PHYTOREMEDIATION OF ABANDONED MINING LAKE BY WATER HYACINTH AND WATER LETTUCES IN CONSTRUCTED WETLANDS

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Graphical abstract

Propagation of Aquatic Plants

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Constructed Wetland Setup

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Effluent Charaterization – Day 0th

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Plant Phsyical Observation: Day 0th & Day 28th

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Weekly Effluent Evaluation in 28 Days
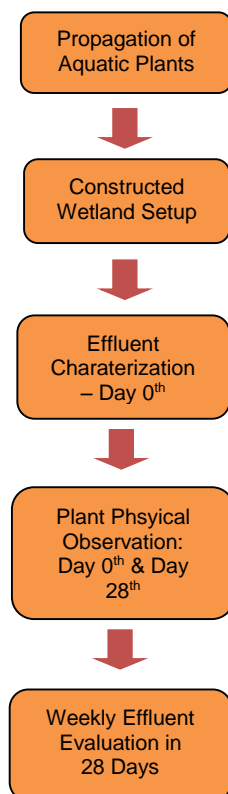
Abstract

Tasik Puteri is a recreational lake for few activities such as scuba diving, kayaking and swimming during the dry season. However, this lake was an iron ore mining site with the remaining contaminants and heavy metal of the lake can harm the people who directly get into contact with the lake water. The present study focused on investigating the phytoremediation potential of locally available floating aquatic plants in the treatment process of water from Tasik Puteri, which was contaminated with mining effluent. The effluent was treated with water hyacinth (*Eichhornia crassipes*) and water lettuces (*Salvinia molesta* and *Pistia stratiotes*) in a constructed wetland for a period of 28 days. The effluent treatment efficiency was determined by measuring the effluent quality over the experimental period. Five water parameters such as the total iron (TI), total phosphorus (TP), chemical oxygen demand (COD), electrical conductivity (EC) and turbidity were examined using standard laboratory procedures. The results indicated that the three plants were able to remove the contaminants. After 28 days, the physical observation shows that the water hyacinth *was* healthier than the water lettuces. Considerable decrements in concentration were recorded in TI, TP and EC, but fluctuation in COD and turbidity value were observed. Comparison results by the plants indicated that after 28 days, water hyacinth was the most effective plant in removing phosporus, COD and EC of the mining lake with 97.3%, 70.5% and 22.2% removal, respectively. Furthermore, water lettuce (*P. stratiotes*) was the most effective plant in removing Iron (96.0%) and the turbidity (50.0%) of the mining lake. *S. molesta* showed the lowest removal capability for all experimental parameters. In conclusion, water hyacinth and water lettuces had shown better capability in removing heavy metals and other contaminants with *E. crassipes* has the highest survivability in the lake water.

Keywords: Phytoremediation, rhizofiltration, abandoned mining lake, heavy metal, floating plants

Abstrak

Tasik Puteri merupakan sebuah tasik rekreasi untuk beberapa aktiviti seperti menyelam, berkayak dan berenang ketika musim kering. Walau bagaimanapun, tasik ini yang merupakan bekas tapak perlombongan bijih besi yang mana sisa bahan pencemar dan logam berat dari tasik ini boleh membahayakan orang yang terdedah secara langsung dengan air tasik ini. Kajian ini tertumpu kepada penyelidikan keupayaan fitorimediasi oleh tumbuhan akuatik terapung yang diperoleh di kawasan tempatan dalam proses rawatan air daripada Tasik Puteri, yang telah tercemar dengan efluen perlombongan. Efluen tersebut telah dirawat dengan keladi bunting (*Eichhornia crassipes*) dan kiambang (*Salvinia molesta* dan *Pistia stratiotes*) dalam pembinaan tanah bencah untuk jangka masa 28 hari. Prestasi rawatan efluen tersebut telah ditentukan dengan mengukur kualiti efluen sepanjang jangka masa eksperimen tersebut. Lima parameter air seperti jumlah besi (TI), jumlah fosforus (TP), keperluan oksigen kimia (COD), konduktiviti elektrik (EC) dan kekeruhan telah diperiksa menggunakan prosedur piawai makmal. Keputusan menunjukkan bahawa ketiga-tiga tumbuhan mampu menyingkirkan bahan pencemar tersebut. Selepas 28 hari, pemerhatian fizikal menunjukkan bahawa keladi bunting lebih sihat berbanding kiambang. Penurunan yang ketara direkodkan bagi kepekatan TI, TP and EC, tetapi turun naik bagi nilai COD dan kekeruhan telah diperhatikan. Keputusan perbandingan bagi tumbuhan tersebut menunjukkan bahawa selepas 28 hari, keladi bunting merupakan tumbuhan yang paling berkesan dalam menyingkirkan fosforus, COD dan EC bagi tasik perlombongan tersebut dengan penyingkiran masing-masing sebanyak 97.3%, 70.5% dan 22.2%. Tambahan pula, kiambang (*P. stratiotes*) adalah tumbuhan yang paling berkesan dengan keupayaan menyingkirkan besi (96.0%) dan kekeruhan (50.0%) dari air tasik perlombongan tersebut. *S. molesta* menunjukkan kebolehan penyingkiran yang paling rendah untuk semua parameter eksperimen. Kesimpulannya, keladi bunting dan kiambang telah menunjukkan kebolehan yang lebih baik dalam menyingkirkan logam berat dan bahan pencemar lain dengan *E. crassipes* mempunyai ketahanan yang paling tinggi untuk terus hidup dalam air tasik tersebut.

Kata kunci: Fitorimediasi, penurasan rhizo, tasik perlombongan terbiar, logam berat, tumbuhan terapung

1.0 INTRODUCTION

Bukit Besi is a small ex-mining town that located 85 km from Kuala Terengganu and formerly known as 'Permata Bumi' or literally the "Jewel of the Earth". It was first opened for the iron-ore mining activity in 1916 by the Japanese and closed in 1970s by the British when the metalliferous ore depleted [1]. The abandoned blasted site has formed an open lake when the natural rainwater soon filled it.

Over the years, the lake has turned naturally to such a spectacular and breathtaking place which currently known as Tasik Puteri. With the blue-green clear water and unique underwater terrain, Tasik Puteri has now become a popular recreational place for few water activities such as scuba diving, kayaking and swimming during the dry season [2].

However, due to the remaining contaminants such as heavy metal in Tasik Puteri, long-term activities that directly involve water contact can be carcinogenic, affecting central and peripheral nervous system and circulatory effects to human beings. High concentration of heavy metal may result harmful health effect on humankind, while only several of them are significant to human life [3]. Many conventional methods have been found to be so effective in order to reduce the concentration of heavy metal to its allowable limits or below, as well as to lessen the toxicity effect [4]. The treatment methods on heavy metals-water involved such as reverse-osmosis, ion exchange, electro dialysis, adsorption by activated carbon, chemical precipitation, disinfection as well as nano-filtration [5]. However, these treatments are mostly expensive, labour-intensive, always produce secondary waste or sludge, energy-intensive and metal specific [6]. Thus, phytoremediation should be a cost effective and an environmental friendly approach to treat water [7].

Phytoremediation comes from the Greek word 'phyto', meaning plant, and the word 'remedium', in Latin, meaning balance or remediation. Phytoremediation is defined as the efficiency of biologically and chemically selected plant activities to remove, detoxify or incapacitate environmental

contaminants in soils, sludge, and water [8]. Phytoremediation can thus be applied to the environment to reduce high concentrations of several pollutants, such as organic compounds and metals [9]. Although it is a relatively recent technology, beginning in the 1990's, it is already considered as a green alternative solution to the problem of metal pollution, with great potential, since over 400 plant species have been identified as potential phytoremediators [10].

Constructed wetlands (CWs) is an engineered wetland created for treating pollutants discharge which originating in human activity such as industrial wastewater, and land reclamation after mining or refineries. CWs is a human-made system developed by the combination of soil, plants and microbial to control the operation and efficiency of CWs. Biotic and abiotic purification mechanisms of CWs are according to the following processes; (a) mechanical screening and sedimentation, (b) microbial degradation, (c) biochemical nutrient removal of plant rhizomes, (d) adsorption through ligand exchange, (e) precipitation and chemical fixation of reactive soil ingredients [11].

Plants can be used for phytoremediation via different physiological processes that allow metal tolerance and absorption capacity [12,13]. *Eichhornia crassipes* (water hyacinth) of a *Pontederiaceae* family, a native of South America, is one of the free floating macrophytes found in the aquatic environment such a lakes, pond and ditches. This plant is mostly studied for the purposes of phytoremediation because of its simple cultivability under heavy metal stress and could produce high biomass in aquatic environment without showing much toxic symptoms [14].

Pistia stratiotes (a water lettuce), is well known for its ability to enhance microbial activity, absorb nutrients and remove suspended solids. The ability of this plant to use nutrients from the sewage to elaborate an important phyto mass added to exacerbate any imbalance in nutrient supply has made it one of the most suitable plant to be used in wastewater phytoremediation in tropical areas [15].

Salvinia molesta (another water lettuce), is a species of *Salviniaceae*, an aquatic plant that have three circles of leaves held by a short stalk and oval. It also has a lot of hair that spread with megaspore or microspores. *S. molesta* also lives in paddy filed, canals and ditches. It can be used for the treatment of black water effluent in eco-friendly sewage system [16].

Hence, the listed plants show great activity in absorbing contaminants and heavy metals thus it is essential to study their effectiveness in removal of total iron (TI), total phosphorus (TP), chemical oxygen demand (COD), electrical conductivity (EC) and turbidity [17]. Therefore, the objective of this study is to phytoremediate the iron ore mining contaminated lake which is conducted within 28 days in dry season which considering physical observation and five other water parameters.

2.0 METHODOLOGY

2.1 Description of Research Site

The site was an old iron mines that was filled up with ground water to form a huge lake, Tasik Puteri. It is located at coordinate of 4.7667° N, 103.2000° E in Bukit Besi, Terengganu, Malaysia. The general weather of the area is 30°C with the 74% of relative humidity. The study was performed between January to March 2016, during a dry season. The natural blue-green color of the lake water is expected due to the contribution of residue of iron metal. The water used in the CWs was collected at the waterfall notch area of the mining lake.

2.2 Propagation of Floating Aquatic Plants

Three floating aquatic plants of *E. crassipes, S. molesta,* and *P. stratiotes* were obtained from a local pond in Bukit Besi. The plants were propagated by pulling their roots apart by hand. The old leaves and roots were cut off before replanting in the individual containers filled with Tasik Puteri water. The habitat propagation took about two weeks before the plants were transferred to the CWs.

2.3 Constructed Wetlands Setup

Three CWs chambers, approximately 0.5 m in length, 0.36 m in width and 0.34 m in depth, were set up as shown in Figure 1. Each chamber was filled with approximately 13 cm height of river sand. The sand was obtained from Chemerong Waterfall and they were washed prior to be placed into the wetland. Approximately 40 L of lake effluent was poured into the chambers.
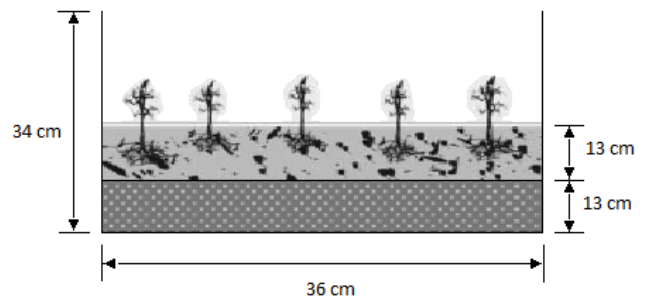

Figure 1 Constructed wetland set up

Floating aquatic plants with mass of approximately 250 g for each species were transferred from the propagated containers to the CW chambers. The CWs were placed in a greenhouse and exposed to the ambient. The percentage removal efficiency was calculated using Equation (1):

$$\text{Removal (\%)} = \frac{\text{Initial reading } (C_o) - \text{Final reading } (C_f)}{\text{Initial reading } (C_o)} \times 100\% \qquad \textbf{Eq. (1)}$$

2.4 Characterization of Lake Water

Three different chambers were used for three replicates of each plant species. The water samples were taken from each chambers on the 0th, 7th, 14th, 21th and 28th days of the experiment. To analyse the concentrations of the contaminants, water samples from the growth medium (100 mL each) from each chamber were collected periodically in clean glass bottles on the sampling days for all treatments. The water sampling was performed based on APHA method (1992) [18].

On the treatment day, to restore the water lost due to evaporation, demineralized water was added to the CW [19]. The parameters analyzed were TI, TP, COD, EC, and turbidity. The TI, TP and turbidity were analysed using HACH procedure (USEPA methods) using DR 900 colorimeter, USA, while COD was analysed using DRB 200 reactor, USA. The EC parameter was determined using conductivity meter equipment, model HQ40d, USA. The reagents used for water analysis were FerroVer Iron Reagent Powder Pillow, PhosVer 3 Phosphate Powder Pillow and COD Digestion Reagent for TI, TP and COD analysis respectively. No reagents involved for turbidity and EC analysis.

2.5 Plant Physical Observation

Plant physical observation was conducted to examine the survivability of the floating aquatic plants in the lake water. The plant physical were visually observed between 0th and 28th days. The observations includes the freshness, color of the plants, and the level of petals survived [20].

3.0 RESULTS AND DISCUSSION

3.1 Characterization of Tasik Puteri Lake Water

Table 1 shows the acceptable conditions of lake water according to the National Water Quality Standards for Malaysia for Class IIA (Recreational use Body Contact) and the characterization of Tasik Puteri effluent at 0th day [21]. For the lake water quality characterization, a few set of data was collected based on three replicates of water samples. The sampling procedure was conducted for three times and the values shown in Table 1 are the average value of the data.

Table 1 Comparison between standard and 0th day value characterization of effluent sample

Parameter	Units	Standard [13]	0th day Tasik Puteri)
Total iron	mg/L	1.0	2.0
Total phosphorous	mg/L	0.2	3.0
Chemical oxygen Demand	mg/L	25	17
Turbidity	NTU	50	6
Electrical conductivity	μs/cm	1000	265

The permissible limit of iron and phosphorous content in lake effluent should be 1 mg/L and 0.2 mg/L, respectively. However, for day 0, the reading of Tasik Puteri water showed that both total iron and total phosphorous content exceeded the water quality standard thus requires further treatment. Otherwise, a long-term exposure to the iron and phosphorous toxicity may cause serious health problem to the lake users. On the other hand, COD, EC and turbidity of Tasik Puteri water showed lower readings compared to the permissible limit hence causing no effect on human being in terms of these parameters.

3.2 Physical Observation

Table 2 shows the differences of physical observation of the three species on day 0 and day 28. The three species were all healthy on day 0. However, *P. stratiotes* showed rapid reaction with the lake water and turned yellowish at the end corner of their leaves after a few days. On day 28, the quantity of live *E. crassipes* decreased due to dead plants but compared to the other two species, it showed better survival level. *S. molesta* and *P. stratiotes* withered; (the leaves turned to yellowish) and were mostly died at day 28. The plants died due to contaminant toxicity either in the roots or upper part of the plants which hinder the plants to grow [22].

Table 2 Plant physical observation

Day	*E. crassipes*	*S. molesta*	*P. Stratiotes*
0	All plants were healthy	All plants were healthy	All plants were healthy
28	Plants were healthy, a few were died	Plants were withered, several were died	Plants were withered, many were died

3.3 Total Iron

The concentration of TI during the 28-day exposure showing a reduction trend in TI concentration over the period of experiment. Based the data collected, the concentration of iron reduced from 2.00 mg/L to 0.19 mg/L, 0.22 mg/L, and 0.08 mg/L for *E. crassipes, S. molesta* and *P. stratiotes,* respectively. Figure 2 represents the percentage removal of TI with the

highest removal percentage was achieved by *P. stratiotes* with 96.0% followed by *E. crassipes* and *S. molesta* with 90.5% and 89.0% removal, respectively.

It was known that, two stages are involved in the removal of heavy metals by *P. stratiotes*. The first stage involved process like adsorption, chelation, and ion exchange. Second stage involves heavy metal precipitation induced by root of Pistia [23] to rhizofiltration. They are natural hyper accumulators of many heavy and toxic metals [24].

On the other hand, *E. crassipes* plant uses their root to absorb water and also the contaminants in the water. The presence of carboxyl groups at the roots system had induced a significant cation exchange through cell membrane and this might be the mechanism of moving heavy metal in the roots system where active absorption takes place [25].
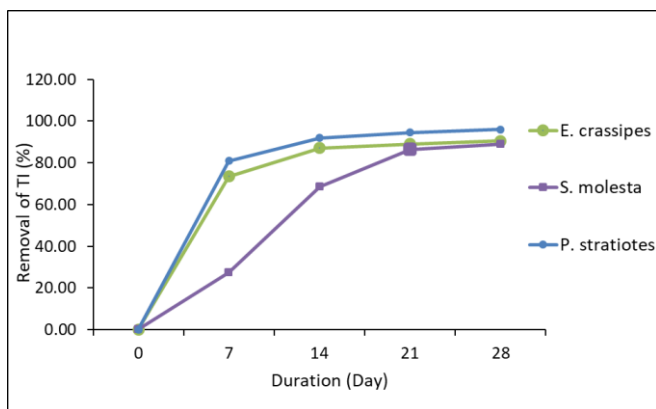

Figure 2 Percentage removal of TI

3.4 Total Phosphorus

The initial and final concentrations of TP indicates that the removal of TP occurred. The reduction of TP concentrations were from 3.00 mg/L to 0.08 mg/L, 0.12 mg/L and 0.10 mg/L for *E. crassipes*, *S. molesta* and for *P. stratiotes*, respectively. Figure 3 represents the percentage removal of TP with the highest removal percentage achieved by *E. crassipes* with 97.3% followed by *P. stratiotes* and S. molesta of 96.7% and 96.0% removal, respectively. The effectiveness of wetlands in removing the TP might be influenced by the adsorptivity of the substrate which placed in the wetlands [26].
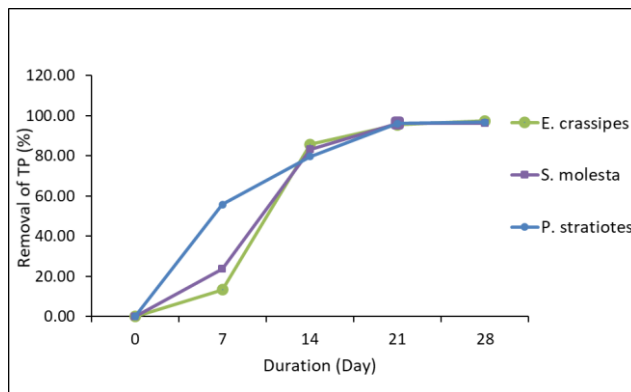

Figure 3 Percentage removal of TP

3.5 Chemical Oxygen Demand

For the parameter of COD, it shows a fluctuated profile. The COD concentration was 17.00 mg/L on the day 0. Figure 4 represents the highest COD removal percentage was achieved by *E. crassipes* with 70.6% followed by *S. molesta* and *P. stratiotes* of 58.8% and 52.9% removal, respectively.

Temperature gave a significant influence to the COD removal efficiency [27]. In this study, the CWs were placed in an exposed area and the environment temperature was fluctuated whereby inconsistent temperature value were recorded throughout the sampling days. The increasing temperature is believed can enhance the adsorption capacity. Greater temperature might increases the kinetic energy of the adsorbent, which caused the pores to expand. The expansion increased the adsorbent active site thus increasing the effeciency of COD removal. Besides, available adsorption of contaminates depends on large number of pores and rough structures of the plants root [28].
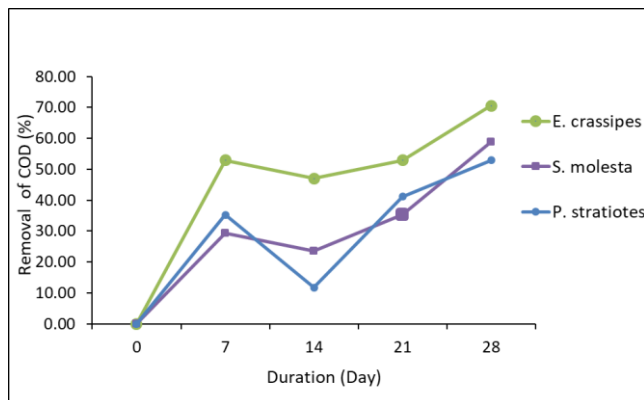

Figure 4 Percentage removal of COD

3.6 Electrical Conductivity

The initial value of EC was 265.0 μS/cm on 0th day. The final EC value for *E. crassipes* was 206.2 μS/cm. It showed the highest removal efficiency which 22.2% removal as in Figure 5. The least reduction in EC was by *S. molesta* with final EC value was 210.3 μS/cm. The decreasing value of EC was due to the salt removal through absorption process by plant, causing the decreasing value of EC using water lettuce [26]. Normally, the existence of inorganic dissolved solids such as chloride, nitrate, sulfate, and phosphate anions or sodium, magnesium, calcium, iron, and aluminum cations will effect the conductivity of water.
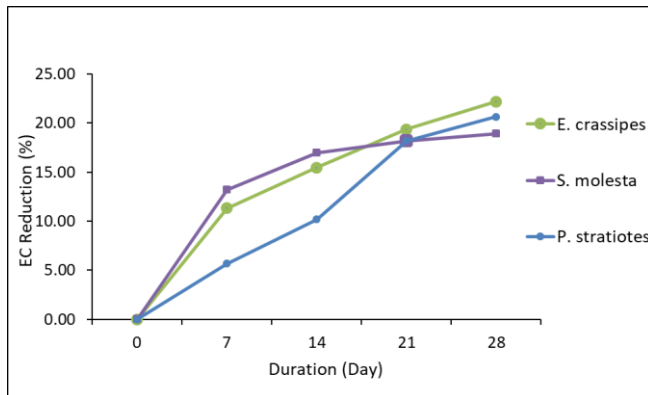

Figure 5 Percentage reduction of EC

Temperature also contibute to substantial effect in water conductivity where the warmer the water, the higher the conductivity [29]. For this reason, a conductivity measurement was reported at 30°C. However, from the previous research, reduction of EC was related to the reduction of total iron in water sample during the process [30] illustrating that the three species of aquatic plants contributed significantly to the removal of iron.

3.7 Turbidity

The CWs in the experiment were batch system, therefore fluctuated trend of removal efficiency was observed due to the quantity of dead plant in the CW. The most efficient turbidity removal was by *P. Stratiotes,* with a reduction from 6 NTU to 3 NTU resulting in 50.00% reduction as shown in Figure 6. Turbidity concentration depends on the concentration of total suspended solid (TSS) value. However the reduction of turbidity depends on the longer the retention time of the process. The increasing turbidity concentration may due to fragmentation of root by plant [22].
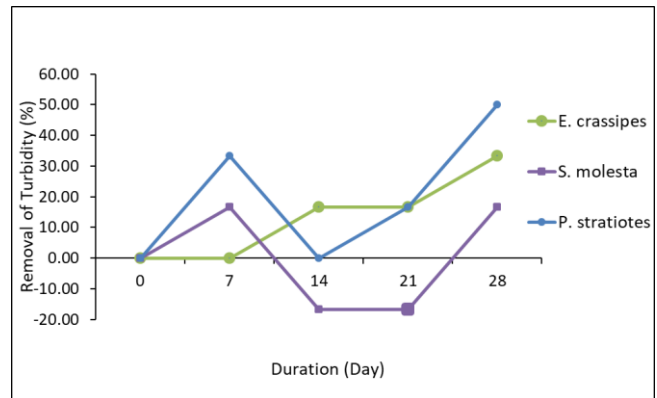

Figure 6 Percentage removal of turbidity

4.0 CONCLUSIONS

The study purposely aims to study the efficiency of aquatic plants to remediate Tasik Puteri water. After 28 die, *E. crassipes* survived well in the lake water, but *P. stratiotes* and *S. molesta* turned yellowish and prone to died. *E. crassipes* was the most efficient phytoremediator to reduce the concentration of TP, COD and EC with 97.3%, 70.5% and 22.2%, respectively. Meanwhile, *P. stratiotes* exhibits as the most efficient removal for TI and TSS with 96% and 50%, respectively. Although *S. molesta* shown the lowest efficiency removal for all parameters, it was still able to reduce contaminants in the lake. Among all, *E. crassipes* exhibits the highest survivability and adaptability in lake water of iron ore mining site in Tasik Puteri.

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