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CAPABILITY OF SEA CUCUMBER *Holothuria scabra* TO REMOVE NITROGEN AND PHOSPHOR WASTE FROM SHRIMP PONDS CULTURE

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ABSTRACT

Solid organic waste (PSW) in shrimp ponds contains relatively high levels of nitrogen and phosphorus and can endanger the ecological balance of the waters. This study evaluates the ability of sea cucumber *Holothuria scabra* to remove nitrogen and phosphorus loads from shrimp pond sediment waste in water and sediment. Sea cucumbers were reared for 40 days with a density of 20 individuals/m^2 (average body weight 2.65±0.09 g) and a double-bottom recirculation system. Five levels of PSW accumulation were inserted into the aquarium substrate and were the sole source of nutrition for sea cucumbers without additional feeding: 10%, 20%, 30%, 40%, and 50% (with three replications). The results showed that increasing the PSW content in the aquarium research substrate significantly increased the substrate's TOC, TN, and TP content and increased the concentrations of TOM, DOC, NH_3, NO_2, and PO_4 in the water column. The activity of sea cucumbers in utilizing PSW nutrients in all treatments up to a PSW level of 50% significantly reduced TOC, TN, and TP in sediments. This activity also substantially removes the concentration of TOM, DOC, NH_3, NO_2, and PO_4 in the water. It is estimated that every kilogram of *H. scabra* can remove up to 12.65-12.73 g of nitrogen/day and 2.57-2.60 g of phosphorus/day contained in the solid organic waste of shrimp ponds. Therefore, this study concluded that *H. scabra* has great potential to be used as integrated multi-trophic aquaculture (IMTA) species, especially to remove nitrogen and phosphorus loads from shrimp pond sediment waste in waters.

KEYWORDS: *Holothuria scabra*; nutrients removal; phosphorus; solid organic waste

INTRODUCTION

Organic sludge waste, also known as pond solid organic waste after settling at the bottom of the pond, in semi-intensive or intensive shrimp farming activities has been widely highlighted by the public because it endangers the ecological balance of water (Hatje *et al*., 2016). The accumulation of this waste has systematically and negatively impacted the health of aquatic organisms, cultivated fish and shrimp, and various wild fish living in the surrounding public water. Organic sludge waste will settle to the bottom of a pond; thus, it triggers anaerobic conditions at the bottom of the pond, increases the concentration of ammonia, nitrite, and hydrogen sulfide, providing a suitable medium for bacterial and viral pathogens. When removed from ponds and accumulated in rivers surrounding the pond area, organic waste rich in nitrogen and phosphorus would enhance eutrophication (Mawi *et al*., 2020; Das *et al*., 2018; Yang *et al*., 2017). Algae bloom inhibits oxygen diffusion and increases oxygen demand by decomposer bacteria to decompose the organic waste. Therefore, lack of oxygen in the water may occured (Herbeck *et al*., 2013).

The waste material released by the shrimp is directly affected by the total feed the shrimp gets. It has implications for the volume of pond waste, which exponentially increases with the increasing age of shrimp. Shrimp cannot convert feed into the meat by ±17% (Primavera, 1994), so most feed nutrients are wasted in water. Approximately 3.009 ha of semi-intensive white shrimp ponds in Brazil are estimated to annually release 830 tons of nitrogen waste and

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69.25 tons of phosphorus waste (Lacerda *et al*., 2008). Meanwhile, 2.57 million ha of shrimp ponds in China released 47.700 tons N year^{-1} and 3750 tons P year^{-1} (Yang *et al*., 2017). Moreover, around 15.700 ha of intensive white shrimp ponds in Indonesia is estimated to annually release 82.111 tons of nitrogen waste and 23.236 tons of phosphorus waste (Sabilu *et al*., 2021). Therefore, innovative and integrated shrimp farming with other species to reduce the organic N and P waste such as integrated multi-trophic aquaculture (IMTA) need to be explored.

IMTA emphasizes the utilization of the waste generated by the first cultivated species as a source of nutrition for the second cultivated species (Resuge *et al.,* 2020). IMTA can be developed in parallel patterns or cultivated in different ponds to avoid competition for space (Rusage *et al.,* 2020). This method, known as the module or block system, has been adopted to manage Pacific white shrimp culture waste. Several fish species, such as seaweed, oysters, and milkfish, use mangrove plants to manage shrimp pond waste. These species are important to remove organic carbon, nitrogen, and phosphorus from shrimp pond sediment waste and convert them into biomass (Biswas *et al.,* 2019).

The IMTA study on sea *cucumber Holothuria scabra* was carried out by Bell *et al.* (2007) and employed the co-culture with *Litopennaeus stylirostris*. However, this study was not successful, characterized by high mortality of *H. scabra*. This phenomenon indicated that space competition at the bottom of the pond causes a high mortality rate for sea cucumbers. It is unknown whether other specific interactions, including the likely adverse impact of using drugs contributed to the mortality. The survival and high growth rate of *H. scabra* reared in monoculture using shrimp ponds of *L. stylirostris* has confirmed the suspicion that the sea cucumbers could utilize the accumulation of organic matter released by activities of *L. stylirostris* farm (Bell *et al*. 2007). Our previous study showed that two months old juveniles of *H. scabra* (a density of 20 individuals m^{-2}) that reared for 40 days in a recirculating aquaculture system, without supplementary food had the highest production performance in 40% shrimp pond sediment and 60% beach sand, with growth and survival rates of 0,41 ± 0.05 g.day^{-1} and 70 ± 10%, respectively (Sabilu *et al*., 2020).

Nutrition sources of *H. scabra* are determined by the characteristics of the ingested substrate. They extract organic particulates, detritus, and plankton in the sand or mud that passes through their intestines. *H. scabra* digests the food in the intestine by involving its mucosa and various digestive enzymes, such as protease, maltase, amylase, and esterase (Sembiring *et al*., 2022). Ecologically, the amount of food digested and absorbed from the accumulation of organic matter obtained from the substrate decreases the concentration of nitrogen and phosphorus in the substrate. This process may be facilitated because *H. scabra* feces are composed of coarser sand and organic matter particles and tend to be hollow. *H. scabra* is employed as a bioturbator against nitrogen and phosphorus loads owned by shrimp pond sediment waste. The sea cucumbers are expected to utilize the nutritional components of shrimp pond sediments as the primary energy source for their growth. Meanwhile, knowledge of the capacity of sea cucumbers to remove nitrogen and phosphorus loads from shrimp pond waste is expected to support the efficiency and productivity of the IMTA aquaculture system, especially between sea cucumbers and Pacific white shrimp. This study aims to evaluate the removal ability of *H. scabra* on nitrogen and phosphorus loads possessed by shrimp pond waste in the sediment and water.

MATERIALS AND METHODS

Research location

The research was conducted at the Laboratory of IPB University Fisheries and Marine Observation Station (IFMOS) Ancol, Jakarta, Indonesia, for 40 days, from October to November 2019. Water and sediment samples in the experimental aquarium were measured at day 5, 20 and 35. Chemical analysis of water and sediment samples was carried out at the laboratory of environmental aquaculture FPIK-IPB, Bogor.

Materials and sea cucumber acclimatization

Sediment waste was obtained from intensive Pacific white shrimp ponds in Langensari Village, Blanakan Sub-District, Subang District, West Java, Indonesia. The sea sand used as a substrate was a commercial Bali's beach sand available from aquarium store in Bogor, West Java, Indonesia. Juvenile sea cucumbers aged two months with an average weight of 2.65 ± 0.09 g were obtained from the Hatchery of the Institute for Marine Research and Fishes Extension (IMRAFE), Gondol, Bali. Juvenile sea cucumbers were acclimatized to the research environment using two tanks for 14 days. Salinity was maintained at 30 ppt and a mixture of *Sargassum* and seagrass flour, as much as 10% of sea cucumber biomass, was given daily as feed. The acclimatization study employed a recirculation water tank (150 cm in diameter and 70 cm in height). The bottom of the water tank was filled with sand as a substrate with rigorous aeration.

Research method

The sea cucumbers were reared in 15 aquarium glass units (dimension: 100 × 50 × 50 cm). Sea cucumbers were reared in a double-bottom recirculation system (Figure 1) following Hastuti *et al*. (2022) for 40 days with the same stocking density of 20 individuals/m^2 (equals to 10 individuals/aquarium).

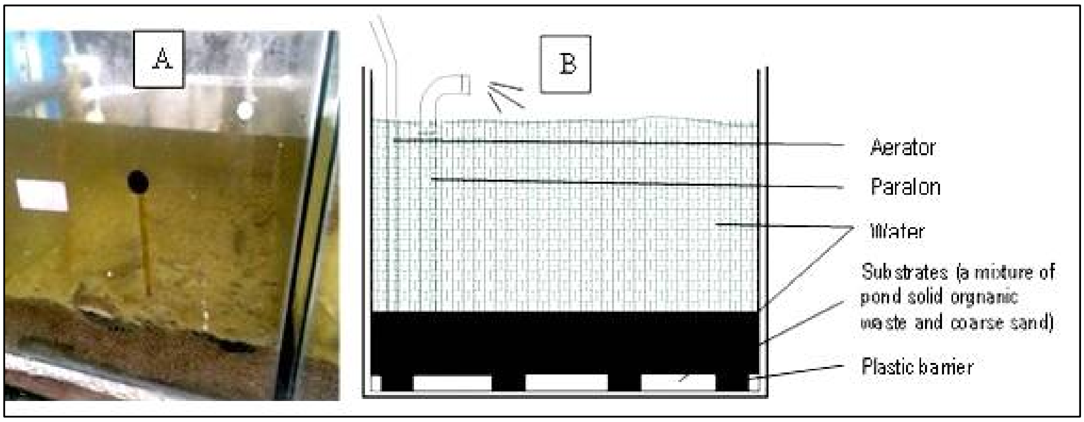

Figure 1. A. Experimental tank with double bottom system; B. Double bottom system scheme applied in experimental tank.

Shrimp pond sediment inserted into the substrate was the sole source of nutrition for sea cucumbers without additional feeding. The study used a completely randomized design (CRD) consisting of five levels of shrimp pond solid sediment accumulation: 10% (PSW10), 20% (PSW20), 30% (PSW30), 40% (PSW40), and 50% of the total weight of the substrate (PSW50). Each treatment consisted of three replicates.

Dissolved nutrient analysis

The removal patterns of total organic matter (TOM), dissolved organic carbon (DOC), total ammonia nitrogen (TAN), nitrite, and orthophosphate in water were periodically analyzed. All water samples were analyzed in the laboratory of Environmental Aquaculture FPIK-IPB, Bogor, by following the standard method (APHA, 2017).

Sediment nutrient analysis

The removal patterns of total organic carbon (TOC), total nitrogen (TN), total phosphorus (TP), and organic matter (OM) in sediment were evaluated by periodic observations. The initial study showed that the addition of pond sediment waste into the system deteriorated water quality and stressed the sea cucumbers, therefore, the initial measurement was taken at day 5 of the culture trial. The TOC analysis referred to Walkley and Black method (Horwitz, 2010). Meanwhile, the sedimentary TN referred to the Kjeldahl procedure (APHA, 2017), and the sedimentary TP referred to the ammonium molybdate method with a spectrophotometer (APHA, 2017).

Statistical analysis

Differences in the concentration of chemical parameters included TOC, TN, TP, OM, ammonia, nitrite, orthophosphate, TOM, DOC, and TAN in the five research treatments were analyzed using the ANOVA and Duncan's test. Whereas the amount of removable concentration of each chemical parameter by sea cucumbers was determined by calculating the difference between the initial and final concentrations in each chemical parameter measured. The significance of chemical parameter removal by sea cucumbers was examined using the repeated measures ANOVA test. In this test, if the assumption of similarity of variance had not met (sig. Shapiro-Wilk <0.05), the significance of the omitted variables was tested with nonparametric statistics using the Friedman test and continued with the Wilcoxon test. A significance level of 95% ($\alpha = 0.05$) was set for all tests.

RESULTS AND DISCUSSION

Dissolved nutrients

Total organic matter (TOM)

This study revealed that the TOM concentration in PSW10, PSW20, and PSW30 aquariums during the sea cucumber rearing period not significantly increased until day 20. After that, the concentration of TOM in the three treatments decreased significantly on day 35. TOM concentration in PSW40 and PSW50 aquariums continued to decline from the initial TOM concentration to the end of the study (Figure 2).

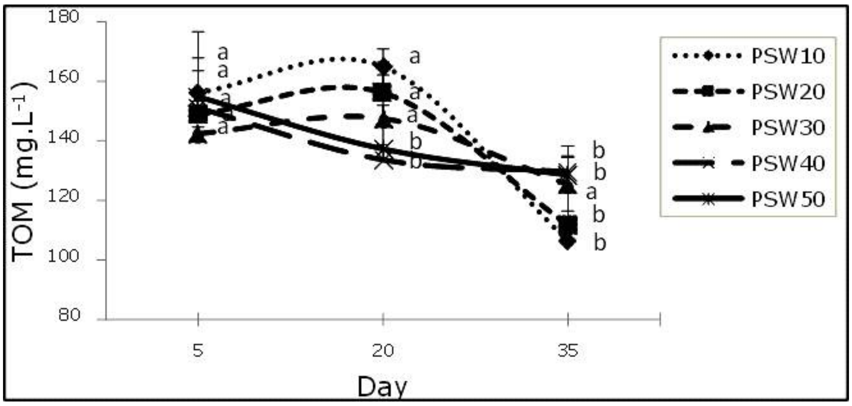

Figure 2. TOM removal capacity by *H. scabra* at five accumulation levels of shrimp pond solid waste (PSW), Different superscript letters at three-time intervals measurement showing significantly different concentrations ($p<0.05$).

Dissolved organic carbon (DOC)

The DOC concentration decreased significantly until the completion of this study ($p<0.05$). The most significant decrease in DOC values was in PSW50 treatment at 25.39 ± 1.04 mg L^{-1}. Whereas the smallest decline was found in PSW20 treatment at 19.91 ± 0.85 mg L^{-1} (Figure 3).

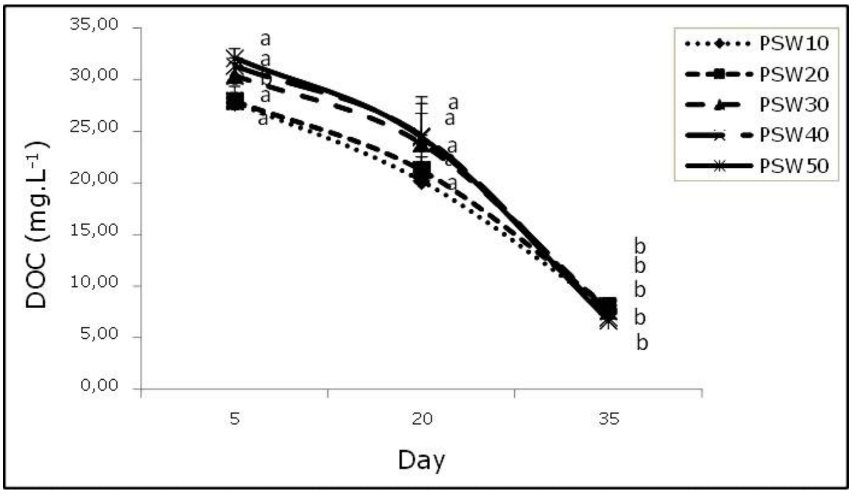

Figure 3. DOC removal capacity by *H. scabra* at five accumulation levels of shrimp pond solid waste (PSW), Different superscript letters at three-time intervals measurement showing significantly different concentrations ($p<0.05$).

Total ammonia nitrogen (TAN)

The concentration of TAN in all treatments until the end of the study decreased significantly ($p<0.05$). The most significant decrease was in PSW50: 0.76 ± 0.11 mg L^{1}, and the smallest was in PSW30: 0.54 ± 0.02 mg L^{-1}. TAN concentrations in the initial period of all treatments ranged from 1.12 ± 0.04 to 1.33 ± 0.01 mg L^{-1}. TAN concentrations at the end of the study ranged from 0.40 ± 0.01 to 0.61 ± 0.02 mg L^{-1} (Figure 4).

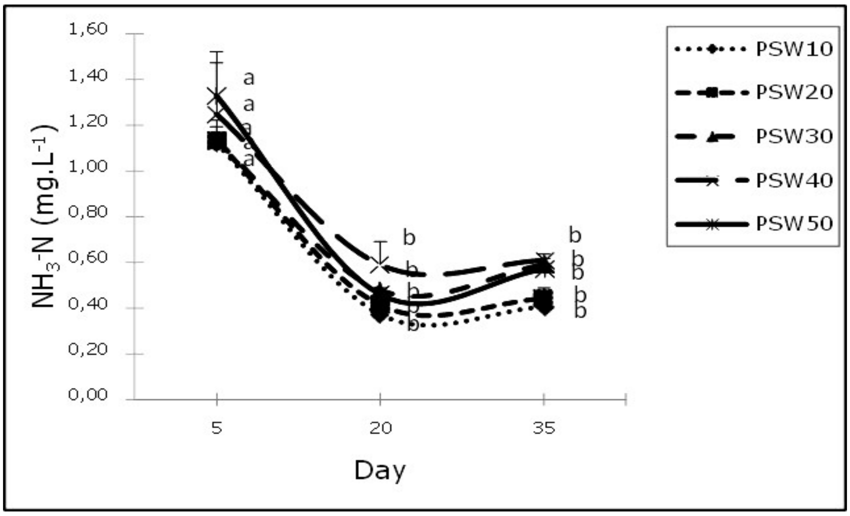

Figure 4. TAN (NH_3-N) removal capacity by *H. scabra* at five accumulation levels of shrimp pond solid waste (PSW), Different superscript letters at three-time intervals measurement showing significantly different concentrations ($p < 0.05$).

Total ammonia nitrogen (TAN)

The concentration of TAN in all treatments until the end of the study decreased significantly ($p<0.05$). The most significant decrease in TAN was in PSW50: 0.76±0.11 mg L^1, and the smallest was in PSW30: 0.54±0.02 mg L^{-1}. TAN concentrations in the initial period of all treatments ranged from 1.12±0.04 to 1.33±0.01 mg L^{-1}. Meanwhile, TAN concentrations at the end of the study ranged from 0.40±0.01 to 0.61±0.02 mg L^{-1} (Figure 4).

Nitrite (NO_2-N)

Nitrite concentration of all treatments until day 20 did not increase significantly compared to the measurement on day 5, ranging from 0.04±0.03 to 0.34±0.06 mg L^{-1}. In the final phase of the study (day 35), the nitrite concentration decreased. The most significant decrease is found in PSW40 at 0.35±0.023 mg L^{-1}. The smallest decline was found in PSW20 of 0.19±0.02 mg L^{-1} ($p<0.05$) (Figure 5).

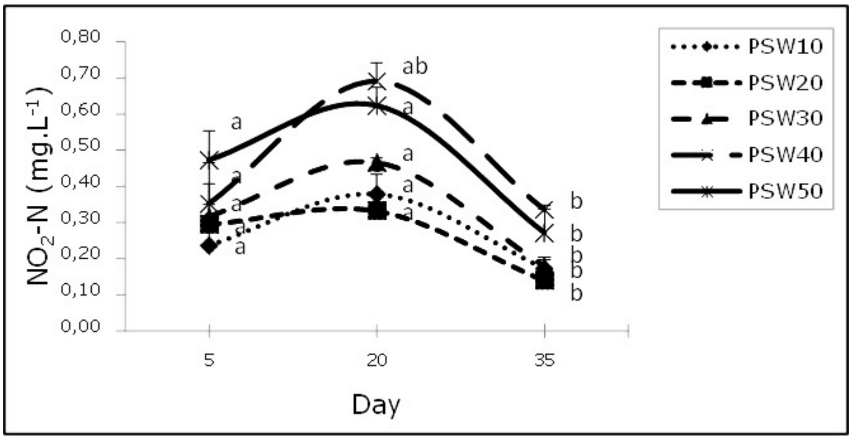

Figure 5. Nitrite (NO_2-N) removal capacity by *H. scabra* at five accumulation levels of shrimp pond solid waste (PSW), different superscript letters at three-time intervals measurement showing significantly different concentrations ($p < 0.05$).

Orthophosphate (PO_4-P)

Orthophosphate in all treatments decreased significantly on day 35 than on day 20, ranging from 0.12±0.02 to 1.30±0.01 mg L^{-1}. In the early phase of this research, the orthophosphate data could not be measured due to laboratory procedure errors (Figure 6).

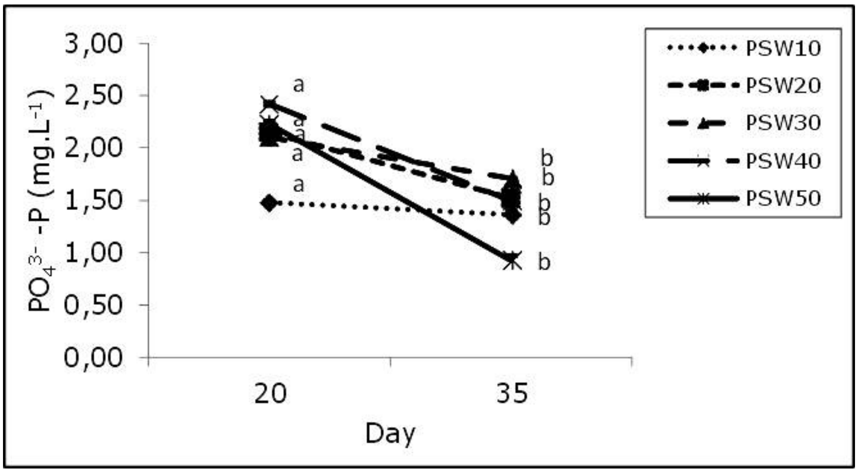

Figure 6. Orthophosphate (PO_4^{-3}-P) removal capacity by *H. scabra* at five accumulation levels of shrimp pond solid waste (PSW), different superscript letters at three-time intervals measurement showing significantly different concentrations ($p < 0.05$).

Different amounts of solid organic waste loaded into the substrate significantly impacts different content of TOC, TN, and TP in the sediment as well as the concentrations of TOM, DOC, ammonia, nitrite, and orthophosphate in water. In the first five days of this study, the negative effect of the accumulation of shrimp pond waste on water quality is shown by high concentrations of TOC, TN, and TP in the sediment (Table 1) as well as DOC, TAN, nitrite, and orthophosphate in water. The interaction between the nutrient components of pond sediment waste loaded into the substrate with the water component above it and microbial decomposition activities on the organic ingredients of the waste encouraged the diffusion of sedimentary nutrients into the water column (Hatje *et al*., 2016). In addition, the reversal of water mass by the double-bottom recirculation system was expected to significantly impact the movement of nutrient particles from the sediment to the aquarium water. It, in turn, influences the conditions of the chemical parameters of the water, which changes or becomes unstable, decreases the water clarity, reduces the solubility of oxygen, and improves the concentration of TOM, ammonia, nitrite, DOC, and orthophosphate. The discharge of the shrimp pond is an anthropogenic waste source that changes the carbon, nitrogen, phosphor, and other nutrient stocks on the public water substrate (Tian *et al.,* 2019). Also, TOM and TOC concentrations in co-culture of *Fenneropenaeus chinensis* and *Apostichopus japonicus*, that reared for six months, showed that in the first three months period (May-August), TOM concentrations increased. After three months (August-November), TOM concentration decreased. These results indicated that sediment bioturbation and shrimp feces production can be a natural food for sea cucumbers *A. japonicus* (Zhou *et al.,* 2017).

TOM, TAN, DOC, nitrite, and orthophosphate profiles in the three measurement periods are follow-up effects of the removal of nutrients from shrimp pond waste in sediments by *H. scabra*, which absorbs nutrients in pond sediment waste and converts the sediment waste into biomass (Sabilu *et al*., 2020). The input of shrimp pond sediment waste made the TOM in the initial phase of the study higher. TOM in PSW10, PSW20, and PSW30 treatments increased on day 20 and was influenced by the increase in the suspended fraction and the decrease in water clarity. Treatment with less input of shrimp pond sediment waste contained less clay fraction in the substrate so that the ability of PSW 10, 20, and SPW30 to bind dissolved and suspended organic matter is lower compared to higher amount of pond sediment waste. The same pattern occured with nitrite concentration, which became higher until day 20. When the organic matter is widely available, the brightness of the water decreases, and the oxygen solubility automatically decreases or tends to be anaerobic. Such conditions enhanced the abundance of *Nitrosomonas* bacteria, further encouraging the oxidation rate of ammonia to nitrite then the oxidation of nitrite to nitrate (Preena *et al*., 2021; Zhang *et al*., 2015). The TOM removal at the end of study strongly influenced by the amount of carbon, nitrogen, and phosphorus in the sediment because it is converted into sea cucumber biomass, as shown by Sabilu *et al.* (2020).

Nutrient sediments

The results of nutrient removal data in the sediment include OM, TOC, TN, and TP for each treatment are presented in Table 1.

Table 1. Nutrient removal of OM, TOC, TN, and TP in the sediment by *H. scabra* at five accumulation levels of shrimp pond solid waste (PSW)

Variables	Treatment	Concentration changes in the sediment nutrients, day- (%)				Nutrient removal (%)
		Initial	5	20	35	
OM	PSW10	7.99[a]	5.81±0.182[a]	4.50±0.365[a]	3.39±0.189[a]	4.59±0.189*
	PSW20	15.99[b]	6.74±0.062[b]	5.41±0.281[b]	3.85±0.113[a]	12.11±0.113*
	PSW30	23.98[c]	7.39±0.296[c]	5.47±0.203[b]	5.14±0.258[b]	18.81±0.258*
	PSW40	31.98[d]	10.23±0.22[d]	8.08±0.135[c]	6.78±0.136[c]	25.14±0.136*
	PSW50	39.97[e]	13.13±0.076[e]	8.08±0.312[c]	6.61±0.286[c]	33.29±0.286*
TOC	PSW10	6.37[a]	5.20±0.064[a]	4.40±0.362[a]	2.58±0.161[a]	3.79±0.160*
	PSW20	12.74[b]	5.57±0.111[ab]	5.21±0.281[ab]	3.69±0.114[b]	9.05±0.114*
	PSW30	19.11[c]	5.84±0.290[b]	5.32±0.197[b]	5.02±0.257[c]	14.09±0.257*
	PSW40	25.48[d]	7.26±0.151[c]	6.88±0.139[c]	6.66±0.134[d]	18.82±0.134*
	PSW50	31.85[e]	9.86±0.077[d]	6.90±0.315[c]	6.46±0.297[d]	25.39±0.297*
TN	PSW10	1.34[a]	0.47±0.033[a]	0.08±0.003[a]	0.08± 0.01[a]	1.26±0.011*
	PSW20	2.68[b]	1.10±0.058[b]	0.18±0.003[d]	0.15±0.003[c]	2.53±0.003*
	PSW30	4.02[c]	1.37±0.093[b]	0.12±0.003[b]	0.11±0.02[bc]	3.91±0.02*
	PSW40	5.36[d]	2.87±0.186[c]	1.18±0.003[d]	0.10±0.003[ab]	5.26±0.003*
	PSW50	6.70[e]	3.07±0.067[c]	1.15±0.003[c]	0.13±0.013[bc]	6.57±0.014*
TP	PSW10	0.27[a]	0.028±0.001[a]	0.016±0.001[a]	0.004±0.0001[a]	0.27±0.0002*
	PSW20	0.54[b]	0.063±0.009[b]	0.025±0.001[ab]	0.009±0.0001[b]	0.53±0.0002*
	PSW30	0.81[c]	0.082±0.001[c]	0.031±0.007[b]	0.008±0.0001[c]	0.80±0.00003*
	PSW40	1.08[d]	0.101±0.001[d]	0.027±0.002[b]	0.011±0.0002[d]	1.07±0.0001*
	PSW50	1.35[e]	0.139±0.002[e]	0.028±0.0003[b]	0.011±0.0002[d]	1.34±0.0002*

Description: The concentration value represents the means ± standard deviation (n=3). Different superscript letters in the same column for each parameter showing significantly different responses (Duncan's Test with $p < 0.05$). *) Significant effect of nutrient removal by sea cucumbers (repeated-measures-ANOVA test and Friedman test with $p < 0.05$)

This study examines the ability of sea cucumbers to remove nutrients from shrimp pond waste, especially chemical compounds derived from nitrogen and phosphorus generated by heaps of solid waste from semi-intensive white shrimp aquaculture. The results of this study denote that *H. scabra* can significantly remove nitrogen and phosphorus from shrimp pond sediment waste in the media (Sabilu *et al.,* 2020).

The OM, TOC, TN, and TP contents of the sediments decreased significantly after five days, and their numbers declined considerably until the end of this study ($p<0.05$). Sea cucumbers substrate with a higher amount of shrimp pond sediment showed higher decrease in OM, TOC, TN, and TP content than lower shrimp sediment waste concentration (Table 1). The biomass of sea cucumbers increases when they are kept in the aquarium, while, at the same time, the number of OM, TOC, TN, and TP in the sediment decreases (Table 1). Finally, this study has revealed the amount of nutrient removal from shrimp pond waste in the substrate of five treatments; they are an organic matter of 4.59-33.29%, organic carbon of 3.79-25.39 mg L^{-1}, the nitrogen of 1.26-6.57%, and phosphorus of 0.26-1.34%. When the accumulation level of PSW in the substrate was higher, the capacity of sea cucumbers to remove PSW nutrients until the end of the study was greater (Table 1). The comparison of the sediment nitrogen concentration (TN) in the five treatments on day 20 has revealed that the five treatments have significantly different sediment nitrogen content. This finding indicates that the nitrogen accumulation level still influences nitrogen availability in the sediment until day 20. This condition is different from the nitrogen concentration on day 35. The sediment nitrogen content of PSW 30, PSW 40, and PSW50 treatments on day 35 was not significantly different, indicating that sea cucumbers have effectively promoted nitrogen removal in the sediment. The phosphorus content of PSW20, PSW30, PSW40, and PSW50 was not significantly different (p

> 0.05) with the highest accumulation level was found on day 20. This finding indicates that sea cucumbers more significantly accelerated the removal of phosphorus than of nitrogen. When organic matter was abundantly available in the sediments, the nutrient removal activity of sea cucumbers continued to obtain energy (Slater & Jeffs, 2010; Bell *et al.,* 2007).

The results of this study also support the use of sea cucumbers as a biological agent to mitigate the negative impacts of the accumulation of sediment waste from shrimp ponds in public water. *H. scabra* can convert PSW nutrients to body weight. Moreover, their body weight increases by 16.41 ± 1.97 g after being reared for 40 days with a growth rate of 0.41 ± 0.05 g/day (Sabilu *et al.,* 2020).

CONCLUSION

This recent study successfully demonstrated the use of *H. scabra* to control the accumulation of shrimp pond solid waste and maintain optimal water quality parameters in research aquariums. *H. scabra* effectively removed nitrogen and phosphorus loads from shrimp pond sediment waste in water and sediment. Based on the conditions of this study, *H. scabra* whose substrate was injected with PSW with a higher level of accumulation (50% PSW) resulted in a decrease in the contents of TOC, TN, and TP. The activity of sea cucumbers utilizing PSW nutrients up to 50% PSW accumulation rate significantly reduced nitrogen and phosphorus in sediments. In addition, this utilization greatly reduces the concentration of TOM, DOC, NH_3, NO_2, and PO_4 in water.

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Declaration of competing interest

The authors declare that they have no conflict of interest.

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