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Research Article

Assessing Bioavailability of Typical Lakes and Reservoirs in Northeast China Based on Phosphorus Fractions

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Abstract: To explore the eutrophication degree in the typical lakes and reservoirs of the northeast region of China, the bioavailability of dissolved organic phosphorus (DOP) of the lakes has been examined in this study. The laboratory incubation was carried out at 20 °C for 55 days and the concentrations of total dissolved phosphorus (TDP), dissolved reactive phosphorus (DRP), DOP and the microbial biomass have been detected. Results showed that, during the process of incubation, the concentrations of TDP and DRP were increased, whereas the DOP was decreased, which leads to the decreased mineralization rate. In addition, the changes of microbial biomass were fluctuant, but they had significantly positive effects on the concentration changes and mineralization rate of DOP ($p < 0.05$). The correlation analysis among the phosphate fractions showed that the TDP significantly promoted the DRP concentration, mineralization rate of DOP and the cumulative mineralization of DOP. The kinetics model was conducted to predict the further mineralization of DOP and to analyze the pollution degree of the eight lakes and reservoirs. Accordingly, the lakes with high DRP and TDP had worse water quality and are prone to algae blooms.

***Keywords*:** lakes and reservoirs, dissolved organic phosphorus, mineralization, microbial biomass, correlation analysis, kinetics model

1. Introduction

Northeastern China is the area where water is scarce [1]. The lakes and reservoirs here are vital links between the environment and humans, which mainly provides drinking water, fisheries, irrigation and, generates electricity [2]. However, crop farming, aquaculture, sewage discharge and any other misconducts are threatening the water environment [3-4]. It may also lead to the eutrophication threatening to the freshwater ecosystems in the northeast region of China. Lake Five-linked (LF), Lake Xingkai (LX), Lake Jingpo (LJ), Dahuofang Reservoir (DR), Lake Songhua (LS), Hongqipao Reservoir (HR), Lake Lianhuan (LL) and Taoshan Reservoir (TR) are the typical lakes and reservoirs. Because they cover a wide area, and remain the main freshwater source for people's life in Northeast China. Therefore, evaluating their ecological health is critical to prevent and govern water contamination in the northeast region of China.

Dissolved organic matter (DOM) derived from the aquatic environment can provide the energy source for the heterotrophic bacteria that participate in many vital nutrient recyclings in the Earth's biosphere [5-7]. The dissolved

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organic carbon (DOC), dissolved organic nitrogen (DON) and DOP are the most important components involved in DOM [8]. These components, especially the DOP, are often used as indicators to evaluate water quality [9-10]. Phosphorus has been implicated traditionally as playing a central role in controlling freshwater primary production [9]. In an aquatic environment, excessive total dissolved phosphorous (TOP) concentration may cause harmful algal blooms [11]. Because DRP and DOP as its main components are available to promote phytoplankton and bacteria growth [12]. In addition, the dissolved inorganic phosphorus is also the phosphorus source for plankton. When the DOM is mineralized by the microbe derived from lakes and rivers, it will supply phosphorus source for phytoplankton [12]. Given that, this process might bring about eutrophication [13-15]. Accordingly, as DOP is one of the parameters that can be measured to assess water quality conditions in freshwater ecosystems, it is important to study the bioavailability potential of DOP in the water environment for evaluating water quality and governing water contamination.

Organic carbon is rich in DOP, which can be mineralized as the first component to form inorganic phosphorous by releasing CO_2 and H_2O [16]. The mineralization of DOP provides not only energy sources but inorganic salt for microbial metabolism in the aquatic systems [17]. Under natural conditions, the DOP in overlying water mainly is derived from the sediments for maintaining ecological equilibrium [18]. However, once the freshwater is polluted by agriculture, it may result in overloaded phosphorus, bringing about the occurrence of cyanobacteria bloom [19]. The treatment of cyanobacteria bloom is a persistent and long-term process [20]. But in addition to artificial treatment, the freshwater can also recover by itself [21]. Therefore, the recovery ability of freshwater itself is another significant item to assess water quality [22]. The recovery ability of freshwater has been assessed by incubation essay. Besides, the various DOP concentration in different latitudes and depths of these lakes and reservoirs may also affect their recovery ability [23]. Therefore, typical lakes and reservoirs of different latitudes were selected to test their recovery ability.

Firstly, the quality of the eight typical lakes and reservoirs have been investigated by determining the concentration of TDP, DRP and DOP. Additionally, a bioavailability experiment was conducted to assess the recovery capacity of these lakes and reservoirs. In this study, to test the dynamics of phosphorus fractions during the incubation process, a theoretic reference for water-quality control has been provided.

2. Materials and methods

2.1 *Study sites and sampling collection*

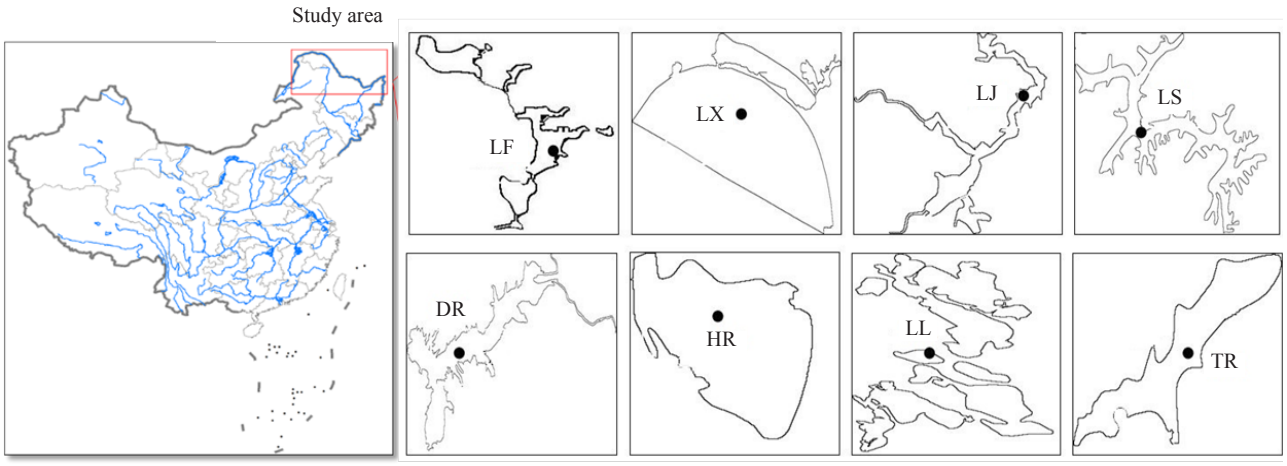


Figure 1. Locations of the eight typical lakes and reservoirs of the northeast region of China

Water samples were taken from 8 typical lakes and reservoirs located in the northeast region of China (Figure 1). These lakes and reservoirs can support agriculture, forestry, animal husbandry, fishery and tourism in Northeast China. Their basic information is presented in Table 1. Samples were collected during the summer high-rainfall period

when the hydrologic variation changed severely. The water samples were taken from the surface (0.5 m above the underwater). 10 L of water was collected in acid-washed bottles and filtered through pre-combusted and pre-washed glass fiber filters (Whatman GF/F 0.7 nominal pore size) to remove large particles in the water. The collected samples were kept in cold and dark storage during the process of transportation.

Table 1. Basic information of each lake and reservoir

Name	Location	Date	Longitude and Latitude	Temperature/°C	pH
Lake Five-linked	Heihe	In September, 2009	126°13′55.6″E, 48°43′50.3″N	10.3	8.03
Lake Lianhuan	Daqing	In September, 2009	124°01′22.6″E, 46°41′52.3″N	11.8	8.56
Hongqipao Reservoir	Daqing	In September, 2009	125°13′55.9″E, 46°38′22.7″N	11.5	8.05
Taoshan Reservoir	Qitaihe	In September, 2009	131°2′27.05″E, 45°48′13.49″N	10.9	8.20
Lake Xingkai	Mishan	In September, 2009	132°22′01.5″E, 45°18′27.8″N	9.8	7.82
Lake Jingpo	Mudanjiang	In September, 2009	129º00′01.7″E, 43º57′17.6″N	13.2	7.49
Lake Songhua	Jilin	In September, 2009	126°47′40.5″E, 43°39′27.0″N	12.6	7.92
Dahuuofang Reservoir	Fushun	In September, 2009	124º06′41.2″E, 41º52′53.2″N	13.4	7.98

2.2 *Bioavailability experiment and analytical chemistry*

The bioavailability experiment was processed according to Shi et al. [24]. Bacteria inoculum, derived from every single lake and reservoir, was shaken with aquae sterilisata at the ratio of 1: 3 (1 h, 200 rpm). The aquae sterilisata was prepared with 121 °C, 103.4 kPa for 20 mins. Thereafter, they were centrifuged (3000 rpm, 1 min) for removing particulate matter and protists [25]. Prior to inoculation, the water samples were filtered by 0.22 μm Millipore membrane, preventing the hampering of other plankton. The inoculated bacteria abundance was approximately 10^5 cells mL^{-1}. Eight bioavailability experiments were conducted in 5000 mL screw-capped jars for every 4 L waster that was separated from the 10 L system, and these jars were exhibited in a constant temperature incubator for 55 days (20 °C, dark). Subsamples were collected on days 0, 5, 10, 15, 20, 25, 35, 45 and 55, and they were filtered by GF/C filters for TDP, DRP and DOP analysis. Plugs of glass wool were fixed in the bottle to allow clean air into the bottle and keep moderate dissolved oxygen concentration in the water [24]. Additionally, it could also prevent samples from being contaminated.

Temperature and pH meters were utilized to determine the basic properties of the original samples. TDP contents were detected by phosphorus persulfate digestion. The determination of DRP was according to the modified method provided by Dupas et al. [26]. Microbial mass was determined by the dilution multiple method [24]. The DOP was calculated as the difference between TDP and DRP.

2.3 *Statistical analyses*

The concentration changes of TDP, DRP, DOP and microbe mass were analyzed by Origin Pro 2015. SPSS version 19.0 was also used for multivariate statistical analysis (Pearson analyses). The first-order kinetic model of bioavailability experiment was conducted by MATLAB.

3. Results and discussion

3.1 *Initial conditions of the typical lakes and reservoirs*

In the different lakes and reservoirs, the initial TDP concentration ranged from 0.02 ± 0.001 to 0.11 ± 0.02 mg/L. According to Figure 2a, HL possessed the highest TDP concentration, followed by HX. LD presented the lowest TDP concentration, followed by HT and JS. In addition, the DRP (Figure 2b) and DOP (Figure 2c) concentration respectively ranged from 0.017 ± 0.001 to 0.053 ± 0.002 mg/L and 0.021 ± 0.001 to 0.064 ± 0.001 mg/L. JS had the lowest DRP concentration, but the difference among the other seven lakes and reservoirs was insignificant. As for DOP, HJ, LD and HH exhibited less DOP concentration. Comprehensively speaking, the results of TDP, DRP and DOP showed that HL had abundant phosphorus compounds.

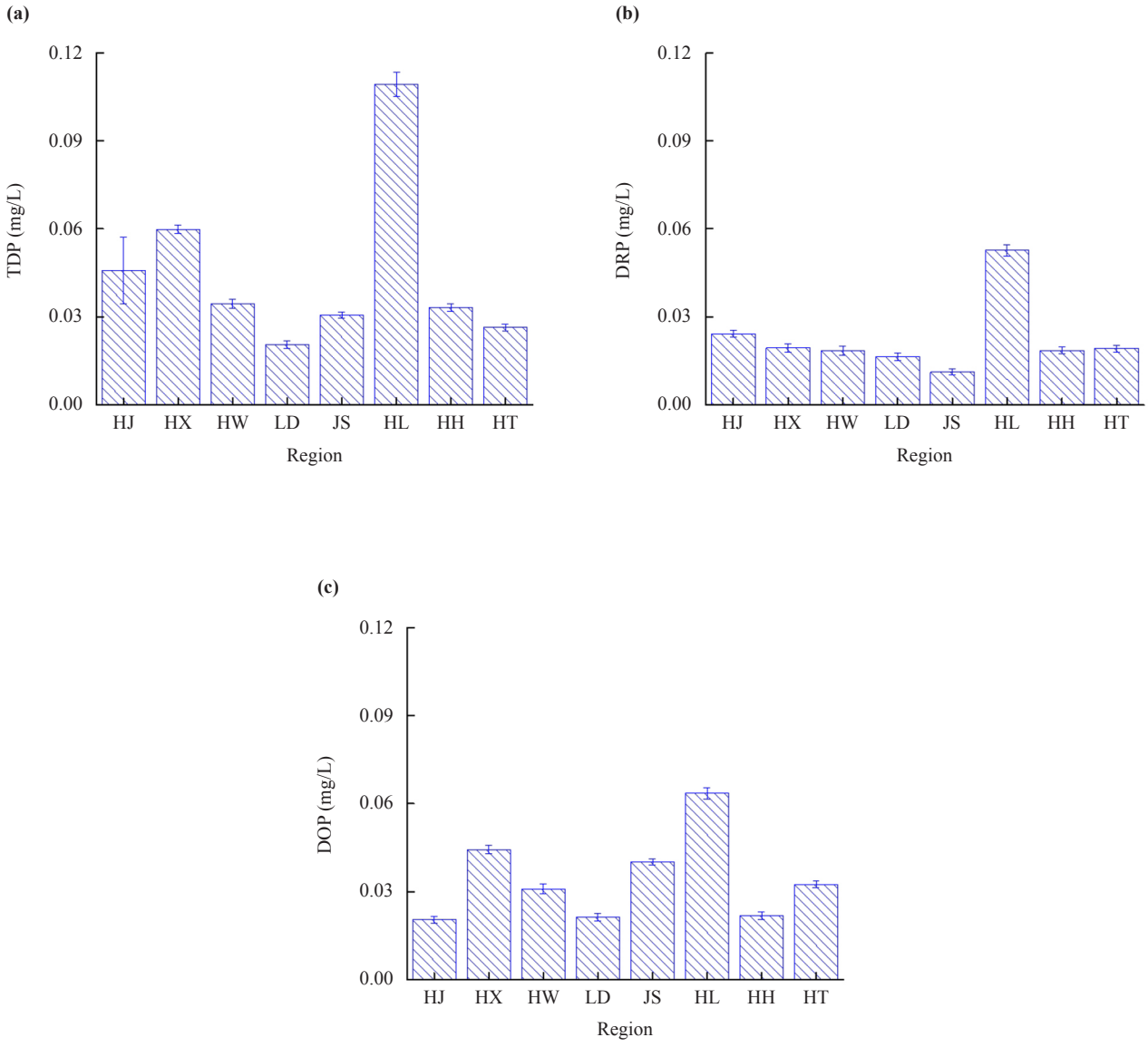
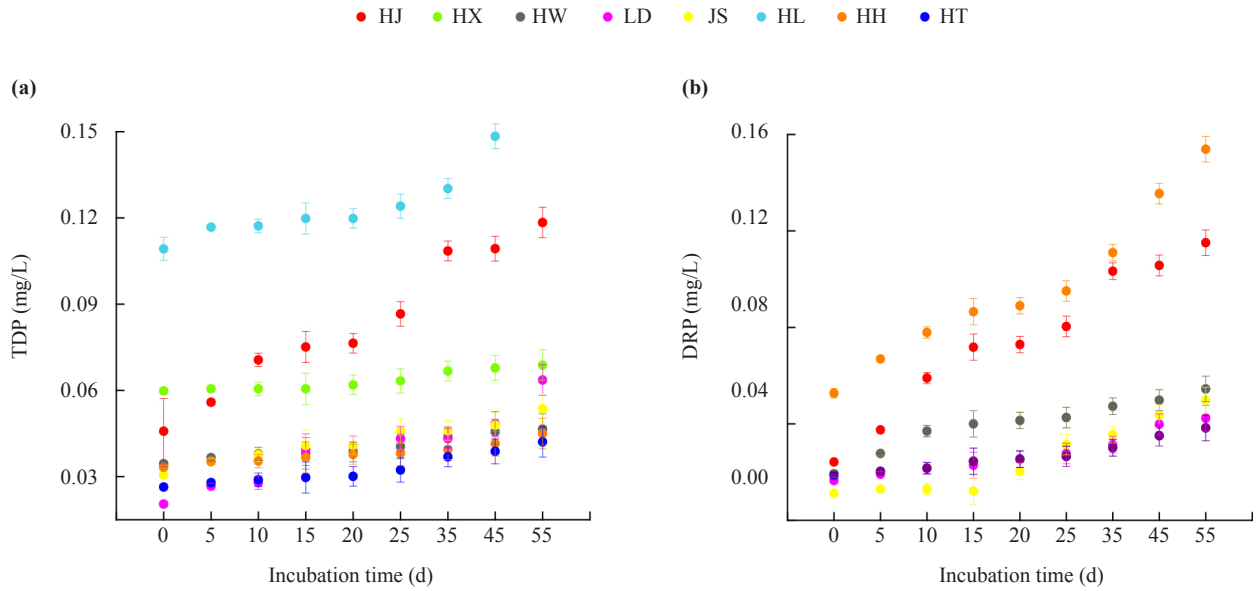


Figure 2. Initial conditions before experiment incubation for eight typical lakes (a: TDP concentration; b: DRP concentration; c: DOP concentration)

3.2 *Bioavailability experiment*

Given the inoculation microbe derived from the original water sample in the collected temperature close to 20 °C, the laboratory culture temperature was set as 20 °C to be close to the actual environment to the greatest extent. Figure 3 showed the concentration changes of phosphorus-related indicators of all locations in the bioavailability experiments. Microbial decomposition over 55 days led to a significant increase in TDP and DRP concentrations (Figure 3a and 3b). At the end of bioavailability experiments, the TDP concentration increase ranged from 159% to 15%. TDP concentration of HJ increased the most, and the lowest increase appeared in HX. Microbial activity is closely related to the TDP concentration [27], which suggested that the microbial metabolic activity in HJ was stronger than the others derived from the other lakes and reservoirs. Similar to TDP, the DRP concentration also increased the most in HJ (376%), but the least increase was at HT (99%). Contrary to TDP and DRP, the DOP concentration decreased during the process of bioavailability experiments (Figure 3c). DOP as the energy source of microbe activity, it will be utilized to support microbe growth [28]. DOP in HJ decreased the most (87%), followed by HH (74.9%), and HX decreased the least to only 40.4%. Figure 3c showed that the final concentration of DOP derived from different lakes and reservoirs is closely related to the initial concentration. Because the ranking of DOP concentration in each period is consistent with the initial value. But the decreasing rate may be in accordance with the microbial activity or the labile structure in DOP. The mineralization rate of DOP decreased rapidly in the process of bioavailability experiments (Figure 3d). In addition, in the earlier stage of bioavailability experiments, the mineralization rate of DOP was relatively high, which ranged from $(14.2 \pm 0.73) \times 10^{-4}$ mg/(L·d) in HJ to $(10.4 \pm 0.62) \times 10^{-4}$ mg/(L·d) in JS, HH and HX. But by the end of this process, the value was almost reduced to zero, the scope was from $(0.99 \pm 0.012) \times 10^{-4}$ mg/(L·d) in HJ to $(0.76 \pm 0.019) \times 10^{-4}$ mg/(L·d) in HL. The high DOP concentration accelerated the mineralization rate, which indicated the high microbial activity [29]. At the end of the bioavailability experiments, HL presented the highest cumulative mineralization of DOP $(27.6 \pm 0.58) \times 10^{-3}$ mg/L and HH had the lowest value in $(16.4 \pm 0.42) \times 10^{-3}$ mg/L (Figure 3e). This result was consistent with the initial conditions of the typical lakes and reservoirs. Taken together, the HL had the highest potential of algal blooming.



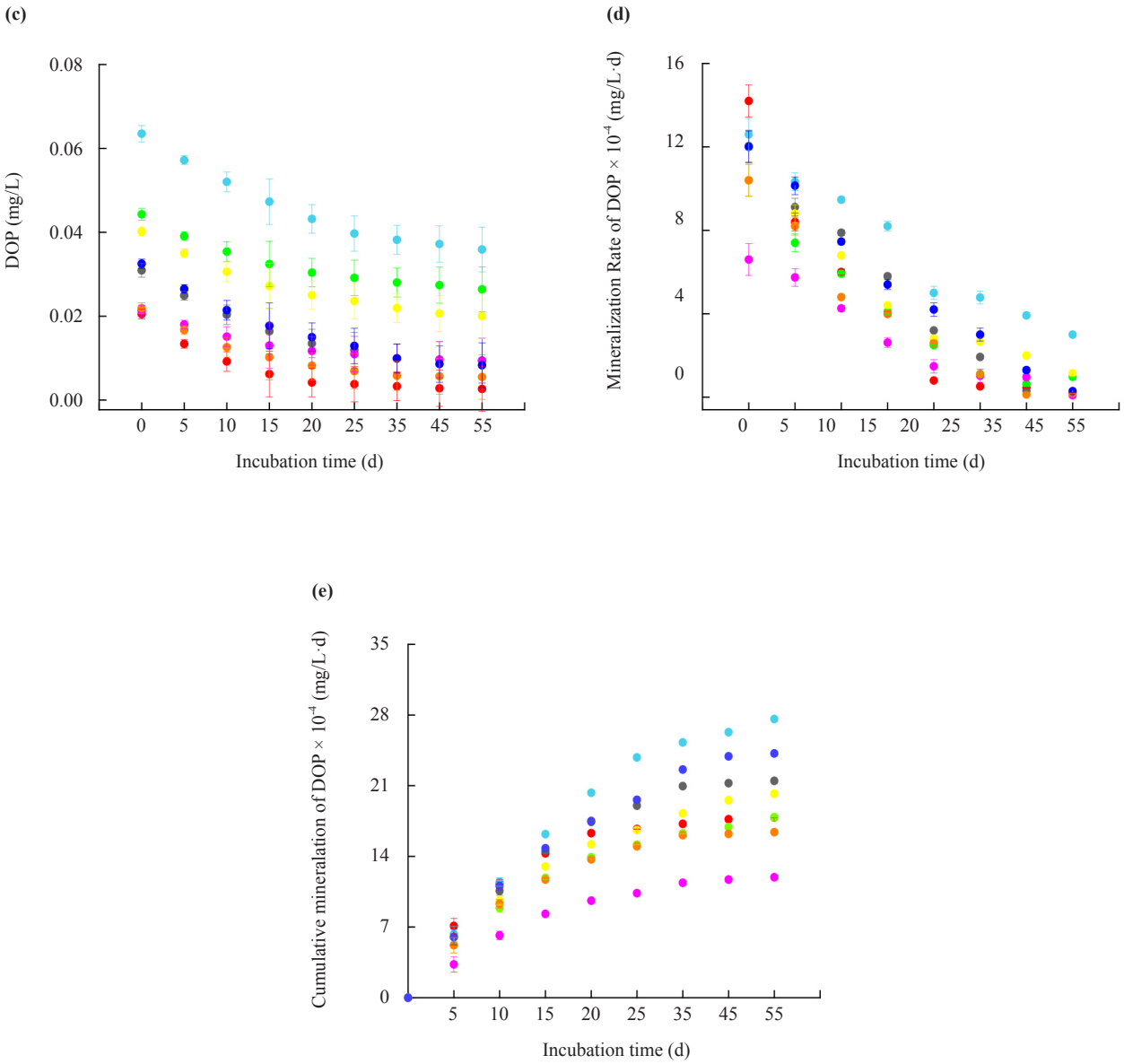


Figure 3. Concentration changes of phosphorus-related indicators derived from the eight typical lakes and reservoirs during the process of incubation (a: TDP; b: DRP, c: DOP; d: mineralization rate of DOP; e: cumulative mineralization of DOP)

3.3 *Microbial mass changes during the incubation process*

Microorganisms are the main driving factors to promote the transformation of organic matter in water [30]. And the microbial mass indirectly reflects organic matter content and microbial metabolic activity in the system [31]. In the first five days, the microbial mass showed a significant increase ($p < 0.05$), which suggested the energy was abundant in the lakes and reservoirs for microbial growth (Figure 4). As the incubation time processed, the microbial mass significantly decreased ($p < 0.05$). HW decreased the most (21.4%), and the least value was from HJ (3.9%) and HL (4.8%). The intense reduction of microbial mass in HW indicated a lack of energy in the water. But it is relatively abundant in HJ and HL.

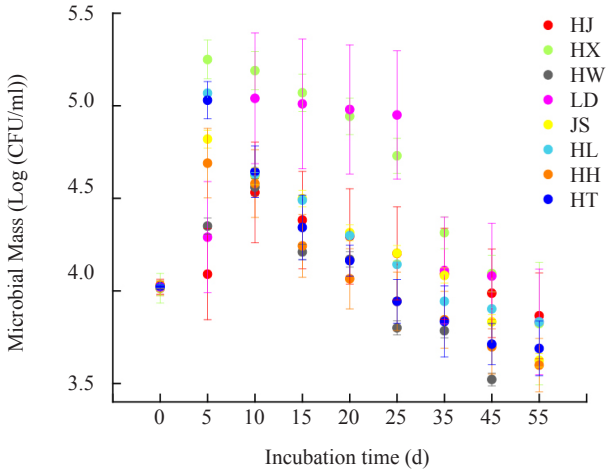


Figure 4. The microbial mass of different lakes in the incubation process

3.4 *Relationship between the microbial mass and phosphorus fraction*

According to Figure 5, the correlation matrix of all indicators showed that the microbial mass had significantly positive effects ($p < 0.05$) on the DOP concentration and the mineralization rate of DOP. During the incubation process, DOP concentration decreased consistently, which might produce the direct source of energy for microbial activity. Additionally, the rapid increase in microbial mass was consistent with the expeditiously cumulative mineralization rate of DOP by day 10, even though the relationship between them was not statistically significant ($p > 0.05$). These results suggested that the microbial metabolism was active and beneficial to the mineralization of DOP. They might also indicate that the faster the mineralization rate of DOP, the greater the possibility that the water environment would suffer blooms [32]. In addition, the concentration of TDP could also significantly affect the changes of DRP and DOP concentration because the phosphorus component in TDP can be transformed into DOP or DRP by microorganisms [33]. This result demonstrated that DRP and DOP might be transformed from TDP. However, section 3.3 has also showed that the degressive trend of microbial mass has not reached stablity at the end of incubation time. Therefore, DOP would continue to be mineralized if the processes would be ongoing.

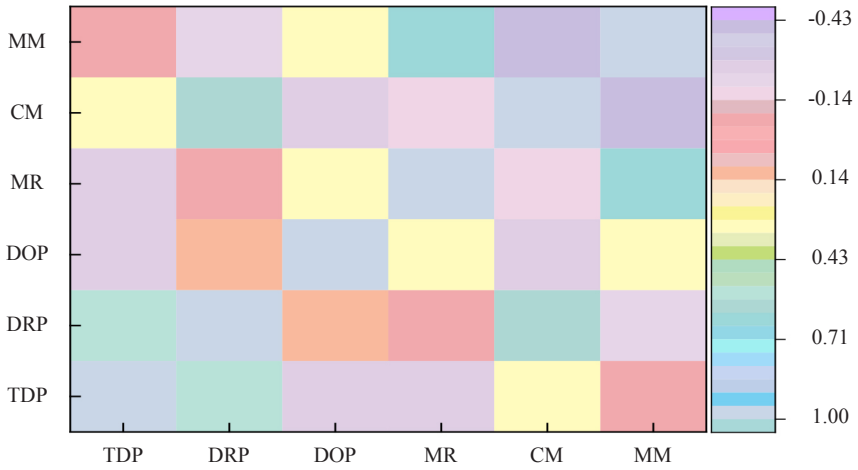


Figure 5. The correlation matrix of all indicators. MM: microbial mass; CM-DOP: cumulative mineralization of DOP; MR-DOP: mineralization rate of DOP

3.5 *Bioavailability kinetics of DOP*

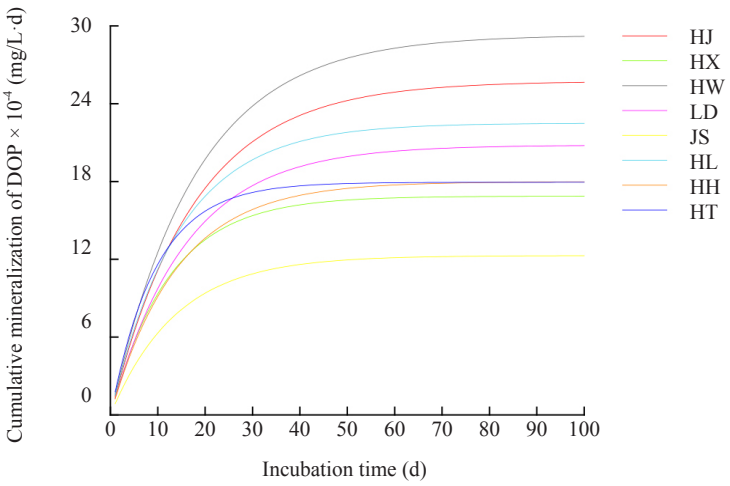


Figure 6. Kinetics model of DOP mineralization of the eight typical lakes

Table 2. Potentially mineralizable S_0 and mineralization rate constant of DOP of each lake and reservoir

Lakes	S_0 (mg/L)	k [mg/(L·d)]	r
HW	0.0212	0.0639	0.9955
HX	0.0243	0.1046	0.9920
HJ	0.0148	0.0924	0.9599
LD	0.0109	0.0551	0.9831
JS	0.0500	0.0180	0.9794
HH	0.0140	0.0447	0.9900
HL	0.0503	0.0430	0.9946
HT	0.0276	0.0432	0.9945

In this study, kinetics models were introduced to better understand the degree of DOP's mineralization by microorganism in a long process [34]. The model showed a continuous increase of accumulative content of DOP within 100 days. Figure 6 showed the kinetics model of DOP mineralization. Results showed that the process of bioavailability is almost stable until 60 days. HT was the first one to get stable and HW was the last one, then HL. Mineralization of DOP was influenced both by the microorganism activity and its initial concentration. The higher initial DOP concentration and lower microbial reduction in HL might be the main reasons leading to a delayed stability of DOP's mineralization. As for HW, the rapid decrease of microbial mass might cause HW to reach mineralization stability later. Therefore, the potentially microbial utilization was another factor to evaluate the quality of lakes and reservoirs. The S_0 and k in Table 2 represent the potential mineralization and mineralization rate constant of DOP, respectively. Accordingly, HL and JS had the maximum mineralization potential, whereas LD, HJ and HH had the least. These results were consistent with the initial concentration ranges of DOP, in which the concentration was highest in HL, HX and JS. These results suggested that DOP in HL and JS was much easier to occur algal blooming than HX [32]. As for the value

of k, the potential mineralization rate in HX was the highest among these typical lakes and reservoirs. This indicated that the structure of DOP in HX was labile and easy to be utilized by microbe. Therefore, controlling the input of DOP is very important for HX, because a high microbial availability signaled a high risk of algal blooming.

3.6 *Water quality evaluation based on the phosphorus fraction*

The initial concentration of phosphorus fraction indicated the potential of algal bloom. Possibly, the polluted situation in HL and HX might be more serious than other lakes. Because the concentrations of DRP and DOP in two lakes were also higher than others (Figure 2b and 2c). Meanwhile, the results also showed the lowest value of TDP, DRP and DOP presented in different lakes (Figure 2), illustrating the water quality in most lakes was good. The results of TDP, DRP and DOP showed that HL had abundant phosphorus compounds, which seriously threatens the safety of the water environment. Furthermore, the bioavailability of DOP fraction also indicated the recovery ability of the typical lakes and reservoirs. During the incubation process, the DOP concentration in HJ and HH decreased the most, same to the mineralization rate of DOP and cumulative mineralization of DOP (Figure 3c-e). These results demonstrated that HJ and HH could more rapidly recover by themselves than others. As for HL and HX, the highest initial concentration of TDP and DOP, and the bioavailability in these lakes were relatively weak. Therefore, HL and HX have the potential to bloom, which deserves more attention to its pollution situation and should limit the discharge of sewage into it.

4. Conclusion

According to the concentration of phosphorus fractions, HL in Heilongjiang province presented the highest mineralization rate. Additionally, the initial concentration and microbial activities were the main reasons to drive the difference among TDP, DRP, DOP in various lakes and reservoirs. They also significantly affected the mineralization rate and cumulative mineralization of DOP. Furthermore, HL and HX in Heilongjiang province had the highest potential of algal blooms than the other lakes and reservoirs. In contrast, the water quality of HW was better, even though its DOP was difficult to be degraded. Therefore, the lakes with high DRP and TDP had worse water quality and were prone to algae blooms.

Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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