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Original Research

Biofilm as a Natural Detector: Monitoring Lead (Pb) Contamination in the Industrial Rivers of Manyar, Gresik

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Abstract

Biofilm is a layer of complex microorganisms that grow and adhere strongly to solid surfaces, such as rocks, sediments, and substrates on the riverbed. This study specifically examines the ability of biofilms to absorb the heavy metal lead (Pb) in the aquatic ecosystem of the Manyar River, Gresik, Indonesia, by comparing them to sediment and water media as the main comparative parameters. Sampling was carried out at three strategic stations along the river flow, which represent variations in the level of exposure to polluting sources: Station I upstream (near industrial activity), Station II in the middle, and Station III downstream (away from sources of pollution). Lead concentrations were analyzed using an accurate Atomic Absorption Spectrophotometer (AAS) method. The comparison between samples was calculated through the BAF (Biofilm Accumulation Factor), BSAF (Biota-Sediment Accumulation Factor), and Kd (Distribution Coefficient) indicators. The results of the analysis revealed that lead content in August 2023 was highest at Station I, reflecting its proximity to the industrial zone, while the lowest value was recorded at Station III. Notably, all measured lead concentrations exceeded the national river water quality standard threshold (0.5 mg/L), indicating severe contamination. The most notable finding is the remarkable accumulation capacity of biofilms: 83-90 times higher than water, and 90-91 times higher than sediments. This fact confirms the role of biofilm as a highly efficient natural biomonitoring agent in the remediation of pollutants in polluted waters.

Keywords: biofilm, biomonitoring, lead

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Introduction

The Manyar River is one of the main rivers located in Gresik Regency, East Java. This river plays an important role in supporting the social and economic life of the people in the surrounding area. The water from the Manyar River is used for agriculture and fisheries, making it a vital resource for various sectors of life in Gresik. The Gresik area is known as one of the largest industrial activity centers in Indonesia. The presence of various industries such as fertilizers, metals, and manufacturing makes a great economic contribution but also poses an environmental threat, especially to the quality of river water. Liquid waste from production processes that are not treated properly can increase the pollutant load in the Manyar River [1].

Aquatic pollution is a complex environmental problem because it involves the interaction between physical, chemical, and biological factors. Domestic and industrial waste that enters rivers carries various types of pollutants, ranging from organic matter, excess nutrients, to heavy metals [2]. The content of these pollutants can disrupt the balance of water ecosystems and reduce the natural ability of rivers to carry out the self-purification process [3]. To understand the impact of pollution on water ecosystems, an accurate and representative water quality assessment method is needed. So far, the most commonly used approach is evaluation based on physico-chemical parameters, such as pH, temperature, dissolved oxygen (DO), conductivity, and concentrations of various metals and solutes. This method allows for rapid identification of changes in aquatic conditions in a given time [4].

The results of parameter measurements are usually summarized in the form of Water Quality Index (WQI) or other water quality indices that describe the status of water quality in one numerical value. This index is widely used in water management policies because it is practical and easy to compare between locations. Nevertheless, this approach has a fundamental drawback as it only reflects physical and chemical conditions at the time of sampling [5]. The physical and chemical parameters of water are volatile and are greatly influenced by time, season, rainfall, and human activities around the river. These fluctuations make the interpretation of the measurement results often unstable and not representative of actual ecological conditions [6]. Therefore, physico-chemical data should be equipped with biological parameters that can reflect the long-term condition of aquatic ecosystems [7].

The biological approach using bioindicators is considered more comprehensive because it is able to show the combined impact of various environmental pressures that occur continuously. Bioindicators represent the real response of living organisms to changes in the quality of aquatic habitats, both due to organic and inorganic pollutants, as well as changes in physical conditions such as flow and temperature [8]. Several groups of organisms such as plankton, macrozoobentos, and microorganisms have long been used as bioindicators in monitoring water quality [9]. Each group of organisms has a different tolerance to pollution levels, so changes in community composition can be an important signal of ecological disturbance. Biofilm is one of the bioindicator candidates that is increasingly studied because it has good adsorption ability [10].

Biofilms are communities of microorganisms that grow attached to the surface and are encased in a layer of Extracellular Polymer Substances (EPS). The layer consists of polysaccharides, proteins, lipids, and extracellular nucleic acids (eDNA) that function to protect and maintain the stability of microbial communities from the influence of the outside environment. EPS also acts as a matrix in which biochemical interactions occur [11].

Biofilms can form in various aquatic ecosystems, both freshwater and seawater, as long as there is a moist surface for microorganisms to attach. Environmental factors such as flow speed, nutrient availability, pH, and light intensity can affect the growth rate and structure of biofilms. Naturally, biofilms play a role in nutrient cycling and maintaining the balance of aquatic systems [12]. In polluted conditions, biofilms are able to adapt to environmental pressures by changing the composition and function of their microorganisms. Communities exposed to certain pollutants, such as heavy metals or high organic matter, generally show changes in microbial diversity and metabolic activity. This makes biofilm a sensitive and stable bioindicator of changes in environmental quality [13].

One type of pollutant that deserves attention is heavy metals, especially lead (Pb). Lead is a toxic metal that comes from various industrial sources such as metal smelting, batteries, paints, and traffic activities. Even in low concentrations, lead can accumulate in the tissues of aquatic organisms and cause toxic effects on the enzymatic and physiological systems of biota [14]. High concentrations of lead in river water are not only harmful to aquatic biota but also risk poisoning humans through the consumption of contaminated fish or other aquatic organisms. The accumulation of lead in the tissues of the human body can lead to disorders of the nervous system, reproductive system, and kidney function. Therefore, the presence of lead in aquatic ecosystems is one of the serious indicators of environmental pollution [15].

Biofilm is known to play an important role in the process of bioabsorption and bioaccumulation of heavy metals, including lead. Active groups in EPS components such as carboxyl (-COOH), phosphate ($-PO_4$), and hydroxyl (-OH) have a high ability to bind to metal ions. This mechanism allows the biofilm to capture and retain pollutants from the water flowing through it, so that it can serve as a natural biological filter [16]. In addition to biofilms, sedimentary components are also often used in studies to study the distribution of heavy metals.

Sediment acts as a storage of pollutants that collect from the water column over a long period of time. Meanwhile, surface water provides an indication of ongoing or temporary pollution conditions. Comparisons between the three can provide a complete picture of the behavior of heavy metals in aquatic ecosystems [17].

This study aims to compare the lead content in biofilms, sediments, and water in the Manyar River, as well as evaluate the ability of biofilms to absorb and accumulate heavy metals. This research is expected to provide comprehensive scientific information on the dynamics of lead distribution in river ecosystems and the potential of biofilms as indicators and natural biomonitoring agents. This study is also expected to be the basis for the development of biofilm-based biomonitoring methods that are more efficient and applicable in Indonesia.

Materials and Methods

Research Location

This research was conducted in the Manyar River, Gresik Regency, East Java, in August 2023 (Fig. 1) and measurements were carried out in the dry season. This study aims to determine the condition of water quality that is suspected to be affected by the surrounding industrial activities. Sampling was carried out at three research stations selected based on differences in environmental characteristics, namely locations adjacent to the main sources of pollution (Fig. 2a)), areas receiving additional domestic waste from nearby settlements (Fig. 2b)), and points located near confluences of large rivers flowing into the sea (Fig. 2c)). The selection of these points is intended so that the data obtained can represent variations in pollution levels along river flows.

The Manyar River has an important role for the surrounding community as a source of life, both for household, agriculture, and fishery activities. However, the rapid development of industrial estates in the Gresik area has led to an increase in the potential for environmental pollution, especially in water bodies such as rivers. Various industrial activities around the area, such as the chemical and manufacturing industries, produce liquid waste that has the potential to contain toxic and hazardous materials. If not properly treated, the waste can pollute the aquatic environment and negatively impact aquatic biota [18]. One of the pollutants that is often encountered due to industrial activities is heavy metals. Heavy metals have distinctive characteristics. They do not decompose easily and can survive for a long time in aquatic environments [19]. In the long term, these metals will accumulate in sediments, aquatic organisms, and enter the food chain. This condition makes heavy metals a serious threat to the balance of the ecosystem because their toxic

Fig. 1. Map of the research location.

Fig. 2. Location of stations: a) station 1, b) station 2, c) station 3.

effects can be chronic and cause biological disturbances in living things [20]. Of the various types of heavy metals, lead (Pb) is one of the most dangerous and often found elements in industrial waste. Lead is toxic, non-biodegradable, and has the potential to cause long-term pollution in the aquatic environment. The accumulation of lead in sediments and organisms can disrupt the balance of ecosystems and significantly reduce the quality of the waters [21].

Water Sample Collection

The bottle is filled slowly directly from the drain without touching the bottom or walls of the drain so as not to mix with solid materials such as leaf litter, sand, or other debris that may affect the chemical composition of the sample (Fig. 3). This technique is applied consistently at three observation points, namely station 1, station 2, and station 3. Each station was sampled three times so that the data obtained could represent water conditions more accurately and reduce the possibility of bias from a single sampling. Once the bottle is fully filled, the lid is immediately tightly closed to prevent outside air exchange that could alter the characteristics of the sample, such as dissolved oxygen levels or water pH. Each bottle is then assigned an identity tag that includes a location code and time of collection so that it can be easily identified during the analysis process in the laboratory. All samples that have been collected are then put into a refrigerated box with a temperature of about ±4°C to maintain the stability of their chemical and physical properties during the transportation process. This step is important to prevent biological or chemical reactions that can alter water quality parameters before laboratory testing is carried out [22].

Sediment Sample Collection

Sediment sampling in this study used a 25 mm diameter PVC pipe as a simple and reliable tool, utilizing the principle of a vacuum to capture the sample without disturbing the natural stratification (Fig. 4). The process began by tightly sealing the bottom end of the pipe to create a vacuum, which was then lowered vertically through the water until it touched the substrate. The seal was gradually opened so that hydrostatic pressure pushed the sediment into the pipe to a depth of 20-30 cm without turbulence. The tip was then resealed to secure the contents during lifting, and the sample was extracted with a thin palette knife, transferred to a fully labeled sterile polyethylene bottle, and stored in an ice cooler with dry ice at a temperature of ±4°C to prevent chemical oxidation during transportation to the laboratory [23].

Biofilm Sample Collection

The biofilm weighing process begins with preliminary cleaning using tweezers to remove sediment and other organisms from the biofilm surface to obtain a clean sample (Fig. 5), followed by the preparation of a closed plastic container containing 80 mL of aquadest and a toothbrush. The container is weighed initially

Fig. 3. Water sampling.

Fig. 4. Sediment sampling.

Fig. 5. Biofilm sampling.

before the biofilm attached to the submerged rock surface is carefully brushed into the container. The hollow rock submerged in river water and covered with biofilm layers is then carefully brushed in the same direction until the entire biofilm is detached and suspended in the water, avoiding material loss or contamination, before the container is closed and reweighed to obtain the final weight; this weight difference is recorded as the weight of the biofilm. From these results, 0.8 g of biofilm were taken as an analysis sample, placed in a clean, dry film bottle labeled with the sample code, date of collection, and location, then sealed with wrap plastic to prevent leakage or contamination, and finally stored in a cold box at ±4ºC to maintain stability until further testing in the laboratory [24].

Sample Preparation of Lead Measurement Using AAS

Water Samples

Analysis of lead levels in river water samples began with the collection of 100 mL of samples which were then filtered using Whatman No. 40 filter paper to separate coarsely suspended particles, resulting in clear filtrate without solid contamination (Fig. 6a)). This filtrate is immediately transferred to the reaction flask and analyzed directly with an Atomic Absorption Spectrophotometer (AAS) to accurately detect lead concentrations. Before entering the instrument preparation, the QA/QC procedure is carried out through instrument calibration, blank and duplo analysis, and a spiked sample recovery test. The Pb recovery value of 85-110% indicates that the method has adequate accuracy and sensitivity. Instrument preparation is carried out by activating the combustion system via the PURGE and IGNITE buttons simultaneously, which is marked by green and blue indicator lights on the control panel, followed by the START command to start sample aspiration. Measurements are automated until stable, with yield data recorded, validated, and stored to support further interpretation of heavy metal pollution levels in rivers (Fig. 6b)).

a)

b)

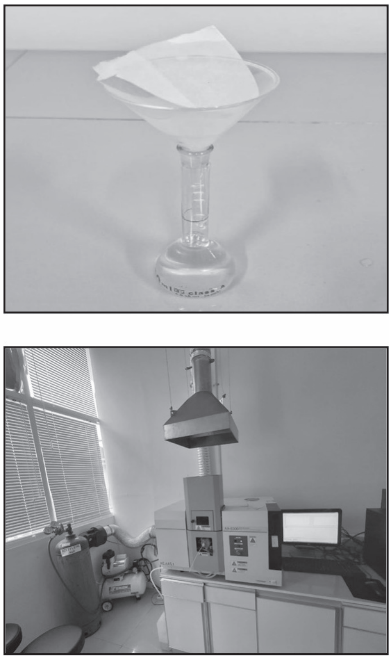

Fig. 6. Measurement of lead concentration in water: a) solution filtration, b) analysis using AAS.

Sediment Samples

Sediment samples are weighed 1 g using an analytical scale, then placed into an Erlenmeyer flask, where 10 mL of concentrated nitric acid (HNO_3) and 10 mL of concentrated sulfuric acid (H_2SO_4) are added to break down complex organic and inorganic matrices in the sediment so that heavy metals such as lead can be released in the form of soluble ions [25]; the mixture is heated on a temperature-controlled hotplate until the solution turns pale yellow (Fig. 7a)), then cooled to room temperature for 24 hours to ensure stability and prevent evaporation of volatile residues, before being transferred to a 100 mL volumetric measuring flask, diluted to the mark with aquades for concentration accuracy, and filtered through Whatman Grade 40 filter paper to separate insoluble solids such as silicates or inert particles (Fig. 7b)). QA/QC procedures are applied by calibrating instruments, blank and duplo testing, and recovery tests on spiked samples. The Pb recovery result of 85-110% indicates that the method used has a high level of accuracy. Furthermore, clear filtrate is ready for quantitative analysis using an Atomic Absorption Spectrophotometer (AAS) at its characteristic wavelength, a sensitive and selective method for detecting heavy metal levels at trace levels in environmental samples.

Biofilm Samples

The biofilm sample was weighed as much as 1 gram using an analytical scale, placed into an Erlenmeyer flask, then added 10 mL of nitric acid (HNO_3) and 10 mL of sulfuric acid (H_2SO_4) to decompose

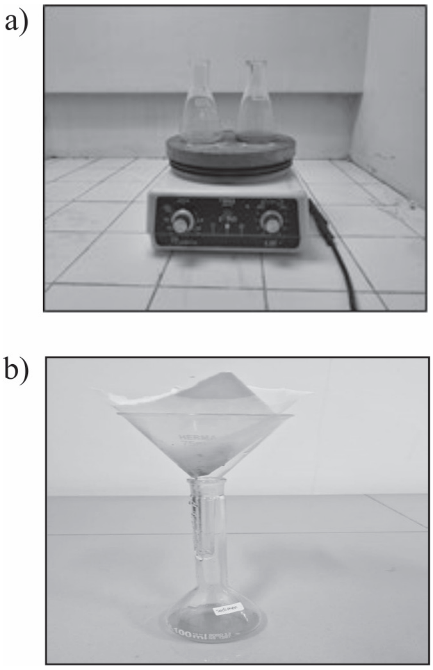

Fig. 7. Measurement of lead concentration in sediment: a) heating of the solution (added HNO_3 and H_2SO_4), b) filtration of sediment samples.

the organic matrix (Fig. 8a)); the mixture was heated on a stirring hotplate with constant stirring until the solution was completely clear, signaling complete mineralization of the organic components, and then cooled to room temperature for 24 hours for chemical stability before being transferred to a 100 mL volumetric measuring flask, diluted to the mark with deionized water, and filtered using Whatman Grade 40 filter paper to separate the insoluble solid residue (Fig. 8b)). The filtrate is then analyzed with an Atomic Absorption Spectrophotometer (AAS) at the characteristic wavelength of lead for accurate and sensitive quantitative determination of the concentration of lead accumulated in the biofilm [26].

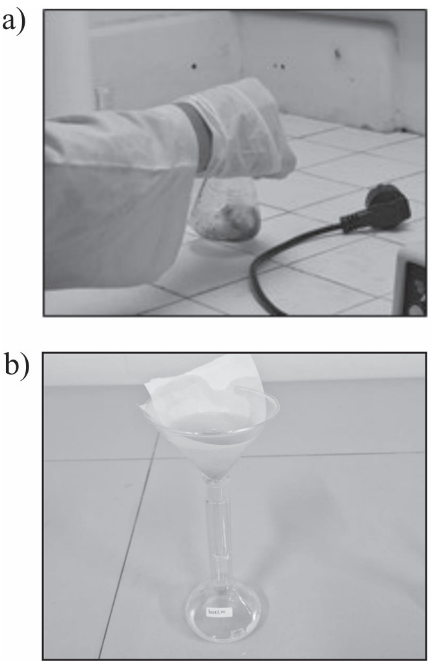

Fig. 8. Measurement of lead concentration in biofilm: a) addition of HNO_3 and H_2SO_4 solutions, b) filtration of biofilm samples.

BAF (Biofilm Accumulation Factor)

BAF (Biofilm Accumulation Factor) is a bioaccumulation factor, which is the comparison of the concentration of a chemical substance in an organism to the concentration of the chemical substance in its environmental medium (usually water), which covers all routes of exposure [27].

$$\text{BAF} = \frac{CB}{CW}$$

CB = level of substances in the organism (mg/kg)
CW = level of substances in water (mg/L)

If BAF>1, it means that bioaccumulation occurs (the levels of substances in the biofilm are higher than in the environment).

BSAF (Biota–Sediment Accumulation Factor)

BSAF (Biota–Sediment Accumulation Factor) is obtained through a comparison between the levels of contaminants contained in biota (biofilms) and the levels of contaminants in sediments [28].

$$\text{BSAF} = \frac{CB}{CS}$$

CB = level of substances in biota (biofilm) (mg/kg)
CS = level of substances in sedimentary media (mg/kg)

If BSAF>10, high bioaccumulation and significant vector contamination occur.

Kd (Distribution Coefficient)

Kd (Distribution Coefficient) in the environment is generally the adsorption distribution coefficient between the sediment phase and the water phase in equilibrium [29].

$$\text{Kd} = \frac{CS}{CW}$$

CS = level of substances in sedimentary media (mg/kg)
CW = level of substances in water medium (mg/L)

If Kd>1, the level in sediment is greater than that in water. If Kd = 1, the level is balanced between the sediment and the water.

Water Quality Parameter

Observations of water quality parameters are carried out with an integrated approach to obtain a comprehensive picture of water conditions. The main parameters observed included water temperature, depth, flow speed, degree of acidity (pH), and dissolved oxygen (DO) levels [30]. Dissolved oxygen measurement is carried out using a PDO-520 type DO meter equipped with a thermometer sensor, thus allowing the recording of DO values and water temperature simultaneously to improve data efficiency and accuracy. The speed of the water current is measured using the Flowatch tool which is able to detect variations in flow speed precisely at certain observation points. Meanwhile, the depth of the waters is measured manually with an iron ruler to ensure high accuracy at each measurement location. Water pH measurements are carried out using Krisbow-brand pH meters, which provide fast and stable results while in the field. Each parameter was observed three times at each observation station in an effort to minimize measurement errors and improve the representativeness of the results to the actual conditions of the waters at the study site [31].

Results and Discussion

Environmental Parameter Results

The pH and temperature range are at a neutral pH – weak base (7.61-7.65) and a temperature of around 29-32ºC so that in general it still supports the life of biofilms [32]. The depth of 29-32 cm indicates that measurements were made in the river edge/surface zone which is generally the main location for biofilm colonization on rocks and hard substrates. The current speed measured (±0.06-0.07 m/s) is also similar to the flow speed of some rivers in East Java such as the Manyar River which ranges from 0.04-0.07 m/s, which means that the hydrodynamic conditions are still relatively calm and allow fine particles and microorganisms to stick to the substrate to form biofilms [33].

A striking difference was observed in the DO value. In many rivers that are still relatively good, DO ranges from 4-8 mg/L, whereas this study obtained a DO value of around 1.5 mg/L, which is close to hypoxia and below the standard threshold for river waters according to Government Regulation No. 22 of 2021. Therefore, this issue needs to be further studied from a policy perspective. Very low DO is usually associated with a high load of organic matter and intense decomposition, as reported in previous studies on China's Gan River, which has very low DO. Conditions like these tend to suppress oxygen-sensitive organisms but can still favor groups of anaerobic or facultative microbes that often dominate the biofilm matrix. In addition, in rivers polluted by industrial activities, biofilms are dominated by groups of opportunistic bacteria, many of which are facultative anaerobes able to survive poor water quality [34] (Table 1).

Comparison of Lead Concentrations in Sediment and Water

The concentration of lead (Pb) in sediment and water in August 2023 (Fig. 9) which ranged between 1.18-1.38 mg/kg (sediment) and 1.27-1.40 mg/L (water) at the three stations showed very polluted conditions because it was much higher than the standard for lead quality in waters which is generally in the range of 0.5 mg/L for water according to national regulations such as the 2021 KEPMEN LH. This value, which far exceeds the quality standard, indicates a strong pollution pressure and has the potential to cause toxic impacts on aquatic organisms, especially sensitive biota that live in the location.

When viewed between stations, the highest concentrations were found at station 1 (1.38 mg/kg for sediment and 1.40 mg/L for water), followed by station 2 (1.22 mg/kg; 1.31 mg/L) and lowest at station 3 (1.18 mg/kg; 1.27 mg/L), indicating a pollution gradient from station 1 to station 3. This pattern is due to the location of station 1 being closer to polluting sources such as industrial or domestic waste disposal activities, and land run-offs that carry lead from fuels, paints, and other industrial activities [35]. The lower value at station 3 is likely due to its greater distance from anthropogenic activities, so the lead pollution pressure is relatively smaller, although the concentration remains very high and unsafe for the ecosystem [36].

The relatively small difference in lead concentration between water and sediment indicates that the system is in a relatively balanced partition state between the dissolved phase (water) and the solid phase (sediment), as reflected by the Kd values of 0.92-0.98 (Table 2). This condition is likely affected by a high degree of turbidity, which can maintain finely suspended particles and increase the presence of dissolved lead as well as bound to particles in the water phase, so that the sedimentation process has not taken place predominantly. Under this condition, the adsorption process, which is the attachment of dissolved lead ions to sediment particles or suspended materials, takes place in balance

Table 1. Water quality parameters in Manyar River, Gresik.

Station	Temperature (ºC)	Depth (cm)	Current Speed (m/s)	pH	DO (ppm)	Turbidity (mg/L)
1	28,2	29	0,066	7,61	1,51	39,82
2	27,9	32	0,057	7,65	1,54	39,82
3	27,8	31	0,073	7,62	1,52	39,82

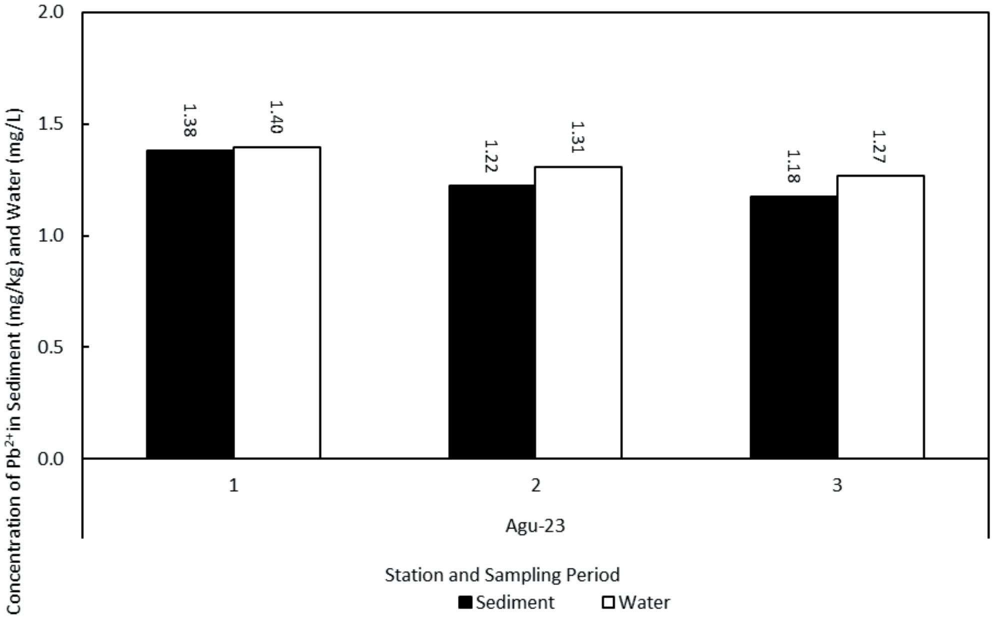

Fig. 9. Pb concentration in sediment and water.

with the desorption process, which is the re-release of lead from solid particles into water. This dynamic balance causes lead levels in water and sediments to tend to adapt to each other without dominance between the two phases. This suggests that aquatic systems are in a chemically stable state, where the exchange of heavy metals between water and sediment occurs continuously and is controlled by environmental factors such as pH, temperature, and microorganism activity [37].

The high turbidity value in Table 1, which is 39.82 mg/L, indicates that the water contains many suspended and colloidal particles. High turbidity can be caused by surface water runoff (runoff) that carries various materials such as soil, mud, and organic materials into the water body. These suspended particles have a high negative charge, thus causing the stability of the suspension in water and inhibiting deposition. As a result, the difference in values between sediment and water becomes insignificant because most particles do not have time to settle and remain in the water column [38].

In addition, the presence of heavy metals such as lead (Pb) also strengthens these conditions. Pb is a metal that is easily soluble in water and has a high affinity for suspended particles. When negatively charged colloidal particles interact with metal ions, complex or stable

Table 2. Distribution value (Kd) between sediment and water.

Station	Sediment (mg/kg)	Water (mg/L)	Kd
1	1,38±0,00	1,40±0,00	0,98
2	1,22±0,02	1,31±0,00	0,93
3	1,18±0,02	1,27±0,00	0,92

bonds can form between the heavy metal and the solid particles. This makes Pb metal not easy to settle with sediment and remain in the water phase, so that the levels of metals in water appear to be balanced with the levels of metals in sediments [39].

Comparison of Lead Concentrations in Biofilms, Sediments and Water

The results showed that biofilms have a significant ability to accumulate heavy metals, especially lead (Pb), compared to sediments and water (Fig. 9). In observations at three research stations, the accumulated lead concentration in the biofilm was recorded at 126.28 mg/kg at station 1, 110.05 mg/kg at station 2, and 106.21 mg/kg at station 3. These values show that the accumulation of lead in biofilms is tens of times greater than that in other media, such as sediment and water (Tables 3 and 4). These findings confirm that biofilm serves as a highly effective biological agent in the adsorption process of heavy metals in contaminated aquatic environments [40].

The lead concentration in biofilm ranged from 106.21-126.28 mg/kg. In contrast, lead concentrations in water and sediments ranged from 1.27-1.40 mg/L and 1.18-1.38 mg/kg, respectively. Considering that the BAF value is in the range of 83-90, this indicates a strong bioaccumulation process from the water medium to the biofilm throughout the study area. The biofilm that grows naturally in the Manyar Gresik River has a specific microbial community and is therefore different from the microbial consortium found in other rivers in Indonesia so that the BAF value produced will also be different. Biofilms are able to bind to and retain pollutants in much greater quantities than the remaining concentrations in the water, so biofilms have the potential to be used as

Table 3. BAF (Biofilm Accumulation Factor) value.

Station	Biofilm (mg/kg)	Water (mg/L)	BAF
1	126,28±13,10	1,40±0,00	90,20
2	110,05±2,57	1,31±0,00	84,01
3	106,21±7,15	1,27±0,00	83,63

Table 4. BSAF (Biota-Sediment Accumulation Factor) value.

Station	Biofilm (mg/kg)	Sediment (mg/kg)	BSAF
1	126,28±13,10	1,38±0,00	91,51
2	110,05±2,57	1,22±0,02	90,21
3	106,21±7,15	1,18±0,02	90,01

biological indicators (bioindicators) to assess pollution levels in waters. While the BSAF value is in the range of 90-91 times, which means that the concentration in the biofilm is much higher than in sediment, it shows that biofilm acts as a strong accumulator against contaminants at all observation stations. The adsorption observed in the Manyar River, Gresik, Indonesia, had much higher BSAF and BAF values when compared to previous studies conducted in rivers in China. This is suspected to be due to the diverse microbial consortia in the biofilm, which can play a role in adsorption [41].

The effectiveness of biofilms in absorbing heavy metals can be explained by the complex structure they have, especially in the extracellular polymeric substances (EPS) matrix. The structure of the EPS plays an important role because it is able to bind metal ions strongly through chemical interactions between the metal and the functional groups contained in it. Some major functional groups such as carboxyl (-COOH), amide (-$CONH_2$), phosphoryl (-PO_4^{3-}), and hydroxyl (-OH) have a high affinity for heavy metal ions, including lead. This interaction takes place through the mechanisms of ion exchange, complexation, and physical adsorption, which overall increases the capacity of biofilms to absorb metals from the aquatic environment [42].

Measurements of heavy metal content such as lead in river water often cannot reflect actual pollution conditions or the amount of waste entering water bodies. This is due to the dynamic nature of the river ecosystem, where water continues to move and flow so that incoming pollutants will be carried away by the current. As a result, despite the large amount of waste discharge at any given time, the concentration of heavy metals in the water can appear low when sampling is performed (Fig. 10). This difference between the time of pollution and the time of sampling is what makes water measurement results alone insufficient to accurately describe the quality of the river water [43].

To overcome these limitations, monitoring methods are needed that are able to record and represent pollution events, even if they occur long before sampling activities. In this context, the use of biofilm as a biomonitoring agent is starting to gain attention. Biofilm is a complex layer composed of microorganisms, organic matter, and inorganic particles that adhere to surfaces in the aquatic environment. Due to its ability to accumulate a variety of compounds, including heavy metals, biofilms can retain traces of pollution for longer periods of time than river water. Thus, the analysis of heavy metal content in biofilms is able to provide a more comprehensive picture of the pollution conditions of river ecosystems [44].

The results showed that the accumulation of lead in biofilms is mostly stored in interstitial water contained in biofilm structures. This condition is important because the heavy metals stored in the biofilm are not entirely stable. When lead concentrations in the waters decrease,

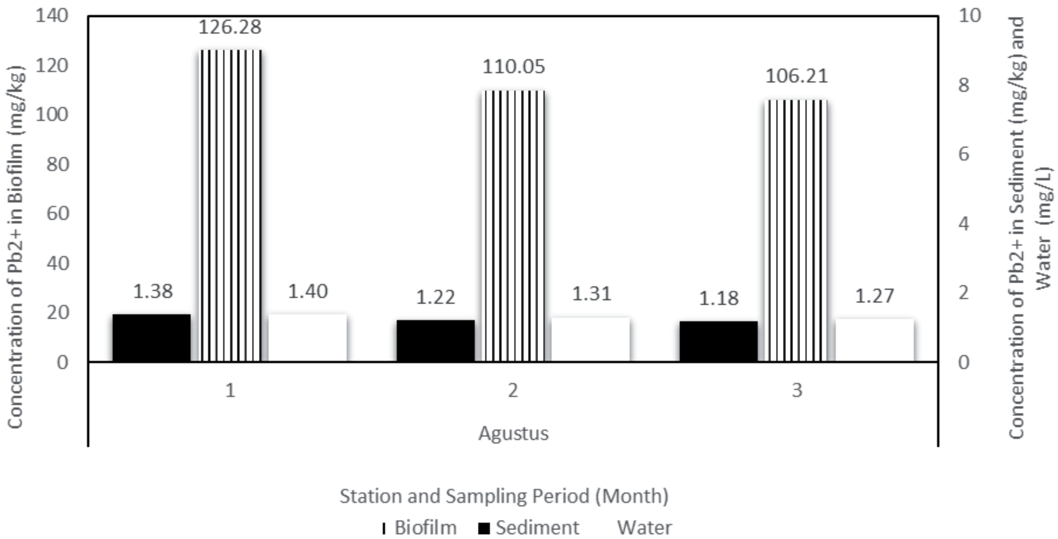

Fig. 10. Pb concentration in biofilm, sediment and water.

lead that has been bound in biofilms can potentially be released back into the water column through the process of desorption or biofilm degradation. The factors that cause desorption are changes in pH that become more acidic, which help the release of lead in the water, and temperature fluctuations, which affect chemical reactions and microbial activity, thereby accelerating the release of lead by biofilms. This phenomenon suggests that biofilms can be a secondary source of pollution, meaning that toxic materials can continue to circulate in the environment even after new waste inputs have stopped. This cycle of lead re-release has major implications for the dynamics of water quality and the health of aquatic ecosystems [45].

In addition to potentially being a source of pollutant release, the presence of lead in biofilms also poses a risk to the aquatic food chain. Lead accumulated in biofilms can enter other organisms through the processes of bioaccumulation and biomagnification, which involve the gradual buildup of heavy metals in organisms and their increasing concentrations at higher trophic levels. Therefore, monitoring heavy metal content such as lead is not enough to be done only in water or sediment, but it is also necessary to involve biofilm as a biological indicator. The results of this study strengthen the argument that biofilm plays an important role in water quality monitoring and can be used as a potential biomonitoring alternative, due to its ability to record the presence of heavy metals over long periods of time and reflect environmental conditions more accurately [46].

Conclusions

This study shows that natural biofilms in the Manyar River, Gresik, play an important role in lead adsorption with a capacity of 83-91 times higher than that of sediments and water. Water quality monitoring policies in the Gresik industrial estate, especially regarding the biological approach in the environmental monitoring system, need to be further studied. High adsorption ability is associated with the production of extracellular compounds (EPS) which exhibit an adsorption mechanism. In addition, the difference in lead concentration between water and sediment is affected by turbidity, while changes in environmental physico-chemical factors also determine the growth and effectiveness of biofilms in the adsorption process. In addition, recommendations related to the potential use of biofilm-based monitoring as a complement to conventional physico-chemical methods by regional environmental agencies need to be considered because of its ability to reflect pollution conditions more accumulatively and ecologically. To strengthen the direction of research development, the priorities for further studies, including seasonal monitoring, characterization of biofilm microbial communities, and exploration of the potential of biofilms for in situ remediation, need to be considered.

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AI Usage Disclosure Statement

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Conflict of Interest

Authors declare no conflict of interest.

Author's Contribution

ITS contributes to the conceptualization and design of research, as well as data analysis and interpretation. US is responsible for the methodology, supervises experimental procedures, and ensures the accuracy of the results. LTS participates in data collection, provides expertise in data analysis, and ensures the accuracy of the results. MY contributes to the collection and processing of data. RK participates in data validation. YP contributes to the development of research instruments. HMR is involved in the coordination of research activities, and MA contributes to the writing and editing of the manuscript.

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