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APPLICATION POLYMERASE CHAIN REACTION (PCR) FOR DETECTION ENVIRONMENTAL POLLUTION (WATER, AIR, & SOIL): A REVIEW

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ABSTRACT

The environment is an important unit in the life of living creatures. The environment provides resources that are beneficial for life such as water, soil, air, minerals, flora, and fauna. Environmental pollution is a problem that often occurs and will impact the survival of living things. The aim of writing this article is to provide information on PCR technology in the living environment. Data collection was carried out by searching literature in the form of international articles for the last 5 years from 2018-2023 and produced 36 research articles related to PCR applications in the environment. Data searches were carried out via search engines, namely Google, PubMed, NCBI, and Publish or Perish 8 software. Based on the article, the PCR method can be used to detect microorganisms that are markers of environmental pollution.

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INTRODUCTION

The environment is everything that surrounds living things and influences the development of life. The influence that the environment has on living things can have an impact both directly and indirectly. The environment is a combination of natural resources, namely water, land, minerals, light, flora, fauna, and so on. The environment can experience pollution due to pollution in land, air, and water (Sompotan & Sinaga, 2022). Pollution is a condition where contaminants enter the natural environment which causes changes in a detrimental direction. Pollution can be in the form of substances or energy. Pollutants, which are components of pollution or particles that cause pollution, can be foreign substances or energy or natural contaminants originating from organic matter in soil, water, and air, which are basic elements or important components in the survival of living creatures. The condition of these three elements can be an indicator of whether an environment is a good or bad place to live in and the condition of these three elements plays an important role in preserving nature. Environmental pollution is a problem that can have an impact on survival. Pollution can occur due to human behavior or naturally from nature. Natural causes of pollution can be volcanic eruptions which cause air pollution from fog, smoke, and volcanic ash, the increase in species of organisms, floods, and so on. However, in general, pollution is caused by human behavior that is inadequate or unable to preserve nature, such as dumping industrial waste into the aquatic environment or on the ground before processing so that it is not harmful to the environment, the behavior of throwing rubbish carelessly, air pollution due to vehicle engines or production machines in factories and other things that have a significant negative impact on the environment (Ukaogo *et al*., 2020).

Advanced technological developments influence various aspects, one of which is molecular biology. PCR is making reforms in the world of research in the fields of health, biodiversity, ecosystems, microorganisms, and the environment. The PCR technique can make sample testing easier because it can facilitate the identification and detection of the characteristics of the entire community of microorganisms in a sample. Polymerase Chain Reaction commonly called PCR is a technique for amplifying DNA in vitro. PCR involves the stages of pre-denaturation, denaturation, annealing (primer attachment), primer extension (extension), and post-extension. The PCR technique can influence the determination of the success of a sample test. PCR methods including RT-PCR, qPCR, and PCR-DDGE can interpret sequences in environmental sample quality analysis effectively. This PCR method can be a promising method for molecular-level testing of microorganisms that cause pollution in the environment. This PCR method has been widely used in various studies to detect the presence and composition of DNA or RNA from microorganisms that cause environmental pollution and then the data will be processed and analyzed so that the best steps to eliminate pollution or pollution can be identified (Cao *et al*., 2020).

MATERIALS AND METHOD

This writing uses a method for preparing article reviews, namely the literature review technique. This technique was carried out by searching literature in the form of international articles for the last 5 years from 2018-2023 and produced 36 research articles related to PCR applications in the environment. Data searches were conducted via search engines: *Google Scholar, PubMed, NCBI,* and *Publish or Perish 8* software. Keywords used in article searches were environmental, bioremediation, pollution, wastewater, and PCR.

RESULTS AND DISCUSSION

Of the 36 research articles found in the article search, they can be grouped into three main parts, namely articles related to water pollution, air pollution, and soil pollution, as presented in **Table 1**, **2**, and **3**, respectively.

Table 1. Water pollution articles resulted from the search.

Title, Author, Year	Method	Results
Metagenomic Sequencing and Quantitative Real-Time PCR for Fecal Pollution Assessment	Real Time PCR	Test results obtained with qPCR showed concentrations of E. coli, enterococci, and human-associated genetic markers increased after rainfall by 1.52-, 1.26-, and 1.11-fold log10 copies per 100 mL, respectively. Taxonomic analysis of the surface water microbiome

in an Urban Watershed (Brumfield *et al*., 2021)		and detection of antibiotic resistance genes, general FIB, and human-associated microorganisms were also employed. Results showed that fecal contamination from multiple sources (human, avian, dog, and ruminant), as well as FIB, enteric microorganisms, and antibiotic resistance genes increased demonstrably after a storm event
Application of Reverse Transcriptase-PCR (RT-PCR) for rapid detection of viable Escherichia coli in drinking water samples (Molaee *et al*., 2015)	Reverse Transcriptase PCR	RT-PCR is able to detect bacteria in different concentrations. Application of EF II primers reduced false positive results compared to 16S rRNA primers. The FHLP hydrophobic filters have higher ability to absorb bacteria compared with HAWB hydrophilic filters. So the use of hydrophobic filters will increase the sensitivity of RT-PCR.
Quantitative PCR Detection of Enteric Viruses in Wastewater and Environmental Water Sources by the Lisbon Municipality: A Case Study (Teixeira *et al*. 2020)	Quantitative Polymerase Chain Reaction	Quantitative Polymerase Chain Reaction (PCR) detection (qPCR) of enteric viruses Norovirus (NoV) genogroups I (GI) and II (GII) and Hepatitis A (HepA) was performed, and also FIB (E. coli, enterococci and fecal coliforms) concentrations were assessed. HepA virus was only detected in one untreated influent sample, whereas NoV GI/ NoV GI were detected in untreated wastewater (100/100%), secondary treated effluent (47/73%), and tertiary treated effluent (33/20%). This study proposes that NoV GI and GII should be further studied to provide the support that they may be suitable indicators for water quality monitoring targeting wastewater treatment efficiency, regardless of the level of treatment.
Application of quantitative PCR for the detection of microorganisms in water (Botes *et al*., 2013)	Quantitative PCR	The quantitative polymerase chain reaction (qPCR) method has proven to be an effective tool to detect and quantify microorganisms in water within a few hours. Different qPCR assays exist depending on whether an internal control is used or whether measurements are taken at the end of the PCR reaction (end-point qPCR) or in the exponential phase (real-time qPCR). Quantitative reverse transcription polymerase chain reaction (q-RT-PCR) is a more sensitive technique that detects low copy number RNA and can be applied to detect, e.g. enteric viruses and viable microorganisms in water, and measure specific gene expression.
Integrating molecular microbial methods to improve fecal pollution management in rivers with designated bathing waters (Karunakaran *et al*., 2023)	Quantitative PCR (qPCR)-aided Microbial Source Tracking (MST) and 16S rRNA gene metabarcoding	This study used a combined molecular approach, along with measurements of water quality, to gain information on pollution sources, and risk levels, in a newly designated recreational bathing site in the River Wharfe (UK). Physico-chemical parameters were monitored in situ, with water quality multiparameter monitoring sondes installed during the 2021 bathing season. The molecular approach was based on quantitative PCR (qPCR)-aided Microbial Source Tracking (MST) and 16S rRNA gene metabarcoding to obtain a fingerprint of bacterial communities and identify potential bioindicators. The analysis from the water quality sondes showed that ammonium was the main parameter

		determining the distribution of FIB values. Lower fecal pollution levels were detected in the main river when compared to tributaries, except for samples in the river located downstream of a wastewater treatment plant. The fecal pollution type (anthropogenic vs. zoogenic) changed the diversity and the structure of bacterial communities, giving a distinctive fingerprint that can be used to inform source.
Molecular Diagnostic Tools Applied for Assessing Microbial Water Quality (Paruch, 2022)	PCR	Continuous technological advances have led to constant improvements and expansions of molecular methods, such as conventional end-point PCR, DNA microarray, real-time quantitative PCR (qPCR), multiplex qPCR (mqPCR), loop-mediated isothermal amplification (LAMP), digital droplet PCR (ddPCR), and high-throughput next-generation DNA sequencing (HT-NGS). These state-of-the-art molecular approaches largely facilitate the surveillance of microbial water quality in diverse aquatic systems and wastewater.
Water Quality Measurements with a Simple Molecular Analysis (PCR- RFLP) of the Microbiome in a Metropolitan River System in Japan (Neneng *et al*., 2020)	PCR- RFLP	Water chemical analyses showed that surface water at the inflow point of a sewage treatment plant had signs of eutrophication. Heavy metal concentrations in surface water were low (< 0.01 ppm) in all sites. The PCR-RFLP analysis showed polymorphisms both in 16S and 18S rRNAs, indicating that the method can detect at least a part of the microbiome changes in a river system. Sequencing of some fragments found the sequence close to a ciliate isolated in wastewater treatment plants, implying contamination from sewage. Principal component analysis (PCA) identified the RFLPs associated with chemical water parameters, which could be bioindicators of environmental pollution. This study also found the RFLPs independent of water quality parameters, suggesting that this simple DNA-based analysis can also detect biological changes in water ecosystems that are not quantified by chemical measurements of water quality.
Assessment of PCR Inhibitor Removal Methods to Monitor Viruses in Environmental Water Samples: DAX-8 Outperforms Competitors (Hamza & Leifels, 2023)	qPCR	The researcher assessed different approaches to mitigate inhibitory impact of complex water samples during RT-qPCR of murine norovirus (MNV), as an inhibition control. The dilution of extracted samples, the use of qPCR additives, a commercial PCR inhibitor removal kit, and polymeric adsorbents such as Supelite DAX-8 and polyvinylpyrrolidone (PVP) were all investigated in this context. Data indicated that the maximum amplification of MNV by RT-qPCR could be obtained by pre-dilution of samples. However, the dilution factor may depend on inhibitors concentration, primer length, probe sequence, and binding capacity. Interestingly, PCR inhibitor removal kits do not seem to be adequate for removing all PCR inhibitors. In comparison to other approaches studied here, the application of 5% DAX-8 led to an increase in MNV qPCR concentrations. DAX-8 can permanently eliminate humic acids from the extracted nucleic acids from the environmental water

		samples, and it has the potential to considerably improve the accuracy of the obtained non-detects and measured concentrations by qPCR. Further research is required to understand the performance of polymeric adsorbents with enveloped viruses.
Chapter 6 - Microbial source tracking: characterization of human fecal pollution in environmental waters with HF183 quantitative real-time PCR (Shanks & Korajkic, 2020)	Real-time PCR	These assays are unable to discriminate between human and other potential animal sources thus limiting rapid identification and remediation of human pollution sources. Recent advances in molecular biology have led to the development of human fecal source identification tools with the potential to dramatically improve water quality and safety management. This chapter describes the development, validation, and implementation of the human-associated HF183 quantitative real-time PCR technology for water quality and public health protection applications.
Microbial pollution of water with special reference to coliform bacteria and their nexus with environment (Some *et al*., 2021)	PCR	Molecular (PCR-based) and enzymatic methods have been applied as a rapid way to detect indicators and other enteric isolates in water samples. Apart from that standard plate count (SPC) of heterotrophic bacteria and biochemical oxygen demand (BOD) techniques also determine the bacterial and organic pollution load in a water sample. Therefore, bacteriological analysis of water indicated that water is polluted by sewage to the extent that it is unsuitable for drinking and also unsuitable for recreation purposes. This is one of the big problems in the twenty-first century is providing everybody with safe drinking or domestic water. The main objective of this article is to highlight the microbial pollution of water with special reference to coliform and its nexus with the environment.

Table 2. Air pollution articles resulted from the search.

Title, Author, Year	Method	Results
Air and environmental sampling for SARS-CoV-2 around hospitalize patients with coronavirus disease 2019 (COVID-19) (Chan *et al*., 2020)	RT-PCR	Air samples taken from the patient's room isolated at AIIR showed negative results for the presence of SARS-CoV-2 RNA. These negative results indicate that the main transmission route of SARS-CoV-2 is not airborne. Environmental samples of 377 from 21 patients showed that 19 environmental samples (5.0%) were positive for the presence of SARS-CoV-2 RNA via RT-PCR. Environmental samples with the most contamination were patient cell phones (6 out of 77 samples/7.8%), bed rails (4 out of 74 samples/5.4%), and toilet door handles (4 out of 76 samples/5.3%). Positive results from the environment show that although the airborne route is not the main route of transmission of SARS-CoV-2, environmental contamination participates in the spread of the virus.
A field indoor air measurement of	RT-PCR	Ten air samples taken from the bedroom of confirmed COVID-19 patients showed negative results for the

SARS-CoV-2 in the patient rooms of the largest hospital in Iran (Faridi *et al*., 2020)		presence of the RNA virus via RT-PCR. These results indicate that transmission of COVID-19 does not occur through the air in a hospital environment. These negative results can occur because air turbulence in the patient's room with negative pressure causes droplets containing virus samples to be retained, thereby affecting the results.
Ambient Air Pollution in Relation to SARS-CoV-2 Infection, Antibody Response, and COVID-19 Disease: A Cohort Study in Catalonia, Spain (COVICAT Study) (Kogevinas *et al*., 2021)	PCR, serological tests, and antigen tests	The results of this study show that there is no specific relationship between long-term outdoor exposure to pollution and SARS-CoV-2 infection. A strong antibody response from SARS-CoV-2 sufferers has a positive relationship with exposure to air pollution such as NO2 and PM2.5. Exposure to NO2 and PM2.5 pollution is associated with high levels of positive IgG antibodies in SARS-CoV-2 sufferers.
Detection of air and surface contamination by SARS-CoV-2 in hospital rooms of infected patients (Chia *et al*., 2020)	PCR	PCR results showed that 56.7% of rooms had one surface contaminated with SARS-CoV-2. The contamination shown from the patient's touch to the surface of the object has a positive result. The contamination results were seen in the first week of SARS-CoV-2 in 10 out of 15 patients and after the first week of illness in 3 out of 15 patients. Air samples showed detection of >4 μm and 1-4 μm particles of SARS-CoV-2 in two rooms even though the rooms had 12 air changes per hour. Air samples taken from patients after the first week of illness showed that SARS-CoV-2 was not detected. Based on these air samples, the presence of SARS-CoV-2 in the air is likely to be highest during the first week of illness.
Existence of SARS-CoV-2 RNA on ambient particulate matter samples: A nationwide study in Turkey (Kayalar *et al*., 2021)	RT-PCR, 3D-dPCR	Analysis was conducted to determine the presence of SARS-CoV-2 RNA in air at various locations in Turkey. The results of the analysis using 3D-dPCR showed that RNA from SARS-CoV-2 was detected in 20 out of 203 samples (9.8%). The virus was detected highest in Tekirdağ, Zonguldak and Istanbul hospital gardens with PM2.5 mode. This means that SARS-CoV-2 may be carried by environmental particles, especially those close to the point of infection. However, the impact on the spread of the virus is still unknown.
Hospital indoor air quality monitoring for the detection of SARS-CoV-2 (COVID-19) virus (Kenarkoohi *et al*., 2020)	RT-PCR	The results of analysis using RT-PCR showed that the potential for airborne transmission of SARS-CoV-2 was found. A total of 2 (14.28%) out of 14 air samples tested positive for SARS-CoV-2. These results indicate the possibility that SARS-CoV-2 can be transmitted through the air.
Long-Term Air Pollution Exposure and COVID-19 Mortality A Patient-Level Analysis from New York City (Bozack *et al*., 2022)	PCR	Analysis was used to determine the relationship between long-term pollution exposure and COVID-19 outcomes in COVID-19-positive patients. Long-term exposure to pollution due to PM2.5 has a high probability of increasing the risk of death and being treated in the ICU if it is associated with COVID-19. This study specifically found that when exposure to PM2.5 increased by 1 μg/m3, the risk of death increased by 11% and the risk of being admitted to the ICU also increased by 13%.

		However, this study did not find an association between NO2 or BC exposure and death or the possibility of being admitted to the ICU due to COVID-19.
Molecular detection of SARS-CoV-2 from indoor air samples in environmental monitoring needs adequate temporal coverage and infectivity assessment (Barbieri *et al*., 2021)	RT-qPCR	The results of analysis using RT-qPCR showed that the air samples contained viral RNA and this was related to the number of patients affected by COVID-19. An experimental model was used to evaluate the infectivity of the samples by growing them on Vero E6 cells (the host of SARS-CoV-2). Viral load above a certain threshold (Ct,24) has the possibility of causing infection in vitro and resulting in potential transmission.
On the airborne trans mission of SARS-CoV-2 and relation-ship with indoor conditions at a hospital (Baboli *et al*., 2021)	RT-PCR	Principal component analysis (PCA) and self-organizing map (SOM) were used to determine air quality parameters in the patient room and their relationship with the presence of SARS-CoV-2 in the samples. The results of this analysis showed that 11.76% of air samples were positive for the presence of SARS-CoV-2 as a result of 2 positive cases in the hallway and 4 positive cases in the patient's room.

Table 3. Soil pollution articles resulted from the search.

Title, Author, Year	Method	Results
Bacterial community structure and abundances of antibiotic resistance genes in heavy metals contaminated agricul-tural soil (Zhang *et al*., 2018)	qPCR, PCR-DGGE	There were different levels of contamination of cadmium (Cd), lead (Pb), mercury (Hg), arsenic (As), copper (Cu), and zinc (Zn) in all samples. The levels of heavy metals decreased with increasing distance from the factory. Five ARGs (sul1, sul2, tetA, tetM, tetW) and one mobile genetic element (inti1) were detected in all soil samples using real-time polymerase chain reaction (qPCR). The bacterial community composition in the soil samples was analyzed using PCR-DGGE. Several dominant bacterial species were identified, including *Ralstonia solanacearum*, Myroides, Pseudomonas, Bacillus, Lactococcus, and Brochothrix.
Increased occurrence of heavy metals, antibiotics and resistance genes in surface soil after long-term application of manure (Guo *et al*., 2018)	qPCR	PCR was used to quantify the abundance of antibiotic resistance genes (ARGs) in soil samples. The qPCR (quantitative PCR) method was employed with the Applied Biosystems 7500 Fast Real-Time PCR System to measure the copy number of tetracycline and sulfonamide resistance genes. The qPCR method is used to measure the copy number of ARGs and obtain quantitative data. The study found that pig manure can lead to heavy metal pollution, especially zinc (Zn) and copper (Cu) pollution, as well as the presence of residual antibiotics. The abundance of ARGs, particularly efflux pump genes, increased with the application of pig manure. The relative abundance of ARGs was influenced by heavy metals, antibiotics, and environmental factors. The study also found a positive

		correlation between the levels of antibiotic residues, heavy metals, and antibiotic resistance genes in the soil.
Manure fertilization increase antibiotic resistance in soils from typical greenhouse vegetable production bases, China (Pu, Zhao, *et al*., 2020)	HT-qPCR	The study used high-throughput quantitative PCR (HT-qPCR) to investigate the effects of manure fertilizers on the diversity and abundance of antibiotic resistance genes (ARGs) in GVP soil samples. HT-qPCR was performed usin g the SmartChip Real-time PCR system by Wafergen Inc. USA. A total of 296 primer sets, including 285 ARGs, 10 mobile gene elements (MGEs), and the 16S rRNA gene, were adopted to detect almost all major classes of ARGs. The study found that manure fertilization significantly increased the absolute abundance of ARGs in GVP soils. The researchers observed elevated ARG diversity and abundance in fertilized soil compared to non-fertilized soil. However, there was no significant difference in ARGs between different types of manure. Redundancy analysis indicated that changes in bacterial community compositions and environmental factors contributed partially to the shift in ARG profiles.
Family livestock waste: An ignored pollutant resource of antibiotic resistance genes (Gu *et al*., 2020)	qPCR, RT-PCR	Qualitative PCR was used to detect the presence of seven classes of antibiotic resistance genes (ARGs) in the samples using specific primers for seven classes of ARGs. The quantification of ARGs and the 16S rRNA gene in the samples was done using real-time PCR. The study revealed a high prevalence and severity of ARGs contamination in family livestock farms. Various common ARGs, including high-risk ones such as bla ampC, bla OXA-1, and bla TEM-1, were prevalent in family livestock waste. Tetracycline resistance genes were identified as the most serious pollution in these farms. The levels of ARGs were higher in family chicken farms compared to pig and cattle farms, with layer waste and sow waste showing more severe pollution than broiler waste and piglet/fattening pig waste.
Changes in soil available cadmium and bacterial communities after fallowing depend on contamination levels (Wang *et al*., 2018)	PCR	The study used PCR to amplify specific regions of the DNA (the V3-V4 hypervariable regions of the 16S rRNA gene for bacteria and the ITS1 region of the fungal ITS gene). The study found that fallowing significantly improved soil nitrogen (N) availability but decreased Cd availability in lightly contaminated conditions. In heavily contaminated conditions, fallowing led to shifts in microbial community composition, indirectly enhancing soil nutrient availability and reducing available Cd. The changes in soil health-related factors after fallowing were influenced by the level of Cd contamination. Fallowing is an agricultural practice aimed at improving soil health and the sustainability of cultivated land. It involves temporarily stopping farming activities and implementing specific measures to enhance soil quality. These measures can include planting green

		manure, applying lime to adjust soil pH, and reducing the availability of contaminants such as cadmium (Cd).
The growth of plants and indigenous bacterial community were signifcantly afected by cadmium contamination in soil–plant system (Du *et al*., 2021)	qPCR	qPCR was used to determine the abundances of bacteria in the samples. The reaction was performed using a RT-PCR Detection System, and the results were analyzed to quantify the bacterial abundances. The study examined that Cd contamination had a significant impact on the indigenous bacterial community in the soil-plant system. Cadmium (Cd) contamination has an impact on the bacterial communities in a soil-plant system. The results showed that Cd significantly affected the bacterial community structure in both the soil and plants. The bacterial communities in the soil were more affected by Cd compared to the bacterial communities in the plants.
Bioremediation of soil long-term contaminated with PAHs by algal-bacterial synergy of Chlorella sp. MM3 and Rhodococcus wratislaviensis strain 9 in slurry phase (Subashchandrabose *et al*., 2019)	Semi-quantitative PCR	Semi-quantitative PCR was used to monitor the survival of R. wratislaviensis strain 9 during the bioremediation process. It was found that the algal-bacterial system was highly effective in removing total PAHs from the soil, with a degradation rate of 12%. The growth of *Chlorella sp*. MM3 and *R. wratislaviensis* strain 9 was also measured, and it was observed that the presence of the algal-bacterial system led to a significant increase in the growth of *Chlorella sp*. MM3.
Enhanced removal of ciprofloxacin and reduction of antibiotic resistance genes by earthworm Metaphire vulgaris in soi (Pu, Wang *et al*., 2020)	High-throughput qPCR	Ciprofloxacin is only partially metabolized and excreted via urine and feces, resulting in its enrichment in soil, which can impact soil microorganisms and fauna. The study found that earthworm activity contributes to the removal of ciprofloxacin in soil, with higher removal rates observed in earthworm cast samples compared to control soil samples.
Assembly of root-associated bacterial community in cadmium contaminated soil following five-year consecutive application of soil amendments: Evidences for improved soil health (Cheng *et al*., 2022)	qPCR	The consecutive application of amendments for five years has led to changes in the root-associated bacterial compositions and rhizosphere functions. These findings suggest that the application of soil amendments is essential for the remediation of Cd-contaminated soil and for sustainable use.
Human fecal contamina tion of water, soil, and surfaces in households sharing poor-quality sanitation facilities in Maputo, Mozambique (Holcomb al., 2020)	qPCR	The study used singleplex qPCR assays to validate candidate assays against each fecal sample. The assays used in the study were EC23S, HF183, Mnif, and GFD. Human targets were detected in 59% of soil samples, indicating that there was widespread fecal contamination in the soil.

A. Water Pollution

In the first article, the result that can studied is that microbial contamination of recreation waters is a major concern globally, with pollutants originating from many sources, including human and other animal wastes often introduced during storm events. Fecal contamination is traditionally monitored by employing culture methods targeting fecal indicator bacteria (FIB), namely E. coli and enterococci, which provides only limited information of a few microbial taxa and no information on their sources. Host-associated qPCR and metagenomic DNA sequencing are complementary methods for FIB monitoring that can provide enhanced understanding of microbial communities and sources of fecal pollution. FIB by culture, qPCR amplification of FIB and host-associated genetic markers, and WMS to detect, identify, and enumerate bacteria, archaea, fungi, protists, and viruses were employed. For future research directions that are fully characterized and considered in the context of water management, WMS is best applied as a complement to established culture and qPCR practices. Water quality assessment has been focused essentially on detecting fecal indicator bacteria (FIB), namely *Escherichia coli (E. coli)* and *Enterococcus* spp (Brumfield *et al*., 2021). FIB are used as surrogates for enteric pathogens in particular for monitoring fecal contamination in environmental waters, relying on the principle that FIB existence is concomitant with pathogen presence, as demonstrated in several studies. However, there are also numerous studies showing that pathogens do not correlate significantly with FIB. Since FIB, like E. coli and Enterococcus spp. are shed in most animal feces, the lack of suitability between FIB levels and human health outcomes may be related to the FIB source (Teixeira *et al*. 2020). In the second article, the information that we get is Polymerase chain reaction (PCR) is preferred to other methods for detecting Escherichia coli (E. coli) in water in terms of speed, accuracy and efficiency. False positive result is considered as the major disadvantages of PCR. The Materials and methods are Specific primers were designed for 16S rRNA and elongation Factor II genes. Different concentrations of bacteria were passed through FHLP and HAWP filters. Then, RT-PCR was performed using 16srRNA and EF –Tu primers. Contamination of 10 wells was determined by RT-PCR in Arak city. To evaluate RT-PCR efficiency, the results were compared with most probable number (MPN) method. It is giving the result that RT-PCR is able to detect bacteria in different concentrations. Application of EF II primers reduced false positive results compared to 16S rRNA primers. The FHLP hydrophobic filters have higher ability to absorb bacteria compared with HAWB hydrophilic filters. So the use of hydrophobic filters will increase the sensitivity of RT-PCR (Molaee *et al*., 2015).

In the third article, the results of the study is supported by the hypothesis that NoV GI and GII might be suitable indicators for water quality monitoring regarding wastewater treatment efficiency, regardless of the level of the treatment—secondary or tertiary. A correlation between NoV (GI) and FIB (fecal coliforms) is observed in untreated effluent, but in subsequent treatment phases, there was no correlation between the targeted enteric viruses and FIB. While positive correlations observed between FIB and NoV in untreated wastewater could be expected and were indeed observed, the absence of correlations between FIB and secondary and tertiary treatment stages points out to a lack of a link between FIB and the targeted enteric virus concentrations. Moreover, in several samples with an apparent total FIB elimination with UV and sand filtration treatment, NoV was still detectable (Teixeira *et al*. 2020). The result of the fourth article is Quantitative PCR assays are applied to determine the quality of drinking water straight from resources and after treatment, the efficiency of wastewater treatment plants and the safety of water from recreational beaches. Research showed that qPCR is a specific, sensitive and rapid tool to determine the presence and numbers of microorganisms in water. It has also proven to be useful for reducing the health risks associated with microorganisms in water and to assist in ensuring a safe supply of water. The US EPA is currently considering qPCR as a rapid analytical tool to detect and quantify fecal indicators in recreational waters. Limitations of using qPCR as an analytical tool in routine monitoring include the inability of qPCR to differentiate between

live and dead microbial cells. The presence of PCR inhibitors in environmental water samples and the need for standardized qPCR protocols remain a challenge. There is potential for the application of high-throughput analytical systems in detection of waterborne pathogens; however, the technology is still in its infancy (Botes *et al*., 2013).

The highlights of the fifth articles are Assessing contamination sources in recreational waters is of utmost importance for directing outreach and mitigation efforts. This study highlights the effective application of microbial genetic analysis, in identifying fingerprints and alternative indicators of fecal pollution. These methods offer a fast and efficient way to quantify new water quality indicators and assess water quality. However, further research is needed to determine if these indicator microorganisms vary across different locations, and if they can accurately assess human health risks, especially when the primary source of fecal contamination is not human-related (Karunakaran *et al*., 2023). The seventh article result shown that present study shows water environmental assessment based on water chemistry and microbiomes in a metropolitan river system in Japan. The results demonstrate that a simple molecular analysis (PCR-RFLP) can sensitively detect at least a part of the microbiome changes. The PCA suggests that the microbiome changes detected by PCR-RFLP are associated with water quality changes caused by the input of treated sewage water, indicating that the method has the potential for developing bioindicators of environmental pollution. Further sequencing analyses of restriction fragments are required to make a list of microbial species as a potential indicator of water environmental changes in the river system. Currently, a high-throughput sequencer can produce massive sequence data of microbiomes from environmental samples, and the cost is decreasing rapidly (Neneng *et al*., 2020).

The eight articles conclusion talk about water and wastewater surveillance is a robust, cost and labor effective, and non-invasive method to follow localized disease outbreaks and monitor the occurrence of virus variants of concern. However, viral detection can be underestimated due to the effect of PCR inhibitors. The presented work aims at providing a baseline comparison of currently available methods to reduce the inhibitory effects of co-concentrated substances in qPCR assays (Some *et al*., 2021). The findings demonstrated that PCR inhibitor removal kits seemed not to be the sole choice for ensuring a minimum of false negative results during sampling campaigns. Besides, their relatively high per reaction cost can be a barrier to their usage in high-throughput scenarios, as well as under resource-scarce conditions. In contrast, the pre-dilution approach as the simplest way to reduce the inhibitory effect of co-concentrated substances has been shown to be powerful since pre-dilution factors of 1:50 and 1:100 could alleviate the inhibitory effect on RT-qPCR. Nevertheless, the approach can be a double-edged blade particularly for non-enteric viruses with high public health relevance, as they are at risk of being completely removed. The information provided in the article, however, did not reveal significant differences between the DTT- and RNase-treated samples. The BSA treatment resulted in a decrease in the Ct values of the qPCR reaction; nonetheless, only 2.5 to 12% of MNV RNA could be detected. Importantly, the addition of polymeric adsorbents such DAX-8 outperforms PVP in the elimination of inhibitors under conditions of high inhibitor concentration and diversity, where ~ 96% of MNV could be detected in DAX-8-treated samples, compared to 6.2% for PVP.

The ninth articles describes the development, validation, and implementation of the human-associated HF183 quantitative real-time PCR technology for water quality and public health protection applications (Shanks & Korajkic, 2020). The tenth articles conclusion is about microbial pollution in the water body is one of the major issues concerning the sanitary quality of drinking and recreational water. The presence of pathogenic bacteria, protozoa, and viruses is one of the serious threats to human health. The dissemination of pathogenic microbes is responsible for several enteric outbreaks. The pollution of water samples by MAR bacteria might become the cause of the severe epidemic of enteric diseases. There is an immediate need for

positive steps to stop further debility and improve the water quality to protect the community from extensive waterborne diseases. Before reaching to the natural water body, wastewater should be treated. The recycled water should be used in an irrigation system for the sustainable agriculture (Some *et al*., 2021). The results of all the articles that have been reviewed, in most cases, the cause of water pollution is human waste which has an impact on the presence of e coli bacteria. The presence of E coli bacteria can be detected accurately using various methods from PCR to the environment.

B. Air Pollution

COVID-19 has various transmission routes, namely transmission from one person to another. Exposure between people will appear to have an impact if it occurs over a long period and interactions between people are not limited by protection such as masks. In terms of preventing transmission of COVID-19, it is recommended to wash your hands several times a day and maintain a distance of at least 1 meter between individuals. Transmission of COVID-19 can also occur through the air around an infected person. Airborne transmission is one of the important transmission routes for agents such as viruses (Noorimotlagh *et al*., 2020). COVID-19, which is included in SARS-CoV-2, can infect other people, especially in closed spaces, through ventilation, air temperature, social distance, and humidity as one way through the air. This is related to the habits of most people who spend 90% of their time in a closed environment (Klepeis *et al*., 2001). Viruses stored in aerosol form in the air can be detected using PCR and then the next step can be taken. Several studies conducted regarding the transmission of SARS-CoV-2 through the air show that the airborne route is not the main route for transmitting the virus.

Patients isolated in a room with AIIR had air samples that were negative for the presence of SARS-CoV-2 RNA (Chan *et al*., 2020). Ten air samples taken from the rooms of COVID-19-positive patients for research by Faridi *et al*., (2020) also showed negative results for COVID-19 virus RNA. This negative result occurred because the air in the patient's environment had been filtered several times so it was possible that the presence of SARS-CoV-2 was not detected. Apart from that, air turbulence with negative pressure in the patient's room also causes droplets with virus samples to be undetectable. However, air samples in the study Kayalar *et al*., (2021) analyzed using 3D-dPCR were detected to contain SARS-CoV-2 RNA in 20 of 203 samples (9.8%). SARS-CoV-2 RNA was detected most highly in hospital gardens close to the point of infection. This means that SARS-CoV-2 can be carried by environmental particles to transmit to other people. Research conducted by Kenarkoohi *et al*., (2020) also showed similar results to research by Kayalar *et al*., (2021), namely that air samples tested positive for SARS-CoV-2 as many as 2 out of 14 samples (14.28%). The principal component analysis (PCA) and self-organizing map (SOM) methods used by Baboli *et al*., (2021) can determine air quality parameters in patient rooms and their relationship with the presence of SARS-CoV-2. The presence of SARS-CoV-2 was not only detected in the patient room or hospital garden but was also detected in the hallway with 2 positive cases. Barbieri *et al*., (2021) conducted an experiment aimed at evaluating the infectivity of air samples obtained from COVID-19-positive patients. The air samples were grown on Vero E6 cells which are the host of SARS-CoV-2.

Transmission of SARS-CoV-2 apart from direct infection by SARS-CoV-2 positive patients may also be through exposure to outdoor pollution. Research conducted by Kogevinas *et al*., (2021) shows that there is no specific relationship between long-term exposure to outdoor pollution and SARS-CoV-2 infection. However, the antibody response from SARS-CoV-2-positive patients has a positive relationship with exposure to air pollution, especially NO_2 and PM2.5. Positive IgG antibody levels are associated with exposure to NO2 and PM2.5 pollution in SARS-CoV-2 sufferers. Bozack *et al*., (2022) have similar results, namely that long-term exposure to pollution has a relationship with COVID-19, especially PM2.5. PM2.5 has a high probability of

being admitted to the ICU as well as a risk of death if associated with COVID-19. When PM2.5 exposure increases by 1 μg/m^3, the risk of being treated in the ICU increases by 13% and the risk of death also increases by 11%. Based on this research, it means that long-term exposure to PM2.5 has a greater chance of contracting COVID-19 compared to exposure to NO_2 and BC.

Transmission of SARS-CoV-2 is not only through the air directly in contact with infected patients but can also be through touching objects that are frequently touched by infected patients. Research conducted by Chan *et al*., (2020) shows that the items with the greatest contamination against COVID-19 are patient cell phones (6 out of 77 samples/7.8%), bed rails (4 out of 74 samples/5.4%), and toilet door handles (4 of 76 samples/5.3%). The patient's cell phone is one of the items with a high level of contamination, possibly because during isolation in the hospital the patient will give news to his family or just play on his cell phone. Chia *et al*., (2020) conducted research with almost similar results, namely that 56.7% of patient rooms had one surface contaminated with SARS-CoV-2. Contamination due to patients touching surfaces of objects mostly had positive results of 66.66% in the first week of patients being confirmed positive for SARS-CoV-2 and after the first week of patients being confirmed positive of 20% with SARS-CoV-2 particles >4 μm and 1-4 μm. However, samples taken a week after the patient was confirmed positive had negative results, namely that SARS-CoV-2 was not detected. This means that the highest presence of SARS-CoV-2 in the air was detected during the first week the patient was confirmed positive.

C. Soil Pollution

Soil pollution refers to the contamination of soil with harmful substances, such as chemicals, heavy metals, pesticides, or industrial waste. This pollution can occur due to various human activities, including improper waste disposal, industrial activities, agricultural practices, and mining operations. These pollutants can have detrimental effects on soil quality, leading to reduced fertility, loss of biodiversity, and potential harm to human health. Efforts to prevent and mitigate soil pollution involve proper waste management, sustainable agricultural practices, and remediation techniques to restore contaminated soils. PCR techniques can be used to detect soil pollution, some of which are semi-quantitative PCR, qPCR, PCR-DGGE, and High-throughput qPCR. A number of studies state that qPCR can be used to detect soil pollution through identifying the presence of heavy metals, ARGs, and bacterial community structures in the soil. The research includes Zhang *et al*. (2018); Guo *et al*. (2018); Gu *et al*. (2020); Du *et al*. (2021); Cheng *et al*. (2022); and Holcomb *et al*. (2020). Zhang *et al*. (2018) states that there were different levels of contamination of cadmium (Cd), lead (Pb), mercury (Hg), arsenic (As), copper (Cu), and zinc (Zn) in all samples. The levels of heavy metals decreased with increasing distance from the factory. Five ARGs (sul1, sul2, tetA, tetM, tetW) and one mobile genetic element (inti1) were detected in all soil samples using real-time polymerase chain reaction (qPCR).

The bacterial community composition in the soil samples was analyzed using PCR-DGGE. Several dominant bacterial species were identified, including *Ralstonia solanacearum*, Myroides, Pseudomonas, Bacillus, Lactococcus, and Brochothrix. Based on the research by Guo *et al*. (2018), states that PCR was used to quantify the abundance of antibiotic resistance genes (ARGs) in soil samples. The qPCR (quantitative PCR) method was employed with the Applied Biosystems 7500 Fast Real-Time PCR System to measure the copy number of tetracycline and sulfonamide resistance genes. The qPCR method is used to measure the copy number of ARGs and obtain quantitative data. The study found that pig manure can lead to heavy metal pollution, especially zinc (Zn) and copper (Cu) pollution, as well as the presence of residual antibiotics. The abundance of ARGs, particularly efflux pump genes, increased with the application of pig manure. The relative abundance of ARGs was influenced by heavy metals, antibiotics, and environmental factors. The study also

found a positive correlation between the levels of antibiotic residues, heavy metals, and antibiotic resistance genes in the soil. Gu *et al*. (2020) also state that qPCR was used to detect the presence of seven classes of antibiotic resistance genes (ARGs) in the samples using specific primers for seven classes of ARGs.

The quantification of ARGs and the 16S rRNA gene in the samples was done using real-time PCR. The study revealed a high prevalence and severity of ARGs contamination in family livestock farms. Various common ARGs, including high-risk ones such as bla ampC, bla OXA-1, and bla TEM-1, were prevalent in family livestock waste. Tetracycline resistance genes were identified as the most serious pollution in these farms. The levels of ARGs were higher in family chicken farms compared to pig and cattle farms, with layer waste and sow waste showing more severe pollution than broiler waste and piglet/fattening pig waste. Du *et al*. (2021) used qPCR to determine the abundances of bacteria in the samples. The reaction was performed using a RT-PCR Detection System, and the results were analyzed to quantify the bacterial abundances. The study examined that Cd contamination had a significant impact on the indigenous bacterial community in the soil-plant system. Cadmium (Cd) contamination has an impact on the bacterial communities in a soil-plant system. The results showed that Cd significantly affected the bacterial community structure in both the soil and plants. The bacterial communities in the soil were more affected by Cd compared to the bacterial communities in the plants. Based on research by Cheng *et al*. (2022), qPCR is used to detect CD pollution in soil obtained the consecutive application of amendments for five years has led to changes in the root-associated bacterial compositions and rhizosphere functions. These findings suggest that the application of soil amendments is essential for the remediation of Cd-contaminated soil and for sustainable use. Research by Hollcomb *et al*. (2020) used singleplex qPCR assays to validate candidate assays against each fecal sample. The assays used in the study were EC23S, HF183, Mnif, and GFD. Human targets were detected in 59% of soil samples, indicating that there was widespread fecal contamination in the soil.

Based on research conducted by Wang *et al*. (2018), PCR can be used to amplify specific regions of the DNA (the V3-V4 hypervariable regions of the 16S rRNA gene for bacteria and the ITS1 region of the fungal ITS gene). The study found that fallowing significantly improved soil nitrogen (N) availability but decreased Cd availability in lightly contaminated conditions. In heavily contaminated conditions, fallowing led to shifts in microbial community composition, indirectly enhancing soil nutrient availability and reducing available Cd. The changes in soil health-related factors after fallowing were influenced by the level of Cd contamination. Fallowing is an agricultural practice aimed at improving soil health and the sustainability of cultivated land. It involves temporarily stopping farming activities and implementing specific measures to enhance soil quality. These measures can include planting green manure, applying lime to adjust soil pH, and reducing the availability of contaminants such as cadmium (Cd).

In addition, there is also high-throughput quantitative PCR (HT-qPCR) which is used for investigate the effects of manure fertilizers on the diversity and abundance of antibiotic resistance genes (ARGs) in GVP soil samples. HT-qPCR was performed using the SmartChip Real-time PCR system by Wafergen Inc. USA. A total of 296 primer sets, including 285 ARGs, 10 mobile gene elements (MGEs), and the 16S rRNA gene, were adopted to detect almost all major classes of ARGs. The study found that manure fertilization significantly increased the absolute abundance of ARGs in GVP soils. The researchers observed elevated ARG diversity and abundance in fertilized soil compared to non-fertilized soil. However, there was no significant difference in ARGs between different types of manure. Redundancy analysis indicated that changes in bacterial community compositions and environmental factors contributed partially to the shift in ARG profiles Pu, Zhao *et al*. (2020).

PCR also has another method, namely semi-quantitative PCR and High-throughput qPCR. Semi-quantitative PCR was used to monitor the survival of *R. wratislaviensis* strain 9 during the bioremediation process. It was found that the algal-bacterial system was highly effective in removing total PAHs from the soil,

with a degradation rate of 12%. The growth of *Chlorella sp*. MM3 and *R. wratislaviensis* strain 9 was also measured, and it was observed that the presence of the algal-bacterial system led to a significant increase in the growth of *Chlorella sp*. MM3 Subashchandrabose *et al*. (2019). High-throughput qPCR was used to detect the presence of Ciprofloxacin that only partially metabolized and excreted via urine and feces, resulting in its enrichment in soil, which can impact soil microorganisms and fauna. The study found that earthworm activity contributes to the removal of ciprofloxacin in soil, with higher removal rates observed in earthworm cast samples compared to control soil samples Pu, Wang *et al*. (2020).

CONCLUSION

PCR can detect and measure the presence of certain microorganisms or genes that can indicate environmental pollution. In research on air contamination, the methods often used are qPCR. Air pollution problems are often found to be related to viruses that cause respiratory tract diseases. This can be explained by the 3D-dPCR method for detection. Soil pollution using semi-quantitative PCR, qPCR, PCR-DGGE, and high-throughput qPCR is often used in analyzing species that cause contamination in soil.

AUTHORS CONTRIBUTION

M. Novenda, N. R. N. Hidayati, & H. Khairunnisa designed and conducted the study, analyzed and interpreted the data, and E. P. Ariyani wrote a draft of the manuscript.

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