JOURNAL of Biotechnology and Conservation in WALLACEA

Volume 01, Number 02, October 2021
Pages: 57 – 65
DOI: https://doi.org/10.35799/jbcw.v1i2.37571

e-ISSN: 2808-4268

Environmental DNA (e-DNA) as a Method for Early Detection of Diesel Oil Pollution: A review

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Abstract

The presence of bacteria in an environment is strongly influenced by pollutants. As a result, there is an opportunity to investigate the presence of these bacteria as a pollutant indicator. Environmental DNA (e-DNA) is one method for rapidly and accurately detecting bacteria in the environment. The purpose of this narrative review is to describe the ability of e-DNA to early detect diesel oil (hydrocarbon) pollution in the environment. This narrative review drew on secondary data obtained from the software Publish or Perish. The results of the analysis indicate that the diversity and composition of bacteria differ between uncontaminated and diesel-contaminated environments. Actinobacteria is the most abundant phylum in an uncontaminated environment, whereas Proteobacteria and Bacteroidetes are the most abundant in a diesel-contaminated environment. According to this review, e-DNA has the potential to be used as an early detection method for diesel oil pollution in a given location.

***Keywords*:** environmental DNA; e-DNA; 16S rRNA gene; diesel oil contamination; bacterial diversity.

INTRODUCTION

Diesel, or diesel oil (petroleum hydrocarbon - PHC), is one of the hydrocarbon derivative products used in mass transportation, such as ships. Diesel oil that is wasted into the sea will affect the surrounding marine ecosystem. These spills are rarely noticed and can pose a serious threat to the environment due to the accumulation of hydrocarbon contamination. The real impact of the presence of diesel oil on the surface of the water is the obstruction of sunlight penetration, which means reducing the rate of photosynthesis in the water. The closure will also reduce the intake of free O_2 from the air into the water. The lack of photosynthesis rate and O_2 input from the air will interfere with the survival of aquatic biota (Cubillos et al., 2014).

Microorganisms have great potential as a tool for monitoring seawater quality and coral reef ecosystem health (Glasl et al., 2017). Changes in the composition and structure of the microorganism community can be used as a proxy for ecosystem pollution. Numerous studies now demonstrate that microbial communities can serve as bioindicators of environmental quality pollution (Abbasian et al., 2016; Mukherjee et al., 2017; Ortiz-Estrada et al., 2019).

One of the methods for detecting the presence of bacteria, both in terms of composition, community structure and diversity, is environmental DNA (e-DNA). Seawater or sediment samples can be used to obtain e-DNA. DNA from bacteria is extracted directly from the environment and amplified using polymerase chain reaction (PCR) techniques, and then sequenced using next-generation sequencing techniques to generate thousands to millions of reads. From this data, the existence of species and their diversity can be determined (Ruppert et al., 2019).

According to the description above, this narrative review will discuss the use of the e-DNA approach to determine the impact of diesel pollution on the bacterial diversity of a diesel-contaminated location, which can be used as an early detection method for hydrocarbon pollution. This e-DNA method identifies bacteria quickly and accurately by utilizing the 16S rRNA gene. To determine the ability of e-DNA to monitor pollution, the diesel fuel content of polluted and unpolluted locations must be compared. Early detection can be accomplished by comparing the community structure and diversity of bacteria in diesel-contaminated and unpolluted samples.

MATERIALS AND METHODS

The data for this non-systematic narrative review was derived from secondary sources such as articles in journals, books, and other libraries, as well as online resources such as database portals and search engines. Secondary data searching is demonstrated in (Figure 1), which is used to locate and access articles in academic journals, institutional repositories, archives, or collections of scientific articles, among others, using the Windows version of the Publish or Perish program (https://harzing.com/resources/publish-or-perish/windows). The keywords selected in the search process included diesel oil, environmental DNA, bacteria, DNA metabarcoding, metagenomic, water quality, and hydrocarbon contamination. Articles that met the criteria were extracted and saved as pdf files. After analyzing and synthesizing the articles, general conclusions were drawn.

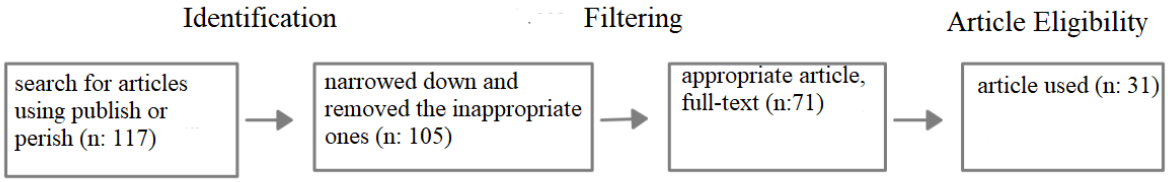


Figure 1. Secondary data searching.

RESULTS AND DISCUSSION

Environmental DNA

Environmental DNA (e-DNA) is defined as genetic material isolated directly from environmental samples such as soil, sediment, or water that shows no evidence of biological origin (Thomsen & Willerslev, 2015). The presence of this e-DNA can be detected using the metabarcoding method, which entails sequencing large amounts of DNA in order to molecularly identify multiple taxa in complex samples (Taberlet et al., 2012). The DNA extracted from the environment is then compared to the GenBank database in order to determine the target organism's identity (Pilliod et al., 2013). Metabarcoding is possible due to Next-generation Sequencing (NGS) technology, which is capable of sequentially sequencing thousands to millions of DNA fragments in a short period of time. Illumina sequencing technology is an NGS technology that has been widely used throughout the world. The advantages of this DNA-based detection method include its high sensitivity, ease of sample collection, and ability to discriminate between species regardless of size or life stage, making it ideal for use as a rapid detection tool (Dejean et al., 2012).

Metabarcoding is a relatively new technique for determining the species composition of a sample in order to use it as a reference for studying how bacteria interact with their environment (Bohmann et al., 2014; Creer et al., 2016; Ficetola et al., 2016; Hering et al.,

2018). This technique is utilized in the e-DNA method, which is used to monitor the presence of a bacterial community in the environment. The NGS technique used in metabarcoding utilizes a bioinformatics approach using software capable of analyzing large amounts of biological data and interpreting it. Metagenomic techniques employ molecular markers such as 16S rRNA (ribosomal RNA) to detect the presence of bacteria in the absence of a culture process (culture-free), allowing for the identification of bacteria that cannot be cultured (Ortiz-Estrada et al., 2019).

The steps involved in conducting e-DNA research for the aquatic environment are detailed in Figure 2. Water samples are collected and filtered in a container. After that, the filter is used as a source of DNA. DNA is extracted from the filter using a commercial DNA extraction kit. The obtained DNA is then amplified using PCR technology and primers for the 16S rRNA gene. The 16S rRNA gene fragment is then sequenced using next-generation sequencing technology. The results of the sequencing in the form of 16S rRNA sequence data from all bacteria in a water sample are then analyzed using bioinformatics tools. The analysis produces taxa at the phylum and species levels. The results are then visualized (Bohmann et al., 2014).

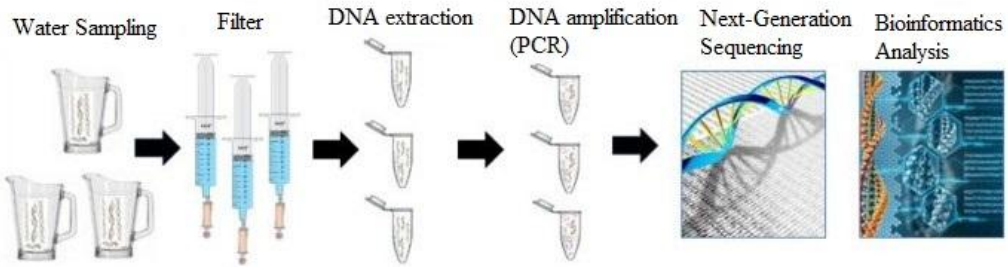


Figure 2. Steps in e-DNA research for aquatics environment.

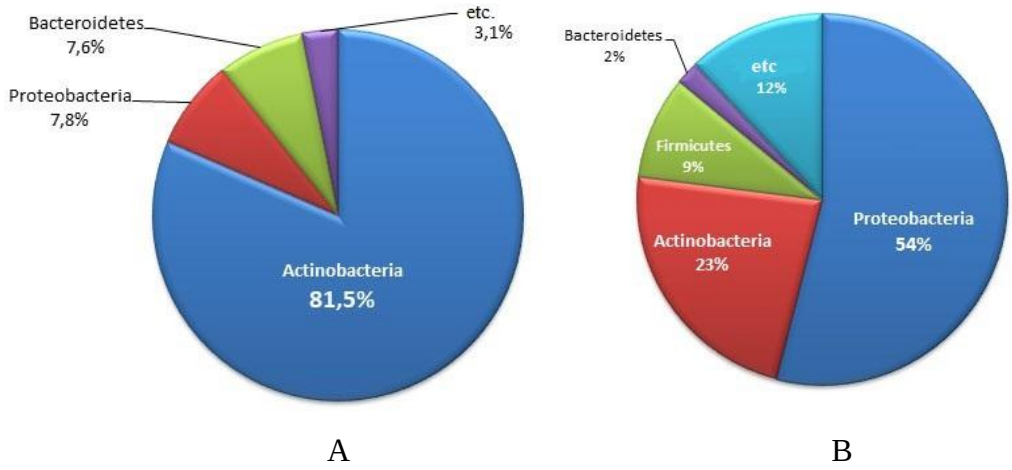
Early Detection of Diesel Oil Contamination Using e-DNA

Diesel oil contamination is a significant source of pollution to natural resources in urban, semi-urban, and industrial areas (Ivshina et al., 2015; Khan et al., 2013). Today, researchers have used microbiome data to compare contaminated and uncontaminated areas. This is because hydrocarbon contamination can cause a shift in the dominance of the microorganism population in the soil or in the aquatic environment. Numerous studies indicate that contaminated soil is a factor in the increase in the diversity of certain bacteria when compared to uncontaminated soil (Abbasian et al., 2016; Peng et al., 2015; Sun et al., 2015; Sutton et al., 2013).

Numerous researchers have used environmental DNA to study the composition of bacteria in the environment. Tardif et al. (2016) discovered that increasing the level of PHC (petroleum hydrocarbons) contamination resulted in a shift in the composition and diversity of microbes, specifically an increase in bacteria that degrade hydrocarbons. Chen et al. (2018) showed that in unpolluted waters, the relative abundance of bacteria was dominated by the phylum Actinobacteria (81.5%), followed by Proteobacteria (7.8%), Bacteroidetes (7.6%), and other phyla (3.1%). Research that has been conducted on the characterization of the composition of the bacterial community in a hydrocarbon-contaminated environment has been carried out by Auti et al., (2019). In their research, they stated that there was an association between soil microbial communities and physico-chemical parameters. In soils contaminated with crude petroleum oil (CP) and refined petroleum oil (RP), Proteobacteria (54%) were the most dominant phylum, followed by Actinobacteria (23%), Firmicutes (9%),

Bacteroidetes (2%), and other phyla (12%). Additionally, Chen et al. (2018) reported an increase in Proteobacteria and Bacteroidetes in waters contaminated with petroleum derivatives. The research data simulated in (Figure 3).

Figures 3A and B are the simulation based on the findings of Chen et al. (2018) and Auti et al. (2019), respectively. By comparing the two images, it is clear that there is a shift in diversity at the phylum level. Actinobacteria was the dominant phylum in unpolluted areas, while Proteobacteria was the dominant phylum in hydrocarbon-contaminated areas. Figure 4 illustrates the comparison of the two results.



A B

Figure 3. Simulation results of relative abundance levels of phyla in unpolluted (A) and hydrocarbon (B) polluted waters.

Manado seaport is the busiest commercial seaport connecting the islands of Sangihe, Talaud, and Maluku which is located in the middle of the business center of Manado city. Diesel pollution is indicated to come from fuel storage tanks or occurs during refueling, embarkation, and docking times. This will lead to increased pollution of diesel oil spills around the port (Abram et al., 2014). The process of a diesel or diesel oil spill includes evaporation, dissolution, emulsification, dispersion, decay of fractions and the total thickness of the oil, as well as the level of long exposure of an area by oil spills (Widhayanti & Ismanto, 2015). OPEC (Organization of the Petroleum Exporting Countries) noted that world ocean oil exploration in 2017 alone reached 95.50 mb/d. This indicates that every week, month and year there is a lot of oil spill pollution in the sea (ILHAM, 2017).

Roy et al. (2018) stated that hydrocarbon refining mud consists of hydrocarbon-degrading microbes, namely Mycobacterium (Actinobacteria), Gordonia (Actinobacteria), Novosphingobium (Proteobacteria), and Geobacter (Proteobacteria). In another study, Al-Dhabaan (2019) found that the bacteria markers of hydrocarbon contamination were Bacillus subtilis (Firmicutes), Pseudomonas aeruginosa (Proteobacteria), and Bacillus cereus (Firmicutes). Some of the findings that have been compiled about bacteria found in hydrocarbon contamination areas can be seen in (Table 1).

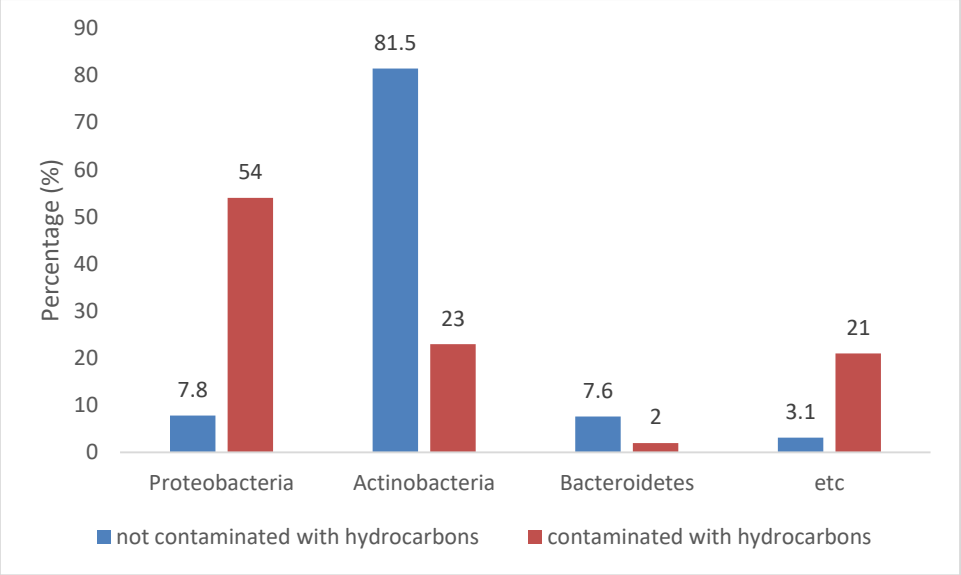


Figure 4. Simulation results of comparison of phyla in polluted (blue) and non-hydrocarbon-polluted (red) environments.

Table 1. Marking bacteria in areas contaminated with hydrocarbons

Phylum	Genus/Species	Reference
Proteobacteria	*Novosphingobium* sp.	Roy *et. al.*, 2018
	Geobacter	Roy *et. al.*, 2018
	Pseudomonas aeruginosa	Al-Dhabaan, 2019
	Pseudomonas spp.	Dealtry *et. al.*, 2018
	Comamonas	Dealtry *et. al.*, 2018
	Ochrobactrum	Dealtry *et. al.*, 2018
	Alcanivorax	Knapik *et. al.*, 2019
	Oleispira	Knapik *et. al.*, 2019
	Cycloclasticus	Knapik *et. al.*, 2019
	Pseudomonas putida	Hakima & Ian, 2017
	Alcanivorax	Knapik *et. al.*, 2020
	Oleispira	Knapik *et. al.*, 2020
	Cycloclasticus	Knapik *et. al.*, 2020
Actinobacteria	*Mycobacterium* sp.	Roy *et. al.*, 2018
	Gordonia sp.	Roy *et. al.*, 2018
Firmicutes	*Bacillus subtilis*	Al-Dhabaan, 2019
	Bacillus cereus	Al-Dhabaan, 2019

The information that has been collected from the results of previous studies shows that there are differences in the diversity, composition, and community structure of bacteria from the phylum and genus/species levels. This information was obtained from studies using the e-

DNA approach. The e-DNA approach offers several advantages that are much greater than traditional methods of monitoring the presence of bacteria associated with diesel pollution. This is due to the ability of the e-DNA approach in terms of accuracy and speed of analysis. The e-DNA approach does not require bacterial culture and all bacterial species in the environment can be detected. Thus, e-DNA can be used as a method for early detection of diesel fuel pollution in the environment, so that mitigation can be carried out quickly in order to prevent further pollution which can result in disruption of the biota ecosystem in the environment.

CONCLUSION

A literature search reveals that using the e-DNA approach, differences in bacterial diversity at the phylum, family, and genus/bacteria levels can be detected in non-polluted locations versus locations contaminated with diesel fuel. The phylum that dominates the uncontaminated waters is Actinobacteria, while the phylum that dominates the location contaminated with hydrocarbons is Proteobacteria. Several bacterial consortiums, including those from the genera *Novosphingobium* and *Geobacter*, can be used as bacteria markers of diesel oil pollution. As a result, it can be concluded that the e-DNA approach can be used to detect diesel pollution early in a location.

ACKNOWLEDGEMENTS

We express our deepest gratitude to the Ministry of Education and Culture of the Republic of Indonesia for its funding support through the 2020 Exact Research Student Creativity Program (PKM-PE). Additionally, we would like to express our gratitude to Sam Ratulangi University for assisting with the implementation of PKM activities.

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