Anchorage and resistance to uprooting forces of eelgrass (*Zostera marina* L.) shoots planted in slag substrates

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ABSTRACT
Different types of slag (air-cooled, granulated and carbonated granulated slag) were employed as basal media of eelgrass, *Zostera marina* L. We investigated how factors such as slag type, particle size and sedimentation of fine particles affected anchorage of eelgrass to the substrates. It was found that eelgrass planted in slag substrates could endure current velocity of 10 to 50 cm s^{-1}. Thus, slag substrates can adequately support and anchor the eelgrass plants even during severe current flow (50 cm s^{-1}). Root establishment and anchorage were also examined by looking at the resistance of the plants to uprooting forces. Results showed that shoots in slag substrates with the finest particle size (<1.18 mm) gave the highest resistance to uprooting among the particle sizes tested (<1.18, 1.18-2.36 and 2.36-4.75mm). Among the slag substrates, shoots in granulated slag pots gave the highest resistance to uprooting. Addition of dredged sediment (DS) or settling of fine particles improved the root establishment, anchorage of eelgrass and root-rhizome extension. However, it was suggested that addition of DS or settling of fine particles to slag may be beneficial to root establishment up to a certain extent only and too much of it might be harmful to eelgrass plants.

Keywords: current velocity, eelgrass, slag, uprooting, *Zostera marina* L.

INTRODUCTION

Slag, a by-product of the iron and steelmaking process, has been regarded as a substitute material for sea sand since a comprehensive ban was placed on the recovery of sea sand in Seto Inland Sea (The Japan Iron and Steel Federation, 2006). Development of various technologies for use of slag in the marine environment remediation has begun ever since (Takahashi and Yabuta, 2002) and one of them is the use of slag as basal media in seagrass restoration and conservation projects (The Japan Iron and Steel Federation, 2006, Hizon-Fradejas *et al.*, 2009).

Seagrass communities are important component of the estuaries and coastal environment. They are among the most productive aquatic ecosystems known. Their decline all over the world has prompted concerns and efforts for conservation of still existing communities and restoration of the lost ones. In the coastal waters of Japan, eelgrass (*Zostera marina* L.) is the most commonly found seagrass (Hoshika *et al.*, 2006). Similar to what is observed globally, decrease of eelgrass communities has also been observed in Japan. For instance, in Seto Inland Sea alone, vast eelgrass beds that existed during the 1950's decreased by 70% in the last three decades (Komatsu, 1996;

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Hoshika *et al.*, 2006). To alleviate these lost of eelgrass meadows, eelgrass restoration efforts are being done all over the world; among them are transplanting, stockpiling and construction of artificial eelgrass beds (Orth *et al.*, 1999; van Katwijk *et al.*, 2000; Borde *et al.*, 2001; Omoto *et al.*, 2005).

It is said that the properties of soil and sediment determine the ease with which plant roots penetrates their substrates (Eavis and Payne, 1969). If slag is to be used as a substrate in eelgrass restoration projects it is important to study if it can sufficiently support the root establishment of the plants that will be planted on it. For instance, in the Dutch Wadden Sea, pilot eelgrass transplants were lost within 3 weeks, presumably due to water dynamics (van Katwijk and Hermus, 2000). Extreme current velocity can lead to decline or even complete loss of submerged aquatic plants (Koch, 2001). But then, if the seagrass is well established and securely planted in its substrate, it could survive even in high current velocity environments (Fonseca and Kenworthy, 1987; Koch, 2001). The physical characteristics of the substrate may affect the anchorage of the aquatic plants planted on them, which in turn, dictates the resistance of the plants to uprooting due to hydraulic forces (Handley and Davy, 2002).

In their study, Handley and Davy (2002) suggested that the size of substrate particles and closeness of packing were likely to affect the rooting of aquatic plants. In our initial study when slag substrates were compared with sediments from various eelgrass beds, it was found that the particle sizes of the slag substrates were within the acceptable range for growing eelgrass (Hizon-Fradejas *et al.*, 2009). But then, the particle size of slag that will be optimum for eelgrass root establishment and growth was not investigated. In our present study, we have looked at the anchorage of eelgrass roots to various type of slag with different particle sizes. Since sizes of slag can be controlled during production, it will be helpful to know the particle size that will be most beneficial for rearing eelgrass. We have also probed on the possible effect of dredged sediment (DS) addition to slag and eelgrass root-rhizome system. DS, which disposal represent one of the principal dilemma in coastal management had been used as a material to create or augment intertidal habitats (Bolam *et al.,* 2004; Streever 2000). In our study, DS was added to slag to simulate the settling of fine particles with organic matter unto slag when used as substrate in artificial eelgrass beds. In doing so, we can see how these fine particles will affect the root-rhizome system of eelgrass and their anchorage to slag, as well as their effect on growth. The objectives of this study are: (1) to investigate whether eelgrass plants grown in slag substrates can withstand different current velocities; (2) to examine whether particle size can affect the ability of slag to anchor the roots of the eelgrass plants and (3) to investigate how sedimentation of fine particles affects anchoring of the eelgrass in the slag + DS substrates.

MATERIALS AND METHODS

Eelgrass collection and acclimatization

The eelgrass plants were taken from well established eelgrass beds of Yoshina tidal flat in Seto Inland Sea, Japan at a depth of about 2m. The collected plants were soon transported to the laboratory where they were cleaned, trimmed and planted in the sediment collected from the same beds where they had grown. Plants were acclimatized before use for a minimum of 2 weeks in a 100-L tank filled with the artificial sea water

(SEALIFE, 30‰) at 20^{o}C with a light regime of 12 h light and 12 h dark and light intensity of 250-280 μmol photon $m^{-2}s^{-1}$.

Current flow experiment

Three types of slag were used in this experiment: air-cooled slag (ACS), granulated slag (GS) and carbonated granulated slag (CGS). The slag samples were sieved to obtain the following particle sizes: <1.18 mm, 1.18-2.36 mm and 2.36-4.75 mm which resulted to nine (9) types of slag substrates. The acclimatized eelgrass plants were planted (3 shoots per pot and 2 pots per type of substrate) and grown in the nine types of slag substrate for 20 days before they were subjected to current flow test.

We used a coastal flow simulator (JSES-500, Japan Aquatic) to examine the resistance to uprooting of the eelgrass shoots in different slag types at different current velocities. The simulator can contain 2m^3 of water and has an observation chamber with the following dimension: 1.0m (l) x 0.5m (w) x 1.0 m (h). Current velocity can be controlled from 0 to 50 cm s^{-1}. In the initial experiment, eelgrass pots were placed inside the coastal flow simulator containing artificial sea water (SEALIFE, 30‰) at 20 ± 1^{o}C. The whole shoots were submerged in water. Putting each slag substrate type pots at a time, they were subjected to gradual increase of current flow rate, using only one-way flow direction, from 10 to 50 cm s^{-1} at 10 minutes interval, increasing by 10 cm s^{-1} at each time. The number of shoots dislodged from the substrates was counted. For the second part of the current flow experiment, the eelgrass shoots were subjected first at 30 cm s^{-1} current velocity for a longer period of 24 hours. The current velocity was then increased to 50 cm s^{-1} for the next 24 hours. One-way flow direction was used throughout this experiment. After the 48 hours experiment, the eelgrass shoots were then exposed to a current velocity of 50 cm s^{-1} for another 24 hours this time using two-way flow direction which changes every 60 seconds during the entire 24 hour experiment. During the entire test, number of shoots that remained or dislodged from the substrate was noted. The same test was done using natural eelgrass sediment (NES) as the substrate which served as the control. NES samples were taken from the same site where eelgrass plants were collected, the Yoshina Tidal Flat in Seto Inland Sea, which has well-established eelgrass beds.

Resistance to uprooting forces

The resistance to uprooting forces of the eelgrass shoots planted in the slag substrates tested above and NES was determined by measuring the pull force needed to uproot the rhizome and roots from the substrate. This was measured 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. The shoots were cut leaving only about 1 cm above the meristem. The upper part of the remaining meristem was then clipped to the digital force gauge, which was pulled up slowly (11 ± 3 cm min^{-1}) until the peak force needed to uproot the plant was measured. Results were statistically analyzed using paired *t*-test.

Addition of silt-clay using dredged sediment

As mentioned above, slag samples which contain very low amount of silt-clay, were added with DS to see how settling of fine particles will affect anchorage and resistance to uprooting of the eelgrass plants. DS used in the experiment were excavated from Ago

Bay, Japan and met the Japanese standard for sea dumping disposal of dredged sediment (MOE, 2005). DS were added to two slag samples (ACS and GS) to achieve mixtures containing 10% and 20% silt-clay content. It was ensured that mixing was homogenously done. Resistance to uprooting was measured for the mixtures using the same procedures described above.

Growth experiment

Growth experiment was done to know the effects of DS addition on growth of plants, in general and the growth of the root-rhizome system, in particular, based on the premise that well established root-rhizome system will correspond to healthy growth of plants. The following methods were employed for the growth experiment using the slag + DS mixtures and NES. Acclimatized plants having almost the same leaf widths were collected and trimmed to around 50 cm shoot length, retaining only the three youngest leaves and three nodes of rhizome. They were then planted at 2-3 plants per pot, using 3 pots per test substrates and were incubated in the same tank and the same conditions used during acclimatization. Growth was evaluated for 20 days by leaf elongation which was taken every 3-5 days using the leaf marking method (modified Zieman, 1974) and compared with that of the NES. Change in the number of rhizome nodes before and after the growth experiment was determined as well as below-ground biomass. Results were statistically analyzed using paired *t*-test.

RESULTS AND DISCUSSION

Effect of current velocity

Table 1 shows the result of the current flow experiment. The data show that no eelgrass shoot was dislodged in any of the substrates used with the experimental conditions employed. This suggests that eelgrass shoots were able to anchor properly during the 20 days that they were cultivated in the slag substrates, enabling them to withstand even the most severe current flow of 50 cm s^{-1}. The data we have obtained is not unusual since once established, many seagrasses are capable of existing in a wide range of energy regimes. For instance, maximum current velocity observed for *Zostera marina* beds is between 120 and 150 cm s^{-1} or even up to 180 cm s^{-1} (Fonseca *et al.*, 1983; Fonseca and Kenworthy, 1987; Koch, 2001). Of course, as suggested by van Katwijk and Hermus (2000), establishment of the eelgrass in the substrate is a prerequisite for them to withstand very severe current flow. As had been reported in the literature, failure of plants to establish in their substrates had caused them to be washed away during severe water dynamics (van Katwijk and Hermus, 2000; van Katwijk *et al.*, 2000).

In Seto Inland Sea occurrence of 50 cm s^{-1} current velocity is very seldom since it is semi-enclosed, but is probable to happen during bad weather conditions (Kobayashi *et al.*, 2006; Kasai *et al.*, 2007). In eelgrass meadows in Hiroshima area, current flow rates seldom exceeded 8 cm s^{-1} (Tamaki *et al.*, 2002). Thus, given the flow regimes in Seto Inland Sea, it is possible that slag can be used as a potential substrate in artificial eelgrass bed constructions in Seto Inland Sea.

Table 1 Current velocity effect on eelgrass planted in different slag substrates with different particle sizes

A. Gradual increase in current velocity (10 to 50 cm s^{-1}, one-way current flow)

Substrate	Particle size (mm)		
	<1.18	1.18-2.36	2.36-4.75
ACS	○	○	○
GS	○	○	○
CGS	○	○	○
NES	○		

B. Current velocity of 30 cm s^{-1} for 24 hour (one way current flow)

Substrate	Particle size (mm)		
	<1.18	1.18-2.36	2.36-4.75
ACS	○	○	○
GS	○	○	○
CGS	○	○	○
NES	○		

C. Current velocity of 50 cm s^{-1} for 24 hour (one way current flow)

Substrate	Particle size (mm)		
	<1.18	1.18-2.36	2.36-4.75
ACS	○	○	○
GS	○	○	○
CGS	○	○	○
NES	○		

D. Current velocity of 50 cm s^{-1} for 24 hour (two way current flow)

Substrate	Particle size (mm)		
	<1.18	1.18-2.36	2.36-4.75
ACS	○	○	○
GS	○	○	○
CGS	○	○	○
NES	○		

Note: ACS = air-cooled slag; GS = granulated slag; CGS = carbonated granulated slag; NES = natural eelgrass sediment; n=6. The symbol ○ means no dislodged shoots.

Resistance to uprooting

While data in the current flow experiment didn't reveal the effect of substrate particle size on root establishment, data for uprooting force showed that eelgrass planted in the finest particle size (<1.18 mm) were most anchored to the substrate among the particle sizes tested (Fig. 1). Moreover, among the slag substrates tested, shoots in GS pots gave the highest resistance against uprooting among the same <1.18 mm particle size samples, possibly because among the slag types used it is the most unconsolidated and closest to natural sand in terms of soil texture having fine, granulated structure (The Japan Iron and Steel Federation, 2006; Hizon-Fradejas *et al.,* 2009). Since eelgrass is a natural entity, they are expected to prefer substrates similar to their natural habitat. ACS and CGS on the other have rounded structure, making roots difficult to anchor due to low sediment cohesion.

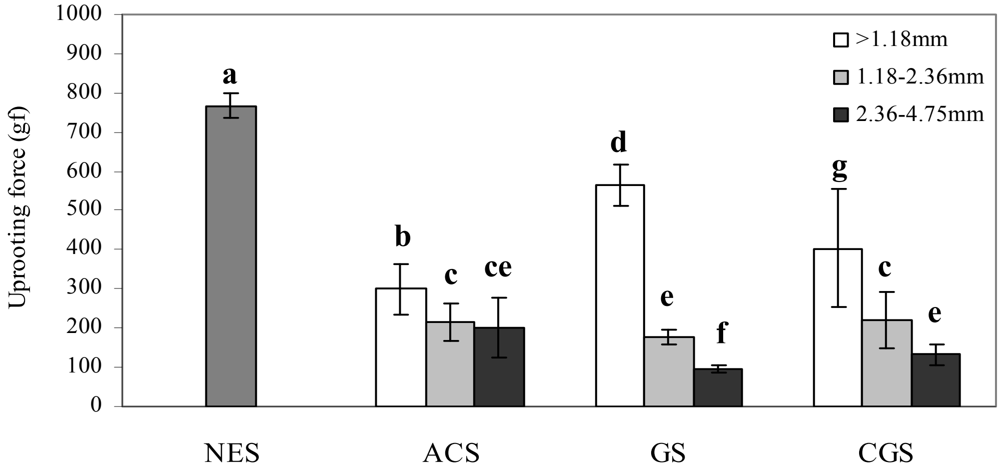

Fig.1 - Uprooting forces of eelgrass planted in slag substrates with various particle sizes.
Data are given as mean ± standard deviation; n=5. Values with common letter (listed above each error bar) are not significantly different (at P<0.05) among samples.

Field observations have identified the existence of eelgrass meadows in a wide variety of sediment types ranging from coarse sand to fine silt (Kenworthy and Fonseca, 1977; Koch, 2001). At first glance, it seems that eelgrass is quite non-selective with regard to colonization in a particular sediment. But then, literatures prove that seagrasses can modify the sediment they colonize, usually leading to the development of fine silt (Kenworthy and Fonseca, 1977; Koch, 2001; Tamaki *et al.*, 2002). It is possible then that silt development in the field is also conducive for growth of eelgrass beds. These suggest that our result is consistent with the literature report; eelgrass root-rhizome system established well and preferred substrates with finer particle size. Nevertheless, the highest uprooting force observed among the slag substrates (<1.18 mm, GS) is still lower compared to NES (Fig. 1). This suggests that aside from particle size effects, some other factors might be operating and preventing eelgrass to achieve its optimum root establishment; for instance, problems related to geochemistry of slag. In the initial evaluation of slag as eelgrass substrate, it was found that slag substrates release possible toxins like sulfide and Zn (Hizon-Fradejas *et al.,* 2009).

Addition of dredged sediment unto slag substrates

As mentioned above, development of fine silt in the field seems to be conducive for eelgrass growth. In the field, fine particles are trapped by the plants leading to development of a particle profile with fine silt (Kenworthy and Fonseca, 1977; Koch, 2001). Thus, we have added DS to slag substrates to replicate this natural settling of fine particles in the field and see how this can affect the root-rhizome system and their anchorage to slag substrates. On the other side, if slag + DS mixtures will be used as substrate for stockpiled or artificially reared eelgrass, we can also see how DS addition to slag can be beneficial or harmful to eelgrass plants.

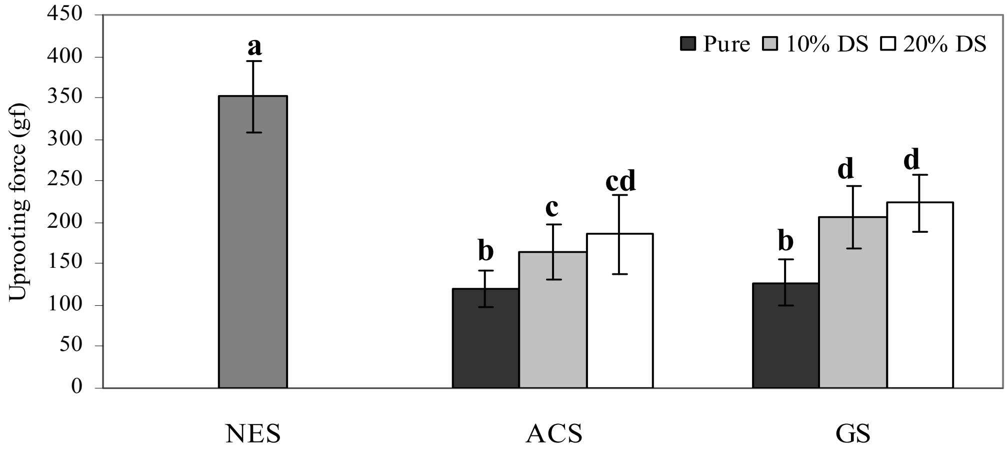

Fig. 2 - Uprooting forces of eelgrass planted in slag + DS mixture substrates.
Data are given as mean ± standard deviation; n=5. Values with common letter (listed above each error bar) are not significantly different (at P<0.05) among samples.

Measurement of the uprooting force for the slag + DS mixtures is shown in Fig. 2. It was noticeable that the uprooting force mean value for NES in Fig. 1 was reduced into half in Fig. 2. It is possible that the difference in the plant material used in these two set of experiments caused the discrepancy. Eelgrass plants used for the experiment of effect of particle size were collected in December, 2005 while plants used for the effect of DS addition were collected in February. In an experiment investigating the uprooting forces of eelgrass taken in different sampling time (with two months interval), it was found that uprooting force values were different for the two sets of eelgrass by 1/3 to 1/2 of the other set (Y. Nakano, unpublished data).

Nevertheless, results in Fig. 2 revealed that addition of DS to both slag type tested improved the anchorage of the shoots. A similar trend was observed for both ACS and GS; that is a big increase in the uprooting force from pure slag to 10% silt-clay mixture which is statistically significant; and just a slight (not statistically significant) increase between the 10% and 20% mixture. The results suggested that the effect of DS addition to eelgrass reaches a maximum and then levels off. This implies that addition of DS to slag or settling of fine particles to slag may be beneficial to root establishment up to a certain extent only. Too much of it may not exhibit significant effect anymore and on the extreme can pose some risks. Naturally, the size of substrate particles and closeness of packing can affect root penetration. As particle size distribution becomes skewed towards silt-clay, the water content decreases resulting to increase sediment cohesion and more resistance to root penetration (Handley and Davy, 2002). Moreover, decreased pore water exchange with the water column can lead to too much nutrients and release of sulfides in the sediment (Holmer and Nielsen, 1997). On the other extreme, coarse substrates also offer great resistance to root penetration since the particle size is relatively large and the density is high (Handley and Davy, 2002). Thus, in adding DS to slag to be used as substrate for artificial rearing of eelgrass, it is important to know the amount that will give maximum benefit and minimum harm. Therefore, we examined the possible effect of DS addition on growth of eelgrass plants.

Effect of DS addition on growth and root-rhizome system of eelgrass

Fig. 3 gives the result of the growth experiment done using the slag + DS mixture substrates. For the ACS+DS mixtures, increase of the silt-clay content to 10% increased the leaf elongation rate (LER) significantly from 0.67 to 0.88 cm $shoot^{-1}$ d^{-1}. But further increase of silt-clay content to 20% did not effect to further increase in LER. On the other hand, for the GS+DS mixtures, the highest LER was exhibited by the plants in the mixture containing 20% silt-clay. Both plants in the 10% and 20% GS+DS mixtures gave LER not significantly different from that of NES. Since data showed insignificant differences between LER of the 10% and 20% mixtures, both for ACS and GS, we can generally say that LER data agrees with the uprooting force data. Similarly, addition of DS to slag may be beneficial to eelgrass growth up to a certain extent only and too much addition may not demonstrate significant effect anymore.

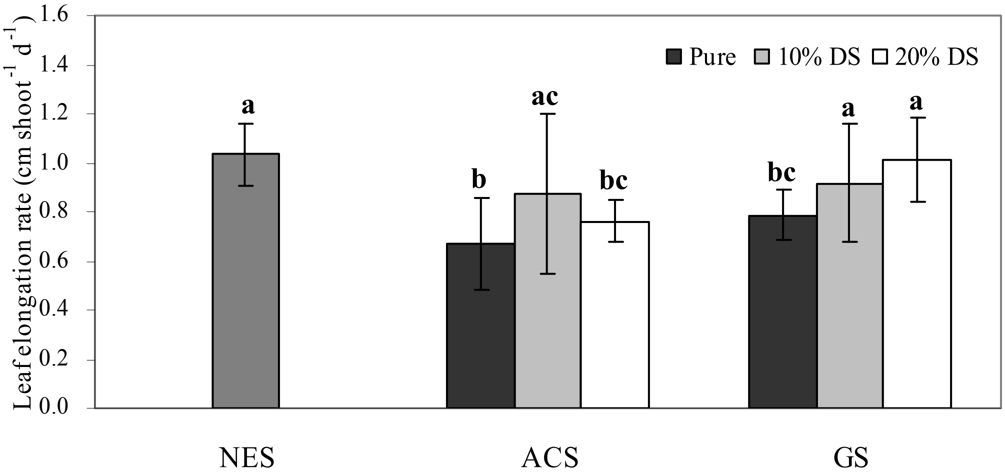

Fig. 3 - Comparison of growth of eelgrass in the slag + DS mixture substrates.

Data are given as mean ± standard deviation; n=5-6. Values with common letter (listed above each error bar) are not significantly different (at P<0.05) among samples.

The anatomical feature which provides seagrasses their ability to grow on their substrates is their extensive root-rhizome system (Fonseca and Kenworthy, 1987). For this reason it is important to investigate the effect of a test substrate on the root-rhizome system of the eelgrass. The change in the number of rhizome nodes and data of below-ground biomass are given in Fig. 4. These data further showed how addition of DS affected the root-rhizome system. For GS, the results clearly showed that addition of DS enhanced rhizome extension as observed from the increase in number of rhizome nodes and increase in below-ground biomass with DS addition. On the other hand, ACS data showed that root-rhizome improvement was evident from the plants in the mixture with 10% silt-clay only, as shown by the significant increase of the rhizome node number and below-ground biomass in this mixture compared to pure ACS substrate. For the 20% ACS+DS mixture, average values of both change in the number of rhizome nodes and below-ground biomass were lower than that of the 10% mixture but their differences were not statistically significant. It seems that disadvantageous effect of too much DS addition was starting to manifest in the 20% ACS+DS mixture. It is possible that the root penetration was perturbed for the 20% ACS + DS mixture, probably due to compactness of the substrate brought about by the combined effects of the high density of ACS and closeness of packing in DS. However, since ACS data for the 20% silt-clay mixture were not significantly different from that of the pure ACS and 10% mixture, this inference cannot be conclusive. Differences in the ACS and GS data could be attributed to differences between their properties. ACS was coarse and dense while GS was finer (Hizon-Fradejas *et al.,* 2009). Generally, data in Fig. 4 showed that healthy root-rhizome system corresponds to good anchorage of plants to their substrate and higher force needed to uproot the plant.

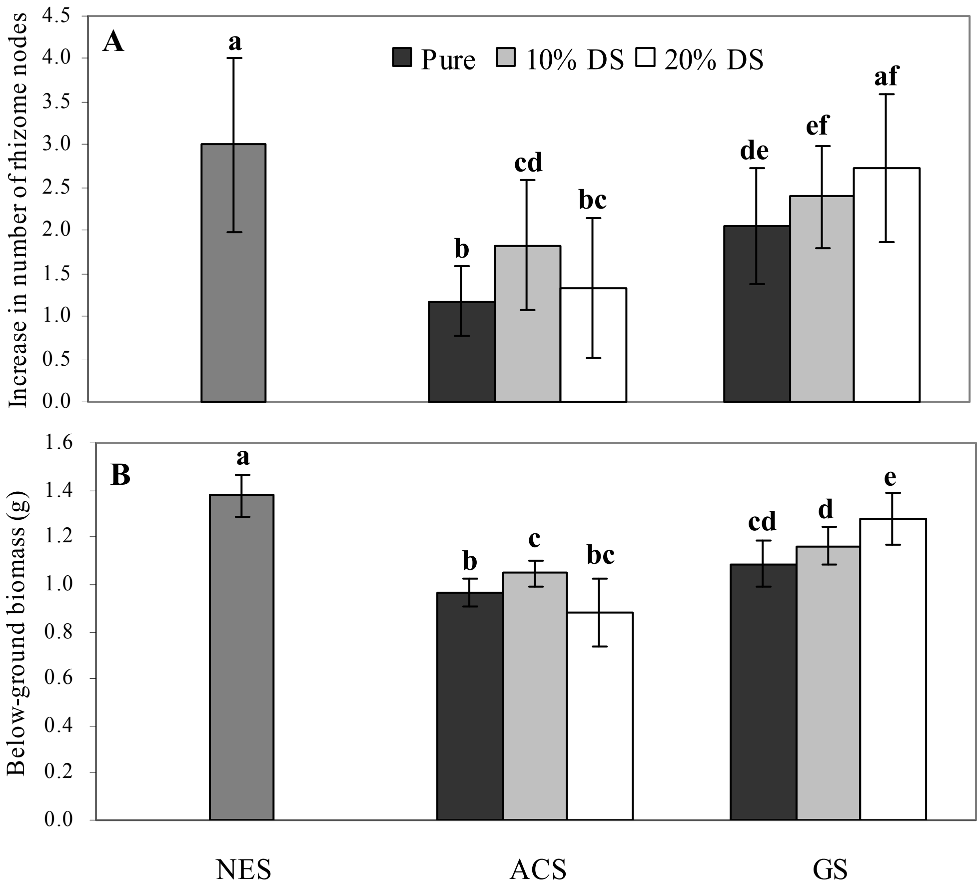

Fig. 4 - Change in the number of rhizome nodes (A) and below-ground biomass (B) of eelgrass planted in slag + DS mixtures.

Data are given as mean ± standard deviation; n=5-6 for A and n=3 for B. Values with common letter (listed above each error bar) are not significantly different (at P<0.05) among samples.

CONCLUSIONS

The following were understood in the present study:

(1) Eelgrass shoots were able to anchor properly to the slag substrates during their 20 days cultivation in the substrates enabling them to withstand even the most severe current flow of 50 cm s^{-1}. Thus, given the flow regimes in Seto Inland Sea, it is possible that slag can be used as a potential substrate in artificial eelgrass bed constructions in Seto Inland Sea.

(2) Data for uprooting force showed that eelgrass planted in the finest particle size (<1.18 mm) were most anchored to the substrate among the particle sizes tested.

Moreover, among the slag substrates tested, it was shoots in GS pots that gave the highest resistance against uprooting among the same <1.18mm particle size samples.
(3) Addition of DS to slag or sedimentation of fine particles to slag may be beneficial to root establishment up to a certain extent and too much of it may not exhibit significant effect anymore and on the extreme can pose some risks.
(4) For GS, addition of DS enhanced rhizome extension as observed from the high increase of rhizome nodes and increase in below-ground biomass with DS addition. Data for ACS is consistent with the growth data, wherein growth of rhizome was enhanced only for the mixture with 10% silt-clay but not for the 20%.

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