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Bacteria as Indicators of Environmental Pollution: Review

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Abstract The environment quality of water, soil and air is degraded increasingly. Therefore we need to raise the prevention of pollution by monitoring environmental quality. There were several monitoring methods on the environmental quality, especially biological method. Biological methods assess the presence of several species, such as plants, insects, fish, bacteria and viruses as environmental indicator. Some species of bacteria have been used as indicators in monitoring environmental quality, e.g. Coliform, *Escherichia coli*, *Streptococcus* sp., *Pseudomonas* sp., *Vibrio* sp., *Clostridia* sp., *Bifidobacterium pseudolongum*, *Arcobacter* sp., *Thiobacillus* sp., and etc. The bacteria act as an indicator of household waste (human and animal feces, household waste and other), heavy metal pollution, crude oils and other pollution.

Keywords Bacteria, Biological indicator, Environment pollution

1. Introduction

Environment is not only spatial place for human but also landfill for their waste which generated from their activity. Environment has the ability to revive the situation and neutralize its own condition and restore their initial state, if the waste is below the threshold of environment carrying capacity. Environment accommodates the waste from households and industry. This leads to the changes on environmental quality of water, soil and air that also affect on the flora, fauna and microorganisms life. The type and number of microorganisms in the environment is influenced by environment characteristic and waste that flow into the environment. It either inhibits or stimulates the growth of microorganisms.

Law No. 32 of 2009 on the Protection and Management of the Environment Article 20, paragraph 2 states environmental quality standards consist of the standard quality of water, waste, sea water, air ambient, emission, disruption, and others in accordance with the development of science and technology. Based on these standards, generally the environment quality measured in chemical, physical and biological parameter [1].

A study reported a contamination of Arsenic (As) and fluoride (F) was associated with chronic kidney disease of infants in Aguascalientes, Mexico [2]. Moreover, the presence of heavy metal contamination such as Zinc (Zn), Lead (Pb) and Chromium (Cr) in water, plankton, fish and sediment in the Cauvery River India is exceed the standard level [3]. Heavy metal pollution on surface and ground water is also found in Patancheru Andhra Pradesh India. Types of heavy metals that contaminated are Sarontium (Sr), Barium (Ba), Cobalt (Co), Nickel (Ni), and chromium (Cr) [4].

Environmental pollution has globally accumulated, recognized by one indicator of global warming which caused by the emission of greenhouse gases (GHG). Several efforts have been attempted, i.e. GHG restrictions on more than 100 countries. In addition, experts have been studying the impact of global warming on the environment of Pacific ocean, the occurrence of El Nino and ocean acidification [5-7].

Several researches in Indonesia evaluate the water pollution, such as heavily polluted river of Jakarta. The researches assess the source and management of the waste in situ. Further, research in the village of Awang Bangka discovered contamination in rivers is based on nutrition value coefficient [8-10]. Monitoring of the environmental pollution in North Sulawesi has also found contamination in Tondano River in the Village of Ternate Baru, Manado, especially on the parameter of Biological Oxygen Demand (BOD), turbidity, and phosphorus content [11, 12].

Based on these research findings, the environment has been degraded due to pollution. Many parameters of environmental pollution were measured, including the biological parameters, especially microorganisms, such as bacteria. This study aimed to reviews the studies on the bacteria as indicators of environmental pollution.

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2. Monitoring of Environment Quality

2.1. Chemical Parameter

Measurements of pH have been performed to evaluate the quality of the marine environment such as the estuary area, aquaculture and watershed [13-17]. In addition to the aquatic environment, pH measurements were also performed to measure air quality, ground water, land mines and household waste which dumped into the environment [18-22].

BOD measurements were also performed in some researches to evaluate the quality of coastal waters, rivers and lakes [23-26]. Besides water quality, BOD was also performed to measure the environmental contamination of soil and water due to the waste in landfills.

COD measurements have also been carried out in several studies as one of the parameters in assessing the quality of waters of the lake, estuary, sea and river [29-32]. Further, COD is also used as a parameter in measuring the quality of waste water treatment and landfills' surroundings environmental [33-36].

2.2. Physical Parameter

Other studies that based on physical parameters measured temperature, pH, and turbidity [37-41]. Study in the Tondano Lake, Minahasa indicated that the temperature of the lake water is 24 ℃ - 33.7 ℃ and pH 6.67 - 7.71. This implies that the water quality of the Tondano Lake was still in accordance with the specified water quality standards.

2.3. Biological Parameter

Currently, many studies have been carried out to measure the environment quality through biological parameters, such as bacteria. Bacteria as an indicator can predict the possibility of the pathogens in water, but cannot accurately predict the level of contamination. The criteria of a species to be indicator are: 1) it must be in the polluted water when polluting sources of pathogenic microorganisms were found, 2) not reproduced within the environment, 3) Must be found in a larger number than the pathogenic microorganism, 4) Must respond to natural environment conditions and water treatment process in the same manner with particular pathogen species, 5) It should be easy to be isolated, identified, and counted, 6) Must be low cost to allow numerous sampling, and 7) not turn into pathogenic microorganisms (to minimize health risks in the analysis) [42].

Several studies have been conducted to assess the ecosystem quality of the river, lake and ocean using sophisticated and expensive equipment thus rarely done by the community. Therefore, several less expensive and easier methods have been developed, e.g. Biolitic method. This method monitors the health of rivers using indicators of macro invertebrates such as benthic, dragonflies, shrimp, snails, and worms. Biota that used in this method can be grouped into intolerant biota (sensitive) against contamination and tolerant (insensitive) biota against contamination. The existence of sensitive organisms to the pollution of a river indicates the good condition (uncontaminated), e.g. larvae of firefly and dragonfly. Conversely, insensitive organisms characterize the polluted river, e.g. earthworms and *cuncum*. Compared to the existing conventional methods, the results of this method can be determined in a maximum of an hour. Whereas the chemical - physical method such as BOD and COD, would takes at least five days for laboratory analysis. However, the results of another measurement using physical and chemical parameters such as pH, temperature, total suspended solid (TSS) and turbidity can be determined immediately. The drawback of these equipments is due to its very expensive cost [43].

The monitoring of environment quality based on biological parameters use the species of plants, animals and microorganisms such as viruses and cyanobacterium. There are several types of indicator plants of pollution in the environment such as *Typha* sp. as an indicator for Cadmium (Cd) and Nickel (Ni) pollutant. Moreover, *Juncus* sp. could be used as indicators of Zinc (Zn) pollution in urban water runoff [44].

The example of animal indicator for environment quality is fish, e.g. Ground fish as indicator for increasing water temperature, *Cathorops spixii* as indicator of heavy metal pollution of mercury (Hg) and methylmercury (CH_3Hg) [45, 46]. In addition to fish, other aquatic species such as *Lamellidens corrianus* used as an pollution indicator for Lead (Pb) and arsenic (Ar), *L. marginalis* for Zinc (Zn) and *Indonaia caeruleus* for Cadmium (Cd) and Copper (Cu), and *Mytilus galloprovincialis* as indicators of marine microbial contamination [47, 48].

Ephemeroptera sp., which is associated with bacteria in assessing the impact of nutrition on the macro invertebrate community of wetlands and honey bees; indicator for the content of *azinphos-methyl* and pesticides [49, 50]. Macrozoobenthos such as *Tubifex* sp., *Lumbriculus* sp., *Haplotaxis* sp., *Branchiura* sp., *Nereis* sp., *Nepthys cormuta*, and *Cossura* sp. is also used as indicators for environment quality. The higher the diversity, the better the quality of the environment [51, 52].

Several studies used unicellular cells, such as periphyton diatom in river and coli phage virus as indicator of water quality assessment in tropical highlands. Pepper Mild Mottle virus (PMMoV) is as an indicator of fecal pollution and the Cyanobacterium such as *Oscillatoria* sp., *Chroococcus* sp., and *Spirulina* sp. is indicator of Lead (Pb) pollution [53-56].

3. Bacteria as Indicators

3.1. Heavy Metal Pollution

Thiobacillus sp. is pollution indicator for mercury (Hg) in marine environment. These bacteria oxidize toxic Hg. These Hg ions enter the food chain and hazard the human. The water-soluble mercury ions (Hg^{2+}) are toxic. These bacteria change the sulfide mercury into mercury ions [57, 58] (Fig.1).

Thiobacillus sp. advanced mechanisms will convert Hg^{2+} into methyl mercury (CH_3-Hg) and dimethyl-mercury (CH_3-Hg-CH_3) through oxidation process. Methylation process requires vitamin B12 as coenzyme. Methyl mercury and dymethyl mercury are stored in fat layers. Methyl mercury is 100 times more toxic than Hg^+ or Hg^{2+}. Sulfide (HgS) called *cinnabar* is the main form of mercury ore. HgS is primary form of Mercury which can be found in anaerobic environments. HgS will be oxidized by *Thiobacilli* then formed mercury ions (Hg^{2+}) through the aeration process. The Hg^{2+} is soluble in water and is toxic [59, 60].

In addition, bacteria have a mechanism to change toxic Hg^{2+} ions into intoxic mercury (Hg^{0+}). This mechanism occurs through the role of enzymes which are sometimes categorized as a plasmid, Nicotinamide Adenine Dinucleotide Phosphate (NADP) reductase. This enzyme is produced by the *Thiobacilli* and will bind Hg^{2+} then catalyze it into intoxic Hg [60].

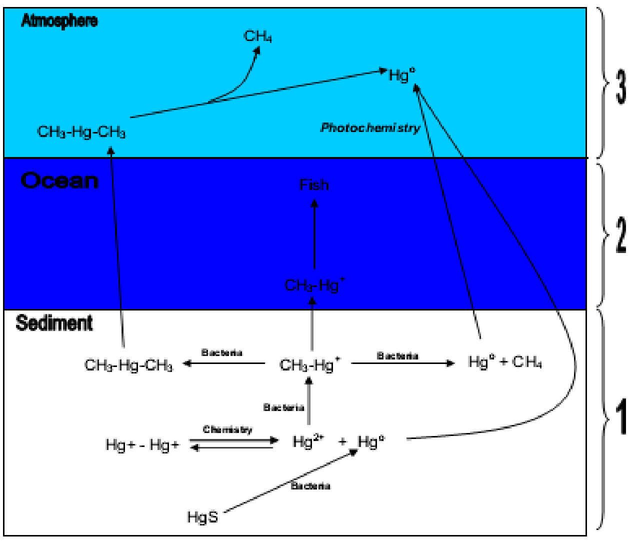

Figure 1. Mercury Cycle in the environment (Modification of Ijong [57]). Cycle of mercury divided in three phase: sediment, water and atmosphere: 1) Mercury cycle began at aquatic sediments in the form of HgS and transformed into Hg^{2+} and Hg^0 ions by bacteria, to be transformed into methyl mercury and dimethyl mercury by bacteria through the oxidation process. 2) Methyl mercury accumulated in fish through the food chain. 3) Dimethyl mercury from water oxidation can be directly released into the atmosphere or through the photochemical process of Hg^0 and CH_4

Further, periplasmic protein Hg^{2+} found in *Pseudomonas* sp. trapping protein that has the ability to bind mercury ions. The principe is that Hg^{2+} bound between two cysteine residues in proteins and form R-S-Hg-S-R bonds. Then, mercury ions are transferred through the plasma membrane, and soon turns into Hg^{0+}. This mechanism prevent the incorporation of Hg^{2+} free ions with cysteine or other proteins that can cause protein denaturation. Several studies have been performed using *Pseudomonas* sp. as an indicator of mercury pollution in the sea [61-63].

There is also *Serratia marcescens* as an indicator for pollution of Cd and Pb. Cristani et al. [64] found that *S. marcescens* can be used in the bioremediation process of toxic metals. Other studies have shown that *Thiobacillus* sp. can also be an indicator of the Pb pollution in the environment and *Vibrio* sp. as an indicator of heavy metal pollution in the sea [61, 62].

3.2. Faecal Contamination

A study using coliform bacteria and *Streptococcus* sp., as an indicator of fecal pollution in coastal areas [65, 66]. In addition to coastal area, coliform can also be used as indicator of human fecal pollution in lakes, rivers, beaches, estuaries and the organisms within, e.g. fishes [67-73].

Fecal indicator bacteria found in lakes, ponds and rivers as parts of the living environment. As long as the fecal coliform bacteria are in low level, then the swimming activity is relatively safe. In contrast, if the bacteria found in drinking water, it is a warning that should be taken for next consideration for fast response. Coliform is common indicator for quality of food and water sanitation. Coliform can be found in the aquatic environment, soil and vegetation. These bacteria are commonly present in large quantities in warm-blooded animal feces. However, coliform itself usually does not cause serious illness [74].

Escherichia coli is an indicator of humans and animals fecal contamination in lake's sediments, organic wastewater, lagoon (artificial pond), rivers, urban areas and river banks, coastal recreation waters, subtropical waters and its sediment [75-81]. Further, these bacteria are found in water sources of Alpine mountains, wild animals such as wild pigeons, regional water reclamation facilities, and beach [82-85].

Escherichia coli are able to respond the signals from the environment such as chemicals, pH, temperature, osmolarity and others in specific manner. Ozone could inactivate *E. coli* which is also particularly sensitive to temperature of 70 ℃ or more. These bacteria can live 1.5 hours up to 16 months in dead surface things [86-88].

Water becomes a contaminant medium of E. *coli* O157: H7 in public facilities, water spring and irrigation water through human or other animals feces [89-90]. These bacteria are also found in dairy farms. Handling of animal feed, litter, insect and microbial interactions in soil and water are environmental factors that affecting the presence of E. *coli* O157: H7 [91].

Soil type also affects the presence of E. *coli* in the environment. Previous study found that the clay improves the endurance and activity of E. coli O157: H7 and other coliforms. In more than 500 days of the frozen stored soil, these bacteria are still present in 37% of tested samples. Moreover, these bacteria stop growing and multiplying in milk wastewater microcosms with or without circulating aerators [92-93].

A number of studies have found that *Streptococcus* sp. as an indicator of fecal pollution in sewage treatment ponds, high rate oxidation ponds in wastewater treatment, coastal, Ganga and Cauvery River India [66, 70, 71, 94, 95]. While, *Vibrio* sp. is an indicator of fecal pollution in the water and sediment of lake [76, 85].

Generally *Vibrio* sp. is mostly found in the estuary, sea water, river estuaries and the marine sediment particles. It is often contaminate seafood such as shrimp, fish, crabs, clams, lobster etc. These bacteria are rapidly growing in the tropical area, especially in dry season. *Vibrio parahaemolyticus* were found in the waters of the bay of Manado, Tasik Ria and the waters surround Bunaken Island [96, 97].

An evaluation study of bacterial standard application for feces bioindicator on Alpine Mountain spring found that C. *perfringens* can act as indicator, however E. *coli* still more reliable [82]. Another study found that the presence of *Clostridia* spores as sulphite reductor could become bio-indicators for quality of reclamation water [84]. *Clostridia* sp. were typically found in soil, sewage, and marine sediments, as well as the intestines of animals and humans. Some *Clostridia* species are used in commercial production of alcohol and solvent industry such as *C. butyricum* and *C. pasteurianum* in producing nitrogen. *Clostridia* spores are produced during pressure times and have the ability to survive in a toxic environment while anaerobic bacteria can not survive [98].

Previous study compared the ability of *E. coli* and *B. pseudolongum* as fecal indicator in the quality assessment of raw milk and cheese. The results showed that *B. pseudolongum* is better than E. *coli* as a fecal indicator [99]. Furthermore, it was found that sorbitol fermenting *Bifidobacterium* sp. act as indicator of human fecal contamination in tropical waters of the East African area. This study suggests that these bacteria could be bio-indicators of human fecal contamination [100].

Arcobacter butzleri, *A. cryaerophilus*, and *A. skirrowii* significantly found in fecal contaminated water. This species entered the sea water through polluted freshwater, where they may be able to coexist with other native species, such as *A. marinus or A. halophilus.* In addition, all waste samples that have been studied were positive for *Arcobacter* sp. and showed a large diversity – even new candidate species such as *A. defluvii*. This indicates that the waste may be an important reservoir for these microbes and the presence of *Arcobacter* sp. in aquatic environments associated with high levels of fecal pollution in freshwater and marine [101, 102].

3.3. Wastewater Pollution

Several studies have been conducted to evaluate the quality of the environment by using bacteria as an indicator of household waste water pollution in tropical estuaries and reclamation water [84, 95, 103]. Heterotrophic bacteria, coliform, *Streptococcus* sp., and *Pseudomonas aeruginosa* was found as an indicator of wastewater contamination [94]. In addition, *Salmonella* spp. and *Streptococcus* sp. can be used as an indicator of bacterial contamination in the wastewater tropical estuaries as well [103, 104].

3.4. Oil and Pentachlorophenol Pollution

One study found that *Chromatium* sp. can acts as be bio-indicators for environmental contamination of crude oil [105]. Moreover, it was found that *Bacillus subtilis* is an indicator of toxicity and pentachlorophenol existence (organochlorine compounds used as pesticides and disinfectants) [106].

4. Conclusions

In monitoring the environment quality, there are some parameters that are commonly used, i.e. physics, chemistry and biology. Specifically, biological methods assess the presence of several species e.g. bacteria as indicators for environmental pollution. The review showed that Coliform, E. *coli*, *Streptococcus* sp., *Pseudomonas* sp., *Vibrio* sp., *Clostridia* sp., *Bifidobacterium pseudolongum*, *Arcobacter* sp., *Thiobacillus* sp., and others various of bacteria is effectively used as pollution indicators for detecting the fecal contamination, human activity waste, heavy metals, and crude oil. The results of this bacteria assessment become the consideration in decision making of policy regards to the environmental quality.

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