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Comparative Assessment of the Bioremediation Potential of Polychaetes (Lug worm-*Arenicola marina sp* and Syllidae Worm-*Syllis prolifera sp)* in Aquaculture Pond Sediment

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Polychaetes play an important role in nutrient cycling and remediation of coastal ecosystems. Large quantities of organic matter that could lead to pollution of pond and coastal waters are generated by aquaculture waste. To assess the remediation prospects of *Arenicola marina* and *Syllis prolifera* species, laboratory sediment microcosm experiments were conducted where large size *Arenicola marina* and *Syllis prolifera* were introduced to sediment in microcosm A, large size *Arenicola marina* to sediment in microcosm B, large sized *Syllis prolifera* to sediment in microcosm C and no polychaetes to sediment in microcosm D. Microcosm A′, B′ and C′ as replicates for small size polychaetes were also set up, respectively. After 30 days, microcosm A, B and C had significant decrease in organic carbon levels with microcosm B being the highest (Total organic carbon (TOC); 27.87%; $p < 0.05$). Both large and small polychaetes promoted significant decrease in sulphur (S) content (mean=62.76±0.21; 62.81±0.21%) and iron(Fe) (mean=49.43±1.47;

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36.28±5.90%) respectively. Increase in pH by 31.15±0.13% was found in the presence of large size polychaetes, most likely associated with the burrowing process involving oxidation of Fe to Fe_2O_3. Large size polychaetes had better survival (mean=92±0.82%) than their small size counterpart (mean=55±4.08%). The extent of biodegradation B>A>C>D observed revealed that large size *Arenicola marina* was a better bioremediator of organic matter (OM), Fe and S enriched aquaculture pond sediment, probably due to its biological characteristics, well suited for the aquaculture than other species of sea worms that produce free swimming larvae. Therefore, large size *Arenicola marina* significantly improved sediment quality as well as increased its pH without compromising their survival. As the search for a better bioremediator of organically enriched sediment continues, our result revealed large size *Arenicola marina* as a more promising candidate compared to other species documented elsewhere in the world. Hence, rearing of large size *Arenicola marina sp* is recommended as their feeding habits are well suited for aquaculture.

Keywords: Bioremediation; aquaculture waste; pond sediment; polychaete; organic matter; macrocosm.

HIGHLIGHT

1. Two species of polychaete were comparatively assessed for bioremediation potential in organically rich aquaculture pond sediment.
2. Treatment of pond sediment with large size polychaete (*Arenicola marina)* in microcosm experiment resulted in higher decrease in organic carbon levels compared to *Syllis prolifera sp* treatment probably due to its unique biological characteristics.
3. Both large and small size polychaetes promoted significant decrease in S and Fe contents regardless of species.
4. Increase in pH found in the presence of large size polychaetes most likely attributed to the burrowing process involving oxidation of Fe to Fe_2O_3.
5. Slight variation in physicochemical properties of overlying water was associated with biogeochemical processes taking place in the pond sediment.
6. Large size *Arenicola marina* significantly improved sediment quality as well as increased its pH without compromising their survival.

1. INTRODUCTION

Aquaculture is among the fastest growing food production sectors in the world as it supplies high quality protein to meet the increasing demand for seafood resulting to an increase in waste generation from the production systems. However, the rapid growth of aquaculture industry has culminated to environmental degradation caused primarily by irresponsible practices and poor management of culture systems [1]. Discharges from aquaculture activities have adversely affected the coastal environment through high amounts of suspended solids and nutrients leading to organic enrichment of the receiving coastal aquatic ecosystems. The accumulation of aquaculture wastes, which include organic sludge, algal and bacterial biomass may alter the physical and chemical composition of the water and sediment that could adversely affect the farmed species and the benthic environment [2-3] Hence, there have been many strategies employed to treat aquaculture wastes such as the use of bivalve filtration and settling basins (Jones et al., 2001), polychaete-assisted sand filters [4] constructed wetlands [5] and artificial substrates [6]. In Australia, settlement ponds were designed to capture nutrient discharges from ponds prior to release and this proved to reduce total suspended solids up to 60% (Preston et al., 2000). The search for ways to decrease the levels of particulate organic matter and convert it to more useable forms by another organism can be a profitable strategy. Polychaetes occupy the benthic sediments of coastal environment and are known to influence the physicochemical properties of the sediment through bioturbation, feeding behavior, sediment reworking and bio-irrigation [7]. Large quantities of aquaculture wastes that contain solid forms such as faeces and uneaten feeds can be converted to polychaete biomass [8]. This could help decrease the amounts of organic matter (OM), available sulfur (S) and iron (Fe) and change the pH of the sediment from acidic to basic. Here, we compared such bioremediation potential of polychaete species used in the present study with those reported elsewhere with the view of ascertaining the most promising bioremediator of OM. The choice of Fe and S was on the premise of its high abundance relative to other heavy metals in the region [9] and that many S-containing compounds are resistant to biodegradation and toxic to resident organisms [10] respectively. Furthermore, when polychaetes grow well in organically enriched sediments, they can be of economic important as supplementary food to high value crustaceans and fin fishes [8]. Different species of polychaete have been reported to remediate organically enriched sediment by speeding-up the decomposition rate of the organic matter of the sediment under aquaculture farms [11], Tsutsumi et al., 2002; [7]. A mechanism of this bioremediation is to reduce, detoxify, degrade, mineralize or transform more toxic pollutants to a less toxic one [12,13]

Although *Arenicola marina Sp* impact on bacteria activity in intertidal sediments has been reported (Grossmann and Reichardt, 1999), this is the first report on the application of *Arenicola marina* and *Syllis prolifera* species (Fig.1a and b) as bioremediators of organically-enriched pond sediment.

These species of polychaetes were chosen in the study on the basis of their biological characteristics. Lugworms (*Arenicola marina Sp)* are burrow dwelling annelid worms with an estimated lifespan of about 5-6 years. They can make up to 30% of biomass of an average sandy beach, making them a very important part of the food web in their habitat. They bioturbate (re-work, re-oxygenate) the sand and serve as a food source for a wide variety of other animals such as flatfish and birds. Lugworms also have a clever way of avoiding predators [14]. Syllidaeworms (*Syllisprolifera sp*) is a family of small to medium sized polychaete worm and are distinguished from other polychaetes by the presence of muscular region of the anterior digestive tract known as the preventable. It ranges in size from 2-3 mm to 14 cm. Syllids are benthic organism that transit to a pelagic epitoke for reproduction. They are found in all region of the ocean and are especially abundant in shallow water. They are found in a range of habitats, moving actively on rock and sandy substrates, hiding in crevice and among sea weeds and climbing in sponges, corals, hydrozoan sea grasses and mangrove. They are generalist feeders [14].

The present study conducted a laboratory experiment to assess the remediation prospects of *Arenicola marina* and *Syllisprolifera* species in changing the chemical composition of the pond sediment after 30 days' treatment. The main objectives were to determine and compare the extent of changes such as the increase or decrease in the levels of OM, S, Fe, and pH of the sediment by the two species of polychaetes (small and large sizes) after being cultured for 30 days in a microcosm. The findings of the study can serve as baseline information using these species to remediate organic rich pond sediments.

2. MATERIALS AND METHODS

2.1 Sample Collection and Preparation

Large and small sizes of *Arenicola marina* and *Syllis prolifera* species of polychaete were collected from brackish water in Ebonyi, state, Nigeria (Latitude 6°20′N and Longitude 8°06′E). They were classified as small and large based on their body weight and peristomium width. Small and large sizes *Arenicola marina sp* had an initial mean body weight, BW = 28.04 ± 6.65mg; mean peristomium width PW = 0.75 ±0.15mm and mean body weight, BW = 127.46 ± 14.64mg; mean peristomium width PW = 1.51± 0.29 mm, respectively, while *Syllis prolifera sp* (small and large sizes) had mean body weight, BW = 25.02 ± 5.85 mg; mean peristomium width PW = 0.55 ±0.24mm and mean body weight, BW = 98.66 ± 22.76 mg; mean peristomium width PW = 1.01± 0.20 mm, respectively.

Fig. 1. Pictorial representation of (a) Lug worm (*Arenicola marina Sp)* and (b) Syllidae worm (*Syllis prolifera sp)*

Sediment was collected by scraping the upper layers of the Eze Azu fish porn. Before use, the sediment was air-dried, pulverized, and passed through <1 mm mesh size to ensure that unnecessary debris (i.e. stones, pond snails, and other animals) was removed. The sediment was mixed homogenously before stocking in each microcosm.

Polychaetes were acclimatized for two weeks in rectangular plastic containers (10 cm height x 20 cm length x 15 cm width) containing sieved sediments (<1 mm) with three liters of aerated water at salinity level of 20-21 ppt. Salinity level of 20-21ppt was prepared in order to simulate the existing salinity level of brackish water.

2.2 Experimental Design

To assess the remediation potentials of *Arenicola marina* and *Syllis prolifera* species, laboratory sediment microcosm experiments were conducted where 5 large size *Arenicola marina* and 5 *Syllis prolifera* were introduced to sediment in microcosm A, 10 large size *Arenicola marina* to sediment in microcosm B, 10 large size *Syllis prolifera* to sediment in microcosm C and no polychaetes to sediment in microcosm D. Microcosm A′, B′ and C′ as replicates for small size polychaetes respectively were also set up. Sediment microcosm experiment was conducted indoor using the 40-watt cool-white fluorescent lamp as light source following the natural light/dark cycle. Aluminium foil was used as liner inside each of the rectangular plexiglass aquaria (height 33 cm, length 24 cm, width 8 cm) filled with 1.25 kg (dry weight) sediment and 3L filtered water (20-21 ppt). Each replicate tank contained about 6 cm height of sediment and water level of 22 cm height from sediment surface. Water in each sediment microcosm was allowed to stand overnight. No feeding was provided and overlying water in each sediment microcosm was kept mildly aerated throughout the 30 days. Every other day, about 80% of overlying water (18 cm water height or about 2.4 L) in each sediment microcosm was siphoned off slowly to prevent the build-up of metabolites. Filtered water of 20-21 ppt was added carefully up to 3L per tank to compensate for salinity increase due to evaporation. Dissolved oxygen, temperature, salinity, and pH of the water in sediment microcosms were monitored daily just before and after water change mainly in the morning. Frequency of water change simulates the water flushing in brackish water ponds during pond preparation. After 30 days, polychaetes were separated from the sediments through sieving. Thereafter, surviving polychaetes were counted. Missing polychaetes were presumed to have died and decayed in the sediment microcosm, as escape would not have been possible. The body weights of polychaetes were not measured after 30 days because they were segmented probably due to stress and if measure could result to unrealistic biomass data. Sediments from each microcosm were spread thinly in trays and allowed to air dry indoor for about two weeks. Dried sediments were pulverized using a mortar and pestle and subjected to final analysis of organic carbon (OC), S, Fe, and pH.

2.3 Sediment and Water Analysis

The percentage total organic carbon (TOC), available sulfur (S), iron (Fe) and pH contents of sediments in each microcosm were analyzed following standard methods [15] before the start (initial) and after 30 days (final) of the experiment. Following the initial decarbonation protocol, TOC was determined as previously reported by Oyo-Ita et al. [16] using flash combustion at 1024^{o}C and thermal conductivity detection in triplicate with a CHNS Elemental Analyser (Carlo Erba 1108).

Available S was determined by wet oxidation/barium sulphate turbidimetry in which the sample was oxidized with a mixture of nitric and perchloric acids in the presence of vanadium where sulfate ions were precipitated by barium chloride and the resulting turbidity measured. Colorimetric method was used to determine the iron concentration by the intensities of the color of sample solutions using a UV/visible spectrophotometer [17].

The pH of sediment (pH_w) was potentiometrically measured in a 1:1 sediment-water ratio after calibrations in buffer solutions of pH 4 and 10 using a pH meter. The probe was inserted, thoroughly shaken and reading obtained after stabilization of the system [18]. Other parameters in water such as dissolved oxygen (DO), salinity and temperature were measured using the multi-probe portable meter kit (Oakton Instruments, Singapore).

2.4 Data Analysis

Besides the deployment of descriptive statistics, percent decrease or increase of TOC, S, Fe, and pH were calculated based on the results of the initial and final measured levels of the sediment in each treatment microcosm after 30 days.

Data were subjected to Pearson correlation analysis in order to understand relationships among paired variables. A t-test was performed to examine the effect of treatment of sediment on percent increase or decrease in TOC, S, Fe, and pH. Percent survival was based on the total number of polychaetes harvested after 30 days over the initial number stocked and multiplied by 100%. Percent survival of polychaetes was compared using T-test at 95% confidence interval. All statistical procedures were done in IBM SPSS Statistics Version 21.

3. RESULTS

3.1 Initial Chemical Compositions of Sediment

Result of the study showed initial TOC, S, Fe and pH levels in sediment as 3.48%, 1359 ppm 201.33 ppm and 6.42 respectively while 11.88 mg/L, 30 ^{0}C and 6.80 were the initial dissolve oxygen (DO) levels, temperature and pH_w respectively. There was observable difference in the appearance of sediment in microcosm experiments after 30 days treatment. The entire treated sediment appeared lighter than the control. The homogenous sediment had significantly higher initial levels of TOC, S, Fe and lower pH(acidic-neutral) than the final sediment levels ($p< 0.05$; Table 1).

3.2 Variations in Total Organic Carbon and Sulphur Contents

The final TOC levels were lower in all treatment microcosms. In general, sediment microcosm with large size polychaetes treatment regardless of species showed a significant decrease(23.37±3.29 %; $p< 0.05$) in TOC levels compared to their small size counterpart (14.37±3.31%) and no polychaete (4.89 %) treatments (Table 2).The decrease in TOC level was highest in microcosm B with large size *Arenicola marina sp* (27.87%) followed by microcosm A with large size *Arenicola marina* and *Syllisprolifera* (22.13%), then microcosm C with large size *Syllisprolifera* (20.11%) and microcosm D with no polychaetes (4.89%).

Amongst the small size polychaetes, microcosm B′ with *Arenicola marina* exhibited the highest decrease of TOC (18.10%), followed by microcosm A′ with *Arenicola marina* and *Syllisprolifera* (14.94%), then microcosm C′ with *Syllisprolifera* (10.06%). There was no significant difference in the decrease of TOC levels among treatments regardless of species ($p > 0.05$; Table 2).

The levels of S significantly decreased with large (62.76±0.21 %) and small (62.81±0.21 %) polychaetes treatments compared to the control (46.01 %) ($p< 0.05$) regardless of species. (Table 2).

3.3 Variations in Iron and pH Contents in Sediment

Similar trend was observed in the case of changes in Fe levels for the large (49.43±1.47 %) and small (36.28±5.90 %) size polychaetes compared to the no polychaete treatments (21.04%) ($p< 0.05$) for both species (Table 2). The sediment treatment with *Arenicola marina* significantly decreased the iron level (51.32% and 44.31 %) compared to *Syllisprolifera* specie (47.74% and 30.29 %) ($p< 0.05$) for both the large and small sizes respectively.

There was a significant difference in the percent increase in pH among treatments regardless of the species of polychaetes ($p < 0.05$; Table 2). The increase in pH in large polychaetes treatments (31.15±0.13%) was higher than the no polychaete treatment (26.48 %) ($p< 0.05$) which was almost similar to that of small polychaete (28.04±0.13 %; Table 2). Correlation analyses revealed a stronger relationship between TOC and Fe ($r^2 = 0.9191$) and pH ($r^2 = 0.8231$) than with S ($r^2 = 0.5798$) while the pH values correlated better with Fe level ($r^2 = 0.8385$) than with S ($r^2 = 0.3602$) (Fig. 2 a, b, c, d, e).

Polychaetes survival, variations in dissolve oxygen with temperature and pH of water.

The extent of polychaete survival appeared higher with large size polychaete (92±0.82 %) compared to the small size counterparts (55±4.08 %) treatments (Fig. 3). However, there was no significant difference between the survival of large *Arenicola marina* and *Syllis prolifera* species in treated sediments ($p> 0.05$). On the other hand, small *Arenicola marina* specie had higher survival (60 %) compared to *Syllis prolifera* (50%) ($p < 0.05$). Regardless of species, large size polychaete (92±0.82 %) survived better than their small size counterparts (55±4.08 %) (Table 2; Fig. 3).

Table 1. Initial/final levels of sediment bulk properties and variations in physicochemical properties of overlying water

Experiment	$pH_{(w)}$	TOC	S (ppm)	Fe (ppm)	DO (mg/L)	$pH_{(w)}$	Tempt. 0C
Initial Concentration	6.42	3.48	1359.72	201.33	11.88	6.80	30
Microcosm A	8.42	2.71	506.37	102.2	11.38	7. 65	31.5
Microcosm B	8.43	2.51	502.9	98.01	11.42	7.73	31.16
Microcosm C	8.41	2.78	509.89	105.21	11.36	7.56	30.78
Mean	8.42±0.01	2.67±0.11	506.39±2.85	101.81±2.95	11.39±0.02	7.65±0.12	31.15±0.29
Microcosm D	8.12	3.31	734.24	158.98	11.68	7.14	30.2
Microcosm A′	8.22	2.96	505.88	132.39	11.36	7.35	30.46
Microcosm B′	8.23	2.85	502.01	112.12	11.36	7.38	30.42
Microcosm C′	8.21	3.13	509.04	140.34	11.32	7.32	30.4
Mean	8.22±0.01	2.98±0.12	505.64±2.87	128.28±11.88	11.35±0.01	7.29±0.13	30.43±0.025

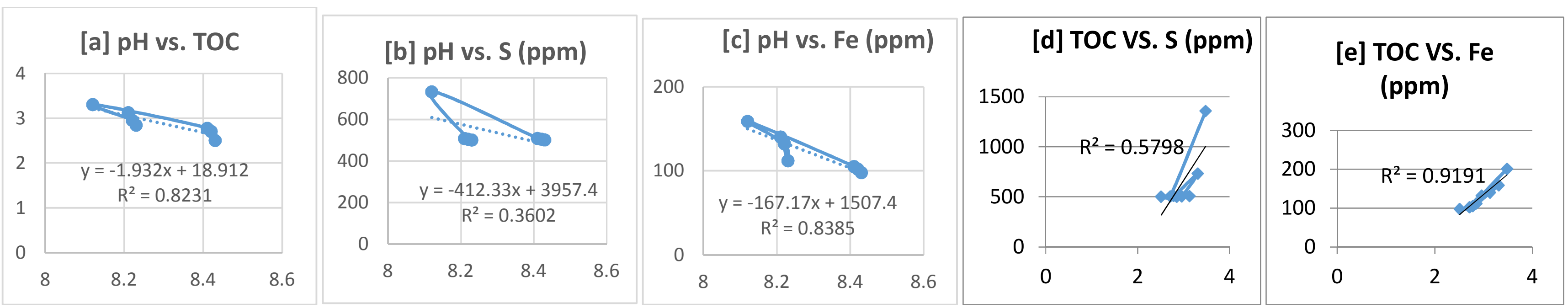

Fig. 2. Correlation among determined parameters

Variations in DO, temperature and pH_w throughout the duration of the experiment (Table 1) were within tolerable limits of the polychaetes (Mandario et al., 2019). Regardless of species of polychaetes, DO levels decreased with increasing temperature with the large size (DO: temperature-11.39±0.03 mg/L; 31.15±0.29 ^{0}C) and no polychaete (control: 11.68±0.03 mg/L; 30.43±0.02 ^{0}C) treatments, whereas, a somewhat different scenario was found for the small size counterparts (11.35±0.02 mg/L; 30.59±0.03^{0}C). The following sequence: Control>Large size>Small size and Large size> small size> Control were observed for mean DO and temperature, respectively (Fig. 3). Changes in pH of overlying water (pH_w) followed a similar trend with that of sediment (Table 1).

4. DISCUSSION

4.1 Variations in Total Organic Carbon and Sulphur Contents

The accumulation of high levels of OM and S in the sediments was most likely due to unconsumed feeds and metabolic wastes of aquatic organisms. This may be aggravated by the continuous stocking of different aquatic species without proper treatment of the pond bottom. After the addition of polychaetes, the TOC levels of sediments in microcosms decreased but the presence of large polychaetes significantly lowered the TOC levels, particularly with *Arenicola marina* specie. Large polychaetes ingested large amounts of sediments so that high amounts of OM were also consumed.

Table 2. Percentage compositions of sediment bulk properties and polychaete survival

Experiment	TOC (%)	S (%)	pH (%)	Fe (%)	Survival (%)
Initial Concentration	**3.48**	**1359.72**	**6.42**	**201.33**	**NA**
Microcosm A	22.13	62.76	31.15	49.24	92
Microcosm B	27.87	63.01	31.31	51.32	93
Microcosm C	20.11	62.5	31	47.74	91
Mean	23.37±3.29	62.76±0.21	31.15±0.13	49.43±1.47	92±0.82
t-test	$p < 0.05$	$p < 0.05$	$p < 0.05$	$p < 0.05$	$p < 0.05$
Microcosm D	**4.89**	**46**	**26.48**	**21.04**	**NA**
Microcosm A′	14.94	62.8	28.04	34.24	55
Microcosm B′	18.1	63.08	28.19	44.31	60
Microcosm C′	10.06	62.56	27.88	30.29	50
Mean	14.37±3.31	62.81±0.21	28.04±0.13	36.28±5.90	55±4.08
t-test	$p > 0.05$	$p < 0.05$	$p < 0.05$	$p < 0.05$	$p > 0.05$

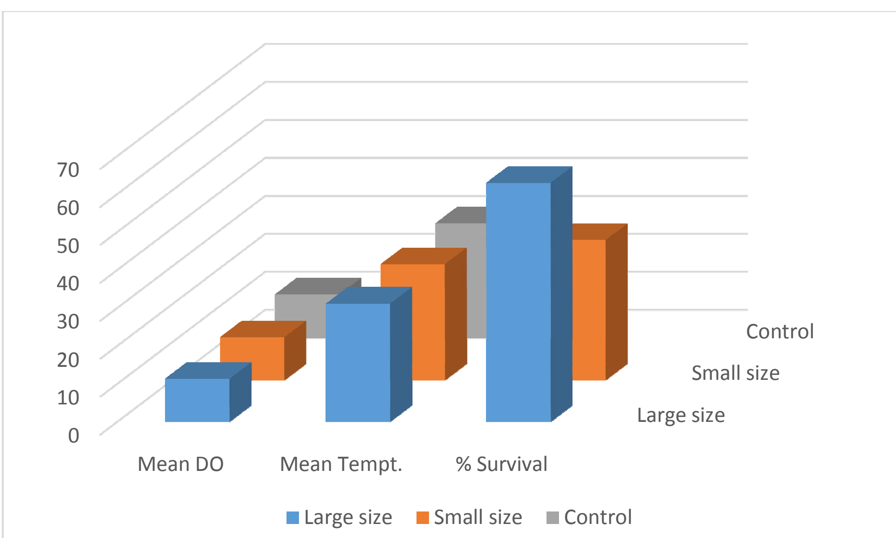

Fig. 3. Percentage survival of small and large size polychaetes and variation in physicochemical properties of overlying water

Table 3. Comparison of remediation effect of different species of Polychaetes in organically enrich aquaculture sediment

Polychaete Species	Duration of Treatment	% Decrease in OM	% Decrease in available S	% Decrease in Fe	% Increase in pH	Location	References
Marphysasp	30 days	27	71	72	12	Dumangas, illoido, Phillipines	Mandario et al., [8]
Perinereisnuntia	60 days		40			Kochi, Japan	Ito et al., [11]
Capitellateleta	60 days		33			Ehime, Japan	Ito et al., [11]
Capitellateleta in-situ	30 days	27.5				Kochi, Japan	Kinoshita et al., [9]
Arenicola Marina sp	30 days	27.87	63.01	51	31.31	Ebonyi State, Nigeria	This study
SyllisProlifera	30 days	20.11	62.5	47.74	31	Ebonyi State, Nigeria	This study

The present study revealed large size *Arenicola marina sp* to be a better bioremediator of organically enriched aquaculture pond sediment than *Syllisprolifera* and other sea worms that produce free swimming larvae, probably due to its biological characteristics well suited for aquaculture. The presence of polychaetes in the sediments obviously improved the sediment quality. Consequently, the decrease in OM levels with the presence of polychaetes indicates that OM is ultimately decomposed by polychaetes either through their own metabolism or through enhancing the microbial processes by sediment reworking activities. Therefore, as earlier reported by Kinoshita et al., [19] the presence of benthic fauna affects OM decomposition inorganically enriched fish farm sediments. Bioturbation activities of polychaetes affects microbial processes in the sediment, thus improved the sediment biogeochemistry [3]. Burrow formation by polychaetes creates an oxidized layer in the sediment providing optimal environment for aerobic bacteria to proliferate [20] and, in the process, increase bioremediation. More importantly, the ingestion of organically-enriched sediments by polychaetes particularly the large size might have decreased OM levels in the treated sediment microcosm. This in consonant with other studies showed that ingestion of sediments by polychaetes can change its chemical compositions [21]. The digestive tracts of benthic invertebrates are known to contain bacteria, enzymes, acids, and surfactants that can aid in breaking down complex polymers in the sediment (Ahrens et al., 2001). The digestive tracts of deposit feeding organisms contain high concentrations of surfactants that are able to solubilize hydrophobic food materials (Mayer et al. 1997). This phenomenon could directly or indirectly aid in the digestive processes of polychaetes. Deposit-feeding polychaetes are highly exposed to high hydrophobic organic contaminants as they ingest large amount of sediment [22]. Thus, OM and contaminants are readily consumed by polychaetes and converted to more useable forms for growth. The observed decrease in OM in sediment without polychaetes indicated that microbial processes may have contributed to the loss [23]. In other words, although microorganisms have significant role in degrading OM, the presence of polychaetes living in sediment could have indirect role in speeding up the bioremediation processes [24-25]. As the search for better bioremediation of organically enriched sediment continues, comparing data in the present study with those reported elsewhere, our result revealed higher % decrease in OM with *Arenicola Marinasp.* (Table 3) suggesting that this specie of polychaete is a more promising bioremediator of organic enriched sediment probably due to its unique biological characteristic.

Similarly, the level S in microcosms A, B, C, D, A', B', C' significantly decreased with the addition of large and small size polychaetes regardless of species. However, with regards to the species used, higher decrease of S was evident in sediment with *Arenicola marina* (microcosm B and B'). A high level of Sin sediment can be reduced in the form of hydrogen sulfide. However, Ross et al. [26] observed that sediments inhabited by burrowing fauna generally have a decrease in S due to irrigation and reworking of sediment particles by the fauna which then agrees with our result. In all microcosm sediments, it seems that the size of *Arenicola marina* and *Syllisprolifera* species did not matter when S decrease was considered although larger animals had greater contribution in the recovery process of contaminated sediments through their reworking activities [27]. Both large and small size species of polychaetes experimented had similar potential to decrease S levels in the sediments. Bio-irrigation by polychaetes through undulatory body movements oxygenated the burrows thereby removing the S from the sediments. The reworking activities of polychaetes can positively decrease the amount of sedimentary S by creating an aerobic condition that prevented the formation of toxic gas that would have been detrimental to aquatic organisms. The reworking of the sediments and ventilation of burrows by polychaetes enhanced the reaction rates and solute fluxes across the sediment-water interface. Hence, the amount of oxygen reaching the sediments increased and made available for aerobic organisms to function well for biogeochemical processes to proceed. Moreover, polychaetes have the ability to oxidize S to less harmful forms such as thiosulfate and switch to anaerobic metabolism when S exceeds their carrying capacity to detoxify [28]. The result indicated that these species of polychaete have the ability to remediate sulphur containing contaminant in sediment.

4.2 Variations in Iron and pH Contents

Fe levels in microcosms A, B, C, D, A', B', C' followed a similar trend as that of S. Since the presence of Fe affects the concentration of OM stored in the sediment, the decrease in Fe was crucial in improving the sediments quality. For instance, Lalonde et al. [29] reported that the higher the amount of Fe in sediment, the higher OM will be preserved. In a similar manner, the decrease in Fe in all the treatments in our study can be in part attributed to the burrowing activities of the polychaetes and increase in oxidation of Fe to Fe_2O_3 in the sediments. In support of this assertion, a report by Kristensen et al. [3] on deeper layers of sediment revealed Fe dominance due to the prevailing anaerobic conditions. The presence of polychaetes irrigates these zones through burrows and oxidizes the Fe by aerobic processes. Therefore, macrofaunal burrow structures have significant role in the Fe and S cycles by facilitating solute transport and continually recycling electron acceptors that is crucial to both the oxidation of OM and coupled nitrification–denitrification [30]. According to these authors, burrow zones improved the Fe cycling by increasing the positive effects of solute transport on coupled nitrification–denitrification by reacting with sulfides and thus, stimulate the removal of nitrogen.

Chemical reactions within the sediment can change the pH into acidic, neutral, or basic conditions [19]. Aquaculture sediments tend to be acidic at high organic matter concentration due to continued deposition of fresh organic matter in the pond bottom. Microorganisms function well in contemporary sediment at pH 7 to 8 [31]. In the study, the initial sediment pH was mildly acidic and on polycheates addition changed to mildly basic with higher values found for large size counterparts. This change in pH most likely reflected the burrowing activity associated with oxidation of Fe to Fe_2O_3 and support that regardless of species employed, polychaetes can efficiently change pH of sediment from acidic to basic state. Generally, the observed good positive correlation between TOC, S or Fe and pH somewhat agree with the findings by Hargrave et al. 2008, that there is a positive relationship between sulphide pools and increased sedimentary OM.

In specific terms, the strong correlation that existed between TOC and Fe supported the idea that the presence of Fe affects the concentration of OM in sediment. Also, the strong correlation that existed between TOC and pH suggested that much of the acidity in sediment was derived from organic acids instead of acidic sulphate compounds. In addition, the moderate correlation that existed between TOC and S suggested that a considerable amount of S containing compounds in sediment existed in their reduced state. The strong correlation between pH and Fe supported the earlier assertion that as the burrowing activity of polycheates oxidized Fe to $Fe_2O_{3,}$ the pH of sediment changed from mildly acidic to mildly basic. The observed weak correlation between pH and S implied that S-containing compounds did not play a major role in acidity or basicity of the sediment.

4.3 Polychaetes Survival, Variations in Dissolve Oxygen with Temperature and pH of Water

Survival of polychaetes after 30 days was crucial in assessing their bioremediation potential. Survival of polychaetes was not only dependent on the quality of the sediment but also on polycheate size and species. In sediment of microcosm A, B and C, survival of large polychaetes was higher than those in microcosm A', B' and C' and survival of *Syllisprolifera* was lower compared to *Arenicola marina*. These results showed that polychaetes should be grown to adult size prior to their introduction to polluted sediment. This strategy aims to maximize their full bioremediation potential without compromising their survival.

Large size polychaetes could withstand pollutants better than their small size counterparts irrespective of species. Many studies showed that young animals usually lack resistance against pollutants because of their underdeveloped organs and weaker immunity [11]. In some pollution studies, for instance, it was also observed that small mollusks and crabs accumulated more α-HCH [16] and PAHs in shrimps [32] than their larger counterparts. This observation was supported by the fact that smaller individuals have faster rates of uptake but slower rates of elimination of hydrophobic contaminants than their larger counterparts [33].

The observed sequence: Control>Large size>Small size for DO implied that regardless of species of polychaetes, DO levels decreased with increasing temperature with the large size and no polychaete treatments but somewhat differ for the small size counterparts. The relative reduced amount of DO observed in the case of small size polychaetes may be attributed to their higher biological decay occasioned by higher death rates. This buttressed the findings that smaller animals have low resistance capacity against pollutants due to their immature organs and weaker [33-34]. The slight change in pH of water observed may also be linked to dilution effect associated with burrowing process involving oxidation of Fe to Fe_2O_3.

5. CONCLUSIONS

Our study compared the remediation effects of *Arenicola marina* and *Syllisprolifera* species of polychaetes in organically-enriched pond sediments. The presence of these polychaetes significantly decreased the levels of Fe and S. While OM was lowered significantly by the large size counterparts, the remediation caused by the small size polychaetes was not significantly different from that of the control. In addition, sedimentary pH improved from mildly acidic to mildly basic regardless of species, while the large size polychaetes showed higher survival to pollution compared to their small size counterparts. The lower mean DO level recorded for the small size relative to the large size treatments was attributed to higher death rates associated with lower resistance capacity of small size polychaetes against pollutants. Rearing and use of large size *Arenicola marina* over *Syllisprolifera* and other species documented elsewhere in settling ponds in the world is highly recommended.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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