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Seasonal dynamics of hexavalent chromium in the Manyar River, Indonesia: Biofilms as sensitive indicators of industrial pollution

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ABSTRACT

Biofilms are communities of microorganisms that are attached to a specific surface and are encased in an extracellular matrix that they produce themselves. These structures create complex microhabitats, allowing for intense biological and chemical interactions among different microbial species. In the context of aquatic ecotoxicology, biofilm has an extraordinary ability to absorb and accumulate pollutants, including heavy metals, so it has the potential to be used as a biological indicator that is sensitive to changes in environmental quality. This study aims to analyze and compare the concentration of heavy metals, especially hexavalent chromium (Cr(VI)), in biofilms, sediments, and water in the Manyar River, Gresik, Indonesia, in two different hydrological periods, namely the dry season and the rainy season. Sampling was carried out at three stations representing different environmental characteristics along the river flow. The physico-chemical parameters measured include temperature, depth, flow velocity, pH, precipitation, and dissolved oxygen (DO) levels. Heavy metal concentration analysis uses the Atomic Absorption Spectrophotometry (AAS) method to obtain precise and reliable results. The data showed that the highest heavy metal content of Cr(VI) was found at Station I which was close to the source of industrial activity, while the lowest value was found at Station III which was further away from the area. All measured Cr(VI) concentration values exceeded the national river water quality standard threshold of 0.5 mg/L. These results prove that biofilm has a heavy metal accumulation capacity of up to 66–98 times higher when compared to water and 67–100 times when compared to sediment, confirming its role as an efficient natural bioaccumulation agent. Thus, biofilm can be used as an effective and sustainable biomonitor to detect and evaluate the level of heavy metal pollution in aquatic ecosystems. These findings have the potential to be the basis for the development of environmentally friendly and low-cost bioindicator-based environmental monitoring systems.

Keywords: biofilm, biomonitoring, hexavalent chromium, heavy metals.

INTRODUCTION

Heavy metal pollution in river ecosystems is a global environmental issue because it is toxic and can accumulate in water, Sediment, and aquatic biota. Among various heavy metals, hexavalent chromium is classified as a priority because it is carcinogenic. mutagenic and has high mobility in waters. thus posing significant health and ecological risks (Sharma et al., 2022). Hexavalent chromium (Cr(VI)) is widely released from industrial activities such as tanning, metal coating, textiles and ill-managed liquid waste disposal. Concentrations of Cr(VI) that exceed quality standards in surface water bodies have been reported in various regions of the world suggesting that the control and monitoring of these pollutants is still an important challenge in water quality management (Xie., 2024).

In the context of rivers in Indonesia. increased industrial activities, urbanization and intensification of land use in watersheds contribute to water quality degradation and increased heavy metal loads. Studies in several watersheds (watersheds) in Indonesia show that Cr(VI) can be detected in

water and sediment (Fadlillah et al., 2023). This condition indicates the need for a monitoring strategy that focuses not only on the water matrix on a momentary basis, but also on other compartments capable of integrating exposure to pollutants over a longer time scale. Biomonitoring approaches are relevant to bridge the limitations of conventional physics-chemical monitoring which is often snapshot and sensitive to short-term fluctuations (Aziz et al., 2023).

Chromium has a complex environmental behavior because it can undergo a redox transformation between the more toxic and the relatively less toxic hexavalent forms influenced by pH conditions, redox potential, organic matter content and microbial activity. In the river environment Cr(VI) is not only in the dissolved phase, but also interacts with suspended particles such as sediments (Balta et al., 2025). Studies in various aquatic ecosystems show that sediments can store Cr(VI) in higher concentrations than water thus serving as an archive of contamination and a secondary source of pollutant release into the water column. Comprehensive Cr(VI) monitoring needs to consider multi-compartment dynamics in order to more accurately describe ecological risks (Sharma et al., 2022).

River biofilms are communities of microorganisms that are attached to the surface of rocks. sediments and other materials encased in the extracellular polymer matrix (EPS) they produce. The complex structure of the biofilm creates microhabitats with sharp chemical and physical gradients allowing for the accumulation and transformation of various pollutants including heavy metals (Moyal et al., 2023). A number of studies have shown that single-species biofilms are able to accumulate metals such as Cr(VI), Pb, Ni and Zn at much higher concentrations than surrounding water thus potentially in the assessment of heavy metal risk in freshwaters. In addition biofilm is a food source for organisms of higher trophic levels, so metal contamination in it can be the starting point for pollutant transfer in aquatic trophic networks (Coclet et al., 2021).

Research on biofilm as a water quality biomonitoring tool has shown promising results in various regions especially in European rivers. Biofilm is known to be able to continuously record heavy metal accumulation and provide signals to environmental changes (Laderriere et al., 2021). However, so far most of the research has been conducted in a geographic and temperate climate context where seasonal dynamics water discharge and pollution patterns differ significantly with tropical conditions. Therefore, there is still a gap in understanding how biofilm characteristics as biomonitors can function consistently in tropical regions that have high hydrological variability such as Indonesia (Anggayasti et al., 2025).

In addition, most biomonitoring studies focus on chemical parameters in water and sediments, while the role of biofilm as a biological indicator is still underexplored in depth. especially for detecting specific pollutants such as Cr(VI). In fact, biofilm is able to reflect chronic exposure and cumulative effects of heavy metals that are not always detected through conventional chemical measurements. This suggests the need to examine the relationship between Cr(VI) concentrations in biofilms and the physico-chemical conditions of the waters in order to obtain a more comprehensive ecological picture (Wu et al., 2024).

In Indonesia itself research related to biofilm as a biomonitor in industrial rivers is still limited including in coastal areas such as the Manyar River in Gresik. These rivers receive pollutant loads from various industrial activities and ports potentially increasing the risk of heavy metal contamination. There is not much information about Cr(VI) especially regarding its spatial and temporal distribution. The lack of biofilm data as a biological compartment in this system creates an important gap in understanding the dynamics of heavy metal-based pollutants particularly Cr(VI) in complex coastal river ecosystems. This limitation is the basis for this study to close the knowledge gap regarding the effectiveness of biofilms in seasonal monitoring of heavy metals in tropical rivers affected by industrial activities.

MATERIALS AND METHODS

Research location

The research was carried out in the Manyar River, Gresik Regency, East Java which is the coastal area of East Java (Figure 1). The area is surrounded by industrial, warehouse and dense residential areas so the river receives a mixed load of industrial and domestic waste from household and commercial activities. The research design uses seasonal monitoring in the dry and rainy periods to capture the dynamics of tropical water quality which is strongly influenced by rainfall

patterns. In the rainy season, increased surface discharge and runoff have the potential to bring pollutant loads from industrial areas and settlements but also cause a dilution effect in the water column. In contrast, in dry seasons decreased discharge tends to increase dissolved pollutant concentrations so this period is important to identify the worst conditions of Cr(VI) exposure in waters (Reyes-Márquez et al., 2025).

One of the main parameters observed is Cr(VI) due to its highly toxic and soluble nature and is widely used in various industrial processes (e.g. tanning, metal plating and chemical industries) which has been reported to be a significant source of pollutants in Indonesian rivers. Sampling locations were purposively determined in three categories of stations: (1) stations near the starting point of entry of pollutant sources to illustrate the initial load of Cr(VI), (2) stations in the middle of the river to record the transport and transformation processes of pollutants along the stream, (3) stations in the downstream segment close to the confluence of large rivers leading to the sea to assess the accumulation and potential release of Cr(VI) into coastal ecosystems. This spatial approach allows the characterization of pollution gradient from upstream to downstream and provides a scientific basis for environmental risk management in the Manyar River area. More details of the station location are presented (Figure 2).

Determination of the season and frequency of sampling

Location and sampling frequency settings in Cr(VI) and natural biofilm studies need to take into account the character of seasonal dynamics as well as adequate temporal representation. The sampling design of the two main periods representing the dry season (23 September–23 October) and the rainy season (23 November–24 January) refers to BMKG data for the Gresik region. In accordance with previous studies of spatial–temporal variations of heavy metals in river and lake waters which emphasizes the importance of capturing climate differences to more accurately describe contamination patterns and microbiological responses (Imsilp et al., 2025). The frequency of these two large seasonal sorting is also in line with the heavy metal contaminant observation approach that prioritizes seasonal limnological and hydrological shifts to assess ecological and bioaccumulation risks in the aquatic matrix. In each season, sampling is carried out every month with

Figure 1. Map of the research location

(a) (b) (c)

Figure 2. Stations location: (a) station 1, (b) station 2, (c) station 3

three repetitions per sampling station the goal of which is to capture fluctuations in heavy metal concentrations every month (Khalifa et al., 2025).

Measurement of the physical-chemical parameters of the waters in situ

Water quality parameters were measured in situ at each station in conjunction with sampling activities so that the recorded physical-chemical conditions of the waters represented the actual state of the biofilm and the concentration of Cr(VI). The parameters observed include temperature, pH, dissolved oxygen (DO), depth and flow velocity as the main environmental factors that affect the solubility, speciation and vertical and horizontal distribution of metals in the water column. This approach to direct measurement in the field is important to minimize changes in sample conditions due to transport and delays in laboratory analysis (Wu et al., 2024).

Dissolved oxygen measurement is carried out using a PDO-520 type DO meter that has been equipped with a thermometer sensor so that the DO value and temperature can be obtained simultaneously. The combination of these two parameters is crucial because the solubility of oxygen and the rate of biogeochemical processes including biofilm activity and Cr(VI) reduction are strongly influenced by the temperature of the waters. Meanwhile, water pH measurement is carried out using Krisbow brand pH meters. The speed of the water current is measured with the Flowatch tool to precisely detect variations in flow speed at specific observation points. Depth measurement is carried out using an iron ruler as a simple but precise vertical measuring tool that allows the determination of the profile of the water column at each station. This combination of current velocity and depth data provides the hydrological context necessary to interpret the spatial-temporal variations in Cr(VI) concentrations and biofilm characteristics formed at the study site.

Figure 3. Water sampling

Water sampling

The water sampling process begins by opening the sample bottle that has been previously tightly closed to ensure that the container is in a sterile condition before use. Water from the observation location is then put into the sample bottle carefully so that it does not mix with litter, mud or other dirt in the waterways. This step is done to guarantee that the quality of the sample remains pure of the actual water conditions at the research site. Sampling was carried out at three observation points namely stations 1, 2 and 3. Each station was repeated three times to obtain more accurate and scientifically accountable results (Adekanmi et al., 2020) (Figure 3).

After all samples are successfully taken, the bottles are sealed again tightly to prevent contamination during the storage and transportation process. Each bottle is labeled according to the location of the collection station so that it can be easily identified at the next stage of analysis. All sample bottles are then placed in a cool box with a temperature of about ±4 °C to maintain the stability of the chemical and biological characteristics of the water during the journey to the laboratory. The samples are then taken to the laboratory for observation and further analysis related to the water quality parameters needed in the study (Garcia et al., 2025).

Sediment sampling

Sediment sampling was carried out using a 1inch paralon pipe as the main tool. In the initial stage one end of the pipe is sealed tightly by hand to create air pressure inside the pipe (Figure 4). After that the exposed end of the pipe is slowly inserted into the water until it reaches the bottom. When the pipe has reached the bottom of the water the hand that covers the other end is slowly opened to allow water and sediment to enter the pipe due to the pressure difference between the inside and outside of the pipe. Once the pipe is removed the sediment that has collected in it is then transferred

Figure 4. Sediment sampling

Figure 5. Biofilm sampling

into a plastic bag that has been labeled according to the sampling station or location. After all the samples are collected a plastic bag containing sediment is put into a cool box with a temperature of about ±4 °C to keep the condition of the sample stable during transportation. Samples that have been stored properly are then taken to the laboratory for observation and further analysis of the characteristics and composition of the sediments that have been taken (Li et al., 2021).

Biofilm sampling

The streamer biofilm sampling process begins with the separation of biofilm from sediment and attached organisms using tweezers to keep the sample pure and free from contamination. This stage is important to maintain the authenticity of the biofilm before the main sampling is carried out. After that a plastic container containing 80 ml of aquadest complete with a lid and toothbrush is weighed first to obtain the initial weight. The streamer biofilm that has been cleaned and still attached to the stone is then brushed in the same direction using a toothbrush into a plastic container containing the aquadest. Brushing is done carefully so that the entire biofilm can be detached without damaging the structures that are important for the analysis (Kurniawan et al., 2025) (Figure 5).

After the biofilm has been successfully collected in aquadest the container that has filled the aquadest and biofilm along with the lid and toothbrush is weighed again to obtain the final weight. The difference between the final weight and the initial weight shows the weight of the streamer's biofilm used which is 0.8 grams. The resulting biofilm solution is then put into a film bottle that has been washed, labeled and sealed using plastic wrap to prevent contamination or evaporation of the liquid. The prepared sample is then stored in a cool box with a temperature of around ±4 °C so that the condition of the biofilm is maintained and the microbial activity in it does not undergo significant changes before further analysis is carried out (Kurniawan and Fukuda, 2023).

Preparation of AAS analysis samples

Water samples

The process of analyzing Cr(VI) levels in river water samples began with taking a sample volume of 100 mL. The sample is then filtered using Whatman grade 40 filter paper to remove coarse solid particles resulting in clear filtrate that is free of particulate contaminants (Figure 6b). The obtained filtrate is transferred to the reaction flask and directly analyzed using an atomic absorption spectrophotometer (AAS). Prior to measurement the fire system is activated by pressing the PURGE and IGNITE buttons simultaneously (displaying green and blue color indicators on the instrument panel) followed by the START command. The data reading process is carried out until it is completed then the results are recorded and stored for further analysis (Figure 6a) (Sumary et al., 2025).

Sedimen samples

A 1 g sediment sample was analytically weighed and put into an erlenmeyer flask then 10 mL of concentrated nitric acid (HNO_3) and 10 mL of concentrated sulfuric acid (H_2SO_4) were

(a) (b)

Figure 6. Measurement of Cr(VI) concentration in water: (a) filtration of water samples, (b) analysis using AAS

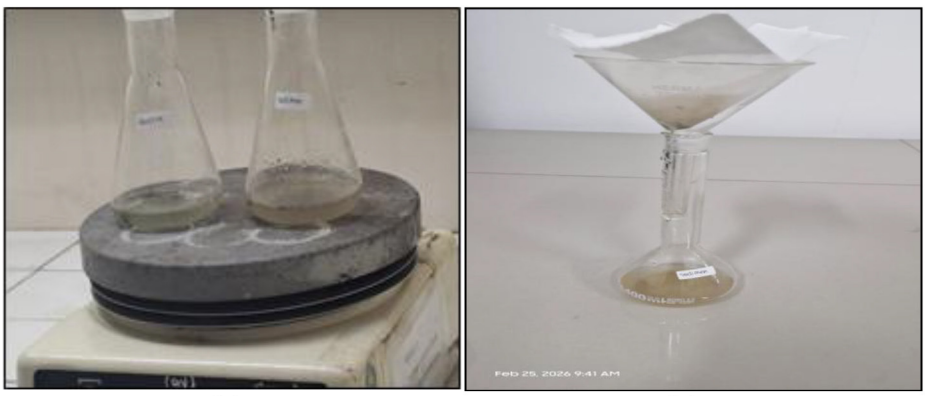
added followed by heating on a hotplate at a controlled temperature until the solution reached a pale yellow color (Figure 7a). After that the solution is cooled to room temperature for 24 hours then transferred to a 100 mL volumetric measuring flask diluted to a mark with distilled water and filtered using Whatman Grade 40 filter paper to separate any insoluble solid residues (Figure 7b). The resulting filtrate was then analyzed for Cr(VI) levels with an atomic absorption spectrophotometer (AAS) at characteristic wavelengths. (Marsay et al., 2025).

Biofilm samples

A 1-gram biofilm sample is precisely weighed using an analytical balance and put into an Erlenmeyer flask. Next, 10 mL of nitric acid (HNO_3) and 10 mL of sulfuric acid (H_2SO_4) were added to effectively decompose the organic matrix (Figure 8a). The mixture is heated on a stirrer hotplate with constant stirring until the solution reaches full clarity signaling complete mineralization of the organic components. After that the solution is cooled to room temperature for 24 hours to ensure chemical stability. The solution was then transferred to a 100 mL volumetric measuring flask diluted to a mark with deionized water (aquades) and filtered using Whatman Grade 40 filter paper to separate the insoluble solids residue (Figure 8b). The resulting filtrate was then analyzed using an atomic absorption spectrophotometer (AAS) at the characteristic wavelength of Cr(VI) allowing for quantitative determination of the concentration of Cr(VI) accumulated in the biofilm with a high degree of accuracy and sensitivity (Kurniawan and Fukuda, 2023).

Biofilm accumulation factor

According to Valdes et al. (2021), biofilm accumulation factor (BAF) is a factor which is the comparison of the concentration of a chemical substance in an organism with the concentration



(a) (b)

Figure 7. Measurement of Cr(VI) concentration in sediment: (a) heating solution, (b) filtration of sediment samples

(a) (b)

Figure 8. Measurement of Cr(VI) concentration in biofilm: (a) addition of HNO_3 and H_2SO_4 solutions, (b) filtration of biofilm samples

of that substance in its environmental medium (generally water) covering all routes of exposure.

$$BAF = \frac{CB}{CW} \quad (1)$$

where: *CB* – concentration of substances in the organism (mg/kg), *CW* – concentration of substance in water medium (mg/L), if *BAF* is > 1 it means that bioaccumulation occurs (the concentration in the biofilm is higher than the environment).

Biota-sediment accumulation factor

According to Krivokapi (2021), biota-sediment accumulation factor (BSAF) is obtained through a comparison between the levels of contaminants contained in biota (biofilms) and the levels of contaminants in sediments.

$$BSAF = \frac{CB}{CS} \quad (2)$$

where: *CB* – concentration of substances in biota (biofilm) (mg/kg), *CS* – concentration of substance in sedimentary media (mg/kg), if *BSAF* > 10 high bioaccumulation and important vector contamination.

Distribution coefficient

According to Bugai et al. (2020), distribution coefficient (Kd) in the environment is generally the coefficient of adsorption distribution between the sediment phase and the water phase in equilibrium.

$$Kd = \frac{CS}{CW} \quad (3)$$

where: *CS* – concentration of substance in sedimentary media (mg/kg), *CW* – concentration of substance in water medium (mg/L), if *Kd* > 1 concentration in sediment is greater than in water, if *Kd* = 1 the concentration is balanced between the sediment and the water.

RESULTS AND DISCUSSIONS

Physico-chemical parameters of the waters

In general, the temperature and pH of the Manyar River listed in Table 1 are still within the quality standard range of PP No. 22 of 2021 for class II river water (maximum temperature deviation of 3 °C from natural conditions pH 6–9). The pH value in all months ranges from 6.5–7.7 so that it remains within a safe range and the DO is around 1.3–2.0 mg/L which means that it has not met the quality standard of class II (≥4 mg/L) so that it is regulated as polluted in terms of dissolved oxygen. In the dry period (September–October) the water temperature is relatively slightly higher (around 28.5–28.9 °C) while the depth tends to be smaller as the river discharge decreases. Under small discharge conditions the low current velocity (approximately 0.044–0.067 m/s) reduces the natural aeration process so that the DO remains low although the pH is stable and neutral–slightly alkaline (7.5–7.7) which is still in line with the quality standard. During the rainy period (November–January) the depth increased (to more than 40 cm) and the current speed at some points increased to about 0.09–0.12 m/s but the DO value remained in the range of 1.7–2.0 mg/L so it was still well below the quality standard.

Increased discharge in the rainy season can carry the burden of organic pollutants from

Table 1. Physico-chemical parameters of the waters

Month	Station	Environmental parameters						
		Temperature (°C)	Depth (cm)	Current speed (m/s)	pH	DO (ppm)	RR (mm)	Turbidity (mg/l)
Sep 23	1	28.7	28	0.058	7.64	1.48		
	2	28.5	31	0.067	7.52	1.51	0.0	40.05
	3	28.9	32	0.063	7.51	1.54		
Oct 23	1	28.5	32	0.044	7.68	1.52		
	2	28.2	31	0.065	7.71	1.44	0.0	40.16
	3	28.6	31	0.056	7.62	1.47		
Nov 23	1	27.8	35	0.098	6.89	1.81		
	2	27.8	36	0.095	6.91	1.83	4.3	51.73
	3	27.9	38	0.091	6.86	1.79		
Des 23	1	27.7	32	0.089	6.85	1.51		
	2	27.1	34	0.092	6.76	1.43	2.2	52.06
	3	27.2	32	0.087	6.73	1.46		
Jan 24	1	27.1	42	0.124	6.49	1.91		
	2	27.4	41	0.119	6.39	1.93	16.78	69.58
	3	27.2	44	0.013	6.54	1.96		

settlements or other activities in the watershed so that oxygen consumption (BOD/COD) increases and DO remains low although the pH remains neutral (around 6.5–7.6) and still according to rule 6–9. Thus compared to the national quality standard the water quality of the Manyar River is mainly problematic with the DO parameters both in the dry and rainy seasons so it is necessary to control the source of organic pollutants along the river (Tong et al., 2025).

Seasonal variations of Cr(VI) in sediments and water

The concentration of Cr(VI) in sediments and water shows a gradual downward trend from the dry season to the rainy season. Based on the observations in the dry season (September 2023–October 2023) the concentration of Cr(VI) in sediment ranged from 0.665 to 0.693 mg/L while in the rainy season (November 2023–January 2024) it ranged from 0.466 to 0.602 mg/L. The same pattern also occurred in water samples where in the dry season the concentration of Cr(VI) was detected between 0.665 and 0.693 mg/L while in the rainy season it decreases to around 0.475 to 0.598 mg/L (Figure 9). This decrease indicates that when rainfall increases there is a process of dilution of Cr(VI) concentrations in the aquatic environment due to the entry of rainwater runoff so that the content of dissolved and sedimented heavy metals decreases gradually (Tabassum et al., 2024). The difference in Cr(VI) concentration between water and sediment that is not too significant indicates that the system is in a relatively balanced partition state between the dissolved phase (water) and the solid phase (sediment) when viewed from the Kd value in Table 2. In this condition, the adsorption process which is the attachment of dissolved Cr(VI) ions to sedimentary particles or suspended materials takes place in balance with the desorption process which is the re-release of Cr(VI) from solid particles into water. This dynamic balance causes Cr(VI) levels in water and sediments to tend to adjust to each other without any 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 takes place constantly and is controlled by environmental factors such as pH, temperature and microorganism activity (Ao et al., 2025).

Effect of turbidity on Cr(VI) concentration balance in water and sediment

The turbidity value recorded from September to January was 40.05; 40.16; 51.73; 52.06; and 69.58 mg/L (Table 1) indicating a fairly consistent increase in turbidity during the observation period. This increase reflects the increasing presence of suspended particles in the water column which are generally fine sediments, organic waste

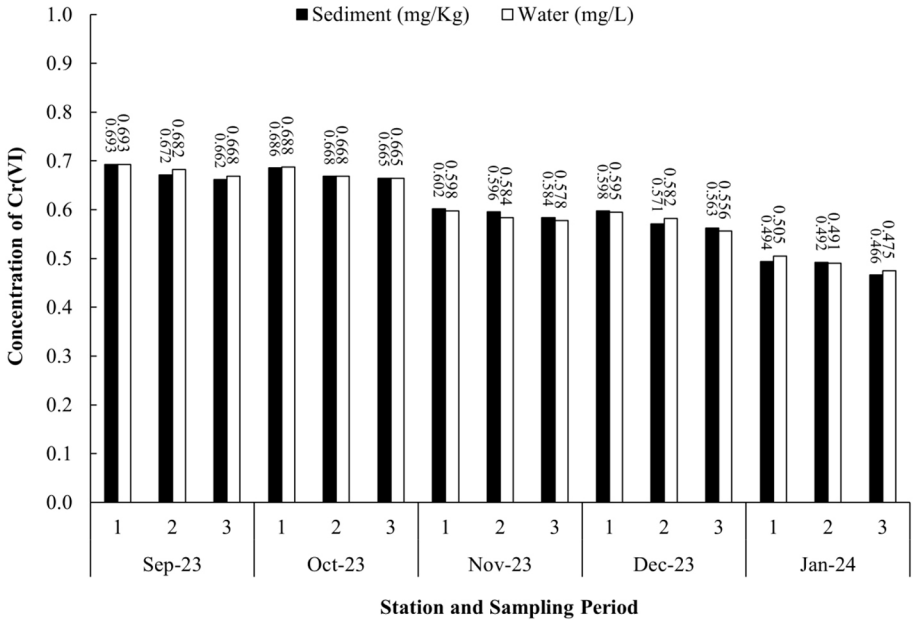


Figure 9. Cr(VI) concentration in sediment and water

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

Month	Station	Sediment (mg/kg)	Water (mg/L)	Kd
September 23	1	0.69	0.69	1.00
	2	0.67	0.68	0.98
	3	0.66	0.67	0.99
October 23	1	0.69	0.69	1.00
	2	0.67	0.67	1.00
	3	0.66	0.66	1.00
November 23	1	0.60	0.60	1.01
	2	0.60	0.58	1.02
	3	0.58	0.58	1.01
Desember 23	1	0.60	0.59	1.01
	2	0.57	0.58	0.98
	3	0.56	0.56	1.01
January 24	1	0.49	0.51	0.98
	2	0.49	0.49	1.00
	3	0.47	0.47	0.98

and minerals from erosion or resuspension from the bottom of the water. The role of turbidity is crucial in determining the distribution of Cr(VI) as these heavy metals tend to associate with fine particles through the adsorption mechanism (Liang et al., 2023). When turbidity is high most of the Cr(VI) is not in a completely dissolved form but rather adheres to the surface of the suspended particles. As a result the difference in Cr(VI) concentration between the water column and the sediment becomes small (Miranda et al., 2022).

In addition to chemical factors physical dynamics such as resuspension and deposition processes also play a major role in maintaining the balance between Cr(VI) content in water and sediment. Cr(VI)-rich suspended particles will settle to the bottom but as current or physical activity increases these particles are lifted back into the water column creating a continuous cycle of circulation (Janssen et al., 2023). This process also strengthens the interaction between the dissolved phase and the solid where Cr(VI) can undergo

adsorption-desorption reactions as well as oxidation-reduction (redox) changes depending on environmental chemical conditions such as pH and dissolved oxygen content. It is this combination of physical and chemical mechanisms that makes aquatic systems tend to achieve dynamic but relatively stable conditions with Cr(VI) concentrations in water and sediments remaining at levels that are close to each other despite turbidity fluctuations over time (Wang et al., 2023).

Comparison of Cr(VI) concentrations in biofilms with water

During the observation period from September 2023 to January 2024 the concentration of pollutants in biofilms at the three stations was recorded much higher than the concentration in the water column. The value of the concentration of Cr(VI) in the biofilm ranges from about 33–68 mg/kg while the concentration of Cr(VI) in water is only about 0.47–0.69 mg/L (Table 3) which suggests that the biofilm compartment plays an important role as a place for the accumulation of pollutants in the waters. This condition is also reflected in the value of bioaccumulation factor (BAF) which is in the range of 66–99 times thus illustrating the occurrence of a strong bioaccumulation process from water media to biofilm along the research site. Consistently high BAF values at almost all months and stations indicate that biofilm-making organisms are able to bind to and retain much larger amounts of pollutants than the remaining concentrations in the water so biofilms could potentially be used as biological indicators (bioindicators) to assess pollution levels in waters (Zhang et al., 2022).

Comparison of Cr(VI) concentrations of biofilms with sediments

The BSAF (biota-sediment accumulation factor) value in the table is the ratio between the concentration of Cr(VI) in the biofilm (biota) to the concentration in the sediment thus describing the ability of the organism to accumulate contaminants from the surrounding sediment. The greater the BSAF value the higher the relative bioaccumulation rate; A value above 10 usually means that the organism is accumulating higher than sediments and important vectors of contamination while a value well below 10 indicates low accumulation. In (Table 4) BSAF was in the range of 68–100 times which means that the concentrations in biofilms are much higher than in sediments indicating biofilms act as strong accumulators against contaminants at all observation stations and moons (Valdes et al., 2021).

Seasonal variations of Cr(VI) in biofilm

The results of the measurement of Cr(VI) concentrations in biofilms in the Manyar River, Gresik using the AAS method showed very high adsorption rates at the three observation stations during the period September 2023 to January 2024 with an average value of around tens of mg/L in each

Table 3. Biofilm accumulation factor value

Month	Station	Biofilm (mg/kg)	Water (mg/L)	BAF
September 23	1	68.35	0.69	98.69
	2	67.28	0.68	98.64
	3	66.46	0.67	99.43
October 23	1	67.71	0.69	98.44
	2	66.46	0.67	99.43
	3	64.03	0.66	96.34
November 23	1	58.87	0.60	98.47
	2	57.76	0.58	98.88
	3	55.89	0.58	96.77
Desember 23	1	51.48	0.59	86.56
	2	50.27	0.58	86.34
	3	49.43	0.56	88.88
January 24	1	33.78	0.51	66.85
	2	33.38	0.49	68.05
	3	33.20	0.47	69.94

Table 4. Biota-sediment accumulation factor value

Month	Station	Biofilm (mg/kg)	Sediment (mg/kg)	BSAF
September 23	1	68.35	0.69	98.69
	2	67.28	0.67	100.17
	3	66.46	0.66	100.42
October 23	1	67.71	0.69	98.71
	2	66.46	0.67	99.43
	3	64.03	0.66	96.34
November 23	1	58.87	0.60	97.81
	2	57.76	0.60	96.95
	3	55.89	0.58	95.76
Desember 23	1	51.48	0.60	86.11
	2	50.27	0.57	88.11
	3	49.43	0.56	87.87
January 24	1	33.78	0.49	68.39
	2	33.38	0.49	67.79
	3	33.20	0.47	71.19

observation month. This value is well above the range of Cr(VI) concentrations generally found in relatively controlled river water bodies where studies in other rivers in Indonesia report that Cr(VI) concentrations in water are generally on the order of less than 1 mg/L (Figure 10). This pattern indicates that the biofilm in the Manyar River acts as a strong accumulator of heavy metals in line with the character of river biofilms which are known to be able to absorb and accumulate various metals including Cr(VI) from the water column and suspended particles. This condition strengthens the indication that there is significant heavy metal pollution pressure in the Manyar area which is known to be close to industrial and coastal activities which were previously also recorded to have accumulated heavy metals in other environmental compartments (Guerrieri et al., 2022).

At station 1 the mean value of the adsorption concentration of Cr(VI) in the biofilm during five consecutive observation times was 68.4; 67.7; 58.9; 51.5; and 33.8 mg/kg. showing a downward trend in concentration from the beginning to the end of the study period. This gradual decline can indicate a change in the input of Cr(VI) to the river body either due to seasonal river discharge variations changes in waste disposal intensity and other hydrological factors such as dilution or sedimentation. Despite the decline all values are still at a very high level when compared to the quality standards of

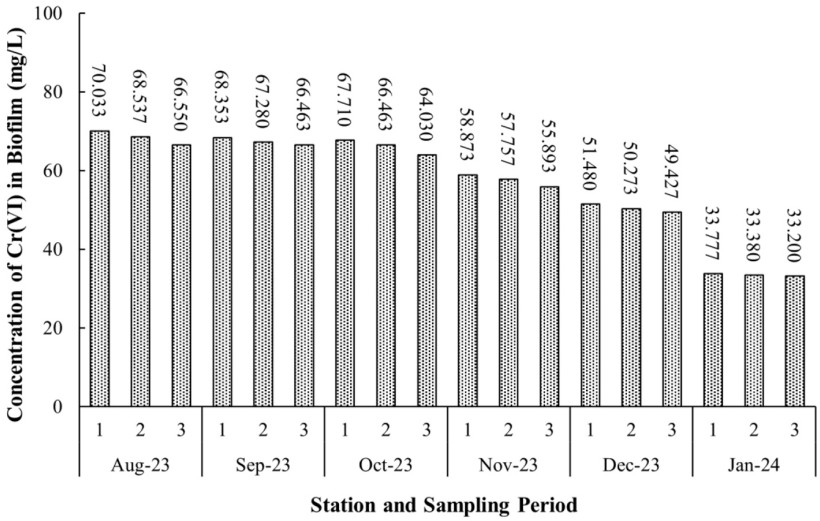


Figure 10. Cr(VI) concentration in biofilm

Cr(VI) in river water so that the biofilm at this station still reflects the conditions of heavy pollution. The phenomenon of high accumulation of Cr(VI) in biofilms is also consistent with the literature that states that river biofilm communities are capable of storing large amounts of metals through adsorption mechanisms in extracellular polymer matrices and cellular uptake (Pagliaccia et al., 2022).

Station 2 showed an average value of Cr(VI) adsorption concentration in biofilm of 67.3; 66.5; 57.8; 50.3; and 33.4 mg/kg while station 3 is 66.5 each; 64.0; 55.9; 49.4; and 33.2 mg/kg for the same observation period. The very close value pattern between stations and the tendency to decrease over time indicate that the source of Cr(VI) pollutants is relatively evenly distributed throughout the river segment studied likely dominated by non-point sources of pollution from industrial and domestic activities in the Manyar area. This uniformity of concentration ranges is also in line with previous research reports showing that heavy metals including Cr(VI) can be distributed along the flow and bound to fine sediment fractions as well as biofilms on the substrate. Thus biofilms at the three stations can be categorized as sensitive and representative bioindicators of Cr(VI) pollution conditions in the Manyar River.

The potential of biofilms to absorb heavy metals such as Cr(VI) can be hundreds of times greater than that of the dissolved phase due to the presence of an extracellular matrix that is very rich in EPS (extracellular polymeric substances) components (Muhammad et al., 2020). EPS is generally composed of polysaccharides, Proteins, lipids and nucleic acids that form a three-dimensional matrix thus providing a large surface and pore space for the biosorption process of metals. This structure allows Cr(VI) ions to interact, diffuse and then bind physically and chemically within the biofilm layer so that the concentration of Cr(VI) accumulated in the biofilm can be much higher than in the surrounding water (Vandana et al., 2023; Kurniawan et al., 2024a).

The high adsorption ability is greatly influenced by the presence of active functional groups in EPS components especially carboxyl (COO^-), hydroxyl (OH), amino (NH_2) and phosphate groups that are charged or easily ionized (Babiak and Krzeminska. 2021; Kurniawan et al., 2024b). These groups act as binding sites that are able to interact with Cr(VI) ions through ion exchange, complexation and coordination bonding mechanisms thereby increasing the capacity and strength of metal bonds in the biofilm matrix. In addition the heterogeneity of EPS composition and micro-environmental conditions in biofilms also optimize the biosorption process so that overall the biofilm can function as a highly effective Cr(VI) biosorbent (Zadeh et al., 2023).

CONCLUSIONS

The results of the study show that the natural biofilm growing in the Manyar River, Gresik. has an excellent ability to absorb the heavy metal Cr(VI) with an adsorption capacity that reaches tens of times higher than water and sediment. This illustrates the important role of biofilm as a natural biomonitoring agent that is able to absorb and retain harmful contaminants in the aquatic environment. The influence of seasons has also been shown to be significant on the concentration of Cr(VI) that is trapped where fluctuations in rainfall and water discharge cause a dilution effect so as to reduce Cr(VI) levels in certain periods. In addition the degree of turbidity (turbidity) is a major environmental factor that affects the distribution and ratio of heavy metals between water and sediment. The high turbidity condition causes Cr(VI) to be more evenly distributed so there is no significant difference in concentration between the two phases. Overall these findings confirm that natural biofilms have great potential in the process of restoring water quality in areas exposed to heavy metal pollution such as in the Manyar River.

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