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Bioremediation of Organically Enriched Sediment under Green Mussel Rafts Using Spionids Genus *Prionospio* (Polychaeta: Spionidae)

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Abstract

Spionid polychaetes, *Prionospio* are one of the dominant polychaetes in the organically enriched sediment of Sriracha Bay, Chonburi, Eastern coastline of Thailand. This study aims at the bioremediation of organically enriched sediment under green mussel rafts by two spionid polychaetes, *Prionospio (Prionospio) membranacea* and *Prionospio (Minuspio) pulchra.* The biomass increment from the two spionids in the bioremediation process revealed that *P. (P.) membranacea* was significantly more efficient in converting the organic waste to biomass in term of body weight than *P. (M.) pulchra.* The efficiency of the two polychaetes in the reduction of total organic matter in the sediment was not significantly different. The potential in bioremediation of organic matter in sediment by the two spionids was better than other polychaetes used in the bioremediation of the organic waste from aquaculture system. This study showed that spionid polychaetes *P. (P.) membranacea,* had the potential for future study as bioremediator species of organically enriched sediment from aquaculture system.

Keywords: bioremediation; organically enriched sediment; green mussel raft; *Prionospio*

1. Introduction

Mussel aquaculture in marine waters has dramaticly increased worldwide. The mussel culture required a fairly productive coastal environment with an adequate production of phytoplankton. This provides benefit of no artificial feed requirement (Grant *et al*., 1995; Christensen *et al*., 2003; da Costa *et al*., 2006; Carlsson *et al*., 2009). Mussel culture is known to have severe local environmental impact due to the increased in sedimentation of biodeposits (feces and pseudofeces) contributing to organic enrichment in the sediment inside the farming area. In some cultures, where currents are not strong enough to transport this material out of the area, the depth of the oxygenated layer of the sediment decreased and bottom oxygen may be depleted, leading to anoxia of the sediment and the overlying water. The aggregation of mussels regulated nutrient fluxes, sedimentation and primary production in the coastal ecosystems (Kaspar *et al*., 1985; Grant *et al*., 1995; Christensen *et al*., 2003; Carlsson *et al*., 2009). Tantanasarit and Bable (2014) reported that the removal rate of carbon, nitrogen and phosphorus by green mussel (*Perna viridis*) were 3302, 380 and 124 mg/year/ind., respectively. Nutrient released, calculated as carbon, nitrogen and phosphorus from mussel faeces were 621, 135, 13 mg/month/ind. This organic enrichment could cause dramatic changes in the benthic community structure, reducing the species to only few tolerant species, lowering both the diversity and community richness (Pearson and Resenberg, 1978). Changes in benthic communities under the mussel farms has been extensively documented (Kaspar *et al*., 1985; Grant *et al*., 1995; Stenton-Dozey *et al*., 1999; Christensen *et al*., 2003; da Costa *et al*., 2006). The macrofauna were found to dominate numerically by opportunistic species, mostly polychaetes. Opportunistic polychaetes, such as *Capitella* spp. in the Family Capitellidae and *Prionospio* spp. in the Family Spionidae, were usually small, short-lived, prolific and capable of exploiting the organically enriched sediment (Pearson and Rosenberg, 1978; Dauer, 1993; Diaz and Rosenberg, 1995; Borja *et al*., 2000)

Many polychaete species were used in the bioremediation process in the integrated aquaculture system and in the coastal area. These polychaete species have simple life histories. They are resistant to toxicity and have the ability to generate an economic return following remediation activities such as polychaetes in the Family Nereididae. Polychaete sand filters were usually integrated in to the prawn and fish farming systems to simultaneously remediate wastewater and produce harvestable polychaete biomass without supplemental feeding (Palmer, 2010; 2011; Brown *et al*., 2011; Van Bruggen, 2012). Not only does this polychaetes are used as bait for sport and professional fishing but they are also considered as a food source in aquaculture. Polychaete worms have been identified as a source of essential fatty acids and have an important role in the development of the gonads (Fidalgo e Costa *et al*., 2000; 2003). Capitellid polychaete, *Capitella* spp., small deposit feeders, have been successfully used as the bioremediator of organic waste from fish farms in Southern Japan (Tsutsumi *et al*., 1990; 2002; 2005; Chareonpanich *et al*., 1994). The techniques of introducing the artificially mass culture of *Capitella* sp.I on the organically enriched sediment below a net pen in the fish farm were developed from these periods. Kinoshita *et al*. (2008) has applied the same method of bioremediation of organically enriched sediment deposited below fish farms in enclosed bay in Japan. They were able to demonstrate that this method of applying mass-culture technique of deposit-feeding polychaete *Capitella* sp.I was promising to enhance the decomposition rate of organic matter markedly in organically enriched sediment below fish farms.

The coastal area of Sriracha Bay, Chonburi Province, eastern coast of the Gulf of Thailand, is one of the important areas for green mussel (*Perna viridis*) rafts culture. Organic enriched sediment in this bay resulted from mussel rafts culture and the domestic effluents from the municipal town of Sriracha. Spionid polychaetes, *Prionospio* are one of the dominant polychaetes in the organically enriched areas of Sriracha Bay (To-orn and Intarachart, 2010). These spionids are similar to the capitellids which were abundantly found in high organic polluted areas with low dissolved oxygen and high sediment sulfide content. They can tolerate these extreme conditions which enables them to rapidly colonize the areas with high levels of organic matters, due to their adaptation in feeding and diverse patterns of reproduction and development (Fauchald and Jumars, 1979; Blake and Arnofsky, 1999; Borja *et al*., 2000). Two dominant spionids, *Prionospio* (*Prionospio*) *membranacea* Imajima, 1990 and *Prionospio* (*Minuspio*) *pulchra* Imajima, 1990, were found in the area. The former species was found 13-655 ind./m^2 while the latter species was found less in the density of 0-68 ind./m^2.

As the bioremediation of organically enriched sediment from fish farms by the deposit feeder polychaete, *Capitella* have been proven successfully. This present study aims to assess the possibility of using two interface deposit feeding polychaetes, *P. (P.) membranacea* and *P. (M.) pulchra* to remediate organically enriched sediment from green mussel rafts. The results can be used as the guideline in the application of spionid polychaetes in the bioremediation of organic enriched sediment from aquaculture system.

2. Materials and Methods

2.1 Collection of polychaetes and sediments

The spionid polychaetes were collected from October to November 2013 from Sriracha Bay, Chonburi Province (13°11′N, 100°55′E), on the east coast of the Gulf of Thailand (Fig. 1). Adult specimens of two spionid species were collected from an area of high organic content (4.66-6.08% TOC) near Sriracha Town Municipality under the influence of domestic waste effluents.

Samples were taken by Ekman grab and washed through a 0.25 mm mesh sieve. Specimens were transported afterward to the laboratory for sorting and identify. The sediment for the experiment was collected from under the floating mussel rafts in the bay using Ekman grab. Each sediment sample was sieved through a 1.0 mm mesh and freeze in the laboratory at least for 24 h to kill living organisms. The seawater from the mussel rafts was filtered through a 1.0 micrometer filter bag for the experimental use.

2.2 Preparation of experimental animals and sediments

In the laboratory, spionid specimens were sorted under a stereomicroscope. The spionid polychaetes, *P. (P.) membranacea* and *P. (M.) pulchra* were identified following the procedures of Imajima (1990a; 1990b). The distinct characteristics of spionids, *P. (P.) membranacea* and *P. (M.) pulchra* are shown in Fig. 2. *P. (P.) membranacea* has four pairs of branchiae on chaetigers 2-5: pairs 1 and 4 are pinnate with digitiform pinnules; and pairs 2 and 3 are apinnate. *P. (M.) pulchra* has 9 pairs of apinnate cylindrical branchiae located from chaetiger 2.

Both spionids were acclimated in aquaria, which added the treated sediment and filtered seawater. The aquaria was mildly aerated. The acclimated polychaetes were individually measured for the body size to estimated the body weight at the beginning of the experiment. The body length and the width of the 5th chaetiger (including the parapodia) of immature specimen were also measured under a stereomicroscope with an ocular micrometer and expressed as body size. The numbers of chaetigers in each worm were counted. Worms were weights to determine the wet weight. Total organic matter in the sediment was determined by the loss on ignition (Nelson and Sommers, 1982) prior to the experiment.

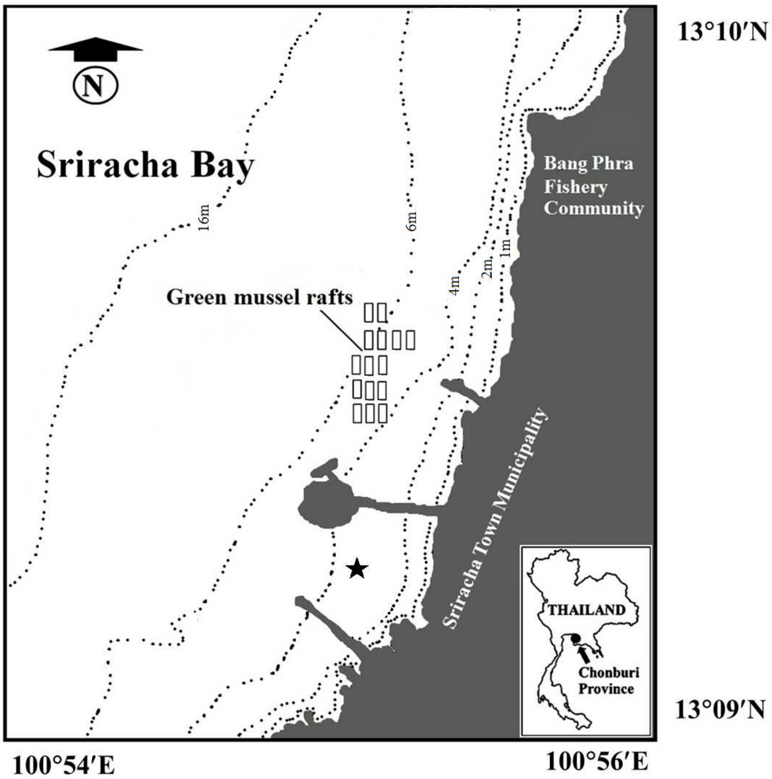

Figure 1. Study area, Sriracha Bay, Chonburi Province, the eastern coastline of Thailand
★-sampling for spionid polychaetes

2.3 Studies on bioremediation of organically enriched sediment by spionid polychaetes

The 12 cultured boxes (20x25x15 cm) were filled with treated sediment at the level of 3 cm from the bottom and they were filled with the filtered seawater at the level of 10 cm above the surface sediment. The filtered seawater in each box was gently aerated. Spionids, *P. (P.) membranacea* and *P. (M.) pulchra* were then added to the cultured boxes in three different treatments: (1) control (without spionid polychaetes), (2) 10 individuals of spionids (normal density based on the average density of the spionid, *P. (P.) membranacea* from high organic content area and the mussel rafts area in Sriracha Bay, 200 ind./m^2 observed by To-orn and Intarachart (2010) and (3) 20 individuals of spionids (twice the normal density, 400 ind./m^2). For each treatment, four cultured boxes were used as replicates. The experiments were carried out for 30 days.

2.4 Assessment of potential spionid polychaetes in bioremediation

At the end of experiment (30 days from the start), the sediment from each cultured boxes were taken by using a plastic hand core with an area of 0.55 cm^2. The sediment was sliced at every 1 cm depth from surface into three layers (0-1, 1-2 and 2-3 cm in depth) for the analysis of total organic content by Ignition loss (Nelson and Sommers, 1982). After that, the remaining sediment in each cultured boxes were washed through a 0.25 mm mesh sieve for the biomass study of the spionid polychaetes. The body length and the body size (width of the 5^{th} chaetiger, excluding parapodia), and the number of chaetigers of the spionds were measured and counted by using an ocular micrometer. The wet weights of the spionids were measured.

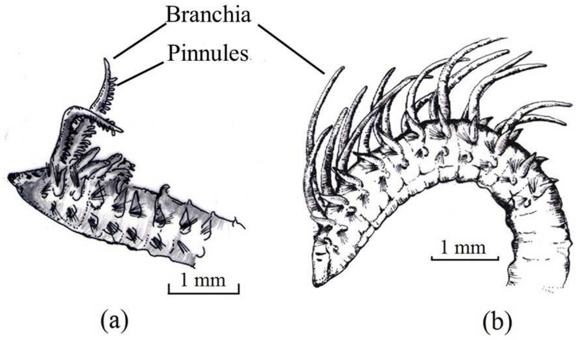

Figure 2. Spionid polychaetes (a) *P. (P.) membranacea* and (b) *P. (M.) pulchra*.

Products in term of the increased of polychaetes biomass and the ability to reduce the organic matter in the enriched sediment from mussel raft culture were assessed as the bioremediation efficiency of the spionid polychaetes. The increase of the polychaetes biomass in percentage were calculated from the differences of body length, body size, number of chaetiger and body wet weight between the initial phase and the final phase of the experiments. The bioremediation efficiency of spionid polychaetes were enumerated from the percentage of total organic matter reduction from the initial phase when compared to the final phase of the experiments.

2.5 Statistical analysis

One-way analysis of variance (ANOVA) at the 95% confidence level ($p<0.05$) was applied to determine significant differences of spionid polychaetes biomass and total organic matter in sediment between treatments. The biomass increment and the total organic matter reduction rate in sediment between the initial and final phases of the experiment, and among three layers between two spiond species were compared using a *t*-test (Zar, 1999).

3. Results and Discussion

3.1 Increment in biomass in spionid polychaetes

The average body length, body size and number of chaetigers of *P. (P.) membranacea* between the initial and final phases of the experiment were significantly different ($p<0.05$). The average body length, body size and number of chaetigers of this spionid after 30 days of the experiment were higher than those in the initial phase about 1.76, 1.27 and 1.34 times in experimental treatment of 10 individuals. The body weight showed the same pattern of increase at the end of the experimental with final body weight was 2.34 times comparing to the initial phase. In the high density treatment of 20 individuals, the lower rate of increased biomass was observed ($p<0.05$). The average body length, body size and the number of chaetigers in the spionid after 30 days of experiment were 1.60, 1.17 and 1.28 times the initial phase, while the final body weight of the polychaetes in the high density treatment was only 1.76 times the initial weight (Table 1).

The biomass increment in *P. (M.) pulchra* in the bioremediation experiments also showed the similar trends as in *P. (P.) membranacea*. The growth in term of average body weight, average body size and the number of chaetigers in the normal density treatment of 10 individuals after 30 days were 1.79, 1.31 and 1.32 times the initial values. The final body weight was 1.85 times the initial weight. Low growth also observed in the high density treatment of 20 individuals ($p<0.05$). The spionid growth was significantly different ($p<0.05$) between the treatments. The average body weight, average body size and the number of chaetigers after the 30 days in the high density of 20 individuals were 1.57, 1.18 and 1.25 times the initial values. The final body weight was 1.17 times the initial weight.

The rate of biomass increment in term of average body length, average body size and the number of chaetigers in the two spionids were not significantly different. However the increment growth rate in term of increased body weight in both treatments of 10 and 20 individuals of *P. (P.) membranacea* were significantly higher ($p<0.05$) than *P. (M.) pulchra* as in Fig. 3.

Table 1. Increment in biomass of spionid polychaetes, *P. (P.) membranacea* and *P. (M.) pulchra* in the bioremediation of enriched sediment under the mussel rafts from Sriracha Bay, Chonburi Province, eastern coast of Thailand

	10 individuals		20 individuals	
	Total increased biomass (30 days)	Increment growth rate per day	Total increased biomass (30 days)	Increment growth rate per day
• *P. (P.) membranacea*				
Body length (mm)	5.920±1.072	0.020±0.035	4.676±1.057	0.156±0.035
Body size (mm)	0.094±0.021	0.003±0.001	0.061±0.030	0.002±0.001
Number of chaetiger (chaetiger)	17.500±1.000	0.583±0.033	14.750±2.217	0.492±0.073
Body wet weight (g)	0.00110±0.00025	0.00004±0.00001	0.00070±0.00032	0.00002±0.00001
• *P. (M.) pulchra*				
Body length (mm)	5.820±0.805	0.194±0.294	4.155±0.294	0.139±0.009
Body size (mm)	0.093±0.009	0.003±0.009	0.053±0.009	0.002±0.001
Number of chaetiger (chaetiger)	17.250±0.957	0.575±2.629	12.750±2.630	0.425±0.087
Body weight (g)	0.00070±0.00013	0.00003±0.00005	0.00010±0.00005	0.000004±0.00001

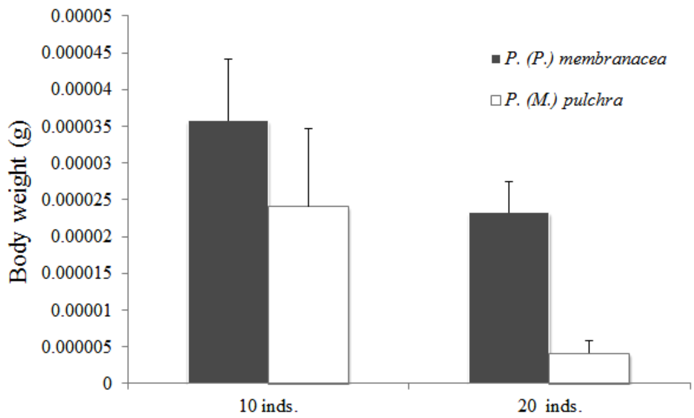

Figure 3. Increment growth rate (g wet weight per a day) of spionid polychaetes, *P. (P.) membranacea* and *P. (M.) pulchra* in the bioremediation of enriched sediment under the mussel rafts from Sriracha Bay, Chonburi Province, eastern coast of Thailand.

The biomass increment in the two spionid polychaetes, *P. (P.) membranacea* and *P. (M.) pulchra* in the bioremediation process revealed that *P. (P.) membranacea* was more efficient in converting the organic waste from the enriched sediment to biomass than *P. (M.) pulchra* (Fig. 3). It was noted that in the normal density treatment, *P. (P.) membranacea* was observed with oocytes. As stated, *P. (P.) membranacea*, the dominant spionid polychaetes, widely distributed in Sriracha Bay in particular near the Sriracha Municipality and mussel raft culture. *P. (M.) pulchra* can also be found in the same area but in low density.

Spionid polychaetes help stabilizing and reworking the surface layers of soft mud and sand bottom through their feeding and burrowing activities (Blake, 1996). These biological activities enhance the bioremediation process in the enriched sediment. Increased biomass in spionids indicated that organic matter in the sediment was consumed as food. Fauchald and Jumars (1979) reported that spionids were generally considered as the surface deposit feeders. Dauer *et al*. (1981) further proposed that spionid polychaetes were interface-feeders species that utilized particles from the sediment surface. Spionid polychaetes have a pair of grooved, ciliated palps that arise from the peristomium. These structures are used to suspension and resuspended the sediment during the feeding. Palps that came in direct contact with the sediment surface picked up particles in the food groove and transported them by ciliary action to the everted pharynx. When particles were resuspended by a current, the polychaetes changed their palp orientation modes and increased their feeding upon suspended particles. Yokoyama (1988) demonstrated the feeding behavior of *Paraprionospio* sp. (form A) in the laboratory. The worms extended their tentacles and two pairs of branchiae onto the surface of the sediment, while laying in their tubes. Worms fed on the surface of the sediment throughout the day using a pair of ciliated tentacles. As a result of feeding, a circular area appeared on the surface of the sediment around the opening of the tube. The radius of the feeding scar was approximately equal to the length of the tentacle, which was approximately 30-40% of the body length. As demonstrated from our result, the biomass increment in the normal density treatment was higher than the high density treatment. The density of the spionid polychaetes affected the food supply and the feeding efficiency. This was true in several deposit feeding polychaetes. Miller and Jumars (1986) reported that spionid polychaete *Pseudopolydora kempi japonica* Imajima & Hartman, 1964 in laboratory experiment had the mean value of 1.4 cm for feeding radius (a circular feeding area of 6.2 cm). At population density of more than 0.16 cm^2, the worm feeding area would overlap with the neighboring feeding areas. Zajac (1986) found that the differences in intra-specific density and food supply in laboratory experiments of the spionid polychaete *Polydora ligni* Webster, 1879 affected the growth and reproduction. In large eunicid polychaete, ragworm *Marphysa sanguinea*, Parandavar and Kim (2014) also found that the density of *M. sanguinea* had impact on the growth. The highest final weight was at the density of 1,000 ind./m^2 and the lowest weight at the density of 4,000 ind./m^2. Van Bruggen (2012) found that the biomass (g dry weight) of *Capitella* sp. that fed on sea bass waste at the density of 100 ind./m^2 had significantly higher biomass than those found at the density of 1,000 and 10,000 ind./m^2.

3.2 Reduction of organic matter in sediment

Comparison on the initial total organic matter in the sediment and the final total organic matter at the end of the bioremediation experiment showed that the two spionid polychaetes had biological activities that would reduce the level of organic matter in the sediment (Fig. 4). The total organic matter in the sediment in each treatment at the start of the experiment were in the range of 7.19±0.70%. No significant changes occurred in the no worm treatment. In *P. (P.) membranacea* experiment, the levels of decreased in total organic matter from the enriched sediment in the three layers of sediment were similar (*p>0.05*). However the decrease of organic matter after 30 days for the treatment of the high density treatment was highest with the reduction rate of 52.48% from the initial total organic matter. The organic reduction rate in the normal density treatment was 38.16% after 30 days.

The reduction of organic matter in the sediment form the bioremediation experiment of *P. (M.) pulchra* also showed the similar trend. No significant differences in the levels of the decreased in total organic matter in the sediment in term of sediment layers. After 30 days, the organic matter reduction rate in the high density treatment was 49.30%, while in the normal density treatment was 37.60%. The ability of the two spionid polychaetes to decrease the level of organic matter in the enriched sediment under the green mussel rafts were similar (*p>0.05*).

Biological activities of deposit feeding polychaetes such as feeding and reworking can apparently promote the decomposition of organic matter in the sediment (Dauer *et al*., 1981; Yokoyama, 1988; Tsutsumi *et al*., 1990; Chareonpanich *et al*., 1994). These spionid polychaetes excreted fecal pellets which were placed outside of their tubes in a neat pile. The bottom currents further eroded and transported fecal pellets that accumulated within the worm's foraging area (Dauer *et al*., 1981). Kinoshita *et al*. (2008) concluded from the application of artificially mass-cultured colonies of a deposit feeding polychaete, *Capitella* sp. as bioremediator of organically enriched sediment deposited below fish farms that the dense culture of *Capitella* sp. increased the decomposition of organic matter. The reworking activities of the sediment by dense patches of *Capitella* including feeding on the subsurface sediment and excreting fecal pellets on the sediment surface as well as burrowing in the sediment, and spouting the subsurface sediment onto the sediment surface had promoted sediment oxidation and provided an oxygenrich environment suitable for aerobic bacteria in the deeper subsurface sediment.

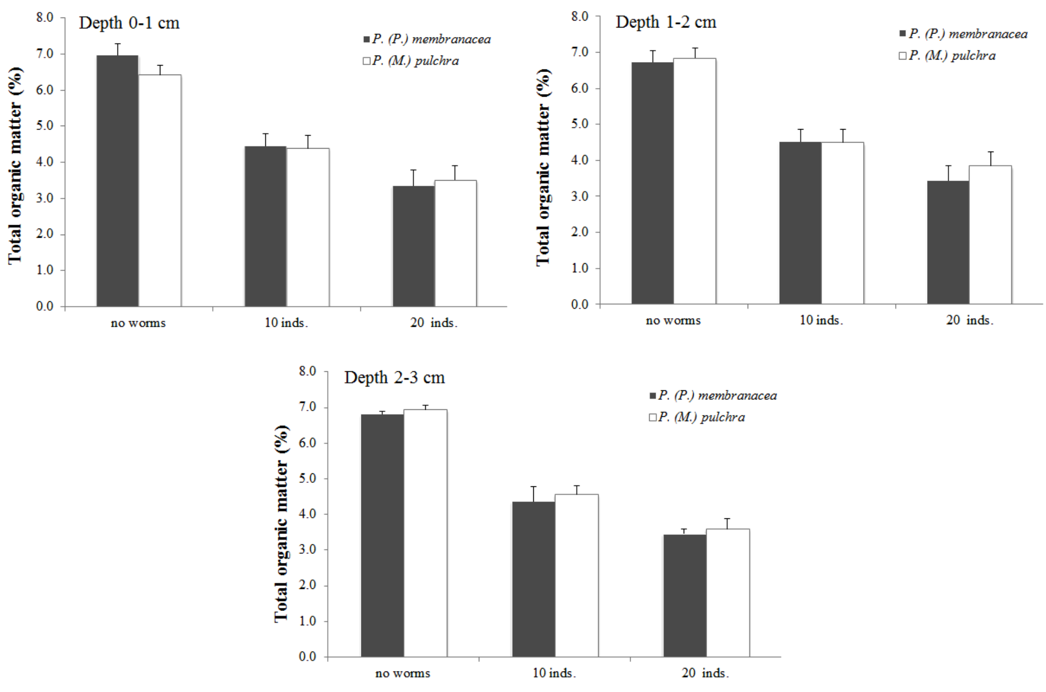

Figure 4. Reduction of total organic matter (%) of three layer of sediment in bioremediation of organically enriched sediment under green mussel rafts by spionid polychaetes, *P. (P.) membranacea* and *P. (M.) pulchra*.

This present study showed the potential of two interface deposit feeding polychaetes, *P. (P.) membranacea* and *P. (M.) pulchra* to remediate the organically enriched sediment from green mussel rafts. The potential in bioremediation of organic matter in sediment of these two spionid polychaetes were high when compared to *Capitella* sp. commonly used in the bioremediation of enriched sediment under fish farms in Southern Japan. Chareonpanich *et al*. (1994) concluded the efficiency of *Capitella* sp. as bioremediator that 0.5 g DW (or 33,139 individuals) of *Capitella* had the ability to decompose organic matter with 1 g of TOC in 1 m^2 of sediment. Tsutsumi *et al*. (2002) conducted the experiments in the outdoor pools filled with organic enriched sediment to measure the bioremediation efficiency from the artificially cultured colonies of *Capitella*. They found that within 16 weeks of experiment period, the outdoor pools with artificially cultured *Capitella* showed the apparent decreased levels of TOC relatively to those of control. During the last nine weeks (from week 7 to week 16) of experimental period, the artificially cultured *Capitella* patches in the experimental pools were able to reduce the TOC ranged from 16.29-30.6%.

We prefer *P. (P.) membranacea* for the bioremediation of organically enriched sediment under green mussel raft in Sriracha Bay, Chonburi Province. This species were more abundant in the coastal area of Sriracha Bay, while *P. (M.) pulchra* was rare. Although the two spionids showed the similar potential in the reduction of organic matter in the sediment, *P. (P.) membranacea* was more efficient in converting the organic waste from the enriched sediment to biomass than *P. (M.) pulchra*. It can be predicted that for further application of this result by the introduction of mass culture of spionid polychaetes in the enriched sediment under the green mussel rafts, the *P. (P.) membranacea* population would increase in density and biomass rapidly. This would inturn increase the decomposition rate of organic matters in the sediment. As shown from the result, the high density of *P. (P.) membranacea*, twice the normal density, would increase the reduction of organic matter in the sediment 1.38 times compared to normal density. The methods on the future application of mass-cultured of spionid polychaetes to treat the organically enriched sediment under the green mussel rafts and in the coastal area should be investigated.

4. Conclusions

The spionid polychaete *P. (P.) membranacea* and *P. (M.) pulchra* can be used as bioremediators for the treatment of organically enriched sediment under the green mussel rafts in Sriracha Bay, Chonburi Province. The two spionids were efficiently converted the organic waste into biomass and reduced the organic matter in the sediment. The spionid polychaetes *P. (P.) membranacea* is the potential species for the treatment of organic waste in sediment from aquaculture system.

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