsergon and Moleleki, 2023).

Furthermore, environmental factors such as soil properties are believed to influence microbial communities (Schlaeppi *et al.*, 2013). Soil texture and plants present in the field are necessary for the interaction between the enteric pathogens introduced via the irrigation of wastewater and the microbial communities at the rhizospheric level. The microbial community in the rhizosphere and inside the plant root are interrelated and are believed to be influenced by the same factor (Compant *et al*., 2005). This warrants the use of metagenomics and meta-transcriptomics to uncover microbial populations and their metabolic activity in the irrigation water, soil, rhizosphere, and crop. From the few studies that have been undertaken, crucial insights on microbial composition and activity have come to light. For example, the work of Lüneberg *et al*. (2018) gave insight into the change in the microbial structure of the rhizosphere as well as microbes associated with the plant under the influence of irrigation water laden with enteric pathogens. In addition, analysis of the 16S rRNA gene recognized possibly harmful human and plant pathogens that could be transferred to humans through the eating of fresh produce. This review aims to present the feasibility of metagenomics and meta-transcriptomics in studying microbial water quality and tracing microbes from irrigation water to crop. For clarification, we provided greater coverage on the application of both techniques in studying microbial communities in water and crops, as well as their advantages and future perspectives in monitoring microbes in light of global climate uncertainty.

An overview of metagenomics and meta-transcriptomics

Metagenomics was first mentioned in a publication by Handelsman and colleagues in 1998 (Handelsman *et al*., 1998). Metagenomics is a comprehensive study of the entire genetic makeup of members of the microbial community in the sample, including viruses, prokaryotes, and eukaryotes (Masenya *et al*., 2024). It provides a set of research techniques, which include many related molecular methods to aid researchers in effectively exploring microbial communities (Oulas *et al*., 2015). Metagenomics studies can use either shotgun sequencing, 16S rRNA gene taxonomic marker, or both (Oulas *et al*., 2015). Shotgun sequencing analyses all the genes in the microbial community, while the 16S rRNA gene taxonomic marker captures bacteria and archaea within the community (Jo *et al*., 2020). Initially, metagenomics used Sanger sequencing technology (Sanger *et al*., 1977), and recently, next-generation sequencing (NGS) technology (Oulas *et al*., 2015). NGS sequencing technology allows the direct sequencing of metagenomic DNA without the need for cloning, thereby eliminating cloning biases (Jo *et al*., 2020). To assign a taxonomic and functional profile of the microbial community, generated data from the sequencing device must be analyzed by comparing the sequence reads to already existing da-

tabases and clustering them based on their similarity using bioinformatics tools (Oulas *et al*., 2015). In the absence of databases, the sequencing reads are clustered against one another (*de novo*) (Somerville *et al*., 2019). New developments that focus on the RNA have emerged providing an insight into the microbial activities via gene expression profiling. This approach assesses mRNA in an environmental sample thereby indirectly gauging gene expression and by so doing, biological activity can be predicted. This approach is called meta-transcriptomics.

For comprehensive identification of the actual activities of a microbial community, an approach that sequences and analyses the transcriptome of all members of the community is required (Aguiar-Pulido *et al*., 2016). The transcriptome is all the RNA content present in a cell at a given time including coding and non-coding RNA species (Wang *et al*., 2024). Whenever genes are expressed, the messenger RNA (mRNA) molecule exports the transcribed sequences from the DNA to the cytoplasm for the synthesis of protein inside a cell (Aguiar-Pulido *et al*., 2016). Therefore, the analysis of various mRNA transcripts gives the functional or gene expression information of a cell. Several low-throughput techniques have been used over the years to study gene expression from a single cell to the entire sample including northern blots, DNA microarrays, quantitative polymerase chain reaction (qPCR), and serial analysis of gene expression (Frye *et al*., 2018). However, these techniques could not analyze the transcriptome of the whole community in a sample, but only selected known genes (Meyers *et al*., 2004). Like metagenomics, the nucleic acid is directly extracted from the sample and sequenced by NGS (Salazar *et al*., 2019). In contrast to metagenomics, which predicts potential functional genes, meta-transcriptomics focuses on the genes that are collectively expressed by the community, which genes are highly expressed, and deferentially expressed genes under different conditions (Levy *et al*., 2018; Salazar *et al*., 2019). Several studies have used metagenomics and meta-transcriptomics independently for microbial community assessment and microbial community activities respectively; others combined both approaches to draw on the individual strengths of the techniques to provide better insights into the microbial microcosm and activities.

Metagenomics and meta-transcriptomics applications in microbial plant populations studies

Plants are exposed to a variety of microbial communities with different species and different biological activities (Andreote *et al*., 2014). Some of the members of the microbial community contribute to the growth, health, and protection of the plant while others are pathogenic (Brader *et al*., 2017). The advancement of molecular technology has provided great opportunities to understand the plant microbiome composition and diversity as well as their functions. With 16S rDNA-based metagenomics, many researchers have explored microbial communities in various plants such as potato (Inceoglu *et al*., 2011), *Tamarix aphylla* (Finkel *et al*., 2016), maize and soybean (Chen *et al*., 2017), wallaby grass, lucerne, kangaroo grass, Rhodes grass and cotton (Qiu *et al*., 2020) as well as variation in microbial composition as influenced by site, season, and host genetics (Goodrich *et al*., 2016), plant organ (Wu *et al*., 2020), roots exude (Zhalnina *et al*., 2018) and biotic and abiotic stress (Prabha *et al*., 2019). Fitzpatrick *et al*. (2018) used Illumina MiSeq to sequence the V4 region of the 16S rRNA gene and observed variation in the root bacterial diversity. A metagenomic study by Dong *et al*. (2019) reported a variation in bacterial diversity on and within different parts of the tomato plant as well as the root-zone soil and the rhizosphere. Similarly, Mitter *et al*. (2017) analyzed the 16S rRNA gene to identify the bacterial community associated with the root of barley and sweet clover planted in sand contaminated with oil. The results revealed a highly diverse bacterial community in the rhizosphere than in the endosphere compartments in both plants, with *Proteobacteria* dominating the endosphere, and *Acidobacteria* and *Gemmatimonadetes* enriched more in the rhizosphere. However, the amplicon sequencing technique has drawbacks in linking biological functions with the identified taxa (Sharpton, 2014).

In some studies, instead of targeting a specific marker gene for analysis, shotgun metagenomics is used where all extracted DNA is fragmented and independently sequenced (Kaushal *et al*., 2020). Shotgun metagenomics has been used to identify plant microbial community members and link the biological important processes such as nitrogen fixation (Eichinger *et al*., 2016) and bioremediation (Kumar *et al*., 2018). It has also been used to identify plant virus biodiversity as well as discover novel plant viruses (Mutuku *et al*., 2018). The metagenomic methods have also provided great insight into the detection of plant and human pathogens in a wide range of crops, both on farms and on shelves of retailers (Leonard *et al*., 2015). For example, a food safety shotgun metagenomic study

by Aw *et al*. (2016), discovered plant pathogenic viruses, and human and animal viruses in lettuce from fields, from the produce in distribution centers and commercial retailers.

For a better picture, an integration of 16S rRNA gene analysis and shotgun metagenomics has been applied to analyze the same microbiota associated with plants such as *Arabidopsis thaliana* (Regalado *et al*., 2020). This combination provides a platform to compare the results of the two approaches regarding relative and variability in abundance and further detects sequences that were missed by either technique (Aw *et al*., 2016; Regalado *et al*., 2020). Similarly, Bulgarelli *et al*. (2015) revealed the composition and active members of the bacterial community living at the root of barley by integration of 16S rRNA gene analysis and shotgun metagenomics sequencing. In addition, they discovered traits that play an essential role in bacterial survival and iteration with other root habitants as well as proteins associated with mobilization and transportation of nutrients. While metagenomics infers the structural and potential functional gene of the microbial community, meta-transcriptomics provides the actual functionality of the microbial community (Conesa *et al.*, 2016; Zulfiqar *et al*., 2024).

A variety of plant-microbe interaction studies has adopted meta-transcriptomics to identify an active microbial community, investigate the interactions or relationships between the microbiome and the host plant, and to a lesser extent the signaling pathways involved (Conesa *et al.*, 2016; Crump *et al*., 2018; Saminathan *et al*., 2018). For instance, meta-transcriptomics was used to compare microbial functional profiles in rhizospheres of wheat, oats, and peas (Turner *et al*., 2013). Similarly, Sham *et al*. (2019) used meta-transcriptomics to explore the active members of the microbial community in the rhizosphere of *A. thaliana*. Rezzonico *et al*. (2017) used an RNA-sequencing approach to identify and compare genes that are differentially expressed by the tomato plant after 24 hours of inoculation with *Phytophthora infestans, Botrytis cinerea,* and *Oidium neolycopersici*. The results showed that *B. cinerea* and *P. infestans* infection elicited the synthesis of 50 and 18 differentially expressed genes respectfully. Through meta-transcriptomic analysis, Sharma and Sharma (2018) were able to identify soil bacterial genera involved in pesticide degradation, detoxifying the heavy metals, degradation of aromatic compounds, and traits associated with transporting phosphate.

The synergic application of metagenomics and meta-transcriptomics approaches has provided a better and clearer insight into plant-microbe associations (Jiang *et al*., 2019). One of the benefits that come with a combination of metagenomics and meta-transcriptomics is the provision of expression ratios information that is obtained by mapping the number of meta-transcriptome cDNA and DNA genes in the metagenome, thereby simplifying the generation of functional and active metabolic pathways data (Morales-Cruz *et al*., 2018). Saminathan *et al*. (2018) used 16S metagenomics and RNA-seq meta-transcriptomics to analyze the diversity, functional fruit-associated microbiome, and gene expression of the ripe fruits from six watermelon cultivars. The result showed the relative abundance of bacterial phyla such as *Proteobacteria Actinobacteria, Firmicutes, Chlamydiae,* and *Cyanobacteria* and fungal phyla such as *Ascomycota, Basidiomycota and Glomeromycota. Proteobacteria* were most transcriptionally active phyla across all cultivars. Similarly, Crump *et al*. (2018) integrated 16S rRNA gene analysis and meta-transcriptomics to assess the interaction of microorganisms and seagrasses (*Zostera marina and Zostera japonica*). Both 16S rRNA analysis and meta-transcriptomics gave similar taxonomic compositions of microbial communities. The bacterial community composition between plant species was not significant, however, a significant difference was observed across leaf, roots, and water column communities. Recently, Zolti *et al*. (2020) used metagenomics to predict functional genes of tomato and lettuce irrigated with waste and freshwater, and further mapped meta-transcriptomic reads to the metagenomic-predicted genes producing comparable sequencing depth between the host and the associated microbiome. Irrigation treatments accounted for 45 and 645 gene significantly differentially expressed genes by tomato and lettuce respectively. The effects of irrigation treatments were more pronounced on the heat-shock transcripts such as *Hsp20*, *Hsp70*, and *DnaJ*. In summary, several studies have shown the importance of both techniques in microbial community identification as well as the microbial activities as impacted by various environmental conditions. After the much-needed background on various metagenomic and meta-transcriptomic studies, the author zoomed on tracing microbes from irrigation water to crops and a very important aspect considering that some water used for irrigation may carry deleterious microbes that should not contaminate ready-to-eat fruits and vegetables.

Tracing microbes from water to crops

Irrigation water is among the contributors to vegetable surface contamination with destructive microbial communities, including human pathogens (Barba-León *et al*., 2024). Traditionally, the microbial water quality has been accessed using fecal indicator bacteria (Briciu-Burghina and Regan, 2023). Most of the studies that observed the prevalence and quantification of plant pathogenic bacteria in irrigation water were based on culture-based methods of bacterial determination (Morris *et al*., 2010). It has been proven that culture-based methods of assessing bacterial compositions in samples are insufficient to delineate species in the sample. For example, the traditional method could not detect *Dickeya* species from water samples taken in many unconnected rivers in South-Eastern England (Toth *et al*., 2011; Parkinson *et al*., 2014). These were later identified as *D. aquatica* by molecular analysis of the 16S rDNA marker gene (Toth *et al*., 2011; Parkinson *et al*., 2014). Since culture-based methods cannot capture all microbes in the samples, metagenomics and meta-transcriptomics have been employed to reveal microbial diversity in marine samples (Ahmed *et al*., 2015) and may be an effective alternative method in tracing microbes from water to crops.

The effectiveness of metagenomics in the water microbiome was observed in the early stages when unprecedented diversity and 70,000 novel genes were discovered from the Sargasso Sea (Venter *et al*., 2004). Metagenome sequencing of water bodies provided an opportunity for the identification and characterization of previously unidentified bacteria (Pedron *et al*., 2019), and the detection of novel human pathogenic viruses (Bibby *et al*., 2019). Recently, the metagenomic analysis identified previously unrecognized viruses and variations in microbial diversity of 11 rivers across three continents using a MinION portable sequencer (Reddington *et al*., 2020). Metagenomic analysis of reclaimed water for irrigation revealed novel viruses related to animal, plant, and insect viruses (Rosario *et al*., 2009). Moreno *et al*. (2018) applied 18S rRNA gene amplicon-based metagenomics to prove that untreated surface irrigation water in open fields could be a potential source of waterborne protozoan parasites such as *Giardia intestinalis*, *Acanthamoeba castellanii*, *Toxoplasma gondii*, *Entamoeba histolytica* and *Blastocystis*. Metagenomics also revealed microbial communities in groundwater wells which are exposed to different amounts of animal manure. The community composition in all wells was dominated by several *Planctomycete* genomes and nano-prokaryotes from the Candidate *Phyla Radiation*, the *Diapherotrites*, *Parvarchaeota*, *Aenigmarchaeota*, *Nanoarchaeota*, *Nanohaloarchaea* and *Thaumarchaeota*, *Aigarchaeota*, *Crenarchaeota*, and *Korarchaeota* (Ludington *et al*., 2017). In another metagenomics study, human pathogenic bacteria, *Legionella* and *Mycobacterium* were discovered in irrigation water (Lequette e*t al*., 2019). Since it has been reported that most microbes can be attached to irrigated crop parts (DiCaprio *et al*., 2015), the use of these waters may subsequently introduce and influence the microbial communities in plants (Moreno *et al*., 2018).

Proving the potential transfer of harmful pathogens from irrigation water to crop, Saab *et al*. (2022) detected *E. coli* in crops irrigated by contaminated river water. Similarly, *E. coli* O157:H7 and *Listeria monocytogenes* were detected in soil and various edible and non-edible parts of potato crops irrigated with contaminated water (Forslund *et al*., 2010). Jongman *et al*. (2017) sequenced bacterial 16S rDNA gene (V1–V3) of irrigation water from the river and leafy vegetables from a small-scale farm and found *Escherichia* and *Salmonella* in both water and cabbage samples. Fernández-Cassi *et al*. (2017) used metagenomics to trace viruses from contaminated irrigation water from Besòs River to parsley leaves. In this study, parsley plants were grown under controlled conditions and irrigated with water from the river that collects waste from 27 wastewater treatment plants. The results showed the abundance of plant viral families in the river viral community, which included *Tymoviridae*, and *Virgaviridae*, and the phage viral families *Myoviridae*, *Siphoviridae*, and *Microviridae* and to a lesser extent by human viral pathogens (1%) including associates of the *Adenoviridae*, *Reoviridae*, *Picornaviridae* and *Astroviridae* families. Highly diverse viral variants were observed on the surface of parsley plants, with most reads assigned to the insect viruses' family *Dicistroviridae*. In addition, plants were also contaminated with human-associated viruses, such as the hepatitis E virus, picornaviruses, human sapoviruses, GIV noroviruses as well as novel strains associated with *Picornaviridae*. The degree of influence of irrigation water on the plant microbiota may depend on the type of water source water microbial quality, and the type of irrigation system (Gu *et al*., 2019). Table 1 provides additional information on microbial diversity and their transition into the crops. Most studies used culture-based methods which could hinder the identification of

non-culturable microbes that could cause health-related problems upon the consumption of the crops.

The advantages of metagenomics and meta-transcriptomics

The introduction of NGS technologies, advanced computing capabilities, and the accessibility of databases have enhanced the use of metagenomics and meta-transcriptomics for monitoring water quality (Hong *et al*., 2020). Both metagenomics and meta-transcriptomics provide a platform to study microbes from their habitat, without the need to culture them in a laboratory (Conesa *et al*., 2016). These techniques capture even the microbial communities that are missed by primitive culturing methods (Conesa *et al*., 2016). Metagenomics and meta-transcriptomics analyze microbes at a community level (Crump *et al*., 2018) without prior knowledge of the gene to be interrogated (Chopyk *et al*., 2020). Meta-transcriptomics provides evidence on viable microbial populations that are actively transcribing their genes to mRNA. Such data (semi-quantitative) can be used to clarify the efficiency of the used water treatment technologies and monitor variations in water quality over a distribution network (Chopyk *et al*., 2020).

However, metagenomics or meta-transcriptomics data can be complemented with data from other techniques such as flow cytometry and quantitative PCR (Chopyk *et al*., 2020; Hong *et al*., 2020). Such information can be used to evaluate the risk against regulatory standards for water intended for crop irrigation (Chopyk *et al*., 2020; Hong *et al*., 2020).

However, to overhaul the quality assurance of the water monitoring system, sustained improvement in metagenomics and meta-transcriptomics is needed in the following areas: improvement in databases, particularly for viruses and protozoa, standardized metagenomics and meta-transcriptomics data analysis protocols that would be benchmarked across different laboratories, lastly, developing bioinformatics tools that identify microbes that occur at a low abundance (Chopyk *et al*., 2020). Currently, most studies mainly focus on monitoring water quality for microbial contaminations and ARGs, overlooking other functional genes such as mobile genetic elements, virulence factors, and metal resistance genes (Zaouri *et al*., 2020), which also affect water safety standards for agricultural purposes.

Future perspectives

The use of metagenomics in studying microbes has closed many research gaps that could not be addressed by the culturing approach. Metagenomics has shown its robustness in studying microbes without introducing bias due to culturing (Compant *et al*., 2005). However, when identifying active functional genes in the microbial community, meta-transcriptomics takes precedence over metagenomic analysis (Aguiar-Pulido *et al*., 2016). Additionally, the meta-transcriptomic analysis also provides the opportunity to investigate gene expression (Levy *et al*., 2018). From the reviewed studies, it is evident that meta-transcriptomics is effective in elucidating active functional microbial communities, regulated genes, and key pathways involved from different samples in agriculture (Sham *et al*., 2019). Moreover, it can assist in closing the gap in identifying key microbial communities whose functionality is not known and novel genes that are regulated under direct environmental conditions. However, irrespective of the robustness of meta-transcriptomics, it has yet to be extensively applied in tracing microbes from irrigation water to crops. Hence, there is a lack of literature on the distribution of microbial genes by irrigation water to edible parts of crops and further clarify transcriptional changes in crops post-irrigation. Therefore, meta-transcriptomics studies in the future will certainly shed more light on microbial community and food safety.

Conclusion

Traditional methods of microbial community identifications provided baseline information on the microbial microcosm associated with various samples. However, their flaws in identifying non-culturable microorganisms and provision of the functional diversity of the microbes led to the need for better technologies. The advent of metagenomics and meta-transcriptomics techniques provided the solution to the weaknesses of the traditional methods of microbial identification. These two techniques provided better insight into the microbial assemblage and functions in the environment. With the application of the two techniques, pathogenic microbes, as well as ARGs can be traced from water used for irrigation of crops to the crops. However, there is scant literature on the use of meta-transcriptomics to track microbial communities harmful to humans from contaminated irrigation water to crops.

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Table 1. Summary of the microbial diversity from the irrigated water to the crops

Method of identification	Type of irrigation water	Name of the crops	Microbial diversity of the irrigation water	Microbial diversity of the crops	References
Cultural method	Secondary treated effluent	Lettuce	Enterovirus	Enterovirus	(Petterson *et al*., 2001)
Culture and molecular methods	Secondary treated wastewater (STWW)	lettuce and spring onion	*E. coli*	*E. coli*	(Farhadkhani *et al*., 2018)
Culture method	Macrophyte treated wastewater	Tomato and eggplant	Faecal coliforms and helminth eggs	faecal coliform (Tomato)	(Akponikpè *et al*., 2010)
Culture method	Secondary-treated urban effluents.	Summer melon	*E. coli*, faecal coliforms, and total coliform bacteria	*E. coli*, faecal coliforms, and total coliform bacteria	(Sacks and Bernstein, 2011)
Culture method	Sewage treatment plant and river water	spinach and cabbage	*E. coli, Salmonella, Clostridium* and *Vibrio*	*Salmonella*	(Rai and Tripathi, 2007)
Culture method	Treated wastewater	lettuce (Lactuca sativa L.)	total and faecal coliforms	total and faecal coliforms	(Mañas *et al*., 2009)
Culture method	Raw wastewater	Alfalfa plant	*Giardia, E. histolytica*/dispar	*Giardia, E. histolytica*/ dispar	(Perez-Mercado *et al*., 2022)
Cultural method	Treated wastewater	Lettuce	helminth eggs, *E. coli* and coliphages	helminth eggs, *E. coli* and coliphages	(Amahmid *et al*., 2022)

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