International Journal of Biological, Life and Agricultural Sciences
ISSN: 2415-6612
Vol:4, No:11, 2010

Utilizing Dredged Sediment for Enhancing Growth of Eelgrass in Artificially Prepared Substrates

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***Abstract*—**Dredged sediment (DS) was utilized as source of silt-clay and organic matter in artificially prepared eelgrass substrates with mountain sand (MS) as the sand media. Addition of DS showed improved growth of eelgrass in the mixed substrates. Increase in added DS up to 15% silt-clay showed increased shoot growth but additional DS in 20% silt-clay mixture didn't result to further increase in eelgrass growth. Improved root establishment were also found for plants in pots with added DS as shown by the increased resistance to uprooting, increased number of rhizome nodes and longer roots. Results demonstrated that addition of DS may be beneficial to eelgrass up to a certain extent only and too much of it might be harmful to eelgrass plants.

***Keywords*—**dredged sediment, eelgrass, eelgrass bed restoration, mountain sand, *Zostera marina*.

I. INTRODUCTION

WORLDWIDE an enormous amount of sediments are dredged for navigation, maintenance and environmental purposes. For instance, in the United States several hundred million cubic yards of sediments must be dredged from ports, harbors and waterways each year for such purposes [1], [2]. Likewise, Japan, having intensive aquaculture areas, uses ecological dredging for the purpose of restoring water environment to a healthier condition [3]. An example of this is Ago Bay in Mie Prefecture, which due to a long history of pearl and oyster culturing caused organically-rich sediments to accumulate in the sea bottom leading to dredging to avoid deterioration of sea water and sediments [3], [4]. Disposal and management of these large volumes of dredged sediment constitute a serious and challenging task.

Recently, beneficial use of dredged material has become a practical option to traditional disposal methods [5], [6]. Broadly, USEPA and USACE [1] categorize these beneficial uses into three: agricultural and product uses, engineered uses and environmental enhancement. The use of dredged material as a potential resource for restoring or recreating intertidal habitats is classified under the third category. A number of studies have been done on the use of dredged material for creating salt marshes [7], [8], mud flats and tidal flats [4], [5], [9]-[11]. On the other hand, there are only few documented examples on the use of dredged material on seagrass habitat development [6].

Due to economic and ecological importance of seagrasses, efforts to conserve and expand existing seagrass communities, restore lost ones and create new ones in the face of continuing coastal development have increased [12]. Eelgrass (*Zostera marina* L.) is the most common angiosperm seagrass found in the coastal waters of Japan [13]. It is said that before 1950's, vast eelgrass beds existed in the Seto Inland Sea, Japan [14]. During the last three decades, the total area of these beds decreased by 70%, just in Seto Inland Sea [13], [14].

Eelgrass restoration efforts being done all over the world are transplanting, stockpiling and construction of artificial eelgrass beds among others [15]-[18]. For most of these restoration projects the substrate used was natural eelgrass sediment or sea sand. However, excavation of sea sand and sediment is banned in prefectures surrounding Seto Inland Sea [19], [20]. For this reason, development of technologies using alternative materials for intertidal habitat restoration projects had started [10], [20], [21].

One possible material that can be used as a basal media for eelgrass beds is mountain sand. Mountain sand (MS) is traditionally used for construction and gardening. In Japan, artificially constructed tidal flats have been constructed using MS [10], [22]. However, as pointed out in [10], MS alone as a material for construction of artificial tidal flats lacks the silt and clay component as well as organic matter necessary to achieve the same physico-chemical and biological structures as that of the natural ones. Addition of another material to MS rich in silt-clay and organic matter component like uncontaminated DS might be necessary to copy the characteristic of the natural habitats. Nevertheless, amount of DS that will be added has to be done with care since too much silt-clay and organic matter can produce adverse effects due to resulting reduced environment and increased shear strength [5], [9]. Therefore, it is the aim of our study to investigate the use of DS as silt-clay

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ISSN: 2415-6612
Vol:4, No:11, 2010

and organic matter source for MS to enhance growth of eelgrass in artificially prepared substrates.

II. Materials and methods

A. Dredged Sediment and Artificial Sand Media

DS used in the experiment was dredged from Ago Bay, Mie Prefecture and had passed the Japanese standard for sea dumping disposal of dredged material [23]. MS used for the study was obtained commercially. Table 1 shows some of the characteristics of DS and MS used in the study.

TABLE I
Characteristics of DS and MS Used in the Study

Characteristics	DS	MS
Silt-clay content (%)	98.0	4.5
Ignition loss (%)	16.29	0.98
pH in sea water	7.25	7.34
Total-N (μg/gDW)	224.16	9.35
Total-P (μg/gDW)	359.75	213.15

DS = dredged sediment; MS = mountain sand; n = 3.

Different amounts of DS were added to the MS samples to achieve mixtures containing different amounts of silt-clay and organic matter. We added DS to achieve mixtures containing 7%, 10%, 15% and 20% of silt-clay. The minimum amount of silt-clay for the prepared mixtures was set at 7% since the natural eelgrass sediment (NES) used in the study has a silt-clay content of around 7% (average silt-clay = 6.7%). NES was obtained from the same site where eelgrass plants were collected. The maximum silt-clay content was set at 20% based from a previous study wherein silt-clay contents of sediments in eelgrass meadows and transplant sites in Seto Inland Sea were found to be below 20% [24].

Prior to mixing with DS, the silt-clay part of MS was sieved off so that all the silt-clay in the mixture will come from DS. It was ensured that mixing was homogenously done. The resulting mixtures were left to stand for at least 24 hours prior to use in the experiments.

B. Plant Material and Growth Experiment

Eelgrass shoots used in the experiments were obtained from well established eelgrass beds of Yoshina tidal flat in Seto Inland Sea at a depth of about 2m. The collected plants, submerged in seawater, were quickly transported to the laboratory where they were cleaned from epiphytes, trimmed and planted in the same sediment where they had grown. Prior to use in experiments, plants were acclimatized for at least 2 weeks in 100-L tanks filled with the artificial sea water (SEALIFE, MarineTech, Japan at 30‰) at 20^{o}C using a light regime of 12h light and 12 h dark and light intensity of 250-280 μmol photon $m^{-2}s^{-1}$.

For the growth experiment, plants were made consistent by using only shoots having almost the same leaf width, trimming the shoots to around 50 cm and retaining only the three youngest leaves and three nodes of rhizome. The shoots were planted in the test substrates at 3 plants per pot and 3 pots per test substrate. During the growth experiment, plants were cultivated in the same tank used for acclimatization employing similar conditions. Growth was evaluated for 20 days by leaf elongation measured every 3-5 days using the modified leaf marking method [25]. Number of leaves and rhizome nodes, above and below ground biomass were recorded at the end of the growth experiment. Interstitial pH was also monitored during the growth experiment. Results were statistically analyzed using paired *t*-test.

C. Laboratory Analyses and Measurements

Particle size distribution and silt-clay content of the resulting mixtures were determined by employing both the wet sieving and dry sieving techniques [26], [27]. Measurements of pH were done from a solution having 1:1 w/v ratio of slag and artificial sea water (SEALIFE). Amount of organic matter by ignition loss was measured from the dry weight of the samples heated in an oven furnace to 450^{o}C for 4 hours. Oxidation reduction potential (ORP) measurements were obtained using a hand-held ORP meter (TOA, Japan).

Total nitrogen (Total-N) was determined using Kjeldahl method while total phosphorus (Total-P) was measured by the molybdenum blue method following the treatment of the samples with nitric acid and perchloric acid [28].

Uprooting force or the force needed to pull and uproot the rhizome and the roots from the substrate was measured to determine how well-established were the root-rhizome system in their substrates. Measurement of uprooting force was done using a digital forge gauge (FGN-B, Nidec Shimpo Corp.) following this method. The whole pot with eelgrass shoots was mounted in the test stand for digital force gauge (FGS-50H, Nidec Shimpo Corp.). The shoots were cut leaving only about 2 cm of meristem from the substrate. The upper part of the remaining meristem was then clipped to the digital force gauge, which was pulled up slowly until the peak force needed to uproot the plant was measured. After uprooting, the number of rhizome nodes was counted and the length of the longest root was measured. Results were statistically analyzed using paired *t*-test.

III. Results and Discussion

A. Physico-chemical Properties

Although it was desired to use MS with no silt-clay content, analysis showed that the sieved MS used has 4.5 % silt-clay. It is possible that the silt-clay component came from the disintegration of MS during sieving in analyzing particle size distribution. Nevertheless, the targeted mixtures of MS and DS were attained based from the result of particle size distribution analysis. Fig. 1 shows the changes in the particle size distribution of pure MS when added with increasing amounts of DS. As expected, increase in the amount of silt-clay content was observed as more DS is added. More or less, the target silt-clay content was achieved based on the result of particle size distribution analysis.

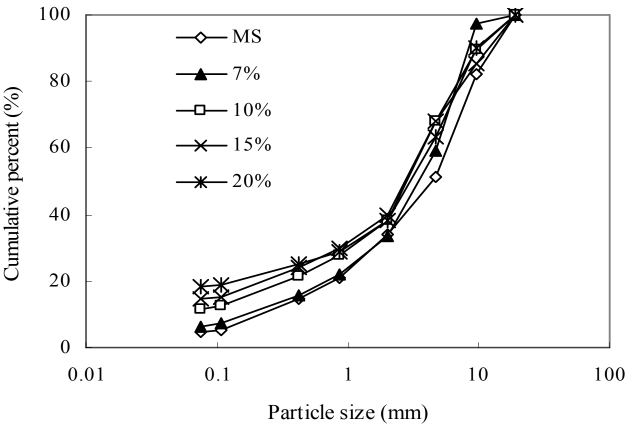

Fig. 1 Particle size distribution of mountain sand (MS) and mixtures of MS and DS. *Percentage values in the legend indicates silt-clay content of the mixture; n = 3.*

Organic matter content based from ignition loss, interstitial pH and average oxidation-reduction potential (ORP) of the resulting mixtures and pure MS are given in Table 2. Data revealed increase in organic matter content with increase in added DS. Indeed DS becomes the source of organic matter for MS which lacks this component. As suggested in [10], in construction of artificial intertidal habitats, silt-clay component as well as organic matter source is needed to establish the same physico-chemical or biological structure as that of the natural environment [29], [30]. The average interstitial pH recorded for all the substrates were lower than that of the overlying water column (average pH=8.3). In natural environment, seagrass beds sediments normally have lower pH than the overlying water column (around pH 8) and pH tends to decrease with increasing depth of the sediment [31].

TABLE II
COMPARISON OF MS AND MS+DS MIXTURES

Parameters	Substrates				
	MS	7% silt-clay	10% silt-clay	15% silt-clay	20% silt-clay
Ignition loss (%)	0.74	1.91	2.63	4.35	4.77
Interstitial pH	7.89	7.95	7.99	7.96	7.93
ORP* (mv)	110.00	102.25	72.00	55.50	-20.50

Values are averages and n=3. *Oxidation reduction potential.

For ORP, decreasing values were measured with increasing amount of DS added (Table 2). Negative ORP value was obtained for the mixture with the highest amount of DS added, which suggest that if more DS is added to MS, this can lead to development of a reduced substrate. In a review [32], it was indicated that as particle size distribution become skewed towards silt-clay, decreased pore water exchange with the water column could occur leading to reducing environments [33] which can be detrimental to seagrasses due to increased concentrations of phytotoxins like sulfide [34].

B. Growth

Fig. 2 shows the LER of eelgrass planted in different mixtures of MS and DS. Increasing LER was observed with increasing amount of DS added up to 15% silt-clay content. Decreased value of LER was obtained for the 20% silt-clay MS+DS mixture, although LER values for the 15% and 20% silt-clay mixture were not significantly different. The values of LER obtained for the 15% and 20% mixture are in same range of data obtained for the natural eelgrass sediment (average LER=1.3 cm $shoot^{-1}d^{-1}$), which suggests that addition of DS to MS achieved the goal of copying the characteristics and functions of the natural eelgrass beds.

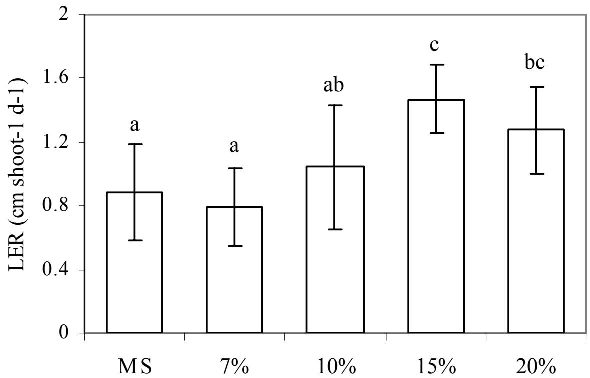

Fig. 2 Growth of eelgrass planted in MS and mixtures of MS and DS with different silt-clay content. *Data are given as mean ± standard deviation; n = 9. Values with common letter (listed above each bar) are not significantly different among samples (P < 0.05).*

It is possible that the increased nutrients in the DS added substrates have caused the increased eelgrass growth. DS from Ago Bay has abundant nutrients that can serve as N and P source (see Table 1) for MS. In another study it was found that addition of DS to MS used for constructing artificial tidal flat, increased Total N and Total P of the substrate leading to increased in benthic populations in the sediments [10]. However, it seems that addition of DS was advantageous to eelgrass growth up to 15% silt-clay and additional DS did not give further increase in growth. Moreover, the lower LER for the 20% silt-clay mixture of MS + DS suggested that too much addition of DS might pose detrimental effects to eelgrass.

Data of the aboveground biomass in Fig. 3 supported the data given by the LER. Increasing the amount of DS added gave increasing aboveground biomass up to mixture with 15% silt-clay. Although plants in the 20% silt-clay gave a lower above-ground biomass data, it was not significantly different from that of the 15% silt-clay mixture. Data of the change in the number of leaves after growth experiment showed almost the same trend as that of LER and aboveground biomass data; wherein increase in the number of leaves up to 15% silt-clay mixture and drop of change in number of leaves for the 20% silt-clay mixture was observed (Fig. 4). Bringing these data all together, further suggests that DS addition up to 15% silt-clay content to MS was favorable for eelgrass growth but too much added DS may not effect to further increase in growth.

Moreover, the decrease in LER, above-ground biomass and change in number of leaves for the 20% silt-clay mixture of MS + DS suggested that too much addition of DS might pose detrimental effects to eelgrass.

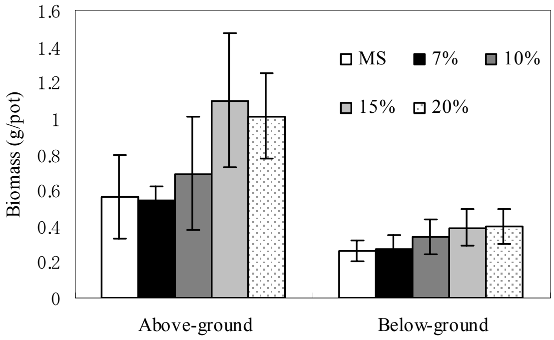

Fig. 3 Aboveground and belowground biomass of eelgrass planted in pure mountain sand (MS) and mixtures of MS and DS with different amounts of silt-clay. *Data are given as mean ± standard deviation; n = 3.*

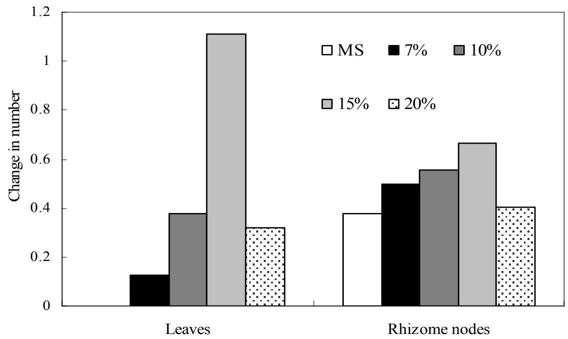

Fig. 4 Average change in number of leaves and rhizome nodes from start to final day of the growth experiment of eelgrass planted in pure mountain sand (MS) and mixtures of MS and DS with different amounts of silt-clay.

C. Root-rhizome System

The extensive root-rhizome system of the eelgrass plants serves as the anatomical feature that enables them to have a stable growth in their substrates [35]. The ease with which plant roots penetrate their substrate will depend on the soil/sediment properties [36]. Thus it is necessary to study the effect of a potential substrate on the root-rhizome system of the eelgrass plants. To investigate the effect of the MS + DS substrates on the root establishment of the eelgrass plants, we measured the uprooting force of the shoots planted in the MS + DS mixtures (Fig. 5). Increase in uprooting force was observed for mixtures with increasing amount of added DS with the maximum uprooting force for the 15% silt-clay mixture. A slight decrease in uprooting force compared to 15% silt-clay mixture was found for the 20% silt-clay mixture, although their difference was found to be not significant.

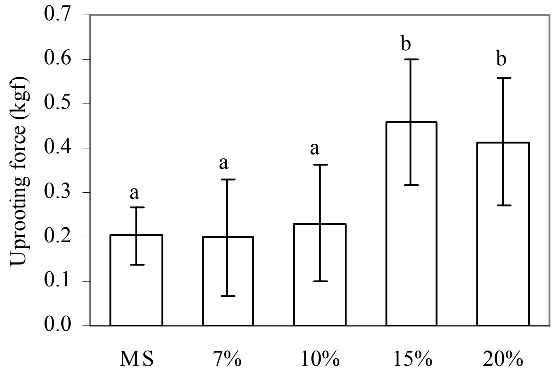

Fig. 5 Uprooting forces of eelgrass in MS and MS+DS mixture substrates containing different amounts of silt-clay. *Data are given as mean ± standard deviation; n = 9. Values with common letter (listed above each bar) are not significantly different among samples (P < 0.05).*

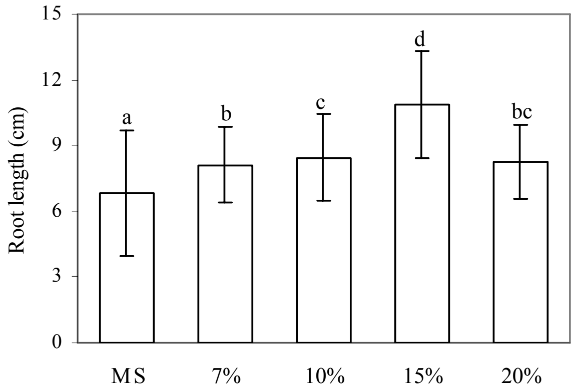

Fig. 6 Length of the longest root of eelgrass shoots planted in MS and mixtures of MS and DS. *Data are given as mean ± standard deviation; n = 9. Values with common letter (listed above each bar) are not significantly different among samples (P < 0.05).*

Addition of DS to MS seems to improve the structure of the sediment as shown by the better establishment of the roots in the substrates with added DS. However, too much added silt-clay can decrease pore water exchange as well as water content in the substrate resulting to increase sediment cohesion which can lead to increase resistance to root penetration [37]. Indeed, measurement of the longest root (Fig. 6) showed that growth of roots of plants in the 20% silt-clay MS + DS mixture was hindered as shown by the decrease in root length in this substrate compared to plants in the 15% silt-clay mixture. Data of the change of rhizome node number (Fig. 4) showed consistent result. Final rhizome nodes number were lower for the 20% silt-clay mixture compared to the 15% silt-clay mixture suggesting the hindered growth of root system in the 20% silt-clay mixture.

Also, decreased pore water exchange in the 20% silt-clay mixture had caused reduced substrate environment in these pots (see ORP data in Table 2) which could have caused stunted growth of plants and root system in these pots. Similar to growth of the shoots, it seems that addition of DS promoted the growth of the root system only up to 15% silt-clay mixture but further addition of DS in the 20% silt-clay mixture didn't show favorable effects anymore. In a previous study [38], almost the same results were obtained when DS was added to slag, another sand media, wherein it was demonstrated that DS addition improved anchorage and root establishments of eelgrass in the DS added pots. Various literature reports a very wide range of silt-clay content in different eelgrass beds (0.8 to 56%, as reviewed in [32]). But it seems that healthy eelgrass beds have silt-clay content below 15% [32], [39], [40]. But then again, a thorough investigation of this observation needs to be done for verification.

IV. Conclusion

Evaluation of DS as silt-clay and organic matter source material for artificially prepared eelgrass substrate was done to develop another beneficial use of dredged material. Addition of DS to the sand media resulted to improved growth of eelgrass. Increased shoot growth was observed with increasing amount of DS added up to 15% silt-clay content. Enhancement of growth was attributed to increased nutrient supply and improved sediment structure of the resulting substrate as shown by the improved root establishment and enhanced growth of the root-rhizome system of the eelgrass plants in the mixed substrates.

Additional DS in the 20% silt-clay mixtures did not result to further increase in growth. Conversely, additional silt-clay in the 20% silt-clay mixture led to reduced substrate environment and resistance to root penetration. This suggests that DS addition may be advantageous to eelgrass up to a certain extent only and too much of it may not be beneficial anymore.

The present study has demonstrated another beneficial use of dredged material in environment enhancement. Use of dredged sediment in eelgrass restoration projects does not only advocate ecological restoration but also promotes resource recycling of this material considered to be wastes long before.

Acknowledgment

The authors would like to acknowledge K. Emoto and T. Yoshimi from Ube College of Technology for their help in this study. We are also grateful to S. Iwasaki for his assistance in eelgrass collection.

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