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Heavy metals concentration in green macroalgae *Spirogyra* sp. of Way Ratai River Pesawaran Regency Lampung

F Alhafizoh¹, E L Widiastuti¹*, Tugiyono¹, G N Susanto¹ and D F Mumtazah¹

¹ Faculty of Mathematics and Natural Sciences, University of Lampung, Indonesia

Corresponding Author: endang.linirin@fmipa.unila.ac.id (E L Widiastuti)

Abstract. The Way Ratai River was affected by heavy metals due to gold and tailings processing activities. Certain metals in high concentrations will be dangerous if found in the environment, including the organisms in them, such as *Spirogyra* sp. Sampling was carried out at 4 observation stations around the Way Ratai River, and this research was conducted in November 2020 – February 2021. Analysis of metal content was performed by using the ICP-OES. The results showed that the heavy metal content in *Spirogyra* sp. namely, Ag, Cd, Cr, Cu, Fe, Mn, Ni, Pb, Zn of station A, B, C were above the predetermined quality standards, but Co content was undetectable. In river water, the content of heavy metals Ag in station A, Cu in all stations, Cd and Fe at in station B-D, Pb in station C, Mn and Zn in station D have exceeded the river water quality standards PP RI No. 22/2021 and ANZECC & ARMCANS 2000. Based on calculating bioconcentration factor (BCF) of heavy metals in *Spirogyra* sp, accumulation of heavy metals was in high to very high category with the highest BCF value of 159381.643 found for heavy metal Mn at station A.

1. Introduction

Ratai Bay is a bay located in Pesawaran Regency and is one of the areas that can be affected by heavy metals due to gold mining activities. The potential for gold in the area has attracted various parties to exploit it. Mining is carried out scattered in several places, either by a company or by local communities on a small scale [1]. Based on field observations, several gold processing places can be seen at several points, one of which is around the banks of the Way Ratai river.

Mining areas, especially gold mines, produce waste that contains toxic compounds such as heavy metals. The presence of heavy metals has potential to cause dangerous environmental impacts on the health of the community around the mining site. Gold mining tailings contain one or more toxic, hazardous materials such as arsenic (As), cadmium (Cd), lead (Pb), mercury (Hg), cyanide (CN) and others [2].

Heavy metals are generally toxic to living things, but some of them are needed in small amounts. Heavy metals are grouped into two types, namely essential metals in the form of heavy metals in certain amounts needed by the body such as zinc (Zn), copper (Cu), iron (Fe), cobalt (Co), and manganese (Mn), and non-essential metals whose presence is not needed by the body and are toxic such as Hg, Cd, Pb, Cr, and others [3].

The factors that cause heavy metals to be classified into pollutants are because heavy metals cannot be degraded through biodegradation, such as those of organic pollutants, but they can accumulate into the environment. The increase in heavy metal levels in the river and seawater will cause an increase in heavy metal levels in the body of aquatic biota [4]. As in the macroalgae group, green algae that live in



water areas by sticking to the substrate of plant roots, rocks, wood, and objects in the water, so it tends to receive more pollutants than other aquatic biotas. The presence of algae has an important role both in stagnant waters and flowing waters, namely as an important food source for invertebrates and some fish, as a biological parameter that provides information for evaluating the quality of water, and can be a vital accumulator of heavy metals. Since macro algae can absorb heavy metals through a biological system known as biosorption.

With the existence of high human activities around the Way Ratai river flows, such as daily community activities and gold mining waste disposal in the Way Ratai river flow which empties into Ratai Bay, it is assumed that the waters of the Ratai Bay have been polluted. In order to determine whether there is any pollution from gold mining activities to the Ratai Bay ecosystem, it is necessary to determine the concentration of heavy metals in the surrounding ecosystem, such as in small rivers that empty into Ratai Bay, one of which is the Way Ratai river. Therefore, this study was conducted to determine heavy metals content in river water of Way Ratai river by looking at the green macroalgae, such as *Spirogyra* sp.

2. Materials and methods

2.1. Study area

This study was conducted from November 2020 to February 2021. Sampling was carried out at 4 observation stations along the Way Ratai river flow from Bunut Seberang Village, Way Urang Village, Khepong Jaya Village, and Sanggi Pematang Awi Village. Sample preparations were carried out at the Biomolecular Laboratory, Department of Biology, Faculty of Mathematics and Natural Sciences, University of Lampung. Analysis of heavy metal content of samples was carried out at the Integrated Laboratory Unit and Technology Innovation Center (LTSIT), University of Lampung, Lampung Province, Indonesia. For more details, the research locations and observation stations can be seen in Figure 1 and Table 1.

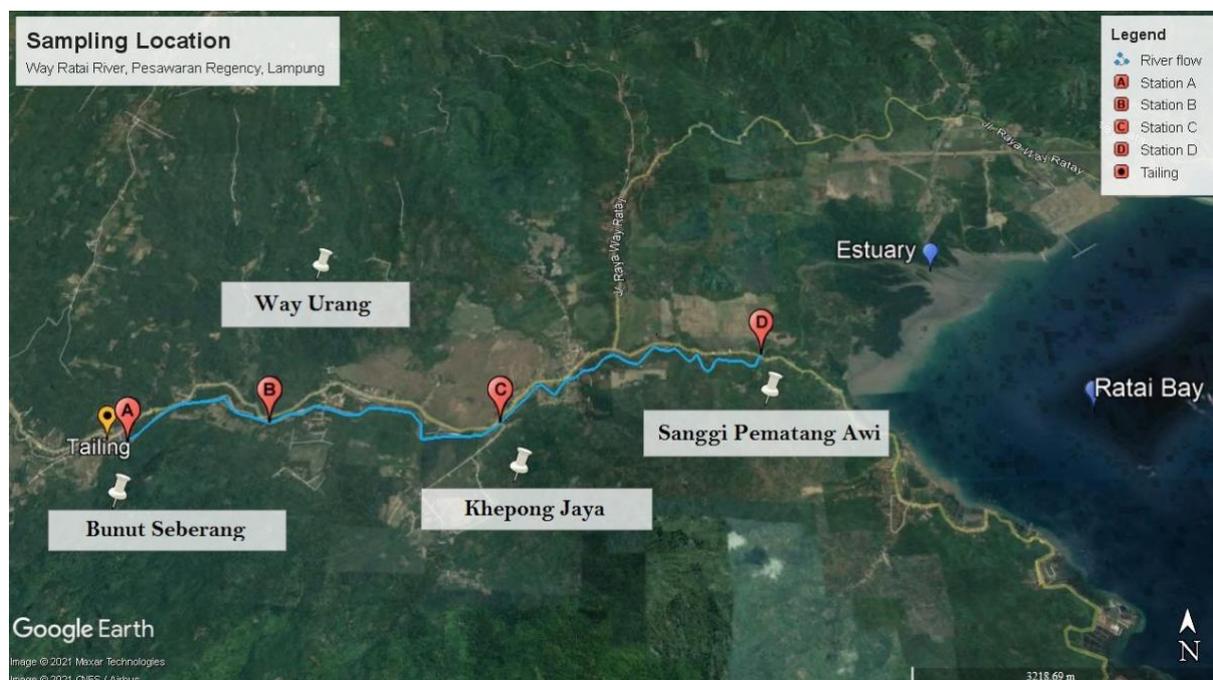


Figure 1. Sketch the sampling location

Table 1. Sampling coordinates from 4 stations (study sites)

Station	Location	South Latitude	East Longitude
A	Bunut Seberang Village River	5 ° 36'39.5 "S	105 ° 05'31.5 "E
B	Way Urang Village River	5 ° 36'24.0 "S	105 ° 06'46.0 "E
C	Khepong Jaya Village River	5 ° 36'30.4 "S	105 ° 06'26.0 "E
D	Sanggi Pematang Awi Village River	5 ° 36'01.0 "S	105 ° 09'29.0 "E

2.2. Materials

The tools used in this research are GPS (Global Positioning System), cool box, plastic zipper bag, plastic bottle, HDPE bottle, stationery, label paper, mortar and pestle, digestion flask, Erlenmeyer, analytical balance, oven, hot plate, measuring cup, metal spatula, volumetric pipette, and Agilent Varian 715-ES Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES) from Rocklin, California, USA. Collected samples were river water and green macroalgae *Spirogyra* sp., 30% HCl solution from Avantor Performance Materials Inc, Thailand, 65% HNO₃ solution from EMD Miliopore Corp. Germany, 30% H₂O₂ solution from Avantor Performance Materials Inc. USA, distilled water, and filter paper Whatman No. 41.

2.3. Research procedure

2.3.1. Research design. The sampling method used was purposive sampling based on the distance between the location of gold mining activities and the input of waste, and the ease of sampling (Figure 1). Water and *Spirogyra* sp. were randomly collected for each station with a distance between stations closed to 500 m.

2.3.2. Sample collection and preparation. A sampling of green macroalgae *Spirogyra* sp. was taken from the surface or bottom of the water, adjusted to the algae condition in the field. Then washed with river water at the sampling location and then put into a sample bag (plastic zipper). The water sampling technique followed Indonesian National Standard number 6989.57/2008 concerning sampling methods for surface water [5]. Water samples were taken by using plastic bottle directly on the river water closed to the surface and they were put in dark sample bottle containers

Algae samples were dried in an oven at 105°C for 24 hours and cooled. The dried samples were then pounded until it became powder and weighed as much as 1 g and then put into Erlenmeyer. 2 mL of HCl, concentrated HNO₃, and H₂O₂ were added, respectively, and then closed and left for 24 hours. Furthermore, the solution obtained was heated on a hot plate at 95°C for 1 hour. After that, the overall volume was increased to 50 mL with distilled water [6]. The concentration of heavy metals in dissolved algae was determined by ICP-OES.

As much as a 10 mL water sample was put into the digestion flask. Then given 0.2 mL of HNO₃ and 0.1 mL of HCl and homogenized. The sample was heated using a hot plate with a temperature of 95°C in 30 minutes. The sample was then cooled and transferred to a 10 mL measuring flask. In order to remove sediment, water sample was filtered by using filter paper in advance. The sample was added with distilled water until the sample volume became 10 mL and transferred to HDPE bottles, and ready to be analysed [7].

2.3.3. Heavy metal analysis using ICP-OES. Samples that have been prepared were analysed using ICP-OES to see the concentration of heavy metals Ag, Cd, Co, Cr, Cu, Fe, Mn, Zn, Pb, and Ni

2.4. Data analysis

The heavy metal concentration data obtained were then analysed descriptively, then they were compared according to the environmental quality standards that had been set. The quality standards used were

based on Australian and New Zealand Guidelines for Fresh and Marine Water Quality year 2000 [8] and refers to the standard water quality standards established by the Indonesian Government Regulation No. 22 the Year 2021 on the Implementation of the Protection and Management of the Environment, Appendix VI National Water Quality Standards [9]. The quality standard for algae was based on the regulations of the National Standardization Agency, regarding the maximum limit of heavy metal contamination in food [10] and national environmental quality standard as set by Pakistan EPA 1993 [11].

2.5. Calculation of bioaccumulation of heavy metals in algae *Spirogyra* sp.

The study of the ability of algae to accumulate heavy metals in the waters of the Way Ratai river was analysed using a bioconcentration factor (BCF). The bioconcentration factor was calculated using a formula [12] as follows:

$$BCF = \frac{c \text{ biota}}{c \text{ ambient media}}$$

Description: c biota: heavy metal concentration in algae; c ambient media: concentration of heavy metals in water.

3. Results and discussion

3.1. Heavy metals in *Spirogyra* sp.

The value of heavy metal content in algae *Spirogyra* sp. can be seen in Table 2 below:

Table 2. Average heavy metal content in *Spirogyra* sp.

Station	Metals (mg/kg)									
	Ag	Cd	Cu	Pb	Fe	Mn	Co	Cr	Zn	Ni
A	10.585	2.073	14.506	41.082	6853.495	2231.343	<0.50*	14.488	197.725	5.695
B	9.791	5.023	19.610	56.474	14615.570	4158.216	<0.50*	11.825	365.735	7.517
C	7.093	4.283	13.264	29.119	14708.750	3520.102	<0.50*	11.641	188.035	6.393
Quality Standard	1.0 ^b	0.1 ^a	1.0 ^b	0.3 ^a	8.0 ^b	1.5 ^b	0.1 ^b	1.0 ^b	5.0 ^b	1.0 ^b

A: Bunut Seberang Village river station.

B: Way Urang Village river station.

C: Khepong Jaya Village river station.

(*): The measured value is below the detection limit, the detection limit value is stated.

^aNational Standardization Agency, regarding the maximum limit of heavy metal contamination in food (SNI 7387: 2009).

^bNational Environmental Quality Standard as set by Pakistan EPA 1993

The result of heavy metal concentrations in *Spirogyra* sp algae was compared with quality standards based on the Regulation of the National Standardization Agency, concerning Maximum Limits of Heavy Metal Pollution in Food and the national environmental quality standard as set by Pakistan EPA 1993. Because the research has not found a quality standard that regulated heavy metal concentrations for *Spirogyra* sp. in river water. So that the quality standard is used by looking at the biomagnification process that occurs, namely the tendency of a pollutant to be concentrated and move from one trophic level to the next trophic level in the food pyramid [13]. So that heavy metals will accumulate more in the next level consumers, namely aquatic biota such as fish that consume algae contaminated with heavy metals.

Based on the analysis of heavy metal content in green macroalgae *Spirogyra* sp. in the Way Ratai River, Pesawaran Regency, obtained the result that exceeds the quality standards, namely Ag, Cd, Cu, Pb, Fe, Mn, Cr, Zn, and Ni metals found at 3 flow stations in A, B, and C. While at stations D, namely the Way Ratai River, Sanggi Pematang Awi Village, samples of green algae *Spirogyra* sp. could not be

found. Because when taking algae samples, station D which is the estuary area is in a state of high tide. Therefore, it was unable to determine the heavy metals content in *Spirogyra* sp at this station (D). The heavy metal content for Co metal was not detected in ICP-OES, where the detection limit or the level of accuracy of this tool was <0.500 mg/kg.

The results of the metal analysis showed that Fe metal at station A-C had a higher average content than other heavy metals. The average content of Fe metal ranged from 6853.495 – 14708.750 mg/kg, with the highest content at station C and the lowest at station A. The second-highest average heavy metal content was found in Mn metal, indicated by the highest Mn metal content at station B, which was 4158.216 mg/kg and the lowest average was 2231.343 mg/kg at station A.

Overall, based on the analysis results obtained, it can be seen that the value of heavy metal content at station B is higher than stations A and C. The difference in the value of heavy metal content between stations B and C is not too big, but the most visible difference is in the value of heavy metal content at station A. The difference between the three stations was presumably caused by where the *Spirogyra* sp. was found in the water. *Spirogyra* sp, collected in station A was floating on the rock surface and not fully submerged in water, unlike those found at stations B and C. At stations B and C *Spirogyra* sp. was found attaching to the rock and submerged in water so that the metal accumulation process would be predicted to be higher. Heavy metal contamination on *Spirogyra* sp. of Way Ratai River presumably occurs due to community activities around the river, such as tailing process from gold mining residue which found at several points around the riverbanks and dumping activity. This tailing activity of gold mining could be found in the riverbank of station A up to station D. The improper disposal of tailings at some gold processing locations will cause environmental pollution. This activity causes the river water to become cloudy, due to sludge sedimentation from the tailings processing process which contains heavy metals. Furthermore, it settles on the riverbed or clings to rocks so that it prevents algae from using light for the photosynthesis process. Furthermore, daily community activities such as washing and domestic waste dump around the banks of the Way Ratai River also could trigger heavy metal pollution.

Biologically, metals affect enzymatic reactions so that they indirectly affect the processes of photosynthesis, respiration, and reproduction so that they will inhibit the growth of algae [14]. Furthermore, if the algae contaminated by heavy metals is consumed by other aquatic biotas such as fish, the heavy metals will accumulate in the water biota network. Furthermore, if consumed by humans who occupy the highest trophic level in the food chain in a food pyramid, the human body will accumulate the highest heavy metals.

Commonly, the heavy metal accumulation process occurs in two biochemical pathways, namely the adsorption of metal ions into the cell surface and the absorption of metals into cells referred to as bioaccumulation. The adsorption process (passive uptake) is fast, back and forth, and occurs in both dead and living cells [13]. This process might occur in the accumulation of heavy metals in *Spirogyra* sp.

Metal ions enter through the cell walls of algae which contain a wide variety of polysaccharides and proteins that have several active sites. These active sites are capable of binding to metal ions through chemical and physical mechanisms, namely ion exchange, complex formation, and adsorption involving functional groups such as carboxylate groups, amines, thiolates, hydroxides, imidazoles, sulfhydryls, phosphodiester, and phosphate groups [15,16]. Furthermore, in the absorption or bioaccumulation mechanism (active uptake), algae will move metal ions bound to the cell wall to the deeper cell organelles. Bioaccumulation occurs as a result of exposure to metal ion materials continuously into the cell, the accumulation of ions will remain in the cell because the rate of release of metal ions is much lower than the rate of absorption into the cell [13].

3.2. Heavy metals in Way Ratai river water, Pesawaran Regency

Analysis of heavy metal content was also carried out on river water samples, the results of the analysis were presented in Table 3 below.

Table 3. Average heavy metals in river water.

Station	Metal (mg/L)									
	Ag	Cd	Cu	Pb	Fe	Mn	Co	Cr	Zn	Ni
A	0.103	0.006	0.052	Nd	0.252	0.014	0.069	0.013	0.009	0.048
B	Nd	0.014	0.057	Nd	0.447	0.030	0.060	0.016	0.034	0.031
C	Nd	0.029	0.041	0.038	0.472	0.027	0.023	0.011	0.035	0.020
D	Nd	0.013	0.041	Nd	0.902	0.112	0.042	0.012	0.151	Nd
River water quality standards	0.003 ^a	0.01 ^b	0.02 ^b	0.03 ^b	0.3 ^c	0.1 ^c	0.2 ^b	0.05 ^b	0.05 ^b	0.05 ^b

A, B, C, D: Observation station.

Nd: Not detected

^a Australian and New Zealand Guidelines for Fresh and Marine Water Quality (ANZECC / ARMCANZ) year 2000.

^b Indonesian Government Regulation No. 22 year 2021 on the Implementation of the Protection and Management of the Environment, Appendix VI National Water Quality Standards class 3.

^c Indonesian Government Regulation No. 22 year 2021 on the Implementation of the Protection and Management of the Environment, Appendix VI National Water Quality Standards class 1.

Based on the results of research on the content of heavy metals in Way Ratai River water at four observation stations, it can be seen that the Ag metal at station A exceeds the predetermined quality standard, which is based on ANZECC/ARMCANZ 2000 (Australian and New Zealand Guidelines for Fresh and Marine Water Quality). Meanwhile, the content of Ag metal at station B-D was not detected by ICP-OES with an Ag metal detection limit of 0.003 mg/L.

The metal content of Cd at stations B, C, and D, Cu metal at all observation stations, Pb at station C, and Zn at station D exceeds the Class 3 water quality standard, which is water intended for freshwater fish cultivation, animal husbandry, and water for irrigation plantations regulated in Government Regulation of the Republic of Indonesia No. 22 of 2021. The content of Fe metal at stations B, C, and D, Mn metal at station D based on PP RI No. 22 of 2021 are above the standard value for Class 1 quality standards, namely water designated as drinking water, where the normal content limit is 0.3 mg/L for Fe and 0.1 mg/L for Mn metal. While the metal content of Fe and Mn is in the Class 3 water group, there is no limiting factor for the designation of that class.

The content of heavy metals Co and Cr in the water in the Way Ratai river for all stations has a value below the quality standard based on PP RI No. 22 of 2021, as well as for Mn, Zn, and Ni at stations A-C. Once again, 5 of the heavy metal concentration in station D which closed to the estuary exceed the predetermined standard values, namely for Cd, Cu, Fe, Mn and Zn. Yet, this still has to be considered carefully, considering that these waters have been contaminated by heavy metals Co, Cr, Mn, Zn and Ni even though they are still below the threshold, since the local activity in riverbank of the Way Ratai is still going on.

The results of the metal analysis showed that the heavy metal Fe in all sampling stations had the highest content compared to other metals in the range of 0.252 - 0.902 mg/L. The highest Fe content is at station D which is the estuary area. The high content of Fe metal in the estuary is thought to be caused by domestic waste disposal, agricultural activities, industrial waste deposits, iron water reservoirs, and corrosion from water pipes containing ferrous metal [17]. The accumulation of the oxidation process that occurs as a result of the gold mining process and the processing of tailings waste from gold mining also contributes to the high concentration of Fe carried by the river flow to the estuary.

Overall, the heavy metal content in water shows a lower level of metal content compared to the heavy metal content in the algae *Spirogyra* sp. One of the factors that could explain the low analysis results of heavy metal content in river water is presumably because several observation stations are located in areas with turbulence, such as at stations A, B, and C compared to those in station D with much laminar flow. This difference in water current then might cause the levels of Fe, Mn, and Zn at station D to be

higher than those at other stations. River currents cause the presence of heavy metals in the water to become unstable [18].

The dynamic of water current causes pollutants such as heavy metals in the water to be evenly distributed in the river water column or undergo dilution. In addition, the deposition process of some heavy metals can also accumulate into the body of these aquatic organisms [17].

Heavy metal contamination in the Way Ratai River can occur due to the processing of tailings from gold mining residue found at several points around the riverbanks. Heavy metal contamination can also result from the disposal of domestic waste or household waste and agricultural activities. Heavy metal contamination in the air will lead to the accumulation of aquatic organisms and ultimately be harmful to humans who consume these aquatic organisms [19]. Moreover, if the metal content exceeds the specified threshold value because the metal can enter the body of aquatic organisms through the air and the food consumed so that the accumulation of food in the aquatic body will be toxic to the life of the air organism. This also applies to humans who consume waters polluted by heavy metals such as will harm their bodies [18].

3.3. Bioconcentration factor (BCF) analysis

In addition to testing the heavy metal content in the green algae of *Spirogyra* sp and river water in the Way Ratai river, Pesawaran Regency, a calculation of the value of the BCF was also carried out to determine the ability of algae to accumulate heavy metals in water. This BCF value can be obtained from the ratio between the metal concentration absorbed into the algae network and the metal concentration in river water. The values of the BCF are presented in Table 4.

Table 4. Value of metal bioconcentration factor (BCF) in *Spirogyra* sp.

Metal Parameters	Station		
	A	B	C
Ag	102	-	-
Cd	345	359	147
Cu	279	344	323
Pb	-	-	766
Fe	27196	32697	31162
Mn	159381	138607	130374
Co	-	-	-
Cr	1114	739	1058
Zn	21969	10757	5372
Ni	118	242	319

Table 5. Categories of heavy metal bioconcentration factor (BCF) [20].

BCF Range	BCF Category
>1000	Very high
100-1000	High
30-100	Moderate
<30	Low

The presence of heavy metals in water causes an accumulation process in the body of aquatic organisms, where this accumulation can occur through direct absorption of heavy metals in water and the food chain. Bioaccumulation of chemicals in water is an important criterion for evaluating the ecology and level of pollution of an environment. In addition to it, determining of the level pollution in water could be done by measuring the value of the bioconcentration of the biota that lives in it [21].

Based on the results, BCF values above 1000 were obtained for heavy metals Mn, Fe, and Zn, so they were categorized as very high accumulative properties. Meanwhile, for Ag, Cd, Cu, Pb and Ni

metals on green algae *Spirogyra* sp. in the high accumulative category because the BCF values are in the range of 100-1000. In this study, the bioconcentration factor for metal Co could not be determined because the metal content in the organism (algae) was not detected. Based on these categories, it can be seen that the accumulation ability of *Spirogyra* sp in accumulating heavy metals is in the high to the very high accumulative category.

The high value of the bioconcentration factor (BCF) proves that the algae *Spirogyra* sp tends to absorb and accumulate heavy metals, so it can be used as a bioindicator of the aquatic environment. The results showed *Spirogyra* sp. in the Way Ratai River, is easier to accumulate Mn than other metals. The sequence of heavy metal accumulation in green algae *Spirogyra* sp. is Mn> Fe> Zn> Cr> Pb> Cu> Cd> Ni> Ag, while the heavy metal Co cannot be determined.

If bioaccumulation continues, biomagnification can occur, which involves the food chain as a link. Biomagnification tends to increase the concentration of pollutants along with the increase in trophic levels in the food chain. So that producers accumulate the lowest toxic materials and the last consumers accumulate the most [13].

4. Conclusion

The heavy metal content of Ag, Cd, Cu, Pb, Fe, Mn, Cr, Zn, and Ni in the green algae *Spirogyra* sp. at each station is above the quality standard that has been set. The accumulation ability of the *Spirogyra* sp. in water is in the high to the very high accumulative category. Heavy metal content in *Spirogyra* sp. and water that exceeded the quality standard indicates that the Way Ratai River waters are contaminated with heavy metals.

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