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# Effectiveness of *Hydrilla verticillata* (L.F.) Royle as a phytoremediation agent in Kaligarang River raw water

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**ABSTRACT**
The main source of pollutants is the impact of domestic (household waste), which pile up due to high population levels. The accumulation of domestic waste produces wastewater containing heavy metals, including iron (Fe), which is then absorbed into the ground and flows into rivers. Phytoremediation is a method that can remove, absorb, destroy, and degrade pollutants, including heavy metals, in water. *Hydrilla verticillata* (L.f.) Royle has become a plant known as a good phytoremediator agent. This research aims to determine morphological changes and the effect of phytoremediation *Hydrilla verticillata* (L.f.) Royle will remediate the water of the Kaligarang River with an exposure time of 5 and 10 days. The testing of iron level (Fe) is done using analysis with atomic absorption spectroscopy (AAS). The results obtained were that the iron (Fe) content was originally 1.2222 mg/L, decreased as much as 0.9539 mg/L in treatment S1 with an average value of 0.1683 mg/L, and in treatment S2 decreased to 0.3557 mg/L with an average value of 0.766.
**Keywords:**
Iron (Fe); Phytoremediation; *Hydrilla verticillata* (L.f.) Royle; Kaligarang River

## Introduction

Water pollution, in general, is the entry of pollutants into water, including chemical, physical, biological, and other elements that can change the original form of water, changing color and giving off an unpleasant aroma (Handani et al., 2017). Water pollution is one of the main environmental problems that occurs in all cities in Indonesia. This is proven by the fact that throughout 2010, there were 79 cases of environmental pollution that polluted 65 rivers in Indonesia (Saputra et al., 2020). The sources of polluted river water consist of several causes, including domestic waste, industrial waste, agricultural activities, fisheries, tourism, and other activities (Yudo, 2006). Saputra et al. (2020) stated that the main source of the largest water pollutants is domestic (household waste). Accumulating domestic waste will cause the absorption of waste water from the ground into waterways, this waste liquid can accumulate and has the potential to cause pollution of the aquatic environment, especially rivers. One of the rivers' pollution caused by domestic waste occurred in the city of Semarang.

One of the largest domestic waste disposal centers in the city of Semarang is located at the Jatibarang TPA. The location of the Jatibarang landfill is close to residential areas and river flows. Harjanti and Anggraini, (2020) stated that every day at least 800-850 tons of waste from Semarang residents is supplied to the Jatibarang landfill. The waste originating from the Jatibarang landfill consists of mostly domestic (household waste) which accumulates to produce wastewater which will accumulate into river water at a distance of approximately 300 meters from the Jatibarang landfill location (Supriyadi et al. 2013).

One of the rivers that is located close to the Jatibarang landfill site is the Kaligarang River, Semarang. Waste data from the Jatibarang TPA on November 11, 2022, exceeds the maximum limit, so the accumulated absorption of pollutants due to waste absorption from the Jatibarang TPA could potentially threaten pollution of the Kaligarang River (Setiawan, 2022). This was proven again in the research of Etnovanese et al. (2021) regarding the water quality of the Kaligarang River; water sampling at points downstream of the Kaligarang River at the estuary showed poor water quality.

Kaligarang River pollution occurs as a result of the accumulation of Jatibarang landfill waste, which carries non-organic substances in the form of heavy metals that are absorbed into the soil and flow through the Kaligarang River. This is reinforced by research from Supriyadi et al. (2013) regarding the distribution pattern of Jatibarang landfill waste, that the liquid flow of Jatibarang landfill waste that is absorbed into the Kreo River is then carried northward to merge with the flow of the Kaligarang River. Heavy metals carried by the Kaligarang River are pollutants whose distribution must be described because they have a big influence on reducing and can damage the sustainability of river waters (Azizah and Maslahat, 2021).

Heavy metals that are often found in open waters such as rivers include lead (Pb), Cadmium (Cd), Iron (Fe), Manganese (Mn), and Nickel (Ni) (Farobi, 2019). Excessive accumulation of heavy metals in water will create negative and toxic impacts on the biological and ecological environment of water (Pratiwi, 2020). Suryani et al. (2018) stated that heavy metals can accumulate in the body of an organism and can persist for a long period of time so that they will become toxic substances for the organism, and can threaten the preservation of a good aquatic environment. A good water environment is usually characterized by water conditions that are colorless, odorless, clean and unpolluted.

One of the efforts to solve the problem of water pollution due to waste containing heavy metals is by phytoremediation. Phytoremediation is considered an easy, practical and effective approach in reducing pollution by utilizing surrounding plants (Martin. N.A, 2019). One type of plant that can be used in the phytoremediation method is the *Hydrilla verticillata* (L.f.) Royle plant. Farobi (2019) stated that this plant has hyperaccumulator abilities, which can accumulate metals.

The research variable that will be used in testing is the heavy metal iron (Fe) found in the Kaligarang River flow. If the concentration of iron (Fe) is high, it can accumulate in the body and poison the bodies of living creatures (Lazulva, 2012). Iron metal (Fe) has toxic properties which can also have an impact on human health; it can cause poisoning with symptoms of vomiting, damage to the digestive tract, premature aging, sudden death, inflammation of the joints, birth defects, bleeding gums, cancer, diabetes, constipation, diarrhea, fatigue, hypertension, hepatitis and insomnia (Parulian, 2009).

Based on research by Aulia (2020) regarding the phytoremediation of Pb and Fe metals in laboratory waste at UIN Maulana Malik Ibrahim using *Hydrilla verticillata* (L.f.) Royle plants, it shows that the Pb content, which was originally 168.067 mg/L decreased on the 10th day to 29.4278 mg/L. L, while the Fe content, which was originally 1615.12 mg/L, decreased on day 5 to 109.9337 mg/L with treatment using an aerator. Research from Puspita et al. (2011) the *Hydrilla verticillata* (L.f.) Royle plant as a phytoremediator agent for Chromium (Cr) metal in factory industrial wastewater is capable of accumulating Cr metal with a percentage of 10.84%. Research from Aminatun and Lestari (2018) shows that the *Hydrilla verticillata* (L.f.) Royle plant with heavy metals (Fe) still needs research. Research from Putriarti et al. (2021) *Hydrilla verticillata* (L.f.) Royle was able to reduce the detergent content of Alkylbenzene Sulphonate (LAS) with a reduction percentage of 99.71%.

Phytoremediation studies using the *Hydrilla verticillata* (L.f.) Royle plant with heavy metals (Fe) are still research needs to be done. Iron (Fe) is a type of essential metal that is needed by every living organism within certain limits, but if it exceeds a certain limit, it can have toxic effects. This is different from non-essential metals such as mercury (Hg), cadmium (Cd), lead (Pb), and chromium (Cr), where their presence is not needed by living creatures and can be toxic (Palar, 1994). Phytoremediation research using the plant agent *Hydrilla verticillata* (L.f.) Royle is still often found in the treatment of non-essential metals, but is rarely carried out on essential metals especially iron (Fe). To determine the level of effectiveness of plants in dealing with iron (Fe) pollution in the Kaligarang River water flow because their hyperaccumulator ability is considered quite effective in phytoremediation.

**Methods**

Kaligarang River water samples were taken twice, namely samples during the dry season and during the rainy season at the Kaligarang River water location, and put into jerry cans, after which they were stored at room temperature for one day (Aulia, 2020). To take samples, *Hydrilla verticillata* (L.f.) Royle plants were taken from cultivators of *Hydrilla verticillata* (L.f.) Royle plants are stored for acclimatization for five days (Mutamainah et al, 2015). Acclimatization of plants is the process of gradually adapting plants to a new environment or set of conditions. After acclimatization, the plants were transferred to transparent containers for exposure to Kaligarang River water samples.

Phytoremediation was carried out in 3 repetitions (triplo) using river water taken during the rainy season. Next, the first three containers or S0 are prepared, containing 1 liter of river water + 15 gr of *Hydrilla verticillata* (L.f.) Royle, exposure time is 0 days, S1 contains 1 liter of river water + 15 gr of Hydrilla verticillata, exposure time is 5 days, and S2 contains 1 liter of river water + *Hydrilla verticillata* 15gr exposure time 10 days. After the sample exposure process, the dissolved solids (TDS) content, pH, and raw water temperature of the Kaligarang River were measured. Next, the water samples were tested for iron (Fe) content using an atomic absorption spectrophotometry (SSA) instrument. All samples resulting from digestion were taken and left at room temperature to cool until the SSA analysis process was ready to be carried out. The Fe content analysis process uses an SSA instrument equipped with an iron hollow cathode lamp (HCL) with a wavelength of 248.3nm based on SNI 6989.4:2009 (Lucyan, 2021).

**Results and Discussions**

Measuring dissolved solids, pH, and water temperature before phytoremediation on Kaligarang River water samples is necessary to compare them with the results of Kaligarang River water samples after phytoremediation. The results of measurements of dissolved solids, pH, temperature, and iron (Fe) content in Kaligarang River water samples during the dry season and rainy season can be seen in Table 1.

**Table 1.** Checking River Water Test Parameters

| Jenis Sampel | Parameter Uji | | | |
|---|---|---|---|---|
| | Kandungan Fe | Zat Padat Terlarut | pH Air | Suhu Air |
| Uji Air Sungai musim Kemarau | 0,6246 mg / L | 200 ppm | 8,05 | 34,3$^0$ C |
| Uji Air Sungai musim Hujan | 1,1222 mg / L | 220 ppm | 7,72 | 32,5$^0$ C |

Based on the results of dry season and rainy season river water characterization tests in Table 1, the iron (Fe) content of the Kaligarang River taken during the dry season is 0.6246 mg/L. The results of these measurements are different from the results of iron (Fe) content in rainy season sampling, namely 1.1222 mg/L. The high Fe content in the rainy season occurs due to the formation of leachate, which is influenced by rainwater, where during the rainy season, the amount of leachate is higher than during the dry season (Faesal, 2022). The high content of heavy metals, including iron (Fe), during the rainy season, is because the leachate flow from the Jatibarang landfill in the leachate disposal area upstream of the Kreo River carries metal (Fe) into the Kreo River flow and merges with the Kaligarang River flow during the rainy season (Wiharyanto, 2008).

The results of river water characterization tests in both seasons showed a higher content than the threshold requirement for iron (Fe) content of 0.3 mg/L (Permenkes, 2010). The high iron (Fe) content occurs because the Kaligarang River watershed area used in this research is the river's downstream area. The lower Kaligarang River is dominated by a population that tends to be dense. This has an impact on the number of residential areas being higher around river basins (DAS) compared to upstream areas, which are dominated by agricultural areas. Rahmawati and Siwiendrayanti (2023) stated that the Kaligarang River Watershed (DAS) in the Semarang city area is in the moderately polluted category.

Measurements of dissolved solids, pH and water temperature of Kaligarang river water samples after being treated with phytoremediation can be seen in Figure 1 and Table 2. Figure 1. Graph of the Effect of Phytoremediation on Iron (Fe) Content in Kaligarang River Raw Water in the Rainy Season.

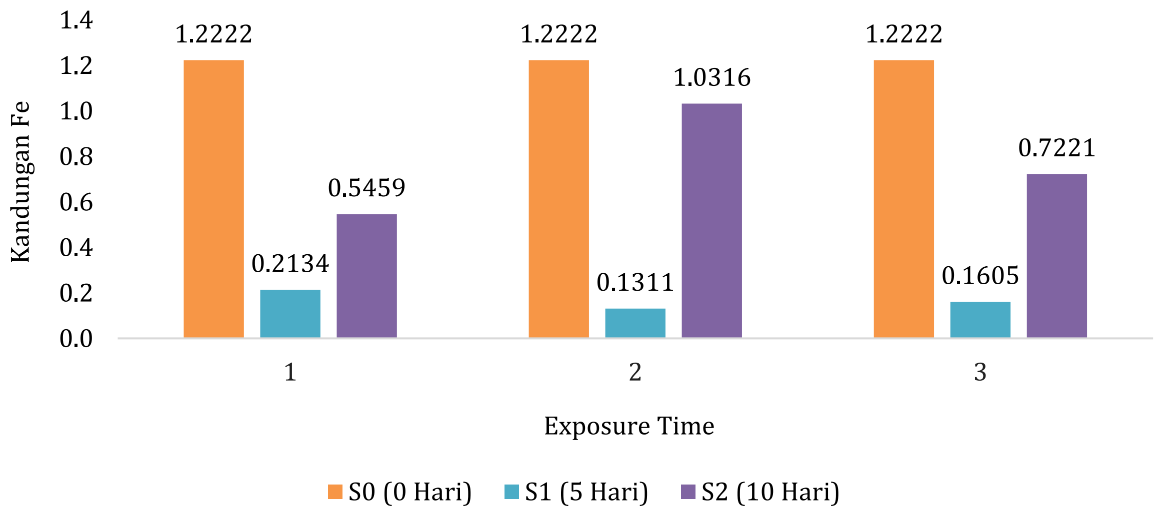


**Figure 1.** Fe content based on phytoremediation exposure time Kaligarang River water

**Table 2.** Analysis of Dissolved Solids, pH, and Water Temperature After Phytoremediation

| Treatment | Observation Parameters | | | | | |
|---|---|---|---|---|---|---|
| | Solid Dissolved (ppm) | Temperature ($^0$C) | pH | Heavy wet | Color stem | Leaf Color |
| S0 | 220 [a] | 32,5[a] | 7,72 | 15 [a] | Light green | Dark Green-Fresh |
| S1 | 143 [b] | 32 [a] | 8,63 | 13,7 gr [b] | Light green-yellowish | Dark Green-wilted |
| S2 | 157 [b] | 33,7 [a] | 8,77 | 19,3 gr [c] | Light green-yellowish | Dark Green-wilted |

The *Hydrilla verticillata* (L.f.) Royle plant has good ability to absorb iron (Fe), so it can be said to be a phytoremediator plant because of its ability to accumulate iron (Fe) in its body tissues. The ability of phytoremediator agents to absorb metals is shown by reducing the metal content in the water. The results of the data analysis in Figure 1 show that there was a decrease in iron (Fe) content in treatment S1 and treatment S2. The iron (Fe) content after phytoremediation in treatment S1 with three repetitions showed an average result of 0.1683 mg/L, while the iron (Fe) content in treatment S2 showed an average result of 0.7665 mg/L. Both data show a decrease in iron (Fe) content, which was originally 1.1222 mg/L, decreased by 0.9539 mg/L in treatment S1, and decreased by 0.3557 mg/L in treatment S2 when compared to S0 treatment. This shows that *Hydrilla verticillata* (L.f.) Royle plants with an exposure time of 5 days (S1) in Kaligarang River water samples have a higher ability to accumulate the heavy metal iron (Fe) than an exposure time of 10 days (S2).

Based on Table 2, the observations of dissolved solids in treatments S1 and S2 show significant results compared to treatment S0. The highest decrease in solutes occurred in treatment S1, with an exposure time of 5 days, compared to treatment S2, with an exposure time of 10 days. This happens because the *Hydrilla verticillata* (L.f.) Royle plant will experience saturation at a certain point, which is thought to be because, on the previous day, the Hydrilla verticillata (L.f.) Royle plant had absorbed almost all of the Fe content in the water to the maximum, so on the 10th day, the substances that are absorbed accumulate, and absorption becomes hampered (Mutmainah et al., 2015). These data show that the exposure time of the *Hydrilla verticillata* (L.f.) Royle plant has a significant effect on the accumulation of the heavy metal iron (Fe) in the raw water of the Kaligarang River.

The significant influence of differences in exposure time during phytoremediation of Hydrilla verticillata plants, is in line with research from Aqli (2019), where exposure times of 1, 3, 5, and 7 days to CuSO4 metal solution with an initial concentration of 3 mg/L showed a significant influence between the length of time exposure to Cu metal absorption by Hydrilla verticillata plants. This statement is also similar to research from Aulia (2020) where exposure times of 5, 10, and 15 days show that there is a significant influence between the length of exposure time on reducing Fe content by *Hydrilla verticillata* plants by the amount of 0.9539 mg/L in treatment S1 and 0.3557 mg/L in treatment S2. The absorption and accumulation of heavy metals by plants is divided into three processes, namely, absorption of metals by roots, translocation of metals from roots to other plant parts, and localization of metals in certain cell parts to ensure that they do not inhibit metabolism (Purnomo et al., 2023). The decrease in solute content was caused by the absorption of dissolved solids by the roots of the *Hydrilla verticillata* (L.f.) Royle plant. Sudiro and Agnes (2013) stated that *Hydrilla verticillata* (L.f.) Royle utilizes all its parts, including leaves, roots, and stems, in the process of absorbing dissolved solid substances in water. The reduction results are in line with research from Safitri et al. (2019) that phytoremediation treatment for 14 days using *Lemna minor* L. and *Hydrilla verticillata* (L.f.) Royle in laundry wastewater was able to reduce the dissolved substance content from 796 mg/L to 764 mg/L - 779.33 mg/L with a reduction percentage range of 2.09% - 4.02%.

The measurement of pH values in this study has an effect on the growth of *Hydrilla verticillata* (L.f.) Royle, where the pH measurement results based on Table 2 show a number that is still in the safe category. In accordance with PP. No. 22 of 2021 Class 1 concerning Implementation of Environmental Protection and Management, with a pH content ranging from 6-9. The increase in pH during the phytoremediation process from 7.72 to 8.63 in treatment S1 and 8.77 in treatment S2 was caused by photosynthetic activity by the *Hydrilla verticillata* (L.f.) Royle plant. Photosynthesis will increase $O_2$ in the water which causes the organic material degradation process to be faster. When organic matter decreases, carbonic acid from the process of diffusing CO2 with water also decreases, causing an increase in the pH value in the water (Safitri et al., 2019). Prasetyo (2022) stated that changes in the pH value of water have a real effect on the chemical, physical, and biological processes of the organisms in it. The neutral pH number is 7, the acid pH range is 1-7, and the alkaline pH is 7-14. Putra (2019) stated that if the pH value in water is less than 6.5, it can cause toxicity, and also the solubility of Fe metal is higher, so that if a reaction occurs with water, ferrous and ferric ions will form, where the ferric ions will precipitate and not dissolve in water. water which causes the water to change color, smell and taste.

Temperature measurements during the phytoremediation process with 3 repetitions did not show significant changes. Treatment S1 has an average value of 32°C, and treatment S2 has an average value of 33.7°C. Normal water temperature is around 3°C from room temperature, namely 27°C (Lestari and Aminatun. 2018). The optimum temperature for photosynthetic activities carried out by the *Hydrilla verticillata* (L.f.) Royle plant is in the range of 28°C to 30 °C but is still able to adapt to temperatures of 43°C for photosynthesis. According to Saputri (2022), photosynthetic activity in plants will not take place if the temperature is below 5°C and above 50°C. Therefore, in the phytoremediation process that has been carried out, temperature does not have much influence on the photosynthesis process of plants. However, the high water temperature during phytoremediation is influenced by environmental conditions, especially by wind conditions, atmosphere, weather, and the intensity of sunlight entering the sea (Patty and Huwae, 2023).

Observations of the physical condition of the plants listed in Table 2 regarding observations of leaf color and stem color of the *Hydrilla verticillata* (L.f.) Royle plant showed a change in color after the phytoremediation process was carried out. The color of the leaves of the *Hydrilla verticillata* (L.f.) Royle plant, which was previously fresh dark green, changed to dark green, with some parts of the plant changing to yellowish in both treatments, both exposure S1 and S2. Color changes also occurred in the stems of the *Hydrilla verticillata* (L.f.) Royle plants, which were originally light green to light green, with some turning yellowish in both S1 and S2 exposures. Color changes in the physical condition of *Hydrilla verticillata* (L.f.) Royle plants indicate symptoms of chlorosis, which occur due to relatively high pollutant loads, causing a decrease in the quality and quantity of chlorophyll in plants (Putriarti et al., 2021). The occurrence of color changes in plant agents is similar to research from Aulia (2020), in whose research the color of the leaves and stems of *Hydrilla verticillata* (L.f.) Royle changed from fresh green to brownish green on the 10th and 15th days of observation.

As a result of the presence of chlorosis symptoms in *Hydrilla verticillata* (L.f.) Royle plants also had an impact on the fresh weight of the plants, as shown in Table 2. A significant decrease occurred in the S0 treatment compared to the S1 treatment. In contrast to the phytoremediation treatment, exposure of 10 days (S2) showed an increase in wet weight. This shows that the *Hydrilla verticillata* (L.f.) Royle plant reproduces quite rapidly (Lestari & Aminatun. 2018), and is able to tolerate water environments with iron (Fe) contents that exceed the limit. This also shows that the Kaligarang River water is not dangerous and does not cause death to *Hydrilla verticillata* (L.f.) Royle.

The longer the exposure time of plant samples to water, the more Fe accumulation by plants will increase to a certain limit that can be tolerated by plants in Syahreza (2012) from Nurlina (2016). So, the response of plants to the accumulation of Fe metal also shows different weights between 5 days and 10 days of exposure. 5 days of exposure showed that the plants had a lighter weight because Fe absorption was higher than 10 days of exposure. This is because when plants experience saturation, they will still be able to adapt to metal-polluted environments by shedding back the accumulated metal that is absorbed. The aborted metal will settle with the substrate in a simpler form, not in the form of dissolved metal as before (Nurlina et al., 2016).

## Conclusion

Phytoremediation using *Hydrilla verticillata* (L.f.) Royle has a significant effect on reducing iron (Fe) content in Kaligarang River water. Both exposure time treatments showed a decrease in iron (Fe) content of 0.9539 mg/L at 5 days of exposure and 0.3557 mg/L at 10 days of exposure. Changes in the morphological condition of *Hydrilla verticillata* (L.f.) Royle, in the phytoremediation process of iron (Fe) pollution in the Kaligarang River, shows symptoms of chlorosis. The stems and leaves of the plant change color to yellowish. Treatment with a 5-day exposure time (S1) showed that plant leaves were shedding and the plant's wet weight also decreased, whereas, with a 10-day exposure time, the plant's wet weight increased.

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