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Bacterial Diversity and Physicochemical Profiles in Pekalongan Waters, Indonesia

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Abstract. Pekalongan City was known as the largest Batik industry area in Central Java. However, most of the waste from Batik production was directly dumped into the rivers around the production site and empties into the Pekalongan beach. This could cause contamination of coastal areas and potentially damage the ecosystems by chemical pollutions. The objective of the study is to analyze the both bacterial diversity and physicochemical profiles of Pekalongan water which is contaminated with the Batik waste. The water samples were collected aseptically using a Nansen bottle. The bacteria from these samples are grown on the marine agar by using the spread plate method and the colonies are counted using Total Plate Count (TPC). Selected bacterial colonies on marine agar media were purified further to identify the microbiological characters. The identification of *Enterobacteriaceae* was using MacConkey agar. Physicochemical profiles were approached based on pH, salinity, BOD, COD, DO, CO_2, PO_4, NO_3, $KMnO_4$, TSS, turbidity, and metal ions (Pb, Hg, Cd). The results gained 25 selected pure isolates, which show high bacterial diversity. The microbiological character showed that 23 isolates were Gram-positive and 2 isolates were Gram-negative with various morphological characters. Coliform bacteria were found in several stations which belong to the family *Enterobacteriaceae*. The number and type of bacteria found in the Pekalongan waters indicate that the bacteria had the potential to degrade Batik industry wastes and provide a balance aquatics state based on physic-chemical analysis. The Gram-positive bacteria have a higher potential than the Gram-negative to adapt and degrade the Batik waste in Pekalongan water.

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INTRODUCTION

Pekalongan is a city known as the City of Batik [1]. The number of batik industries in Pekalongan City in 2014 reached 917 batik industries spread across several sub-districts in Pekalongan City. Escalation of the number of batik industry resulted in environmental pollution due to waste from factories [2]. The presence of batik waste is caused by synthetic dyes which are generally used in making batik. These synthetic dyes are difficult to be degraded by microorganisms so it can lead to a decrease in water quality. Previous research stated that in batik wastewater reservoirs and wells around ditches located in the center of the Jenggot batik industry, Pekalongan City contained heavy metals such as Cd, Cr, and Pb that exceeded the required environmental quality standards [3].

Synthetic dyes used in making batik contain several heavy metals such as Cd, Pb, Cr, Cu, Co, Hg, Ni, Mg, Mn, and Fe [3]. Heavy metals that are polluted in waters may be toxic so that they inhibit the growth of species in the waters. This resulted a decrease in the number of species found in these waters [4]. In addition, synthetic dyes could be an effect for human and animal health problems such as cancer [5]. However, there has not been much research on the condition of batik waste found in river and sea waters.

Waters have an important role in life and are commonly used for agricultural irrigation, drinking water, transportation, power generation through hydropower, and recreation such as swimming and boating. Water quality can be measured based on physical parameters (turbidity, temperature, color, solids), chemical (pH, alkalinity, heavy metals, dissolved oxygen, BOD, COD, organic substances, and biology (bacteria, algae, viruses, protozoa)) [6].

Type of bacteria that often found in water pollution caused by Batik dyes are *Bacillus* sp. and *Pseudomonas* sp. [7]. Research on bacterial diversity in Pekalongan estuary until sea side that contaminated by batik waste has never been done. Therefore, the aims of this study are to see the diversity of bacteria and analyze the physicochemical profiles in the aquatic environment polluted by heavy metals from batik waste.

MATERIALS AND METHODS

Sampling location is located in Pekalongan River, Central Java. Sampling points include river bodies, river estuaries, and the sea which could be seen in Fig. 1. Determination of sampling time is influenced by wind, weather, waves, and sea conditions. Sampling was carried out on Tuesday, June 15, 2021 based on consideration of existing factors. Water sample was taken using a Nansen bottle with direct sampling technique on each sampling points. The sample for microbiology test was put into 1 L sterile dark glass bottle and stored in cool box to maintain the bacteria inside water. Water sample for COD and BOD test was stored into 500 ml dark plastic bottle. Sample for heavy metal, CO_2, pH, salinity, organic compound, nitrate (NO_3), and phosphate (PO_4) test was stored into 5 L jerry can.

FIGURE 1. Sampling location

Water sample was grown on Zobell Marine Agar media dissolved in seawater. 0.9 ml of seawater was poured into a microtube to prepare a 10^{-1} to 10^{-5} dilution, then 0.1 ml of the water sample is poured in stages into each dilution, starting from the 10^{-1} dilution to 10^{-5}. After that, the diluted sample was homogenized using a micropipette, then 0.9

ml of water sample in each dilution poured into Zobell Marine Agar and spreaded using a glass spreader. Incubation was carried out between 24-48 hours at 30^{o} C. The growing colonies was observed and counted.

The growing mixed culture colony will be selected based on macroscopic morphology such as shape, color, texture, margin, and elevation. Selected colony will be purified on Zobell Marine Agar media. Pure culture will be Gram tested to see whether the bacteria belongs to Gram positive or Gram negative. Microscopic observation was done using Opti Lab. The image will be interpreted using Image Raster software to measure the bacteria cell. The pure colony will be grown on MacConkey differential and selective media.

Analysis of organic compound, COD, BOD_5, CO_2, PO_4, and NO_3 in water sample were done in BBTPPI laboratory. The method used to test organic substances refers to SNI 06-6989.22-2004, COD refers to SM 5220 D, 23rd Edition : 2017, BOD_5 refers to SM 5210 B, 23rd Edition: 2017, CO_2 refers to SM 4500-CO2 D, 23rd Edition : 2017, PO_4 refers to MU 2.08 (discrete photometry), and NO_3 refers to MU.2.03 (discrete photometry). Turbidity, Dissolved Oxygene (DO), Total Suspended Solid (TSS), and heavy metal (Pb, Hg, Cd) analysis were done in Central Laboratory, Diponegoro University.

RESULTS AND DISCUSSION

Total Plate Count (TPC) is a method to indicate the number of microbes (fungi, yeast, bacteria) contained in a product by counting bacterial colonies grown on agar media [8]. Microbial colonies that can be counted are those with microbial colonies between 30-300 colonies [9]. Microbial cells that can live and reproduce when grown on agar media and form colonies that can be seen without using a microscope [10]. The highest number of bacteria was observed at station C1, while the lowest number was observed at station C12. TPC results are shown in Table 1.

Mixed cultures grown on Zobell Marine Agar media were selected based on their different macroscopic morphology and 25 isolates obtained had been purified. The selection of isolates was based on shape, color, margin, elevation, and texture (See supplementary 1). The bacterial isolates obtained had circular and irregular shape with transparent, creamy white, neon yellow, orange, white, creamy chocolate, super white, and soft pink colors. Based on Table 1, there were 23 Gram-positive isolates and 2 Gram-negative isolates. The results showed that the presence of Gram-positive bacteria was more dominant when compared to Gram-negative bacteria. Gram-positive bacteria have long been found in marine environments. However, these bacteria are not considered as indigenous bacteria but are introduced from terrestrial habitats. Gram-positive bacteria have unexpected diversity in marine bacterioplankton and marine sediment communities where they account for 13% of the total bacteria [11]. Marine sediments, including deep sea sediments, are the main marine habitats where positive bacteria are found [12]. The results of research in 2019 showed that 12 bacterial isolates that were successfully isolated from Indonesian marine waters in the Lombok Strait and Indian Ocean were Gram-positive bacteria [13].

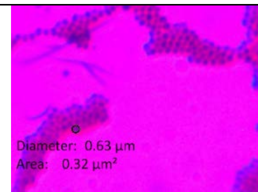
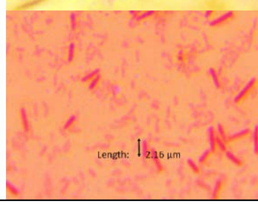
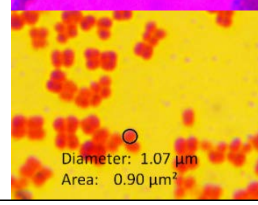
Other research in 2019 that located in Banyumas has identified the genus of Gram-positive bacteria that could degrade batik waste. The identified genus are Xanthobacter, Deinococcus, Sporosarcina, Methylococcus, and Bacillus [14]. Bacteria are able to tolerate water environments that are toxic to heavy metals caused by batik waste because there is NaCl content in seawater which makes these bacteria halophilic and halotolerant. Halophilic and halotolerant bacteria have a good metabolic potential to degrade azo dyes because it could tolerate the high number of oxyanions and heavy metal [15]. Bacteria in batik wastewater enzymatically interact with each other to reduce heavy metal levels. The decrease in the levels of other heavy metals also resulted in decrease of BOD value. A research conducted by Muchtasjar with his colleagues showed that bacteria consortium including Xanthobacter, Deinococcus, Sporosarcina, and Bacillus reduced BOD levels by 85.71%.

The isolates were then observed microscopically and described in Table 2. Based on the result in Table 2, the biodiversity of bacteria originating from Pekalongan water is very high.

TABLE 1. Bacteria test

Bacteria Test		Station C												TOTAL
		C1	C2	C3	C4	C5	C6	C7	C8	C9	C10	C11	C12	
Total Plate Count (CFU/ml)		$1,6 \times 10^7$	$3,3 \times 10^5$	$2,7 \times 10^5$	4×10^6	$3 x 10^5$	$6,4 \times 10^5$	$1,7 \times 10^6$	$4,4 x 10^4$	$1,5 \times 10^5$	$1,1 \times 10^6$	$9,8 \times 10^5$	$2,5 x 10^4$	-
Gram Staining	**Positive(+)**	2	1	4	-	2	1	1	-	3	4	5	-	23
	Negative(-)	1	-	-	-	-	-	-	-	-	-	1	-	2

TABLE 2. Microscopic morphology

Picture	Description	Picture	Description
	Shape : Bacillus Gram : Positive Length : 2,64 µm Code : C6A		Shape : Coccus Gram : Positive Diameter : 0,63 µm Code : C1B
	Shape : Bacillus Gram : Negative Length : 2,16 µm Code : C3D		Shape : Coccus Gram : Negative Diameter : 1.07 µm Code : C1D

Bacterial colonies derived from the purification results were regrown in differential media in the form of MacConkey so that it would be known the presence of bacterial isolates belonging to the group of Gram negative bacteria. MacConkey is a selective and differential medium used to grow species of Gram negative bacteria based on their metabolic ability to carry out lactose fermentation [16]. MacConkey media supports the growth of Gram-negative rods, particularly *Enterobacteriaceae* [17]. On the other hand, this medium inhibits the growth of Gram-positive bacteria and some fastidious Gram-negative bacteria, such as *Haemophilus* and *Neisseria*. This is due to the content in MacConkey media, namely crystal violet dye and bile salts which are able to stop the growth of Gram-positive bacteria [16].

Isolates grown on MacConkey Agar media had various colony colors, ranging from pink to purple, pink, to colorless. The differences in the color of the colonies were caused by differences in the ability of each bacterium to ferment lactose contained in MacConkey media. Gram negative bacteria capable of fermenting lactose will grow with pink colonies [16]. There were 2 bacterial isolates that were successfully grown in the media from station C1 and C3. The isolate code from station C1 is C1D, while at station C3 it is C3D. Both isolates grew on MacConkey media with transparent colonies. Bacteria that cannot ferment lactose will form white or colorless colonies. Several genera of Gram-negative bacteria that cannot ferment lactose are the genera *Salmonella*, *Shigella*, *Proteus*, and *Yersinia*. The appearance of the colonies of *Salmonella*, *Shigella*, and *Proteus* had a flat elevation and the diameter of the three colonies was two to three millimeters. While the size of the *Yersinia* colony diameter is less than one millimeter [16,18].

The genus *Escherichia*, *Enterobacter*, *Klebsiella*, *Salmonella*, *Proteus*, and *Yersinia* are a genus of bacteria belonging to the family Enterobacteriaceae. Several genera from this family are known as opportunistic pathogenic bacteria in waters that cause disease to humans and animals in the waters [19]. A study showed that several genera of *Enterobacteriae* such as *E. coli*, *Serratia* spp., *Enterobacter* spp., *Klebsiella* spp., *Proteus* spp., and *Salmonella* spp. found in the Istanbul Strait has resistance to heavy metals. *E. coli* is known to have resistance to heavy metals such as Ni, Zn, and Cu. Heavy metals in high levels are one of the stress factors for bacteria. Bacteria enlarge the plasmid under conditions of stress. But under certain conditions, the plasmid will shrink and disappear so that bacteria can adapt to these environmental conditions [20].

The waters have a source of nutrients, organic matter, and various other compounds that can support the growth of aquatic bacteria. In addition to the availability of nutrients, waters also contain dissolved oxygen which is essential for the life of microorganisms. Other factors that affect the growth of aquatic bacteria are the level of biochemical oxygen demand (BOD), suspended solid (SS), total phosphorus (TP), total organic carbon (TOC), water temperature (WT), and phosphate phosphorus (PO4-P) content [21].

pH

Acidity (pH) is one of the chemical parameters as an indication of water pollution. Measurement of pH could indicate whether the water is acidic or alkaline. Water pollution could change the pH of the water and have an impact on aquatic biota. Animals, plants, and even microorganisms cannot live stably because of changing pH conditions. In addition, heavy metals will become more soluble in water and will be more toxic [6,22–24].

The average pH at station C is 8.7 and it exceeds the standard set by The Decree of the Minister of the Environment Number 51 of 2004 concerning Seawater Quality Standards which ranges between 7-8.5. It could be influenced by biological activity, photosynthesis, temperature, oxygen content, and the amount of anion cations. In addition, the high pH value also be caused by the entry of organic matter and activities around the land carried by water currents [22,25,26].

Salinity

Salinity is one of the natural limiting factors for living in these waters. Based on the data that has been taken, the salinity value at station C has an average of 23.17 $^{o}/_{oo}$. Based on the Decree of the State Minister of the Environment Number 51 of 2004 concerning Seawater Quality Standards, salinity values can vary at any time (day, night, and season), and allow changes to occur up to <5 $^{o}/_{oo}$ seasonal average salinity. Based on this decision, the salinity values at the three stations were appropriate, with a seasonal average salinity value of 22.73 $^{o}/_{oo}$. At station C, the salinity value is more than the seasonal average salinity value. The low salinity value can be presumed to be influenced by the land, such as mixing with freshwater carried by rivers [27]. The results of research in 2019 shows that the lowest salinity value at the surface of the Java Sea and its surroundings is close to 30.0 $^{o}/_{oo}$, while the highest salinity value reaches 35.0 $^{o}/_{oo}$[28]. The open sea generally has stability and similarity to composition, including total salinity. On the other hand, marine environments near land (coasts) and water bodies such as estuaries and lagoons have dynamic salinity due to tidal influences, fresh water input, evaporation, and increased waste contaminants from human activities [29]. Salinity is known to control the diversity of microbial communities. Salinity is not a barrier to microbial life, but it can limit its diversity [30,31]. Smyth and Ellioth in 2016 also stated that changes in salinity affect community structure and species distribution boundaries [29]. The effect of salinity on bacterial diversity is quite variable. Several studies have shown that salinity reduces bacterial diversity, but peaks when salinity levels are low [32].

Biological Oxygen Demand (BOD)

BOD or Biological Oxygen Demand is an indicator of pollution in waters. BOD is the amount of oxygen needed by an organism to replace an organic component at a certain time. The higher the value, the more organic matter contained, thereby increasing the growth of microorganisms [6,22,24,33]. The results of the observations stated that the BOD value at station C has the average of 5.8 mg/L. Based on the Decree of the State Minister of the Environment Number 51 of 2004 concerning Seawater Quality Standards, the BOD figures at both stations were still below the standard, 20 mg/L. High BOD conditions in a water indicate that the water contains a lot of bacteria. If a water contains a large amount of organic waste, many bacteria are present working to decompose the waste which causes the oxygen demand to be high, so the BOD level will be high. On the other hand, low BOD levels indicate that bacteria and waste in the waters are few in number [34]. Wastewater in Malang shows BOD value of 206.88 mg/L which higher than Pekalongan [35].

Chemical Oxygen Demand (COD)

The amount of oxygen that consumed by the reaction in oxidizing organic compounds could be expressed in terms of COD or Chemical Oxygen Demand. The COD number could calculate the total amount of pollutants that could be oxidized in the water because this test cannot distinguish between organic or inorganic components [6,23,24,33] .At station C, the COD average value is 20.9 mg/L. The COD threshold value based on the Decree of the State Minister of the Environment Number 51 of 2004 is 10 mg/L. This indicates that the COD value at station C exceeds the normal limit that has been set. The COD value will always be higher than the BOD value [6]. COD values in the waters of the Bedog River, Yogyakarta have a range of <5-28 mg/L. The high COD value can be caused by the location close to the disposal of Batik waste. The COD value will also be higher during the day [36]. COD is one of the important factors for the growth of microorganisms because it is related to the nitrification and denitrification processes. The high value of COD has an impact on changes in bacterial groups. These changes are triggered by nutritional competition between groups of bacteria [37].COD is also one of the parameters of water pollution COD is also one of the parameters for the contamination of waters [38].

Dissolved Oxygen (DO)

Dissolved oxygen (DO) is the oxygen content that dissolved in the air which is used as a parameter to measure air quality. The average of DO levels at station C is 5.6 mg/L. The lowest score is at station C4, while the highest value is at station C8. According to the Decree of the State Minister for the Environment No. 51 of 2004, stations C2 (3.9 mg/L), C4 (3.4 mg/L), C6 (4.4 mg/L), C11 (3.5 mg/L), and C12 (4.7 mg/L). /L) does not meet the minimum DO value standard, which is >5 mg/L. DO at concentrations less than 2 mg/L can threaten marine animals known as hypoxia [39]. Indirectly, environmental conditions with concentrations at the threshold can support bacteria to grow well [40]. In the study in Condong River, Cirebon, DO values ranged from 1.93-5.03 mg/L. Stations that have a low DO value indicate that there is high pollution due to organic matter, especially residential waste. Decomposition of organic matter and oxidation of inorganic matter will affect DO value [41].

Carbon Dioxide (CO_2)

The concentration of CO_2 at Station C ranges from 43.63-97.7 mg/L with average of 83.4 mg/L. The highest level is at point C1 of 97.7 mg/L or equivalent to 97.7 ppm and the lowest level is at point C12 of 43.63 mg/L or equivalent to 43.63 ppm. These data indicate that CO_2 concentrations are relatively high compared to CO_2 levels in seawater in general [42]. The high concentration of CO_2 in waters is caused by the formation of carbonic acid which then has the potential to release hydrogen ions which then lowers the pH in these waters. The low pH in certain aquatic habitats can be toxic to organisms that live in them so that only organisms that have a tolerance for low pH can live well [22,42,43]. In the Ampenan River, Lombok, the CO_2 value has a range of 15-50 mg/L. CO_2 can be formed from the activity of microorganisms that oxidize organic substances [44]. CO_2 values that are too high can be toxic to aquatic biota.

Phospate (PO_4)

Phosphate (PO_4) is one of the parameters to identify the level of water pollution. The concentration of phosphate in the waters is present in high amounts, which can be suspected of being polluted [45]. Phosphate levels at station C ranged from 0.002 to 0.006 mg/L with 0.004 mg/L average. The highest phosphate levels were at stations C3, C6 and C12, namely 0.006 mg/L, 0.005 mg/L, and 0.005 mg/L. Station C3 is far from the coast, while C6 and C12 are closer to the coast. The lowest phosphate concentration is at station C1 which is the farthest from the coast. Phosphate pollution occurs a lot on the coast because the beach is the closest location to the source of phosphate from factory waste and household waste [46]. Phosphate levels at station C are below the threshold of 0.015 mg/L based on the Decree of the State Minister of the Environment Number 51 2004 concerning Seawater Quality Standards, which means the waters are not polluted by phosphate. Sources of phosphate in Pekalongan waters are caused by human activities, such as community agricultural waste, household waste, and batik industry waste. In addition, phosphate compounds in waters far from the mainland are came from the results of metabolism in animals and the breakdown of organic matter and phosphate minerals. Natural sources of phosphate in waters could be through weathering, dead organism carcasses, and industrial waste which is broken down by bacteria into nutrients [47]. The increase in phosphate concentration can be caused by the discharge of waste from human activities that are discharged into the waters. The results of research conducted by Yusuf in 2018 showed that the Java Sea contained phosphate in the range of 0.04 to 0.24 mg/L [48]. The highest concentration of phosphate is obtained from the point at the mouth of the river mouth. Meanwhile, the lowest concentration of phosphate was found at a point located in the waters towards the open sea. Microbes generally require phosphorus in high concentrations where this phosphorus requirement is met from the absorption and metabolism of phosphorus-containing organic substrates [49]. The phosphorus available to microbes is in the form of inorganic phosphate and this element is important for use in growth, energy, and nucleic acid synthesis. However, the presence of phosphate in marine areas located in tropical and subtropical latitudes is indicated by low concentrations. Low phosphate concentrations are known to limit microbial productivity [50]. In these conditions marine bacteria will synthesize the enzyme alkaline phosphatase (APase) which allows it to access many organic substrates that contain phosphorus [51].

Nitrate (NO_3)

Nitrate (NO_3) is one of the reactive inorganic nitrogen compounds and commonly distributed in aquatic ecosystems, especially seawater. Nitrates found in seawater ecosystems could be produced due to natural factors such as nitrogen fixation by several types of microbes to human activity factors that produce various types of inorganic nitrogen carried to marine ecosystems [52]. The results of observations of nitrate concentrations at station C ranged from <0.001 to 0.388 mg/L with an average of 0.105 mg/L. The highest nitrate concentration of 0.388 mg/L is at station C12, while the lowest <0.001 mg/L is at station C5. Based on the Decree of the Minister of the Environment Number 51 of 2004 concerning Seawater Quality Standards, it can be concluded that the condition of the waters at station C is polluted with excess nitrate compounds that can endanger aquatic biota because they are at the threshold of 0.008 mg/L. Nitrate could be obtained from bacterial or microbial fixation that converts free nitrogen into dissolved nitrate so that high nitrate concentrations can be correlated with high nitrogen fixation activity by these microbes [53]. At stations C12, C11, and C10, nitrate concentrations were higher than other stations which could be caused by waste from community activities that produced dissolved inorganic nitrogen compounds carried by rivers to accumulate in estuaries (stations C12, C11, and C10). Most of the nitrogen compounds produced from community activities will accumulate in river mouths or estuaries, so it makes sense if this area has a higher nitrate concentration than other stations in the high seas [54].

Organic Matter ($KMnO_4$)

Potassium permanganate ($KMnO_4$) is a substance commonly used as oxidizing agent. $KMnO_4$ will completely dissociates in water to K^+ and MnO_4^-. The MnO_4^- is a strong oxidative agent that could be use to detect Total Organic Matters (TOM) in waters and act as therapeutic and prophylactic agent [55–57]. $KMnO_4$ also known as "disinfectant" that could kill bacterial and parasitic live forms in aquatic habitat [57]. Some research shows that adding $KMnO_4$ to aquaculture could recover and prevent catfishes (*I. punctatus*) from columnar disease caused by *F. columnare*. *Flavobacterium columnare* are parasitic bacteria that could cause aberration on fish's skin and latter systemic disease [56,57].

The content of $KMnO_4$ could be an indicator of water pollution. The concentration of $KMnO_4$ at station C ranged from 3.21-21.26 mg/L with mean value 11.47 mg/L. The highest points are at C9, C8, C5, and the lowest points are at C10, C4, C6, C3, C2, C1, C7, C11, and C12. The levels of $KMnO_4$ at station C are still at a safe limit based on the Decree of the State Minister of the Environment No. 51 of 2004, which is <30 mg/L. $KMnO_4$ are came from terrestrial soil, natural surface waters, and human activities such as industrial waste [58,59].

Moderate concentration of $KMnO_4$ and considering its nature as a strong oxidative agent in water, it has potential to cause oxidative stress and thus damage marine cells of marine bacteria. $KMnO_4$ can be used as disinfectan and has therapeutic value to prevent pathogenic microbes infection [56,57]. It is safe to assume with mentioned concentration of $KMnO_4$ at C station, it could prevent some bacteria and parasitic bloom and contaminate waters within C station area.

Total Suspended Solid (TSS)

TSS (Total Suspended Solids) is a parameter used to monitor water quality and management of air resources [60]. The TSS value is one part that plays a role in determining the quality of the aquatic environment. If the waters have a high TSS value, the productivity of the waters will be lower. This affects the condition of the waters and shows the level of pollution that can damage the marine biota ecosystem [61].Based on the data at station C, the TSS value has an average of 8.67 mg/L. Based on the Decree of the Minister of the Environment Number 51 of 2004 concerning Seawater Quality Standards, the TSS value at station C is less than 20 mg/L which indicates that the water quality based on these parameters is classified as good. In the waters of the Bedog River, Yogyakarta which are also polluted by Batik waste, the TSS value is from 11-29.5 mg/L [36]. This value is below the threshold, the same as Pekalongan waters.

Turbidity

Turbidity describes the lack of brightness of the waters due to the presence of colloidal and suspended materials such as mud, organic and inorganic materials, and aquatic microorganisms [62]. At station C, the turbidity value ranges from 0.00 – 3.83 NTU. The lowest value is at stations C2, C5, C6, C8, and C9, which is 0.00, while the highest value is at station C1 which is 3.83 NTU. The lowest turbidity value from each station still meets the water turbidity quality standard according to the Decree of the State Minister of the Environment No. 51 of 2004, which is <5 NTU. The results of previous studies stated that the higher the turbidity value, the higher the abundance of bacteria in waters [63]. Research in Citarum river showed that the turbidity of its polluted water is 320 NTU [64]. Conditions that exceed normal limits affect bacterial biodiversity, because the cloudier the waters, the higher the colloid and suspended materials such as mud, organic and inorganic materials and aquatic microorganisms. Turbidity of sea water with the highest value dominates coastal waters close to the river mouth and vice versa towards the sea the turbidity of the water decreases. The concentration of organic matter is closely related to the total density of bacteria in the waters of the Baboon River estuary, the higher the concentration of organic matter, the greater the total density of bacteria contained in these waters [65].

Metal Ions (Pb, Hg, Cd)

Lead (Pb) is known as a non-essential heavy metal which if it exceeds the specified quality standard, will cause poisoning (toxicity) in living things and inhibit the growth rate of microorganisms. Sources of lead pollutants (Pb) in the environment include industrial waste, ship paint, and lead-based fuel (Pb) [66]. The results showed that at stations C5 and C7 the Pb levels were 2,318 ppm and 1,854 ppm. Based on the Decree of the State Minister of the Environment No. 51 of 2004, stations C7 and C5 exceed the threshold, which is 0.008 ppm.

Mercury (Hg) is a heavy metal that is very toxic because it could inhibit enzyme function, disrupt cell membranes and denature proteins in microorganisms [67]. Mercury is difficult to decompose in aquatic environments and is generally concentrated in water and sediments [68]. The range of Hg values at station C is 0.77 – 2.257 ppm. This value exceeds the threshold based on the Decree of the State Minister of the Environment No. 51 of 2004, which is 0.001 ppm.

Cadmium (Cd) is a heavy metal that is known as a by-product of various industries such as the battery industry, electrical conductors, alloys, pigments, and plastic manufacturing, and the stabilization of phosphate fertilizers [69]. The presence of Cd contamination in the environment induces toxicity to microorganisms by inducing oxidative damage and damaging cell membranes and DNA structures resulting in functional disorders, enzyme work, and oxidative phosphorylation in exposed microorganisms [67]. The concentration of Cd at station C has exceeded the specified threshold of 0.001 ppm so that station C is contaminated with Cd metal.

Research in the coastal waters of Lhokseumawe City, Aceh Regency has bad conditions due to industrial waste. The values of Hg and Cd were still within safe limits, but the Pb values had exceeded the threshold, which ranged from <0.05-0.70 Mg/L [70]. Excessive amounts of heavy metals will cause toxicity to microorganisms. Heavy metals can cause the enzymatic function of the production of reactive oxygen species, impair ion regulation and can cause mutations as well as physiological and biochemical properties [67]. In a previous study showed that heavy metal pollution in the waters of the Chepelarska river, Bulgaria reduces microbial diversity in it [71].

CONCLUSION

The waters of Pekalongan which are the place for Batik waste disposal are dominated by Gram-positive bacteria. Based on the physicochemical analysis, the bacteria obtained in this study, including the Enterobacteriaceae group, have the potential to adapt and degrade Batik waste.

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