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GROWTH RESPONSE OF PHYTOPLANKTON TO DIFFERENT SOIL EXTRACTS FOR CHOOSING SUITABLE SOIL TYPES FOR POND AQUACULTURE

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Abstract

For choosing suitable soil types for aquaculture pond construction, the density, biomass, and dominant species of phytoplankton were used as dependent variables to test the effect of acid yellow soil, neutral purple soil, and alkaline purple soil of Sichuan Basin, China. The results showed that the acid yellow soil extracts had a low nutrient content and low primary productivity. The neutral purple soil extracts had a low content of major elements, but a high content of microelements. The alkaline purple soil extracts had relatively high nutrient contents and promoted the growth of phytoplankton which enhanced the grazing of zooplankton and planktivorous fish. These results indicate that the alkaline purple soil is better for aquaculture pond construction than the acid yellow soil and neutral purple soil. This work provides an evaluation method for selecting pond bases for aquaculture, and will benefit further research to explore the fishery suitability of soils and developing aquaculture according to the local soil conditions.

Introduction

In China, pond is the major containment for freshwater fish aquaculture (Bureau of Fishery 2009-2014, Cao *et al*. 2007). In 2012, the pond aquaculture area in China accounted for 43.5% of the total freshwater aquaculture areas (Bureau of Fishery 2013). Despite the substantial contribution to aquaculture production, the criteria of site selection for pond culture are largely based on empirical knowledge and administrative decision. The ponds constructed in nutrient deficient soils can cause low production of fishes, especially for the planktivorous fishes, because the later require suitable quantity and quality of phytoplankton species for grazing.

In Sichuan Basin, southwest China, pond aquaculture plays an important role in freshwater aquaculture. In this region, the pond aquaculture area accounted for more than 53% of total aquaculture area in the last five years. And the pond aquaculture yield accounted for more than 54% of total aquaculture yield (Bureau of Fishery 2009-2014). However, the yields of aquaculture ponds varied considerably with the same method and technology. Pond soils may be the impact factor. Sichuan Basin is popularly known as Purple Soil Basin and according to the fertility characters, soils of that area can be divided into 10 different types. Some of those are: Alluvial-, purple-, yellow-, red-, mountain brown- and alpine frozen desert soils.

Purple soil is the most widespread of all soil types in Sichuan Basin. Certainly, yellow soil and red soil are also common at lower elevations. Acid yellow soil, neutral purple soil, and alkaline purple soil are three of the main soil types in this region. Their alternating distribution

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may affect the output of the aquaculture ponds with different pond bottom soils. Because pond bottom soils are the storehouse for many nutrients and metabolites in pond ecosystems, and the chemical and biological processes occurring in surface layers of pond soils influence the water quality and primary productivity (Boyd 1995, Aynimelech and Ritvo 2003). In addition, studies have shown that the biomass of fish community was closely related to the primary productivity of the water body (Gascon and Leggett 1977, Hanson and Leggett 1982, Leach *et al*. 1987). Therefore, the present study has been aimed at investigating the effect of nutrient composition of different categories of soils on the growth of pond phytoplankton.

Materials and Methods

The soils used in the present investigation together with the pH, area of collection and GPS have been provided in Table 1.

Table 1. Description of the soil samples used in the present investigation.

Sample No.	Type	pH	Area of collection	GPS
S_1	Acid yellow	5.84	Jinyun Mountain, Chongqing	N 29°48′45″, E106°22′49″
S_2	Neutral purple	7.06	Purple soil experimental base of the Southwest University	N29°48′32″, E106°24′33″
S_3	Alkaline purple	8.30	Tongnan, Chongqing	N30°6′19″, E105°48′48″

In each sampling station, 20 kg soils were collected from 5 - 8 randomly selected quadrate points. After bringing the samples in the laboratory, they were spread on a plain thin layer of plastic sheet and air-dried at room temperature (22-28°C). After non-soil components such as stones and plant residues had been removed via manual screening, the samples were ground and filtered using a 2 mm aperture sieve. The samples were sealed and stored for further use (Carter 1993).

During laboratory analyses 200 g of sieved sample was placed in a 1000 ml beaker (Shuniu, Chengdu, China) and soil solution was made by adding deionized water at a ratio of soil: water = 1 : 5 (m/v). The solution was stirred at 100 rpm with a magnetic stirrer (AS ONE REXIM RS-4D, Osaka, Japan) for 10 min until there was no precipitation. Then the solution was steeped in the dark for 24 hrs and filtered using a 0.22 µm filter membrane (Millipore, Massachusetts, USA). The supernatant was collected, and the volume was adjusted to 1000 ml. The samples were separated into 250 ml culture flasks (SHUNIU, Chengdu, China), and the mouth was sealed with a membrane. After being sterilized at 121°C for 20 min, the samples were stored at 4°C in a refrigerator for future experimental use (Hesse 1971). The three types of soil extracts were labeled SE_1, SE_2, and SE_3, corresponding to the acid yellow soil, neutral purple soil, and alkaline purple soil.

The pH values were measured using a pH meter (AZ 8651, Taiwan, China). The concentration of total nitrogen (TN) was determined using a UV spectrometer (PERSEE, T6, Beijing, China) after digestion by alkaline potassium persulfate (SEPA, 2002). The concentration of total phosphorus (TP) was measured using the phosphomolybdate blue method after digestion by acidic potassium persulfate (SEPA 2002). The concentration of boron (B) was determined using the curcumin colorimetric method (SEPA 2002). The concentration of sulfate (SO_4^{2-}) was measured using barium sulfate turbidimetry (SEPA 2002). The concentrations of potassium (K), calcium (Ca), magnesium (Mg), cupper (Cu), zinc (Zn), iron (Fe), and manganese (Mn) were determined using atomic absorption spectrometry (Friese and Krivan 1995, Ren *et al*. 2008, Batool *et al*. 2015).

Fresh phytoplankton used in this study were collected from the aquaculture ponds at Southwest University. Concentrated samples of phytoplankton were collected by sieving 20 l of pond water through a plankton net (mesh width 0.064 mm, PURITY Instrument, Beijing, China). The concentrated samples of phytoplankton thus were collected at 5000 rpm (CENCE L550, Changsha, China). The supernatant was washed twice with 15 ppm sodium bicarbonate, and the step was repeated 4 - 6 times. Once there was no zooplankton in the supernatant under microscopic examination, the samples were suspended in 20 ml of distilled water and then used in the experiment.

The collected pond phytoplankton sample was inoculated into culture flasks containing 100 ml of soil extracts. And the cell density for all experiments was 2.25×10^3 cells/ml. The samples were cultured in a light incubator (Ningbo Southeast Instrument Co. Ltd. GXZ380B, Ningbo, China) at 25 ± 0.5°C under a light: dark cycle of 12:12 hrs. The light intensity of the incubator was 2000 lux. Three treatments (SE_1, SE_2 and SE_3) and one control were set for the experiments. And all experiments and control were carried out in three replicates.

During the culture stage, the quality and quantity of phytoplankton in each category of soil extracts were monitored at 1, 2, 3, and 4 weeks of the growth. For this, 1 ml of algal suspension was sampled from the culture and fixed immediately using Lugol's solution. A quantity of 0.1 ml was observed using a microscope (Leica DM500, Germany) at a magnification of 400×. Duplicate counts were made for each sample. The results were considered effective when the difference between each counting result and the average values of the two counting results was within 15%. The phytoplankton were identified after Hu and Wei (2006) and Zhou and Chen (2011).

The biomass of phytoplankton was calculated after Li *et al*. (2008). But the species, those having without average weight was converted directly using the volumetric method (10^9 μm^3 = 1 mg) to calculate the average wet weight.

The software SPSS 17.0 for Window was used for statistical analysis. One-way analysis of variance (one-way ANOVA) with Duncan's multiple comparison tests was applied to test differences in each nutrient, in the cell densities, and in the biomass of different soil extract. A significance level of 0.05% was used in the analysis. Figures were drawn with MS Excel 2010.

Results and Discussion

Data of Table 2 indicate that all of the soil extracts were slightly alkaline in nature. Moreover, the differences in pH of the three types of soil extracts (SE_1, SE_2, and SE_3) were not significant $p < 0.05$) and showed slightly alkaline in nature (Table 2). Sanders (1983) reported that the pH value of a water body affects the charged status of colloid in water to cause the adsorption or release of ions, thus influencing the effectiveness and concentration of nutrient elements. Kozlowski (1984) reported that during flooding conditions, the pH value of the soil comes closer to neutral. The pH value of the soil extracts after 24 hrs was approximately 6.05 - 7.37. After filtering by a 0.22 μm filter membrane, particulate nutrient substances in the extract were removed so that the original acid-base balance might be destroyed (Ponnamperuma 1972). Therefore, the pH values of the extracts were all approximately 7.5.

For the three types of soil extracts, the total concentrations of major elements were SE_3 (29.03 mg/l) > SE_1 (22.6 mg/l) > SE_2 (13.26 mg/l). The total effective concentrations of microelements were SE_2 (0.66 mg/l) > SE_3 (0.43 mg/l) > SE_1 (0.16 mg/l).

The concentrations of the major element nutrients, including TN, TP, K, Ca, Mg and SO_4^{2-}, had significant differences in three types of soil extracts ($p < 0.05$). SE_1 had the lowest TN concentration; SE_2 had the highest TN concentration, which was 1.5 times that in SE_1. The TP concentration in SE_3 was higher than that in SE_2 and was much higher than that in SE_1 (the concentration of TP in SE_3 was approximately 20 times that in SE_1). The K concentration was SE_3 > SE_1 > SE_2. The pattern of Ca concentration was consistent with that of the TP concentration in the soil extracts: SE_1 had the lowest Ca concentration at 0.40 ± 0.01 mg/l; SE_3 had the highest concentration at 2.67 ± 0.12 mg/l, which was six to seven times that in SE_1. The pattern of Mg concentration was also the same as that of the Ca concentration, which was SE_3 > SE_2 > SE_1. The concentration in SE_3 (0.73 ± 0.05 mg/l) was approximately 10 times that in SE_1 (0.06 ± 0.01 mg/l). The pattern of changes in the SO_4^{2-} concentration was consistent with that of the K concentration.

N, P, and K, which are necessary major elements for plant growth, have important influences on the synthesis of protein and lipid in phytoplankton, thus affecting the growth and reproduction of phytoplankton (Higinbotham *et al*. 1964, Kilham *et al*. 1997, Lai *et al*. 2011). Studies have demonstrated that TN and TP in SE_1 were significantly lower than those in SE_2 and SE_3 ($p < 0.05$). The TP concentrations in SE_2 and SE_3 were 20 and 350 times that in SE_1, respectively. This phenomenon not only was associated with the nutrient composition of the soil itself but also might have been caused by the different dissolution rates of soil nutrients in the extracts (unpublished data). Overall, the S_2 and S_3 soils had better TN and TP supply ability for ponds. Ca, Mg, and S are also necessary elements for plant growth. Ca is an important component of the cell wall and plays an important role in protein synthesis, cell permeability, and the absorption and transformation of carbohydrates, nitrogen, and phosphorus. Mg is a component of chlorophyll and plays an important role in glucose metabolism, nitrogen metabolism, and calcium absorption. S is an indispensable component of proteins and enzymes and combines with organic matter to participate in the oxidation-reduction process in the cells of organisms (Ewald and Schlee 1983). Therefore, the concentrations of Ca, Mg, and S in soil extracts will influence the growth and reproduction of phytoplankton. SE_3 had the highest concentrations of Ca, Mg, and S among the three different types of soil extracts. This result indicates that S_3 soil had a stronger ability than S_2 and S_1 to supply the Ca, Mg, and S required for algal growth. That is, the alkaline purple soil had a stronger ability than neutral purple soil and acid yellow soil to supply Ca, Ma and S, which is conducive to the algal growth.

As for the microelements, the effective Fe concentrations among the three types of soil extracts were not significantly different ($p > 0.05$). SE_2 had the highest Mn concentration while the concentrations of SE_1 and SE_3 were not significantly different ($p > 0.05$). Cu was not detected in SE_1 while the Cu concentrations of SE_2 and SE_3 were not significantly different ($p > 0.05$). The patterns of changes in Zn and B concentrations were the same, with the highest concentration in SE_2 and the lowest in SE_1.

Fe, Mn, Cu, Zn and B, which are microelements necessary for algal growth, influence the formation and function of chloroplasts and the photosynthesis and respiration of cells; furthermore, these microelements participate in enzymatic reactions that mediate biological activities of cells (Fisher 1986, Knauer *et al*. 1998, Danilov and Ekelund 2001, Gobler *et al*. 2007, Nguyen-Deroche *et al*. 2012). In this study, SE_1 had lower concentrations of microelements. SE_2 had the highest concentrations of Mn, Zn, and B. SE_3 had the highest concentrations of Fe and Cu. However, the Zn concentration in SE_2 reached 0.25 mg/l, which may have an inhibitory effect on the growth of some phytoplankton (Knauer *et al.* 1998, Danilov and Ekelund 2001, Hörnström 2002). The concentrations of microelements in the soil extracts were associated not only with the background concentrations of soil nutrients but also with the acidity of soils. The dissolution rate of the microelements revealed that the microelement dissolution rate in alkaline purple soil was higher (unpublished data). Therefore, the construction of ponds based on alkaline purple soils was conducive to the increase in the primary productivity of the water body.

In summary, for the three types of soil extracts, the nutrients in SE_1 were all lower, SE_3 had higher concentrations of major element nutrients, and SE_2 had higher concentrations of microelements.

Table 2. Physicochemical properties of the soil extracts used in the present investigation.

Measurements	Samples		
	SE_1	SE_2	SE_3
pH	7.52 ± 0.06^{a}	7.55 ± 0.01^{a}	7.37 ± 0.10^{a}
Major elements (mg/l)			
TN	1.46 ± 0.02^{a}	2.23 ± 0.08^{c}	1.98 ± 0.04^{b}
TP	0.004 ± 0.001^{a}	0.08 ± 0.01^{b}	0.14 ± 0.01^{c}
K	1.2 ± 0.1^{b}	0.6 ± 0.1^{a}	1.6 ± 0.1^{c}
Ca	0.40 ± 0.01^{a}	1.13 ± 0.06^{b}	2.67 ± 0.12^{c}
Mg	0.06 ± 0.01^{a}	0.37 ± 0.02^{b}	0.73 ± 0.05^{c}
SO_4^{2-}	19.52 ± 0.01^{b}	8.84 ± 0.27^{a}	21.89 ± 0.27^{c}
Total	22.66	13.26	29.03
Microelement (mg/l)			
Fe	0.08 ± 0.01^{a}	0.09 ± 0.01^{a}	0.10 ± 0.01^{a}
Mn	0.04 ± 0.01^{a}	0.10 ± 0.01^{b}	0.04 ± 0.01^{a}
Cu	—	0.05 ± 0.01	0.08 ± 0.01
Zn	0.01 ± 0.01^{a}	0.25 ± 0.04^{b}	0.10 ± 0.02^{a}
B	0.03 ± 0.01^{a}	0.17 ± 0.03^{c}	$0.11 \pm 0.02^{b\,c}$
Total	0.16	0.66	0.43

Superscript letters on data on the same line indicate a significant difference ($p < 0.05$).

After the samples of phytoplankton were inoculated in different soil extracts, the changing trends of the cell density and biomass were not completely consistent (Figs. 1-2).

Fig. 1 shows that in all the four treatments (control, SE_1, SE_2 and SE_3) the densitites of phytoplankton reached their maximum after one week. The control group showed a density of 2.27×10^6 cells/l in the first week which gradually decreased to 0.03×10^6 cells/l via 2 – 4 weeks of the growth period (Fig. 1). SE_1 treatment yielded a maximum 4.71×10^6 cells/l in the first week. In this treatment the cell density fell drastically in second week but rose a little in the third week after which it fell further. SE_2 treatment group also gradually decreased after reaching the maximum value of 5.26×10^6 cells/l. In this group, the density after four weeks reached to 2.01×10^6 cells/l. The density of phytoplankton in the SE_3 treatment group also decreased after reaching the maximum value of 3.18×10^6 cells/l and then gradually increased. At week four, the density was 2.01×10^6 cells/l. A comparison of the density between the control and the treatment groups revealed that the increase in the SE_1 and SE_2 treatment groups at week 1 was larger and that the density was approximately 5×10^6 cells/l after one week. However, the decrease was larger at the late stage, especially in the SE_1 treatment group; the density in the SE_3 treatment after one week was lower than those in the SE_1 and SE_2 treatment groups but was higher than that in the control group. The density pattern of the groups after two weeks was SE_2 > SE_3 > control > SE_1. After three weeks, the density in the control group was significantly lower than those in the three treatment groups. After four weeks, the density pattern was SE_3, SE_2 > SE_1 > control.

Fig. 2 reveals the changing trend of the biomass of the phytoplankton for different treatments over the four weeks of growth period. The trend is almost identical with that of phytoplankton density pattern as presented in Fig. 1 except SE_3. In SE_3 treatment, the biomass after it attained a peak in the first week dropped to a value of 2.25 mg/l. Thereafter the biomass started increasing and reached a value of 5.74 mg/l in fourth week of the growth period (Fig. 2).

The above mentioned results showed that the density and biomass of phytoplankton in the control group were the lowest, indicating that the soil extracts could maintain the algal growth to a certain extent. However, because the different types of soil extract had different types and concentrations of nutrients, they had different influences on the algal growth. Hörnström *et al*. reported that alkaline soil was suitable for the growth of phytoplankton, whereas acid soil had a low nutrient content and low primary productivity (Hörnström 2002, Lessmann and Fyson 2003, Schagerl and Oduor 2008). The algal biomass in SE_3 during the experimental period was higher, indicating that the nutrient composition in SE_3 facilitated algal reproduction. That means the soil condition of ponds with alkaline soil (S_3) was conducive to the generation of higher population density of phytoplankton. Soil ponds with neutral purple soil (S_2) had a shorter duration time for maintaining conditions suitable for algal growth, whereas soil ponds with acid yellow soil (S_1) had unstable population density of phytoplankton. This finding is consistent with the analytical results of nutrient contents in soil extracts.

Based on the nutrient contents of the soil extracts, the N/P ratio was 365 : 1 in SE_1, 28 : 1 in SE_2, and 14 : 1 in SE_3. The TP concentration in SE_3 not only was higher than those in SE_1 and SE_2 but also conformed to the Redfield N/P ratio (16 : 1) during its absorption and utilization by phytoplankton. By contrast, SE1 and SE2 had a lower TP content and higher N/P ratio, indicating that the growth of phytoplankton in the SE_1 and SE_2 treatment groups could easily be limited by phosphorus.

Among the collected pond phytoplankton, the dominant species was *Tribonema minus*. During the experiments, the results revealed that the changes in dominant phytoplankton in different types of soil extract were different (Table 3). The dominant species in the control, SE_1 treatment, and SE_2 treatment groups after the first three weeks were Xanthophyta. After four weeks, the dominant species were *Anabaena azotica* in the control group, *Staurastrum inconspicuum* and *Chroococcus minor* in the SE_1 treatment group, and *Schroederia nitzschioides* and *Synedra acus* in the SE_2 treatment group. In contrast with the other groups, in the SE_3 treatment group, *Cryptomonas ovata* was already growing rapidly at week 2, and *Navicula simplex* and *Synedra acus* became the dominant species at week 3; finally, the algal phase dominated by Cryptophyta and Bacillariophyta was formed. These results indicated that pond soils could influence the community structure of phytoplankton of soil ponds (Table 3).

An analysis of the changes in dominant species showed that the dominant species *Tribonema minus* and *Botrydiopsis arhiza* belong to *Tribonema* and *Botrydiopsis* of Xanthophyta, respectively. After reaching a large biomass, they float on the water surface in a yellow-green flocculent shape. However, these two types of phytoplankton are not closely associated with aquaculture. The dominant species *Anabaena azotica* in the control group at the late stage belongs to the *Anabaena* of Cyanophyta. The dominant species *Chroococcus minor* in the SE_1 treatment group at the late stage belongs to the *Chroococcus* of Cyanophyta. These two types of phytoplankton easily become dominant species in the summer. They prefer water bodies that are slightly alkaline and rich in nutrients; however, they are not easily digested by fish. The dominant species *Gonatozygon monotaenium* and *Staurastrum inconspicuum* in the SE_1 treatment group at the late stage belong to the *Cosmarium* of Chlorophyta. They commonly favored acid bogs and lakes. The dominant species *Synedra acus* and *Navicula simplex* in the SE_2 and SE_3 treatment groups at the late stage belong to the *Synedra* and *Navicula* of Bacillariophyta. They are excellent baits for filter-feeding fishes, zooplankton, crustaceans, shellfish, and their larva. The dominant species *Cryptomonas ovata* in the SE_3 treatment group at the late stage belongs to *Cryptomonas* of Cryptophyta. They are also excellent baits for aquatic animals (Hu and Wei 2006). Thus, it can be speculated that the community structure of phytoplankton is influenced by soils in soil ponds; further, the nutrient composition of alkaline purple soil is suitable for the growth of Bacillariophyta and Cryptophyta and is conducive to forming the community structure of phytoplankton preferable for fish consumption.

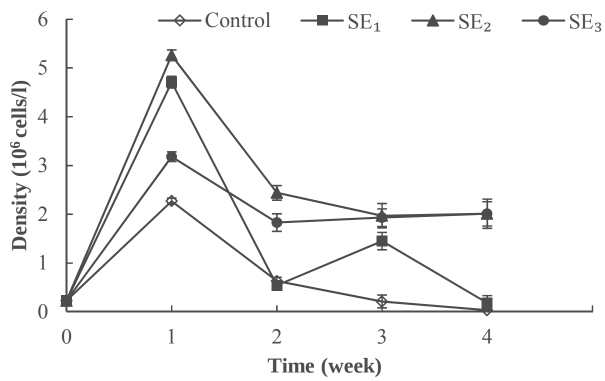

Fig.1. Showing the density of phytoplankton versus time in weeks for the three different soil extracts and the control. In the control group, the soil extract was replaced with deionized water.

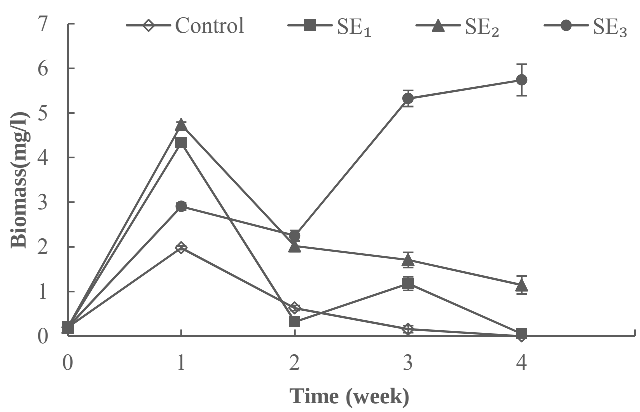

Fig. 2. Biomass (wet weight) of phytoplankton grown in three different types of soil extracts and control group versus time in weeks.

Various soil ponds are suitable for the production of different phytoplankton, which are largely influenced by the nutrient supply. Therefore, there is an idea that the aquaculture pond might be regulated by rational fertilization to reconstitute the poor soil condition, thereby forming the community structure of pond phytoplankton that is preferred for fish consumption.

Table 3. Dominant species of phytoplankton recorded after 1 - 4 weeks of culturing in treatments with different soil extracts and the control.

Dominant species	Control				SE_1				SE_2				SE_3			
	Weeks															
	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
Anabaena azotica Ley.				+												
Botrydiopsis arhiza Borzi	+					+			+	+			+			
Chroococcus minor (Kütz.) Näg.								+								
Cryptomonas ovata Ehr.														+		+
Gonatozygon monotaenium De Bary							+									
Tribonema minus (Will.) Haz.	+	+	+		+	+	+		+	+	+		+	+		
Melosira granulata var. *angustissima* O.Müller					+											
Navicula simplex Krasske															+	
Schroederia nitzschioides (G.S.West) Korschikoff												+				
Staurastrum inconspicum Nordstedt								+								
Synedra acus Kützing												+			+	+
Tetraselmis incisa (Nygaard) Norris		+														

"+" means that the species were the dominants which could reach 10% of the total number (Judit *et al*. 2003, Tang *et al*. 2014).

The results of the nutrient composition, algal density, biomass, and dominant species of three types of soil extracts revealed that acid yellow soil extracts had a low nutrient content and unstable primary productivity. The neutral purple soil extracts had a lower nutrient content of major elements, a higher nutrient content of microelements, and a shorter duration of maintaining conditions suitable for algal growth. The alkaline purple soil extracts had relatively high nutrient contents and were conducive to the formation of higher primary productivity; and the resultant community structure of the pond phytoplankton was the ideal phytoplankton for fish consumption. These results indicate that the alkaline purple soil is better for aquaculture pond construction. However, there are many different types of soil besides the three ones. Nevertheless, the nutrient composition of soil and the potential to support algal growth can be used as indicators for the site selection of soil for construction of fish ponds. This study provides scientific bases for the site selection of aquaculture soil ponds.

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