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EVALUATION OF RIVER WATER QUALITY BASED ON BIOTIC INDEX OF BENTHIC MACROINVERTEBRATE AS BIOINDICATOR (CASE STUDY IN GENJONG RIVER, EAST JAVA, INDONESIA)

EVALUASI KUALITAS AIR BERDASARKAN INDEKS BIOTIK DARI MAKROINVERTEBRATA BENTOS SEBAGAI BIOINDIKATOR (STUDI KASUS DI SUNGAI GENJONG WLINGI BLITAR JAWA TIMUR, INDONESIA)

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ABSTRACT

This research aims to determine the water quality profile of Genjong River based on physicochemical parameters of water and benthic macroinvertebrates as bioindicators. Sampling was carried out at four different locations based on difference of human activity in surrounding. The sampling was done in triple repetition for each station. The activity of Station 1 is ecotourism (as a reference site or positive control in this study), Station 2 (livestock I), Station 3 (livestock II), and Station 4 (plantation). The physicochemical water parameters were measured, including water and air temperature, water current velocity and discharge, conductivity, pH, DO, BOD, TSS, nitrate, and orthophosphate. The result from the identification and calculation of the benthic macroinvertebrates density was used to determine some diversity and biotic indices. The difference in the value of each water quality parameter was tested by One-way ANOVA. Based on the abiotic water quality profile, Genjong River water was categorized as the Class IV category based on Indonesia Government Regulation No. 22 of 2021 with a BOD value of 3.61 – 7.22 mg.L⁻¹. Human activities along the Genjong River greatly impact decreasing water quality as indicated by increasing nitrate levels from 0.52 ± 0.07 mg.L⁻¹ at Station 1 up to $0.85 \pm 0.07 \text{ mg}.L^{-1}$ at Station 4. Also, orthophosphate levels from $0.02 \pm 0.01 \text{ mg}.L^{-1}$ at Station 1 to 0.18 \pm 0.02 mg.L⁻¹ at Station 4. Meanwhile, based on benthic macroinvertebrates as bioindicators, Genjong River was classified as lightly (S1, H = 1.74) to moderately polluted (S4, H = 1.24) with toxic materials and slightly contaminated with organic matter (S4 with FBI value = 5.38). The decline in water quality was also shown by the decreasing ASPT value from 4.20 at Station 1 to 3.68 at Station 4.

Keywords: benthic macroinvertebrate, Genjong River, water quality

ABSTRAK

Tujuan penelitian ini adalah menentukan profil kualitas air Sungai Genjong berdasarkan parameter fisika kimia air dan makroinvertebrata bentos sebagai bioindikator. Pengambilan sampel dilakukan secara triplo pada 4 titik aliran sungai yaitu Stasiun 1 (aktivitas ekowisata untuk reference site), Stasiun 2 (peternakan I), Stasiun 3 (peternakan II), dan Stasiun 4 (perkebunan). Parameter fisika kimia air yang diukur meliputi suhu air, suhu udara, kecepatan arus, debit, konduktivitas, pH, DO, BOD, TSS, nitrat dan ortofosfat. Hasil identifikasi dan penghitungan kerapatan makroinvertebrata bentos digunakan untuk menentukan beberapa indeks diversitas dan indeks biotik. Perbedaan nilai tiap parameter kualitas air diuji dengan One Way ANOVA. Hasil penelitian menunjukkan bahwa berdasarkan profil kualitas abiotik air, Sungai Genjong termasuk dalam kategori Kelas IV berdasarkan PP No 22 tahun 2021 dengan nilai BOD 3,61 -7,22 mg/L. Aktivitas manusia di sepanjang Sungai Genjong telah berdampak pada penurunan kualitas air yang ditunjukkan oleh peningkatan kadar nitrat dari 0.52 ± 0.07 mg/L di Stasiun 1 menjadi 0,85 ± 0,07 mg/L di Stasiun 4, dan juga kadar ortofosfat dari $0,02 \pm 0,01$ mg/L di Stasiun 1 menjadi $0,18 \pm 0,02$ mg/L di Stasiun 4. Sedangkan berdasarkan makroinvertebrata bentos sebagai bioindikator, Sungai Genjong termasuk dalam kategori tercemar bahan toksik ringan (stasiun 1, H = 1,74) hingga sedang (stasiun 4, H = 1,24) dan tercemar bahan organik sedikit (di stasiun 1 dengan FBI = 4,31) sampai agak banyak (di stasiun 4 dengan nilai FBI = 5,38). Penurunan kualitas air juga ditunjukkan oleh menurunnya nilai ASPT dari 4,20 di Stasiun 1 menjadi 3,68 di Stasiun 4.

Kata kunci: kualitas air, makroinvertebrata bentos, Sungai Genjong

INTRODUCTION

Rivers are freshwater ecosystems that are important for living things to maintain their lives [1]. Genjong River is a river that crosses some villages in Wlingi District. Genjong River is the main river that used by the surrounding community for several purposes, including tourism, animal husbandry, and irrigation sources for rice fields. Based on the visibility characteristics of the Genjong River, there are indications of a decrease in water quality which is indicated by a change in the color of the water to become cloudy.

Water quality evaluation can be determined by some parameters, including physics, chemical, and biological [2]. One of the biological parameters that can be used as a bioindicator is benthic macroinvertebrate because it can show the specific conditions of the waters and complete information on the physicochemical parameters of water using several biotic indices such as the Hilsenhoff Biotic Index (HBI), Family Biotic Index (FBI) and Average Score Per Taxa (ASPT) [2, 3, 4].

This study aims to evaluate the water quality of the Genjong River based on physics, chemical parameters, and benthic macroinvertebrates as bioindicators. The evaluation results can be used as a basis for determining the management of the Genjong River ecosystem.

METHODS

Site. The study was conducted from July to December 2021. A sampling of benthic macroinvertebrates was carried out at four points (Figure 1) in the Genjong River channel, district of Wlingi, Blitar, East Java, Indonesia, based on human activities around the river. Station one is a stream with human activities in the form of Sirah Kencong Tea Plantation. Station two is a stream after Telogosari Village and a dairy farm. Station three is the stream after Genjong Village with residents' farms. Station four is a stream after human activities like coffee plantations, sengon plantations, and coffee processing factories. The identification of benthic macroinvertebrates and data analysis were carried out at the Laboratory of Ecology and Tropical Ecosystem Restoration, Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Brawijaya.

Benthic macroinvertebrate sampling. The benthic macroinvertebrate sampling was conducted by using a Surber net. The frame root of the net was put in the opposing directions. The substrate contained in the root frame was stirred carefully by hand so benthic organisms attached to any substrate, like rocks, could be rinsed, washed away, and collected in a Surber net. The obtained samples were sorted and preserved with formalin 4%. The identification of benthic macroinvertebrates was assisted by a stereo microscope.

physicochemical Water parameter measurement. The physicochemical water parameters measured included water temperature, air temperature, flow velocity, water discharge, conductivity, pH, dissolved oxygen (DO). biological oxygen demand (BOD). total suspended solids (TSS), nitrate level. orthophosphate level, and substrate composition. Water and air temperature were measured with a digital thermometer in Celsius. The river current (flow) was measured by buoy and stopwatch with units of m.s⁻¹.



Figure 1. Water and benthic macroinvertebrates sampling location

The water discharge was calculated based on the depth and width of the river with units of dm³.s⁻¹. Conductivity was measured by a conductivity meter with units of mS.m⁻¹. pH was measured with a pH meter. DO and BOD were measured by DO meter, TSS was measured by TSS meter, nitrate and orthophosphate levels were measured by spectrophotometry with units of mg.L⁻¹. Substrate composition was measured by assessing the ratio (%) between rock, sand, and mud of the riverbed.

Data analysis. Descriptive analysis was held for the water physicochemical parameters. The difference in the value of each location was tested with One-way ANOVA followed by the Tukey HSD test if the variance value was homogeneous and the Brown Forsythe and Games Howell test if the variance value was heterogeneous with Sig. 0.05. Water quality groupings and interactions between parameters were tested by Principal Component Analysis (PCA)/biplot analysis using the PAST program.

The benthic macroinvertebrates community structure was analyzed by some indices. There was abundance, important value index (IVI), Shannon-Wiener diversity index (IV), Simpson diversity index (D), Margalef diversity index (dMg), Evenness index (E), Simpson dominance index (Id), Family Biotic Index (FBI), Hilsenhoff Biotic Index (HBI) also Average Score Per Taxa (ASPT) [4, 5, 6, 7, 8, 9]. The benthic macroinvertebrate abundance (ind.m⁻²) was calculated by the following formula [4].

$$N = \frac{O}{S} \times 10.000$$

Where N was the number of benthic macroinvertebrates per m^2 , O was the number of benthic macroinvertebrates counted per sample, and S was the transverse area of Surber Net in m^2 .

Important value index (IVI) was calculated by the following formula [4].

IVI = *Relative density* + *relative frequency*

Shannon-Wiener diversity index (H') and Simpson diversity index (D) were calculated by the following formula [6].

$$H' = -\sum_{i=1}^{s} Pi^{2} \log Pi$$
$$D = 1 - \sum_{i=1}^{s} (Pi^{2})$$

Where Pi was the proportion of species-i to the total number, s was the total number of the community. The H' and D values indicated toxic pollution. The H' was classified into four categories, 2 was no apparent pollution, 2–1.6 was

slightly polluted, 1.5–1 was fairly polluted, and <1 was severely polluted. D value was also classified into three categories, >0.8 was slightly contaminated, 0.6–0.8 was moderately contaminated, and <0.6 was severely contaminated.

Margalef diversity index (dMg) was calculated by the following formula [<u>5</u>].

$$dMg = \frac{S-1}{\log N}$$

Where S was the total number of identified species, N was the total number of individuals recorded. The dMg value was categorized into three classes, <3.5 was low diversity, 3.6–4.9 was moderate diversity, and >5 was high diversity.

The Evenness index (E) and The Simpson dominance index (Id) were calculated by the following formula [$\underline{6}$].

$$E = \frac{H'}{S}$$
$$Id = \frac{\sum Ni(Ni - 1)}{N(N - 1)}$$

Where Ni was the total number of species-i, N was the total number of individuals. The E value was classified into three categories, <0.4 was low evenness, 0.4–0.6 moderate evenness, and>0.6 high evenness. The Id was also classified into three categories <0.4 was low domination, 0.4–0.6 moderate domination, and >0.6 high domination.

Family Biotic Index (FBI) and Hilsenhoff Biotic Index (HBI) were calculated by the following formula [8].

$$FBI/HBI = \sum \frac{x_i t_i}{n}$$

Where x_i was the total number of species-i, t_i was the tolerance score for every species, and n was the total number of individuals. The index values of FBI and HBI were classified into seven categories with different values.

 Table 1. Evaluation of water quality using FBI [8]

FBI values	Water Quality	Degree in Organic Pollution		
0.00-3.75	Excellent	Organic pollution unlikely		
3.76-4.25	Very good	Possible slight organic pollution		
4.26–5.00	Good	Some organic pollution probables		
5.01-5.75	Fair	Fairly substantial pollution likely		
5.76-6.50	Fairly poor	Substantial pollution likely		
6.51-7.25	Poor	Very substantial pollution likely		
7.26–10.00	Very poor	Severe organic pollution likely		

 Table 2. Evaluation of water quality using HBI
 [8]

Biotic Index	Water Quality	Degree in Organic Pollution		
0.00-3.50	Excellent	No apparent pollution		
3.51-4.50	Very good	Possible slight organic pollution		
4.51-5.50	Good	Some organic pollution		
5.51-6.50	Fair	Fairly significant organic pollution		
6.51-7.50	Fairly poor	Significant organic pollution		
7.51-8.50	Poor	Very significant organic pollution		
8.51-10.00	Very poor	Severe organic pollution		

The Average Score Per Taxa (ASPT) index was calculated by the following formula $[\underline{8}]$.

$$ASPT \ Index = \sum \frac{(BMWP \ score \ \times n)}{\sum n}$$

Where BMWP score was the Biological Monitoring Working Party, n was the total number of individuals. The index values for ASPT were classified into four categories, (>6: clean water, 5–6 doubtful water, 4–5 probable moderate pollution, <4 probable severe pollutions) [4].

RESULTS AND DISCUSSION

Based on the measurement of physicochemical parameters (water temperature, pH, DO, TSS, nitrate, and orthophosphate levels) from Genjong River, the water quality was categorized as the third class of water quality standards based on Indonesian government regulation (Table 3). However, the BOD of Genjong River water meets the fourth-class water quality standard. Overall, Genjong River is included in the fourth-class/ fourth category according to Indonesia government regulation No. 22 Year 2021. The physicochemical quality of water from upstream to downstream was getting worse, as indicated by the decreasing value of DO and BOD as well as increasing TSS, nitrate and orthophosphate levels which could still be used for crop irrigation.

The substrate composition of each station (Figure 2) consisted of rock, sand, and mud. The first station has the highest percentage of rock. The second station has the highest percentage of sand. While the mud at the third and fourth stations. The highest percentage of mud is at the last station. The substrate will affect the presence of macroinvertebrates species. The substrate was associated with changes in water temperature and flow conditions [9].

The cooler streams were generally dominated by sand and rock and had more variable flow and occasional high flow, which could remove the fine sediment from the stream. The warmer stream has stable to moderate flow conditions that allow sedimentation of fine particle accumulation. It was also dominated by mud or other fine substrates [9]. The rocky substrate was mostly inhabited by arthropods, while the sand and mud were mostly inhabited by annelids and mollusks [10].



Figure 2. Substrate composition of each station

Physicochemical	Station 1	Station 2	Station 3	Station 4	Quality standard**	
lactors					3 rd class	4 th class
Water temperature (°C)	17.67 ± 0.46^a	22.07 ± 0.42^{a}	21.67 ± 0.58^a	23.67 ± 0.58^a	Dev 3	Dev 3
Air temperature (°C)*	19.13 ± 1.53^{a}	25.20 ± 0.87^{b}	25.33 ± 3.21^{b}	24.67 ± 0.58^{c}	-	-
Flow velocity (m.s ⁻¹)	$0.75\pm0.16^{\rm a}$	0.59 ± 0.07^{a}	$0.72\pm0.11^{\text{a}}$	0.61 ± 0.26^{a}	-	-
Water discharge (dm ³ . s ⁻¹)	496.75 ± 82.29^{ab}	928.40 ± 240.35^{b}	958.66 ± 274.02^{b}	343.84 ± 179.97^{a}	-	-
Conductivity (mS.m ⁻¹)	$8.34\pm3.15^{\mathrm{a}}$	9.54 ± 3.89^{a}	$9.19\pm3.19^{\mathrm{a}}$	10.89 ± 3.67^a	-	-
pH*	7.60 ± 0.23^{a}	$7.86\pm0.09^{\rm a}$	7.81 ± 0.02^{a}	7.72 ± 0.06^{a}	6-9	6 – 9
DO (mg.L ⁻¹)	4.96 ± 0.18^{bc}	$5.29 \pm 0.12^{\circ}$	4.79 ± 0.15^{ab}	$4.49\pm0.06^{\mathrm{a}}$	min. 4	min. 3
BOD (mg.L ⁻¹)	3.61 ± 0.77^a	6.19 ± 0.51^{b}	5.61 ± 1.51^{ab}	7.72 ± 0.52^{b}	6	12
TSS (mg. L^{-1})	1.95 ± 0.22^{a}	2.51 ± 0.12^{bc}	2.19 ± 0.10^{ab}	$2.82\pm0.00^{\circ}$	100	400
Nitrate (mg.L ⁻¹)	0.52 ± 0.07^a	0.71 ± 0.09^{ab}	0.74 ± 0.09^{b}	0.85 ± 0.07^{b}	20	20
Orthophosphate (mg.L ⁻¹)*	0.02 ± 0.01^{a}	0.04 ± 0.01^{a}	0.09 ± 0.01^{b}	$0.18\pm0.02^{\rm c}$	-	-

Table 3. Water physicochemical profile of Genjong River

Desc: Different notations for each parameter indicated a significant difference between locations based on the One-way ANOVA test followed by Tukey HSD with Sig. 0.05, *difference test based on Brown Forsythe followed by Howell Games with Sig. 0.05, ** based on Indonesia government regulation No. 22 Year 2021.

Based on benthic macroinvertebrates found in Genjong River (Figure 3), the fourth station had the least abundance and taxa richness (family). It was caused by the fourth station located downstream of the river, where a place of the pollutants accumulated. It was also indicated by a low DO value, and it was related to organic pollution [11]. Low dissolved oxygen levels affected benthic macroinvertebrate assemblages as it depended on oxygen availability. Also, the high concentrations of nitrate and orthophosphate might indicate eutrophication of the water body [12].

The first station also had low abundance and taxa richness due to the low levels of nutrient input. It was indicated by the lowest levels of nitrate and orthophosphate among all stations. The low level of nitrate and orthophosphate also indicated low primary productivity and biomass (Figure 3). The increasing value of DO was influenced by increasing water depth, which caused decreasing in water temperature [13]. The first station was also located near the spring, so based on the information provided, the first station was classified as oligotrophic waters [13, 14].



Figure 3. The abundance and taxa richness of benthic macroinvertebrate each station

A total of 18 benthic families were found in all stations. Based on the IVI calculation (Figure 4), all stations were dominated by Hydropsychidae. Hydropsychidae have a wide range of tolerance to organic contamination based on their species. However, the tolerance range usually varies based on the longitudinal distribution due to the combined effect of several abiotic, biotic, and geographical factors. The crucial role that formed the wide range of Hydropsychidae distribution is the annual temperature range, flow velocity, and the size of suspended food material. Based on the each habits of species, the upstream Hydropsychidae had a shorter tolerance range than downstream Hydropsychidae [15].

Based on the calculation of the Shannon-Wiener diversity index (H') (Figure 5), it indicated a change in water quality from station 1 to station 4 due to toxic pollution. Station 1 was lightly polluted with toxic materials because it was close to tourist attractions. Stations 2, 3, and 4 were moderately polluted with toxic materials because there were residential areas and plantations along the river.



Figure 4. The Importance Value Index (IVI) of each benthic macroinvertebrate family found in each station



Figure 5. Shannon-Wiener diversity index (H') for each station. Description: — classification of H'

Tourism activities led to an increase in garbage which increased water pollution [16]. The residential areas and plantations allowed the entry of pollutants such as detergents and pesticides into water body [17]. Also, a big-scale farm located between stations 2 and 3 could make different

pollution levels. Based on the analyses of Simpson diversity index (D) (Figure 6), stations 1 and 4 indicated moderate pollution, while stations 2 and 3 were waters with severe pollution. The calculation of D showed little value to rare taxa. Stations 2 and 3 had more taxa that did not show up at stations 1 and 4. That was why the D value at Stations 2 and 3 was higher than others [18]. Based on the calculation of Margalef diversity index (dMg) (Figure 6), all of the stations had low dMg values (<3.5). Margalef diversity index was measured taxa richness and highly sensitive to the sample. The value of dMg was influenced by the taxa richness found, in which the greater sampling effort, the more diverse benthic got, so the higher the Margalef index value [19, 20].



Figure 6. Simpson diversity index (D) and Margalef diversity index (dMg) of benthic macroinvertebrates in each station. Description: — classification of D, — classification of dMg

Based on the calculation of evenness (E) (Figure 7), Stations 1 and 4 were stations with moderate uniformity (0.4 - 0.6). Stations 2 and 3 were stations with low uniformity (<0.4). The value of E was related to taxa richness. If the value was high, the benthic macroinvertebrates were evenly distributed in the waters [20].

The calculation of the Simpson dominance index (Id) (Figure 7) showed that partial dominance occurred at Stations 1 and 4. While Stations 2 and 3 had moderate partial dominance. The value of Id was influenced by the diversity of benthic macroinvertebrates, where the low diversity indicated the high dominance that occurred [21].



Figure 7. Evenness (E) and Simpson dominance index (Id) of benthic macroinvertebrates in each station.

Description: — classification of E; — classification of Id

Based on the calculation of the HBI value (Figure 8) showed the level of organic matter pollution. Stations 1 and 2 were classified as very good water with some organic pollution probable. While stations 3 and 4 were classified as good water with some organic pollution based on HBI and indicated the presence of Tubificidae. Tubificidae had high pollutant tolerance values (8–10) [14]. The low HBI value was obtained from the low tolerance score of dominated benthic macroinvertebrates, which were intolerant to organic matter contamination at stations 1 and 2.





Description: — classification of water quality based on HBI values.

Based on the calculation of FBI values, stations 1, 2, and 3 were classified as good quality waters (4.26–5.00) with probable organic pollution (Figure 9). Station 4 was classified as fair waters with substantial organic pollution likely. This was influenced by more families that were tolerant of organic pollutants. In addition, Station 4 was downstream of the river, so it was affected by pollution along the stream, such as domestic waste and livestock waste directly discharged into the river from settlements around the river [14].



Figure 9. The FBI value and classification water quality of each station.

Description: — classification of water quality based on FBI values.

Based on the calculation of ASPT, it could be seen that there was a degradation in water quality (Figure 10). Stations 1, 2, and 3 were classified as waters with probable moderate pollution by organic matter. Station 4 was classified as probable severe polluted waters. This was indicated by the presence of several benthic macroinvertebrate families found with low BMWP values (Figure 6) [8], such as Tubificidae, Hirudinidae, and Lymnaeidae. The low BMWP value indicated a high level of tolerance to pollutants [8].



Figure 10. The ASPT value and classification of water quality in each station.

Description: — classification of water quality based on ASPT values

The result of the biplot analysis showed that there was a water quality shift at each station (Figure 11). Station 1 was characterized by a high abundance of Limnephilidae. Stations 2 and 3 were characterized by high ASPT values and an abundance of Hydropsychidae. While Station 4 was characterized by high BOD, nitrate levels, orthophosphate levels, and a high abundance of Hirudinidae.

The three families had the potential to be specific bioindicators for the water quality of the Genjong River. Limnephilidae was Trichoptera which indicates good water quality. Hydropsychidae also indicates good water quality with a wide distribution. Both of them were sensitive to metal pollution and insecticides [22]. While Hirudinidae could live in different trophic levels, they usually prefer a polluted organic environment [17].

According to the correlation test using biplot analysis through PCA, Hydropsychidae correlated significantly with almost all the families found because it had a wide tolerance range [21]. Hirudinidae did not correlate with Limbephilidae and Planariidae because Hirudinidae was quite tolerant of organic pollution. Limnephilidae and Planariidae were bioindicators of clean waters, although their abundance was still influenced by abiotic environmental factors [17, 22, 23, 24].

biotic indices Several had different classification bases. The water evaluation results showed water quality degradation from stations 1 to 4 (Table 4). Based on several physicochemical parameters and biotic indices, it was found that Station 1 was a station with poor nutrition, lightly polluted by toxic materials, and moderately polluted by organic matter. Stations 2 and 3 were stations with moderate pollution by toxic and organic materials. Station 4 was a station with moderate toxic contamination and heavy pollution by organic matter.



Component 1 (55.45%)

Figure 11. The correlation between water quality and benthic macroinvertebrates community structure at each station was based on biplot analysis using PCA

Table 4. Resume of Genjong River water quality based on physicochemical parameters and biotic index

Biotic indices	Station 1	Station 2	Station 3	Station 4
ShannonWienerdiversity index (H')	Contaminated with light toxic materials	Moderately polluted with toxic materials	Moderately polluted with toxic materials	Moderately polluted with toxic materials
HBI	Very good (possible slight organic pollution)	Very good (possible slight organic pollution)	Good (some organic pollution)	Good (some organic pollution)
FBI	Good (some organic pollution probable)	Good (some organic pollution probable)	Good (some organic pollution probable)	Fair (substantial pollution likely)
ASPT	Probable moderate pollution	Probable moderate pollution	Probable moderate pollution	Probable severe pollution
Conclusion	Contaminated with light toxic materials and slight organic pollution	Moderately pollutes with toxic material and organic pollution	Moderately pollutes with toxic material and organic pollution	Moderately pollutes with toxic material and probable severe organic pollution

CONCLUSION

Based on the study results, it was concluded that the physicochemical quality profile of the Genjong River water showed degradation of water quality in the downstream area, which was indicated by an increase in nitrate. orthophosphate, BOD, TSS, and conductivity levels. The results of benthic macroinvertebrates identification showed the degradation of water quality in downstream parts reflected by the increasing abundance of Lymnephilidae, Hydropsychidae, and Hirudinidae. Calculation of the biotic index showed that station 1 was contaminated with light toxic materials and slight organic pollution. Stations 2 and 3 were moderately polluted by toxic and organic pollutants. Station 4 was moderately polluted by toxic material and probable severe contaminated by organic pollution.

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