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## Analysis of biofilms as biomonitoring agent for $\text{Cu}^{2+}$ and $\text{Pb}^{2+}$ pollution in the lotic ecosystem

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**Abstract.** Contamination of heavy metals such as  $\text{Cu}^{2+}$  and  $\text{Pb}^{2+}$  have become serious problems in the aquatic ecosystems. Various efforts to prevent the negative impacts of pollution are being conducted. One of the crucial efforts is to monitor the concentration of heavy metals in aquatic ecosystems. However, the monitoring the heavy metal concentration faces various obstacles, particularly in the lotic ecosystems such as river due to the water flow. This study aims to analyze the utilization of biofilm matrix as a biological agent in the monitoring of river ecosystems. The study focuses on  $\text{Cu}^{2+}$  and  $\text{Pb}^{2+}$  in the Cokro River of Malang City. The concentration of  $\text{Cu}^{2+}$  and  $\text{Pb}^{2+}$  were measured in the river water, sediment and biofilm. The environmental parameters those analyzed were temperature, pH of bottom sediment, pH of water, current speed and water depth. The results of this study reveal that the concentration of  $\text{Cu}^{2+}$  and  $\text{Pb}^{2+}$  inside the biofilms were much higher compared with those of in the sediment and river water. According to the results of this study, the biofilm matrix is a promising biological agent to monitor the  $\text{Cu}^{2+}$  and  $\text{Pb}^{2+}$  concentration in the lotic ecosystems such as Cokro River.

### 1. Introduction

Aquatic ecosystems can be contaminated by various pollutants such as heavy metals [1,2].  $\text{Cu}^{2+}$  and  $\text{Pb}^{2+}$  were the heavy metals that largely use in various industrial activities such as in the paper factory. The excess amount of  $\text{Cu}^{2+}$  and  $\text{Pb}^{2+}$  may cause various health problems such as cancer, kidney disorder and liver disorder [3,4]. Therefore, the presence of  $\text{Cu}^{2+}$  and  $\text{Pb}^{2+}$  in the aquatic ecosystems such as river should be monitored continuously.

The monitoring of pollutant such as heavy metals in the lotic ecosystems faces various problems mostly due to the water flow [5,6,7]. The movement of the water makes the pollution assessment in a specific area of the river such as near the industrial factory is complicated. The pollutant will immediately move to the other areas after being discharged into the river. The concentration of the



pollutant in the water solely is not sufficient to access the pollution condition [8]. Thus, the utilization of other agents to monitor the presence of the pollutant is required.

Biomonitoring is one of the alternative methods to assess the presence of the pollutant in the lotic ecosystem such as river [9,10]. This method uses a biological agent to monitor the pollutant in the ecosystems such as in the river. The primary key success in the biomonitoring is the biological agent selection [11,12]. The biological agents should be ubiquitous in the river, easily form and able to accumulate the heavy metals. In this case, the microbes that live in almost all parts of the river show the potentiality to be used as a biomonitoring agent. Microbes live by form the microbial community structure which is named as biofilm [13,14,15]. Biofilm is a predominant habitat of microbes and ubiquitous in the aquatic ecosystems including river [16,17]. The ability of the biofilm to accumulate heavy metals have been reported in the previous studies [18,19]. However, the study analyzing the utilization of the biofilms as biomonitoring agents for the heavy metals particularly  $\text{Cu}^{2+}$  and  $\text{Pb}^{2+}$  in the lotic ecosystems is quite limited.

This study aims to analyze the utilization of the biofilm as a biological agent in the monitoring of  $\text{Cu}^{2+}$  and  $\text{Pb}^{2+}$  in the Cokro River as an example of the lotic ecosystems. Cokro River is located in the Malang Regency, East Java, Indonesia. There are a paper factory and residential housing areas around this river. These activities are suspected to cause the entry of pollutants such as  $\text{Cu}^{2+}$  and  $\text{Pb}^{2+}$  into the river. The water of the river used to be used by the people for the various daily live activity. However, due to the low quality of the water, the river water is not used for the daily need anymore. In this study, the concentration of  $\text{Cu}^{2+}$  and  $\text{Pb}^{2+}$  were measured in the biofilms, bottom sediment and river water. The environmental parameters (temperature, pH of bottom sediment, pH of water, current speed, river depth, dissolved oxygen) were also measured. The results of this study suggest that the biofilms can become an effective biological agent to monitor the presence of heavy metals such as  $\text{Cu}^{2+}$  and  $\text{Pb}^{2+}$  in the lotic ecosystems.

## 2. Materials and Methods

### 2.1. Sampling sites

Samples in this study were biofilms, bottom sediments and river waters collected from the Cokro River in Malang Regency, East Java, Indonesia. The biofilms were the natural forming biofilms formed on the stones. In the around of this river, there are a paper factory and domestic activities. The sampling sites to collect the samples were the area near the outlet of the paper factory (Site 1), the area that relatively still natural (surrounded by plants and bamboo forest) (Site 2), and the area near the domestic activities just before the Cokro River get water input from the Lebak Water Source (Site 3). In each sampling sites, the samples (biofilm, sediment, river water) were collected in the middle of the river and both banks of the river.

### 2.2. $\text{Cu}^{2+}$ and $\text{Pb}^{2+}$ measurement

The stones covered by biofilm, the sediments and the surrounding river waters (ca. 15 cm from the biofilm surface) were brought back to the laboratory in plastic containers. The temperature of the container is maintained at around 4 °C. The biofilms were removed from the stones using a sterilized toothbrush in 80 mL of distilled water. The concentrations of the  $\text{Cu}^{2+}$  and  $\text{Pb}^{2+}$  in the suspension are assumed as the diluted concentration of the ions inside the biofilms. The  $\text{Cu}^{2+}$  and  $\text{Pb}^{2+}$  concentrations in the samples (i.e., biofilm, sediment, river water) were measured using an Atomic Absorption Spectroscopy Shimadzu AA-6800 (Shimadzu Corporation, Japan). The measurements were repeated three times independently.

### 2.3. Environmental parameters measurement

Environmental parameters investigated in this study are temperature, pH of bottom sediment, pH of water, current speed, river depth and dissolved oxygen. Temperature and dissolved oxygen were measured by integrated Thermometer and DO Meter Lutron DO-5509 (Lutron Electronics, Taiwan). pH

was measured using a digital pH meter (Krisbow, Indonesia). The current speed of the river was measured by the Floating Method. The river depth was measured using an iron ruler. Measurements were conducted three times independently.

### 3. Results

#### 3.1. Sampling sites and environmental parameters

This research was conducted at Cokro River, Malang Regency, Indonesia. This river is located between the residential settlements and the paper factory. The Cokro River has rocky riverbed and sandy soil. In the past, the residents used water from the Cokro River for daily life. However, the residents do not use the river water again since the Cokro River is suspected to be contaminated by waste from various activities including the paper factory.

Three sampling sites in the Cokro River were investigated in this study (Fig. 1). Sampling site 1 (SL 7°58'21" – EL 112°43'59") is the area near the paper factory (Figure 1a). Sampling site 2 (SL 7°58'19" - EL 112°43'56") is located in the middle between the sampling site 1 and the area before the Cokro River get water input from the Lebak Water Source (Figure 1b). This site is the area surrounded by the plant and bamboo forest. The Sampling site 3 (SL 7°58'16" - EL 112°43'55") is located in the area just before the Cokro River get water input from Lebak Water Source (Figure 1c). Site 3 is the area near the domestic activities of the residents.

**a) Site 1**



**b) Site 2**



**c) Site 3**



**Figure 1.** Sampling sites in the Cokro River, Malang Regency (Fig. 1a, 1b, and 1c are Site 1, Site 2 and Site 3, respectively)

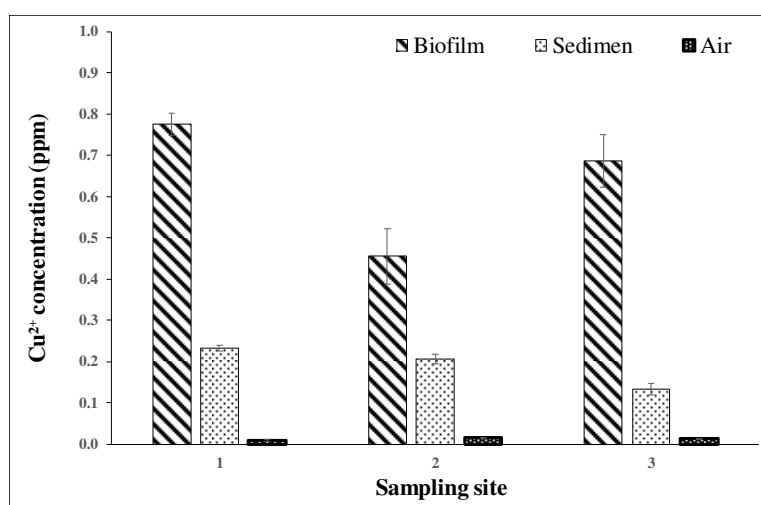
The temperature, pH of bottom sediment, pH of water, current speed, water depth and dissolved oxygen in each sampling sites in the Cokro River were investigated (Table 1). The temperatures of the river water ranged between 22.5-23.07 °C. The pH of the bottom sediment and water ranged between 4.1-4.8 and 7.1-7.2, respectively. The current speed of the water flow in this river ranged from 0.4 m/s until 0.6 m/s. The water depth of the sampling sites in this study range between 41.8-52.8 cm. The dissolved oxygen range from 11.9 to 12.3 mg/L. The characteristics of the sampling sites in this study are generally almost the same. The parameters those mostly different among the sampling sites are the current speed and the river depth.

**Table 1.** Environmental parameters in 3 sampling sites in the Cokro River. Measurements were repeated 3 times independently. ± represents Standard Error.

Sites	Temperature (°C)	pH of bottom sediment	pH of water	Current speed (m/s)	River depth (cm)	Dissolved oxygen (mg/L)
1	22.5 ± 0.17	4.8 ± 0.12	7.1 ± 0.11	0.4 ± 0.07	41.8 ± 0.011	11.9 ± 0.39
2	23.07 ± 0.38	4.3 ± 0.11	7.2 ± 0.13	0.6 ± 0.08	52.8 ± 5.4	12.03 ± 0.43
3	22.8 ± 0.019	4.1 ± 0.11	7.1 ± 0.12	0.5 ± 0.07	50.8 ± 3.7	12.3 ± 0.12

### 3.2. $\text{Cu}^{2+}$ concentration

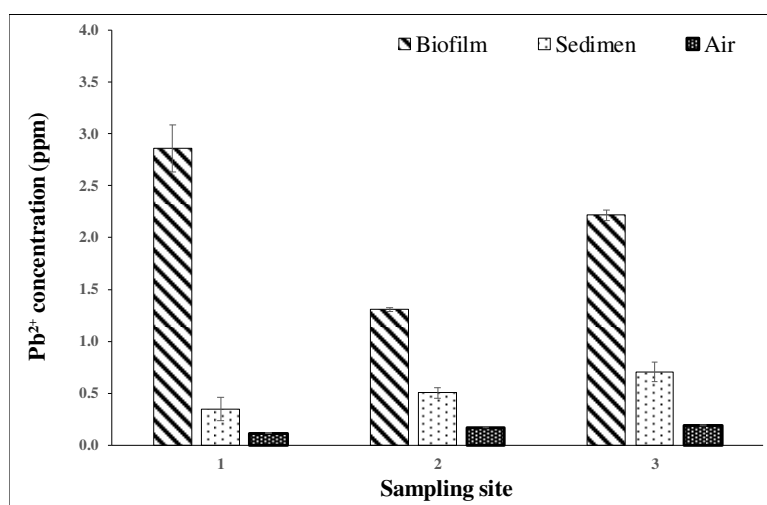
$\text{Cu}^{2+}$  concentrations in the water, sediment and biofilm were investigated (Fig. 2). The concentration of  $\text{Cu}^{2+}$  inside the biofilms (0.46-0.78 ppm) were much higher compared with those of in the sediments (0.13-0.23 ppm) and the river water (0.0087-0.015 ppm). In the case of biofilms, the highest concentrations of  $\text{Cu}^{2+}$  was founded in the Site 1 (0.78 ppm) which is followed by Site 3 (0.69 ppm) and then Site 2 (0.46 ppm). The highest concentration of  $\text{Cu}^{2+}$  in the sediment was in Site 1 (0.23 ppm) followed by the Site 2 (0.21 ppm) and then the Site 3 (0.13 ppm). The highest  $\text{Cu}^{2+}$  concentration in the water was in Site 2 (0.015 ppm), and then followed by the Site 3 (0.012 ppm) and the Site 1 (0.0087 ppm).



**Figure 2.**  $\text{Cu}^{2+}$  concentration in the water, sediment and biofilm. Measurements were repeated 3 times independently.  $\pm$  represents standard errors.

### 3.3. $\text{Pb}^{2+}$ concentration

$\text{Pb}^{2+}$  concentrations were also investigated in this study (Fig. 3). The concentrations of  $\text{Pb}^{2+}$  inside the biofilms (1.31-2.86 ppm) were much higher compared with those of in the sediment (0.35-0.71 ppm) and the river water (0.12-0.19 ppm). The orders of  $\text{Pb}^{2+}$  concentrations inside the biofilms from the highest concentration were 2.86 ppm (Site 1), 2.22 ppm (Site 3) and 1.31 ppm (Site 2). For the case of  $\text{Pb}^{2+}$  in the sediments, Site 3 showed the highest concentration (0.71 ppm) followed by Site 2 (0.51 ppm) and Site 1 (0.35 ppm). The highest concentration of  $\text{Pb}^{2+}$  in the water was found in the Site 3 (0.19 ppm) that followed by the  $\text{Pb}^{2+}$  concentration in the Site 2 (0.17 ppm) and the Site 1 (0.12 ppm).



**Figure 3.**  $Pb^{2+}$  concentration in the water, sediment and biofilm. Measurements were repeated 3 times independently.  $\pm$  represents standard errors.

#### 4. Discussion

The concentrations of  $Cu^{2+}$  in the water of the Cokro River are lower than the maximum threshold (0.02 ppm) while the concentrations of  $Pb^{2+}$  are higher than the maximum threshold (0.03 ppm) [20]. These results indicate that based on the concentration of  $Cu^{2+}$  and  $Pb^{2+}$  in the river water, the Cokro River are have been polluted by the  $Pb^{2+}$ . Unfortunately, Indonesia does not have the standard threshold for the concentration of  $Cu^{2+}$  and  $Pb^{2+}$  in the sediment, and thus, the pollution status of the ions cannot be assessed from the concentration of the ions in the sediment.

The concentration of  $Cu^{2+}$  and  $Pb^{2+}$  inside the biofilms, the sediments and the river water were different in each sampling sites. For the case of the sediment and the river water, there is no particular trend of the order of the concentration. It seems that the environmental conditions particularly the hydrodynamics of the water such as a current speed significantly affect the ion concentrations [8] resulting in the complex trend of the concentrations. On the other hands, the concentration of  $Cu^{2+}$  and  $Pb^{2+}$  inside the biofilms show the similar order trends (Site 1 > Site 3 > Site 1). The activity around the river seems to become the main factor affected the ion concentrations. Site 1 is an area near the paper factory plant while Site 3 is near the domestic activities. These two kinds of activity have been reported to become sources of heavy metals such as  $Cu^{2+}$  and  $Pb^{2+}$  in the aquatic ecosystems. Site 2 is an area that relatively more natural (surrounded only by three and bamboo forest) than other sites. These results indicate that the heavy metal concentrations inside the biofilm matrix may more represent the influence of the surrounding area to the river ecosystems than the heavy metal concentrations in the water or the sediment.

The  $Cu^{2+}$  and  $Pb^{2+}$  concentrations inside the biofilm were hundreds until thousands times higher than the concentrations in the sediments and waters. The biofilms may accumulate the  $Cu^{2+}$  and  $Pb^{2+}$  from the surrounding water resulting in the ion-rich microenvironment inside the biofilms. Our previous study indicates that the biofilm matrices have polymers carrying the negatively charged sites those can attract, and then, retain the ions inside the biofilms [21]. The ions including heavy metals such as  $Cu^{2+}$  and  $Pb^{2+}$  may be retained both in the charged sites of the biofilm polymers and the regions between the biofilm polymers.

The ion rich microenvironment inside the biofilm dynamically adapts with the concentration of the ion in the surrounding environment [22]. Hence, if the ion concentration in the surrounding environments increases, the concentration of the ion inside the biofilm will also increase. The biofilm may also desorb the ion when the concentration of the ion in the surrounding environment decrease. These abilities make the biofilm can actively adsorb and desorb the ions such as  $Cu^{2+}$  and  $Pb^{2+}$  from and

to the surrounding environments. This characteristic makes the  $\text{Cu}^{2+}$  and  $\text{Pb}^{2+}$  concentration inside the biofilm may represent the existence history of those ions in the river for an extended period.

One of the primary difficulties in the biomonitoring of the lotic ecosystems such as the Cokro River is the presence of the water flow. The movement of the water leads to the difficulty to detect the pollution in the specific area of the river. For example, it is difficult to detect the discharge of the pollutant from the specific industrial factory if the used samples are the river water. The difficulty will be increased if the waste is disposed in the specific times such as in the dawn or in the rainy time. Therefore, another agent to monitor the presence of the pollutant more precisely is needed. Biofilm matrix is a predominant habitat of microbes. This microbial community is ubiquitous and easily form on almost all substrates in the aquatic ecosystems. These characteristics and the characteristics related to the heavy metals accumulations as explained above indicates that the biofilm matrix meets the requirements to become a biological agent in the monitoring of heavy metals such as  $\text{Cu}^{2+}$  and  $\text{Pb}^{2+}$  in the aquatic ecosystems.

The standard to assess the heavy metal pollution level in the aquatic ecosystems in Indonesia is only the concentration of the heavy metals in the water. Therefore, the serious obstacles are faced when the pollution in the specific area in the lotic ecosystems want to be assessed. Some countries have used the concentration of the heavy metals in the sediment as a standard. Even this standard has shown the improvement, the dangerous cycle of the heavy metals which can be accumulated and magnified through the food webs is still not accommodated well. Thus, the utilization of biological agents appears as a promising alternative. Taking the biofilm characteristics as discussed above as a primary consideration, the biofilm matrix is one of the most promising alternative biomonitoring agents to monitor the presence of the heavy metal pollutants such as  $\text{Cu}^{2+}$  and  $\text{Pb}^{2+}$  in the lotic ecosystem.

## 5. Conclusion

This study analyzed the  $\text{Cu}^{2+}$  and  $\text{Pb}^{2+}$  concentration in the biofilm, sediment and river water in the Cokro River, Malang Regency, Indonesia. The results indicate that the concentration of  $\text{Cu}^{2+}$  and  $\text{Pb}^{2+}$  inside the biofilms were hundreds until thousand times higher than the concentrations in the sediments and waters. The biofilms may accumulate the  $\text{Cu}^{2+}$  and  $\text{Pb}^{2+}$  from the surrounding water resulting in the ion-rich microenvironment inside the biofilms. The  $\text{Cu}^{2+}$  and  $\text{Pb}^{2+}$  concentration inside the biofilm seem to be able to represent the influence of the surrounding activities to the river ecosystems for an extended period. According to the results of this study, the biofilm matrix is one of the most promising alternative biomonitoring agents to monitor the presence of the heavy metal pollutants such as  $\text{Cu}^{2+}$  and  $\text{Pb}^{2+}$  in the lotic ecosystems.

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