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The nitrogen and phosphorus release in the sediment by the *Perinereis aibuhitensis* bioturbation effect

D. Yang [1,2,3], C. Cao[2,3], F. Chen[2,3], Y. Zhou[2,3], Z. Xiu[1*]

[1]*College of Life science and Technology, Dalian University of Technology, 116021,Dalian, China;*
[2]*Key Laboratory of Marine Bio-resources Restoration and Habitat Reparation in Liaoning Province, Dalian Ocean University, 116023,Dalian,China*
[3]*Key Laboratory of North Mariculture, Ministry of Agriculture, Dalian Ocean University, 16023,Dalian,China*

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Eutrophication in sediment is harmful to the marine ecology system. Polychaetes are important marine benthic animal populations; their bioturbation can promote the release of nutrients to the upper water. In the current study, using a simple, self-made test device, we studied the influence of *P. aibuhitensis* on the regular release of nitrogen and phosphorus from the sediments. The experiment was designed in three groups: low concentration (354 ind/m^2, 293.81 g/m^2), high density (708 ind/m^2, 587.61 g/m^2) and control group. The results of a test in a 10-day experimental period showed that released concentrations of NH_4^+-N, NO_3^--N, and PO_4^{3-}-P in worms' sediment were significantly higher than those in the control group. Among them, the change of NH_4^+-N and PO_4^{3-}-P was the most significant. The NH_4^+-N concentration reached the highest value at the sixth day in the high density group (708 ind/m^2) and its value was 11.4365 mg/L. The released concentration of PO_4^{3-}-P reached a peak at the third day with a value of 0.1574 mg/L. The experimental results proved that *P. aibuhitensis* could improve the sediment quality.

Key words: Bioturbation, nitrogen, phosphorus, *Perinereis aibuhitensis*.

INTRODUCTION

Eutrophication-induced changes to an ecosystem can cause reduced water quality, shifts in species composition, and a bottom dead-zone. Eutrophication in aquatic systems is caused by the input of excess nitrogen, phosphorus, and other nutrients, and therefore, the fate of these nutrients in aquatic systems has attracted wide attention. For example, while nitrogen and phosphorus are two essential nutrient elements in the ecosystem, excess input of nitrogen and phosphorus in aquatic systems can lead to eutrophication, which may destroy the balance of an ecosystem [1-4]. As an example, the release of nitrogen from the sediments, as one of the essential limiting nutrients, facilitates the growth of plankton in the water column. However, excessive deposition of nitrogen can cause sediment pollution, especially under anaerobic conditions, with the generation of toxic ammonia through de-nitrification resulting in a bottom dead-zone. Therefore, the cycle of nitrogen, especially regarding the release of nitrogen from sediment to the water column, plays an important role in the health of the sediment environment [5]. Phosphorus has no direct toxic action, but it is also an important facilitator of phytoplankton growth. Therefore, the release of phosphorus from the sediment is a major limiting factor on the growth of phytoplankton. Dynamic nutrient equilibrium exists between the sediment and the water column [6]. Nitrogen and phosphorus in the sediments will gradually diffuse into the water column down a concentration gradient, which can cause secondary pollution [7, 8]. Therefore, it is very important to understand the mechanisms behind sediment nutrient release.

Bioturbation refers to the sedimentary structure change caused by organisms, mainly through the turnover of deposited particles by crawling, feeding, burrowing, and habitat construction. Different forms of bioturbation exist, such as cave-making, irrigation, diffusive and advective mixing, and other dynamic processes, which redistribute the sediments [9]. The direct effect of bioturbation is the vertical transport and mixing of sediments, or "turnover." Turnover of sediments can accelerate the material exchange between interstitial water and the overlying water column, thereby facilitating the decomposition, mineralization, and metabolism of organic matter [10]. Studying the mechanisms behind biological disturbance may allow an understanding of the load release from the sediments to the water column, thereby assisting the prediction of algal blooms and incidents of red tide. Furthermore, this knowledge provides the basic parameters and conceptual understanding for ecological modeling and water

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* To whom all correspondence should be sent:
E-mail: zhilxiu2008@163.com

treatment [11].

Polychaetes are a dominant benthic fauna in the marine environment and as such, they play a key role in energy flow and material recycling in the marine ecosystem. Some polychaetes are of economic importance, such as the adults and larvae of the family *Nereidae*, reported to be food for many economically important fishes, and also used as bait for recreational fishing. Due to their wide distribution and sensitivity to pollution, *polychaetes* play an important role in biomonitoring of the marine environment for effects of toxic materials and pollution. *Polychaetes* also facilitate bioremediation of eutrophic, toxic and heavy metal polluted sediment [12]. The application of aquaponic systems using *nereis-paralichthys* and *nereis*-shrimp are good examples of using bioremediation to improve eutrophication, through improving the exchange between sediment-water interfaces, purifying water quality, and generally improving the environment. Furthermore, *polychaetes* can reduce the pollution from aquaculture wastewater, thereby reducing the effect on the ocean and the cost of breeding, and therefore, promote sustainable aquaculture [13]. However, few studies have reported the role of *nereis* in nutrient cycling and the release of nitrogen and phosphorus from the sediments. The current study reports on a laboratory study using *Perinereis aibuhitensis*, a widely distributed and economically important *polychaete* in coastal China [14], to determine the effect of its bioturbation on the release of nitrogen and phosphorus from sediments.

MATERIALS AND METHODS

Animal collection and experimental installation

Perinereis aibuhitensis individuals were collected from a coastal area in Dalian City, China. The collected specimens had an average body length and weight of 10.55 ± 2.86 cm and 0.83 ± 0.29 mg/ind, respectively. After collection, the experimental animals were immediately brought to the laboratory and placed into a temporary culture for 72 hours, then, individuals of similar size and seemingly good health were selected as the experimental animals.

Sediment samples were collected from the Dalian Heishijiao coastal area. Sediment was separated from the surface mud, placed into a closed tank, and shipped to the laboratory, where it was frozen for future use. The experimental system used was a 1,000 ml glass beaker. A total of 300 g of sediment sample, creating a bottom layer of approximately 7.5 cm thickness, was placed into each beaker. Sand filtered seawater was then added to the 1,000 ml scale line of the beaker. The seawater was filtered by a 0.45 µm fiberglass microfiltration membrane. A plastic hose was used to input air into the system when adding water without stirring the sediment. Water samples were collected through the siphon method using another plastic hose. The equipment used in the experimental system is shown in Fig. 1.

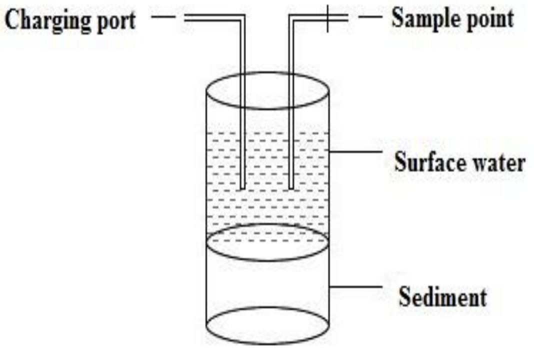

Fig. 1. Equipment used in the sediment release experiment.

Experimental methods

The experimental system was divided into three different groups by worm density: 1) control group; 2) high density group; and 3) low density group. Three repeated experiments were set for each group. The control group did not contain any worms. The low density group contained four worms with a density of 354 ind/m^2 and a total worm mass of 293.81 g/m^2. The high density group contained eight worms in each beaker with a density of 708 ind/m^2 and a worm mass of 587.61g/m^2.

The experimental period was 10 days; sampling for phosphorus occurred twice a day at 8:00 am and 8:00 pm. Ammonia nitrogen (NH_4^+-N), nitrite (NO_2^--N), and nitrate (NO_3^--N) were sampled once a day at 8:00 am. The environmental variables measured in each beaker during the experimental period are shown in Table 1.

Table 1. The environmental variables of each system during the experiment.

	Control	Low	High
Density (/m^2)	0	354	708
Biomass (g/m^2)	0	293.81	587.61
Temperature (°C)	17 ± 1	17 ± 1	17 ± 1
Dissolved oxygen (mg/L)	7.87-9.67	8.03-9.67	7.44-9.67
pH	7.78-8.06	7.76-7.98	7.76-7.91

Method for determination of nitrogen and phosphorus

Ammonia nitrogen (NH_4^+-N) was determined by a colorimetric method using Nessler's reagent;

nitrite (NO_2^--N) was measured by the naphthyl ethylenediamine dihydrochloride spectrophotometric method, nitrate (NO_3^--N) was measured using a cadmium reduction column, and soluble phosphate (PO_4^{3-}-P) was determined by the molybdenum blue photometric method. Dissolved oxygen (DO) was determined by the iodometric method. Ammonia nitrogen, nitrite, nitrate, and phosphate were measured using a 721 spectrophotometer [15].

RESULTS

Variation in ammonium (NH_4^+-N) concentration between the different groups

The variation in ammonium concentration between the groups is shown in Fig. 2. The control group, high density group, and low density group all showed a similar general trend: the concentration of ammonium rapidly increased at the start of the experiment, reached a peak value, after which the concentration stabilized. The change of ammonium concentration in the high density group was 0.4724 mg/L at the start, reaching a peak of 11.4185 mg/L on the sixth day, achieving a steady state for about 2 days, following which the concentration began to decline. The concentration of the low density group changed from 0.3228 mg/L up to 8.9540 mg/L, reaching a peak on the eighth day, after which the concentration of NH_4^+-N showed minor fluctuations. In the control, the scale of concentration variation of ammonium was 0.4409-5.5529 mg/L.

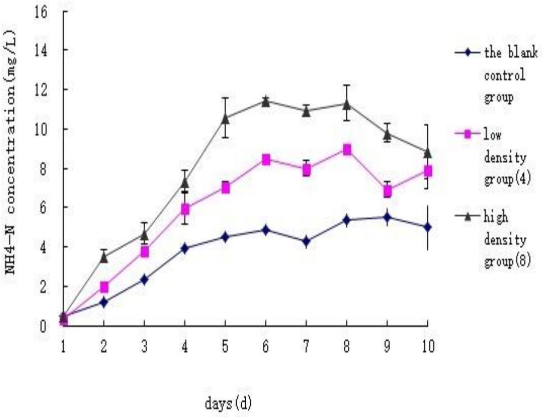

Fig. 2. The changes in NH_4^+-N concentration over time in the different worm density groups.

Fig.3 shows the ammonium concentration regressed against time for the first 6 days for the different groups, representing the period when the ammonium concentration showed a linear increasing trend (see Fig. 2). According to the data, the trend line was calculated in the different density groups. The concentration/time (days) curve in the first 6 days was linear. The highest to lowest slopes of the regression lines referred to the high density group, the low density group, and the control group. Analysis of one-way variance indicated that the effect of worm density in the sediment on the amount of ammonium released was very significant ($P < 0.001$). The worms' action played a significant role in promoting ammonium release.

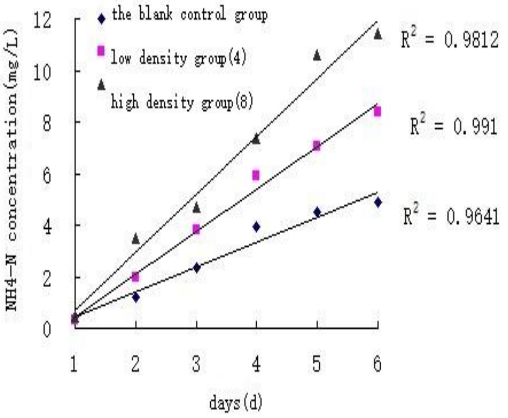

Fig. 3. The NH_4^+-N changes in the different groups over the first six days of the experiment.

The variation in nitrite (NO_2^--N) concentration among the different groups

As shown in Fig. 4, the fluctuation in nitrite concentration between the different density groups was very small. The nitrite concentrations in all groups showed a declining trend until the sixth or seventh day, after which the concentrations began slowly to rise. The ranges of nitrite concentrations were 0.6244-1.8880 mg/L, 0.4814-1.7423 mg/L, and 0.4763-1.6762 mg/L in the high density group, low density group, and control group, respectively. Statistical analysis showed that there was no significant difference in the concentration of NO_2^--N between the different density groups ($P > 0.005$).

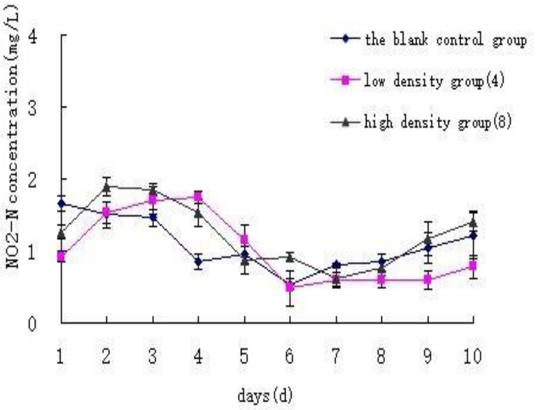

Fig. 4. The changes in NO_2^--N concentrations between the different density groups.

The variation in nitrate (NO_3^--N) between the different groups

The changes in nitrate (NO_3^--N) concentration over time for the different groups can be seen in Fig. 5. A slow rising trend during the entire experimental period is evident for all groups. The nitrate concentrations in the groups ordered from highest to lowest were the high density group, the low density group, and the control group. The range of concentration changes was 0.6643-1.2356 mg/L, 0.6577-1.12184 mg/L, and 0.6217-0.9214 mg/L in the high density, low density, and control group, respectively. Statistical analysis showed that nitrate concentrations in the different groups were significantly different ($P < 0.005$).

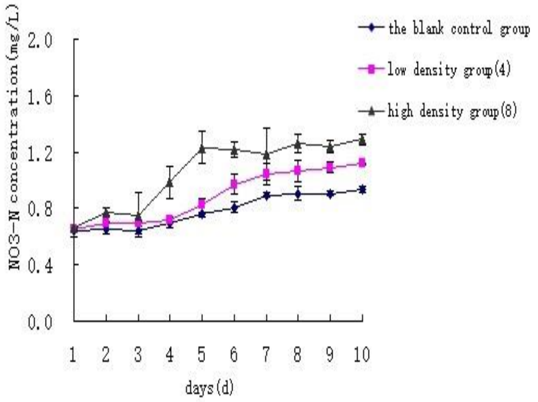

Fig. 5. The changes in NO_3^--N concentration between the different groups over time.

Variation in phosphate (PO_4^{3-}-P) between the different groups

The concentrations of soluble phosphate PO_4^{3-}-P showed a similar trend in the high density and low density groups (Fig. 6): the concentrations rapidly increased from low starting values to the peak values within 3 days, after which a gradual decrease was observed. A final steady state was reached after 6 days.

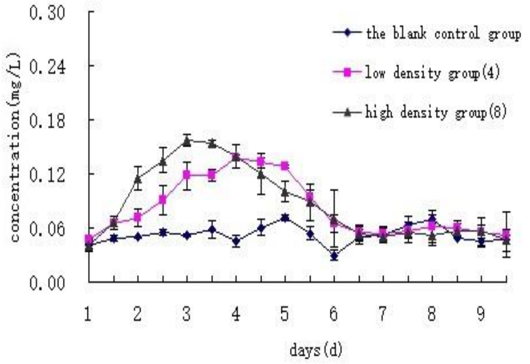

Fig. 6. The changes in PO_4^{3-}-P between the different groups over time.

The concentration in the high density group was higher than that in the low density and control groups. The day when peak values were obtained was different for the two density groups: peak values of 0.1574 mg/L and 0.1378 mg/L occurred at day 3rd and day 4th for the high density and low density group, respectively. The range of fluctuation of soluble phosphate concentration during the experimental period was 0.0463-0.0703 mg/L, 0.0521-0.0645 mg/L, and 0.0295-0.0717 mg/L in the high density group, the low density group, and the control group, respectively.

Using the same method as for ammonium, we made a concentration/time (days) figure using data of the first 3 days (Fig. 7). It displayed the effect of worm density on the release speed of soluble phosphate. In the first 3 days, the relationship of time and concentration was linear for the high and low density groups, with an R^2 of 0.9770 and 0.9603, respectively. An R^2 of 0.7384 was obtained for the blank group. The slope of the regression line from highest to lowest was as follows: high density group, low density, and control group. Using analysis of variance, the concentrations of soluble phosphate in the different density groups showed very significant differences ($P < 0.001$).

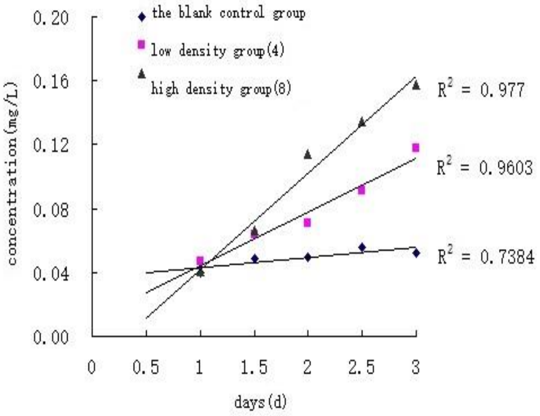

Fig. 7. The PO_4^{3-}-P changes of each group for the first 3 days of the experiment.

CONCLUSIONS

In aquatic ecosystems, different forms of nitrogen are involved in a variety of reactions and processes. Important forms of nitrogen are ammonium (NH_4^+), ammonia (NH_3), nitrate (NO_3^-), nitrite (NO_2^-), and nitrogen gas (N_2). Ammonium, nitrate, and nitrite constitute the soluble inorganic nitrogen (DIN). The chemical species of nitrogen are consumed by the phytoplankton and are used by bacteria as electron acceptors. Organic forms of nitrogen consist of organic detritus, which tends to settle as dead organic matter to the sediment.

Organic nitrogen in the sediment can be converted to inorganic nitrogen by anaerobic bacteria. The sediments can not only recycle nutrients and other chemical compounds back to the water column, but also act as a sink for nutrients. This is the reason why sediments play a major role in controlling eutrophication [16]. If the release of inorganic nitrogen back to the water column occurs slowly, major eutrophication of the sediment could take place while the primary productivity of the surface water would be low. Therefore, the release of nitrogen from the sediment is very important [17]. In the current study, the release of DIN in the control group also showed an increasing trend from the start of the experiment. Because the concentrations of DIN in the water and the sediment were different, diffusion from the high to the low concentration gradient could occur. The concentration of nitrate and ammonium in the overlying water was obviously higher in the groups containing worms than in the control group. The average concentrations of NH_4^+-N and NO_3^--N in surface water were 5.9252 mg/L and 0.8869 mg/L, respectively, in the low density group; 7.8902 mg/L and 1.0608 mg/L, respectively, in the high density group; 3.7451 mg/L and 0.7832 mg/L, respectively, in the control group. The reason for these differences was mainly due to the effect of the worms on bioturbation, thereby facilitating the mixing of sediments, interstitial water, and surface water, allowing inorganic nitrogen to quickly spread to the overlying water [18].

The release of NH_4^+-N and NO_3^--N which was obvious higher in the groups containing worms than in the control group, occurred mainly during the initial days of the experiment. The concentrations in the high density group reached a peak value at day 6th and were stable for 2 days, after which the concentrations began to decrease. The concentration of NH_4^+-N increased during the initial experimental days, but later decreased, mainly because of two possible reasons: 1) building of caves occurs when the worms arrive at a new environment, following which, stable concentrations are obtained; and 2) bioturbation by the increased intensity of the worms can produce a larger amount of nitrogen in an aerobic environment, strengthening nitrification, while decreasing denitrification. The degradation of organic matter in an oxygen environment will consume a large amount of $NH4^+$-N, which is partially converted into NO_3^--N [19]. The low density and high density groups showed a similar trend with only a time delay (approximately two days). It also follows from the results that the release rate of ammonia nitrogen strongly correlates with worm density. The concentration change of NO_2^--N was similar in each group because NO_2^--N is an intermediate chemical species of nitrogen between NH_4^+-N and NO_3^--N, so it is very unstable under oxidizing conditions, and is soon converted to NO_3^--N. The concentration of NO_3^--N always maintained an upward trend during the experimental process. This may be because the surface water was always in aerobic conditions, and therefore, the nitrification process exceeded the denitrification one. During nitrification, a proportion of NH_4^+-N and NO_2^--N was oxidized into NO_3^--N, causing a continuous accumulation of NO_3^--N [20,21].

Under natural conditions, phosphorus in sediments is subjected to simultaneous adsorption and desorption. The rate of phosphate release, which is accelerated by wind and biological disturbance, can exceed the adsorption rate of phosphate in sediment [22]. The apparent release rate of phosphate in the sediments in the current study was therefore different from the amount of released and adsorbed phosphate. It is evident from Fig. 7 that worm bioturbation promotes the release of soluble phosphate from the sediments in the initial period (3 days). The bioturbation action of the high density group was higher than that of the low density group, and therefore, the day of phosphorus peak appearance of the high density group was slightly ahead of that of the low density group (about 1 day). After reaching a peak, soluble phosphate in the high and low density groups began to decline. This is because the *nereis* continued to effect bioturbation beyond the phosphate peak, but the release rate of phosphorus had little effect, or the content of phosphorus in the sediment declined. This demonstrates that during the experiment, the effect of bioturbation on the released phosphorus was only a short-term and therefore, limited effect. Phosphorus in sediments undergoing *nereis* bioturbation may become depleted over time [23, 24]. At this point, adsorption and release of phosphorus to and from the sediments achieves a dynamic equilibrium. Therefore, while the phosphorus concentration in the overlying water may decrease, the release of phosphorus from the sediments continues.

Nereis bioturbation is a physical process that affects dissolved phosphate concentrations by resuspension of particular phosphorus and accelerating the diffusion of phosphorus from the sediments through the sediment-water interface, causing a sharp rise in soluble phosphate concentration. Therefore, the more intense the physical disturbance, the more obvious is the phosphorus release. The experiments showed that the quantity of phosphorus released and the density of *P. aibuhitensis* have a very significant correlation.

In this decade, bioturbation has been studied widely for different species and environments. In the ocean, various animal groups cause bioturbation effects, such as sea cucumber [25], shrimp [26], crabs [27], clams [28], shellfish [29], and polychaete [30]. In 2012, Kristensen defined bioturbation in aquatic environments to include all transport processes carried out by animals that directly or indirectly affect sediment matrices. These processes include both particle reworking and burrow ventilation [31]. Particle reworking occurs through burrow construction and maintenance, as well as ingestion and defecation, and causes biomixing of the substratum. Organic matter and microorganisms are thus vertically and laterally displaced within the sediment matrix. Burrow ventilation occurs when animals flush their open- or blind-ended burrows with overlying water for respiratory and feeding purposes, causing advective or diffusive bio-irrigation exchange of solutes between the sediment pore water and the overlying water body. Many bioturbating species simultaneously perform reworking and ventilation.

From the results of the current study, it is evident that *P.aibuhitensis* can significantly promote the release of nitrogen and phosphorus in sediments through churning the sediments and accelerating the migration processes of nitrogen and phosphorus in the sediments from the subsurface and finally to the water. Meanwhile, its eating, caving, excreting, and other metabolic and ecological habits may affect the process of water management. This biological disturbance will bring changes to the physical and chemical properties of sediments, and affect the structure and various dynamic processes of the sediment-water interface through simultaneous reworking and ventilation of the sediments [32]. Theoretically speaking, the bioturbation of Nereis can increase the dissolved oxygen of the sediment-overlying water interface, which should not promote the release of nitrogen and phosphorus from the sediments, but bioturbation can promote the mixing and exchange between sediment and the water interface, and the effects of mixing and exchange is more obvious than the impact of simple dissolved oxygen on nitrogen and phosphorus release in the sediment [21]. Indeed, polychaetes bioturbation has been widely used in sediment environment bioremediation or multiculture. Zhou found that Nereis can remediate the sediments through its feeding and living behavior. A Nereis biomass of 200 g/m^2 can process sediments of up to 762.6 g/m^2/d, which is equivalent to the amount of 558.9 cm^3 of sediment [33]. This means, in three months, Nereis can remediate the entire sediment surface of 5 cm in a shrimp pond. A study on the benthic animals of Tamar estuaries in the UK showed that in regions with *Neanthes diversicolar* densities of 4,000 ind/m^2, the flux of soluble ammonia from sediment to water is 1,565 μmol/m^2/d; while the flux is -134 μmol/m^2/d in polychaetes sparse regions [34]. It is therefore obvious that bioturbarion can greatly improve the efficiency of material circulation and transformation and therefore, polychaete bioturbation can improve the quality of sediment and the state of the marine environment [35].

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ОТДЕЛЯНЕ НА АЗОТ И ФОСФОР ОТ СЕДИМЕНТИТЕ ОТ *PERINEREIS AIBUHITENSIS* ЧРЕЗ БИОТУРБАЦИОНЕН ЕФЕКТ

Д. Янг[1,2,3], Ч. Цао[2,3], Ф. Чен[2,3], Й. Жу[2,3], Ж. Ксю[1*]

[1]*Колеж за науки за живота и технологии, Университет в Далиан, Китай*
[2]*Ключова лабораторияпо възстановяване на морски биоресурси и подобряване на жилищната среда в провинция Ляонинг, Океаноложки университет в Далиан, Китай*
[3]*Ключова лабораторияпо северни морски култури, Министерство на земеделието, Океаноложки университет в Далиан, Китай*

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(Резюме)

Еутрофикацията на седиментите е вредна за здравето на морските екологични системи. Polychaetesca важни за животинските популации в бентоса. Техните биотурбации могат да предизвикат отделяне на нутриенти в горните водни слоеве. В настоящата работа използвахме прост, собствен тест за изследване на *P. aibuhitensis*и ролята му за редовното изхвърляне на азот и фосфор от седиментите. Изследването е остроено на три групи: при ниски концентрации (354 ind/m^2, 293.81 g/m^2), висока плътност (708 ind/m^2, 587.61 g/m^2) и контролна група. Съгласно тестовете, проведени в продължение на 10 дни отделяните концентрации на NH_4^+-N, NO_3^-N и PO_4^{3-}-P в седименти от червеи за значително по-високи, отколкото в контролната група. Сред тях изменениетона NH_4^+-N и PO_4^{3-}-P е най-значимо.Концентрациите на NH_4^+-N достигат най-високи стойности при групата с висока плътност (708 ind/m^2) и неговата стойност беше 11.4365mg/L. Концентрацията на отделения фосфор стига до максимум на третия ден със стойност 0.1574 mg/L. Опитните резултати доказват, че *P. aibuhitensis*може да подобри качеството на седимента.