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ECOSYSTEM-LEVEL MICROCOSM-WET ASSESSMENT OF ADSORPTION AND REMOVAL BY CALCIA-IMPROVED SOILS FOR *Ulva* spp. ELUATE

Sosuke Sato[1] and *Kazuhito Murakami[2]

[1]Graduate School of Engineering, Chiba Institute of Technology, Chiba, Japan; [2]Department of Life Science, Chiba Institute of Technology, Chiba, Japan

*Corresponding Author, Received: 29 May 2025, Revised: 13 Mar. 2026, Accepted: 15 Mar. 2026

ABSTRACT: An ecosystem-level evaluation was conducted to assess the adsorption and removal characteristics of Calcia-improved soil for eluate derived from decaying green tide algae (*Ulva* spp.) using a microcosm-based whole effluent toxicity (WET) test. Calcia-improved soil, produced from steelmaking slag, is recognized as a useful recycled material; however, due to its chemical characteristics, evaluation of its potential environmental impact is required prior to practical application. The microcosm (N-system) is a flask-scale model ecosystem composed of three phytoplankton species as producers, four zooplankton species as consumers, and four dominant bacterial species as decomposers, and is known for its high reproducibility and stability. In the microcosm-WET test, dissolved oxygen (DO) as a functional parameter was continuously monitored, and changes in microbial abundance as structural parameters were observed microscopically following the addition of treated or untreated eluate obtained from unsolidified Calcia-improved soil. Statistical evaluation was performed using a branched-type analysis of variance (ANOVA). For both parameters, the highest concentration showing no statistically significant effect was defined as the microcosm no-observed-effect concentration (m-NOEC), and the toxicity unit (TU) was calculated based on the dilution factor. The m-NOEC for the treated eluate was determined to be 2.5%, corresponding to a TU value of 40, indicating a lower impact compared with the untreated eluate. Considering the reported safety factor of 200 for extrapolating microcosm results to real environments, Calcia-improved soil was not expected to have a significant environmental impact.

Keywords: Calcia-improved soil, Microcosm, Microcosm-WET test, Green tide alga, Ulva spp.

1. INTRODUCTION

With increasing environmental awareness in Japan, Calcia-improved soil has been evaluated as a recycled material that reduces environmental burdens from the perspectives of resource and energy conservation and has been applied as a reclamation material for marine bottom sediments [1]. Calcia-improved soil is produced by mixing Calcia-modified material with dredged soil (Fig. 1) and is characterized by its ability to enhance sediment strength and improve water quality. Owing to these properties, it has attracted considerable attention as a material for bottom sediment improvement in coastal environments and has been increasingly considered for practical applications in coastal restoration and sediment management projects. However, due to the chemical characteristics of its raw materials, the potential impacts of Calcia-improved soil on water quality and aquatic ecosystems must be carefully evaluated prior to widespread application. In particular, the dissolution of certain components from modified materials may influence water chemistry and potentially affect aquatic organisms. Therefore, comprehensive environmental assessments are necessary to ensure that the application of recycled materials does not lead to unintended ecological risks in coastal environments. At present, most safety assessments of sediment remediation materials rely primarily on chemical analyses that quantify the concentrations of specific substances. Although such analyses provide useful information regarding potential contamination, they do not necessarily reflect the actual biological effects on aquatic organisms. Even when biological evaluations are conducted, they are often limited to single-species toxicity tests. While these tests are useful for detecting acute toxic effects, they do not account for interactions among organisms or the complexity of ecological processes occurring in natural ecosystems. As a result, the ecological relevance of these assessments remains limited when predicting environmental responses at the ecosystem level [2]. To overcome these limitations, whole effluent toxicity (WET) testing has been increasingly adopted as an integrative approach for evaluating environmental impacts. WET tests assess the overall biological effects of complex mixtures rather than focusing on individual chemical substances. When combined with microcosm systems—controlled experimental ecosystems that incorporate multiple trophic levels—this approach allows researchers to examine ecological responses while considering interactions among different organisms [3–6]. Microcosm-based experiments can simulate simplified ecosystem structures that include producers, consumers, and decomposers, thereby enabling the evaluation of both structural parameters, such as community composition and population dynamics, and functional parameters, including ecosystem metabolism and energy flow. Such ecosystem-level assessments are particularly important in coastal environments, where biological communities are strongly influenced by multiple environmental stressors. In tidal flat ecosystems, for example, large-scale macroalgal blooms known as green tides frequently occur and can substantially alter ecosystem processes. Green tides, typically dominated by species of the genus *Ulva*, are characterized by rapid algal proliferation under eutrophic conditions and often lead to the accumulation and decomposition of large amounts of biomass. The decomposition of macroalgae can result in oxygen depletion, the release of organic matter and odor compounds, and significant changes in water quality. These processes may affect the structure and functioning of benthic and pelagic communities and can influence the ecological balance of tidal flat environments. Because tidal flats are highly dynamic systems influenced by tidal cycles, sediment–water interactions, and biological activities, evaluating the ecological impacts of sediment remediation materials in such environments requires approaches that consider ecosystem complexity. Microcosm-based WET testing provides a useful experimental framework for addressing this challenge by enabling the simultaneous observation of multiple ecological responses under controlled yet environmentally relevant conditions. In this study, we focused on the adsorption and removal capabilities of Calcia-improved soil and aimed to evaluate both its effectiveness in bottom sediment improvement and its potential impacts on surrounding ecosystems. The assessment was conducted using a microcosm-based WET testing approach under conditions simulating a tidal flat environment affected by green tide. By integrating multi-trophic biological interactions and ecosystem responses, this study provides a comprehensive framework for evaluating both the environmental effectiveness and ecological safety of Calcia-improved soil prior to field application. The findings contribute to environmental risk assessment and support decision-making in coastal management projects, promoting the appropriate use of recycled materials and the sustainable restoration of tidal flat ecosystems. The remainder of this paper is organized as follows. Section 2 describes the experimental design and microcosm setup. Section 3 presents the results of structural and functional ecosystem responses. Section 4 discusses the implications for environmental risk assessment and sediment remediation. Finally, Section 5 summarizes the main conclusions.

2. RESEARCH SIGNIFICANCE

This study introduces an ecosystem-scale microcosm whole effluent toxicity framework to evaluate adsorption and removal performance of Calcia-improved soils under green tide conditions. Unlike conventional chemical or single-species assessments, it integrates multi-trophic biological interactions with functional and structural ecosystem responses. This approach simultaneously quantifies pollutant mitigation efficiency and ecological risk, enabling mechanistic interpretation of material–ecosystem feedbacks under simulated tidal environments. It establishes a reproducible evaluation method linking recycled-material performance with ecosystem-level safety thresholds, advancing predictive environmental risk assessment. The framework provides transferable scientific evidence supporting sustainable sediment remediation and informed coastal resource management decisions worldwide while strengthening nature-based restoration strategies.

3. MATERIALS AND METHODS

3.1 Calcia-Improved Soil

Calcia-improved soil is a recycled material produced by mixing Calcia-modified material—manufactured from steelmaking slag with controlled particle size and chemical composition—with dredged soil [1]. The incorporation of Calcia-modified material enhances the mechanical

Fig.1 Overview of Calcia-improved soil

strength of the resulting soil and is expected to improve water quality through the adsorption and removal of substances such as phosphorus and sulfides. However, because steelmaking slag is used as a raw material, Calcia-improved soil is known to increase pH, particularly in aquatic environments. This characteristic necessitates careful evaluation of its potential effects when applied under real environmental conditions. The mixing ratio of Calcia-modified material in Calcia-improved soil is generally set at approximately 30% in practical applications [1]. In previous studies, dissolution experiments were conducted using Calcia-improved soil with mixing ratios of 30%, 20%, and 10%. These studies reported a significant increase in pH at a mixing ratio of 30% compared with the lower ratios. Based on these findings, the present study focused on mixing ratios of 20% and 30% to evaluate both the effectiveness of Calcia-improved soil and its potential ecological impacts under conditions relevant to practical use.

3.2 Microcosm

A microcosm is a flask-scale simulated ecosystem in which multiple species coexist under controlled conditions (Fig. 2). By reproducing material cycling and biological interactions characteristic of natural ecosystems, microcosm systems enable ecosystem-level impact assessment, providing a more integrative evaluation than conventional single-species toxicity tests. In addition, microcosms offer advantages such as low experimental cost and high reproducibility. In this study, a gnotobiotic-type microcosm (N-system) maintained by serial passaging was used.

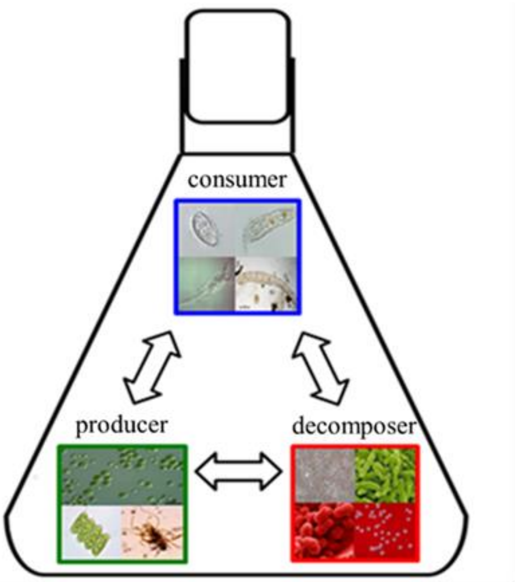

Fig.2 Outline of microcosm

All constituent species of the N-system are known and include primary producers, consumers, and decomposers. The producers consisted of the green algae *Chlorella* sp. (1.1×10^5 cells mL^{-1}) and *Scenedesmus quadricauda* (5.3×10^3 cells mL^{-1}). The consumers included the filamentous cyanobacterium *Tolypothrix* sp. (8.0×10^2 cm mL^{-1}), the ciliate protozoan *Cyclidium glaucoma* (6.0×10^3 cells mL^{-1}), the rotifers *Lecane* sp. (1.3×10^2 individuals mL^{-1}) and *Philodina erythrophthalma* (1.1×10^1 individuals mL^{-1}), and the oligochaete *Aeolosoma hemprichi* (1.0×10^1 individuals mL^{-1}). The decomposer community consisted of four dominant bacterial species (*Bacillus cereus*, *Pseudomonas putida*, *Acinetobacter* sp., and coryneform bacteria), with a total abundance of 1.1×10^6 CFU mL^{-1}. Values in parentheses indicate steady-state population densities under non-additive conditions. These microbial communities were self-selected and stabilized through repeated subculturing.

Microcosms were established in 300 mL Erlenmeyer flasks containing 200 mL of TP100 medium (Taub medium supplemented with 100 mg L^{-1} peptone). After inoculation with 10 mL of microcosm seed culture, flasks were incubated at 25°C under an illumination of 2400 lux with a 12 h light/dark cycle for 30 days [3,7,8]. Each experimental condition was conducted in triplicate. The coefficients of variation (standard deviation/mean, %) for production and respiration rates were ≤20% during days 0–7 (based on 25 independent experiments) and ≤5% during days 14–21, consistent with previously reported values. Data presented in figures represent the mean values of three independent experiments. Differences between treated and control microcosms were evaluated using branched-type analysis of variance (branched-type ANOVA), in which variability among samples is treated as a random effect.

3.3 Microcosm-WET Test

In the microcosm-based whole effluent toxicity (WET) test, the test stock solution was added to the microcosm by partially replacing the culture

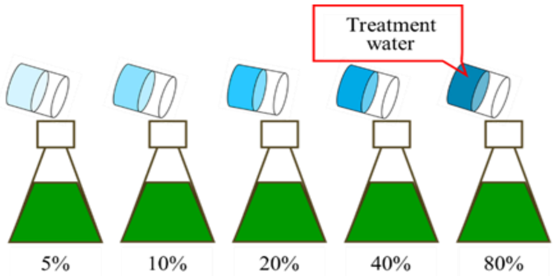

Fig.3 Outline of microcosm-WET test

medium with a diluted test solution, and the resulting biological responses were evaluated (Fig. 3). This replacement approach allowed the effects of the test solution on the microcosm to be assessed while maintaining the overall structure of the simulated ecosystem. The test stock solution was diluted with TP_2 medium, a modified medium with reduced peptone content, in order to minimize secondary effects on microbial abundance arising from differences in nutrient availability rather than from the test solution itself. Prior to the addition of the test solution, the microcosm culture was transferred to centrifuge tubes and centrifuged at 250 × g for 5 min to adjust the biomass. The supernatant was carefully removed using a micropipette, after which the diluted test solution prepared with TP2 medium was added to restore the original culture volume. The test stock solution was added stepwise at final concentrations of 0, 5, 10, 20, 40, and 80% (v/v) in the microcosm. Each concentration was tested under the same experimental conditions to evaluate concentration-dependent effects on the microcosm.

3.4 Preparation of Eluate

The experiment was conducted using *Ulva* spp. collected from the Yatsu Tidal Flat in Narashino City, Chiba Prefecture, Japan (Fig. 4). The Yatsu Tidal Flat is an artificial tidal flat created through land reclamation and has been registered as a Ramsar wetland since 1993. In recent years, excessive growth of *Ulva* spp. has been observed at this site, resulting in odor generation, landscape degradation, and a decline in migratory bird populations [9–12]. Harvested *Ulva* spp. were placed in TP_2 medium, and 2000 mL of TP_2 medium was added to the biomass. The mixture was incubated for 7 days at 30°C under an illumination of 2400 lux to allow decomposition. After incubation, solid residues were removed, and the resulting liquid was collected as the test stock solution (eluate). To evaluate the adsorption capacity of Calcia-improved soil, materials with mixing ratios of 20% and 30% were prepared. Immediately after preparation, 25 g

Fig.4 Location of Yatsu Tidal Flat

Fig.5 A green tide that occurred tidal flat (left) and the *Ulva* spp. that makes up the green tide (right)

Fig.6 Test stock solution preparation method

Table1 Test stock solution water quality analysis results

	Decaying *Ulva* spp. leachate water	Filtered water with dredged soil	Filtered water with 20% calcia-improved soil	Filtered water with 30% calcia-improved soil
COD(mg/L)	76	42.4	44	58
T-N(mg/L)	20.2	66.1	21.1	19.5
NO_3-N(mg/L)	7.3	10.0	14.0	2.1
NO_2-N(mg/L)	0.12	0.07	0.07	0.01
NH_4-N(mg/L)	4.4	3.1	1.5	3.0
T-P(mg/L)	4.0	3.6	0.2	2.1
PO_4-P(mg/L)	1.87	3.02	0.04	0.37
Cl(mg/L)	827.2	1489.0	472.7	236.4
pH	9.19	9.19	11.3	11.6
H_2S-S(mg/L)	0.1	0.1	0.1	0.1

of each Calcia-improved soil was placed into a glass funnel (upper outer diameter: 90 mm; stem length: 90 mm; stem outer diameter: 8 mm). The test stock solution was filtered under gravity through the Calcia-improved soil to obtain 20% and 30% filtered test solutions, respectively (Fig. 6). The Calcia-modified material used for soil preparation was supplied with adjusted chemical composition by the Nippon Slag Association, and its particle size was controlled to ≤2 mm. Mixing was performed based on volume ratios. Water quality parameters of the test stock solution were analyzed, including chemical oxygen demand (COD), total nitrogen (T-N), nitrate nitrogen (NO_3-N), nitrite nitrogen (NO_2-N), ammonium nitrogen (NH_4-N), total phosphorus (T-P), phosphate phosphorus (PO_4-P), chloride (Cl^-),

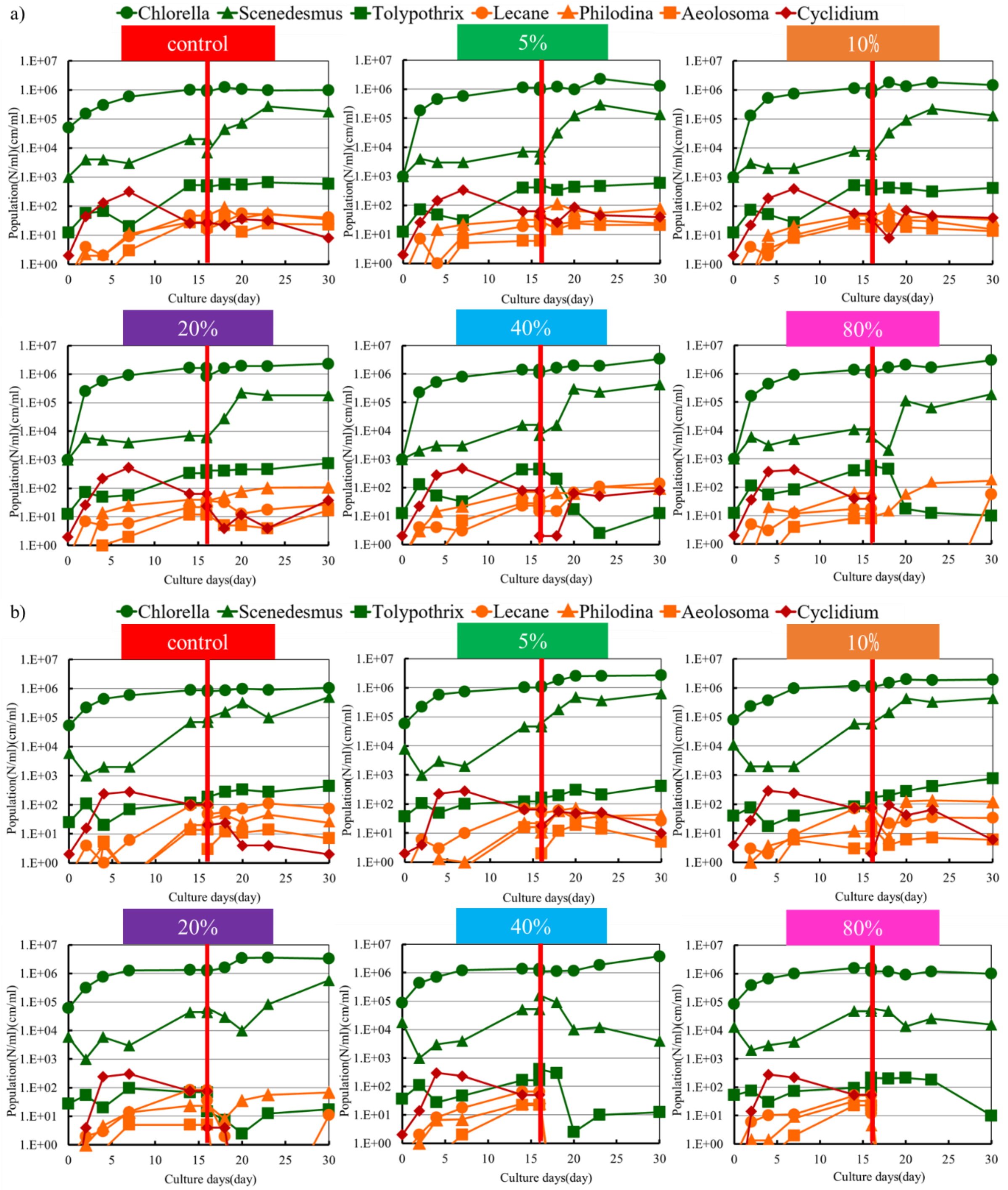

Fig.7 Changes in the population of plankton in the microcosm. a) filtered water with 20% Calcia-improved soil added system (upper), and b) filtered water with 30% Calcia-improved soil added system (lower). (The red vertical line indicates the date on which the test stock solution was added.)

pH, and hydrogen sulfide sulfur (H_2S-S) (Table 1). COD was determined using the potassium by absorbance spectrophotometry following potassium peroxodisulfate digestion. Chloride was quantified by silver nitrate titration, and the remaining parameters were measured using commercial pack test kits.

3.5 Evaluation Method

Impact risk assessment for each concentration of green tide eluate was conducted based on changes in dissolved oxygen (DO) and microorganism abundance in the microcosm. DO was continuously monitored using a DO meter, and

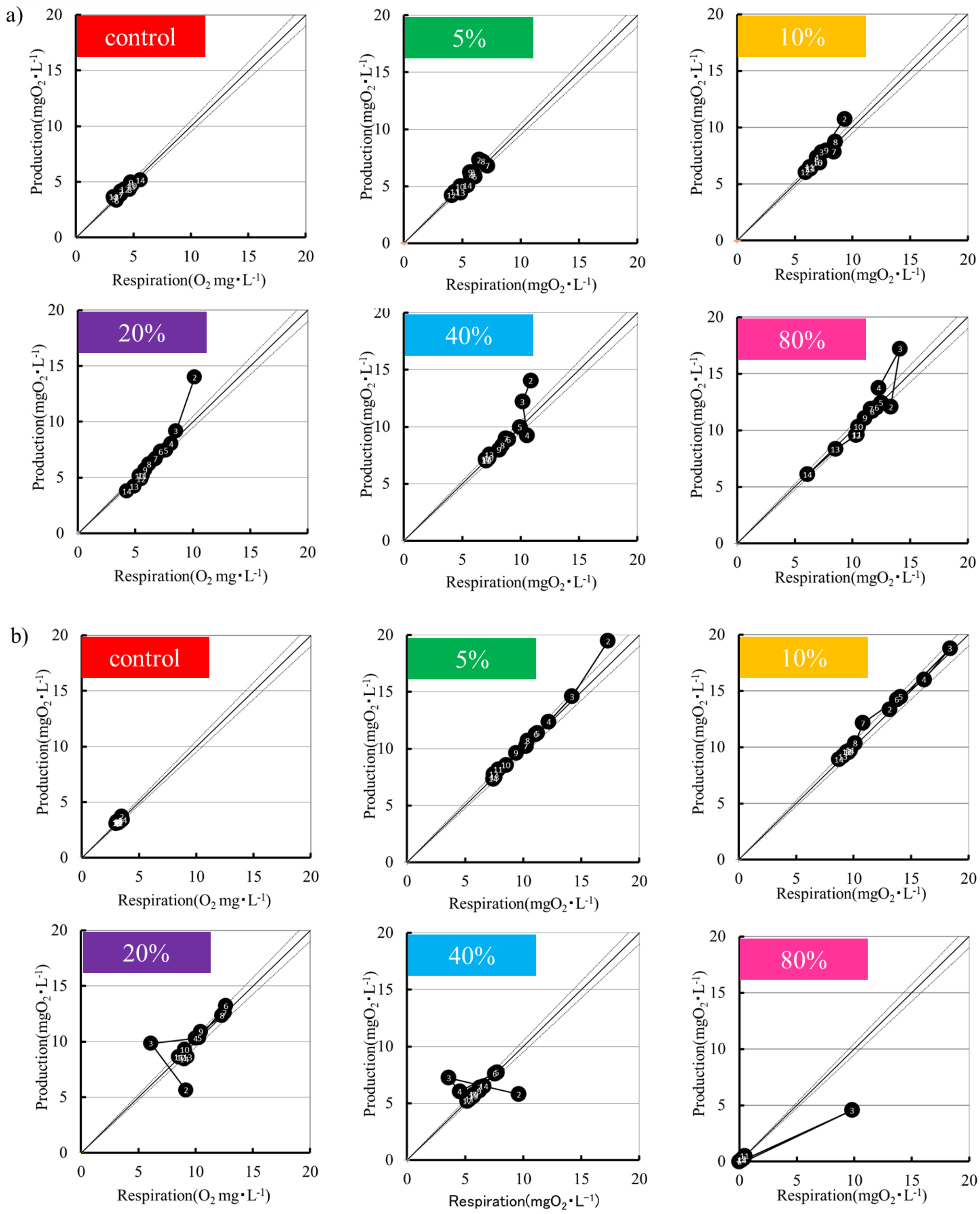

Fig.8 Changes in P/R ratio of microcosm. a) filtered water with 20% Calcia-improved soil added system (upper), and b) filtered water with 30% Calcia-improved soil added system (lower). (The dotted lines in the figures indicate a range of plus or minus 0.05, respectively.)

parameters of ecosystem metabolism. In parallel, structural parameters were evaluated by temporal variations in production (P), respiration (R), and the P/R ratio were calculated as functional parameters of ecosystem metabolism. In parallel, structural parameters were evaluated by quantifying the abundance of microorganisms constituting the microcosm. Subsamples (1 mL) were collected from each microcosm at 0, 2, 4, 7, 14, 16, 18, 20, 23, and 30 days after the start of exposure. Plankton abundance was determined under a light microscope at 40× magnification using a plankton counting chamber, and temporal changes in population densities were recorded. Changes over time in both functional and structural parameters were statistically evaluated using branched-type analysis of variance (branched-type ANOVA). As summarized in Table 2, branched-type ANOVA separates variance into two components: "group," representing differences among treatment systems based on all observed values, and "interaction," representing time-dependent treatment effects [3]. For each functional and structural parameter, the highest test concentration that showed no statistically significant effect in the branched-type ANOVA was defined as the microcosm no-observed-effect concentration (m-NOEC). The toxicity unit (TU) was calculated from the reciprocal of the m-NOEC, as shown in Eq. (1). A safety factor of 200 for he microcosm-based assessment has been proposed in previous studies and was adopted for environmental risk interpretation in this study [3,4,12].

$$TU = \frac{100}{m-NOEC} \qquad (1)$$

4. RESULTS AND DISCUSSION

4.1 Improvement Effect on Water Quality

Table 1 summarizes the results of the water quality analysis for four solutions: decaying *Ulva* spp. eluate, eluate treated with dredged soil alone (prior to the addition of Calcia-modified material), eluate filtered through Calcia-improved soil with a 20% mixing ratio, and eluate filtered through Calcia-improved soil with a 30% mixing ratio. Compared with both the untreated eluate and the dredged-soil-only treatment, filtration through Calcia-improved soil resulted in marked reductions in total phosphorus (T-P) and phosphate (PO_4-P). These results indicate a strong phosphorus removal capacity of Calcia-improved soil, consistent with previous reports attributing such removal to calcium-induced phosphate precipitation and adsorption processes. Although the underlying mechanisms were not directly investigated in this study, the increase in COD observed under the 30% mixing condition likely reflects a balance between

Table 2 Statistical analysis results using branched-type ANOVA. (○ shows no effect, × shows effected.)

		Filtered water with 20% Calcia-improved soil added system				Filtered water with 30% Calcia-improved soil added system			
		P/R ratio	Respiration	Production	Evaluation	P/R ratio	Respiration	Production	Evaluation
5%	Group	○	○	○	×	○	○	○	×
	Interaction	○	○	×		○	×	×	
10%	Group	○	○	○	×	○	○	○	×
	Interaction	○	○	×		○	×	×	
20%	Group	○	○	○	×	○	×	×	×
	Interaction	×	×	×		×	×	×	
40%	Group	○	○	○	×	○	○	○	×
	Interaction	×	×	×		×	○	×	
80%	Group	○	○	○	×	×	○	×	×
	Interaction	×	×	×		×	×	×	

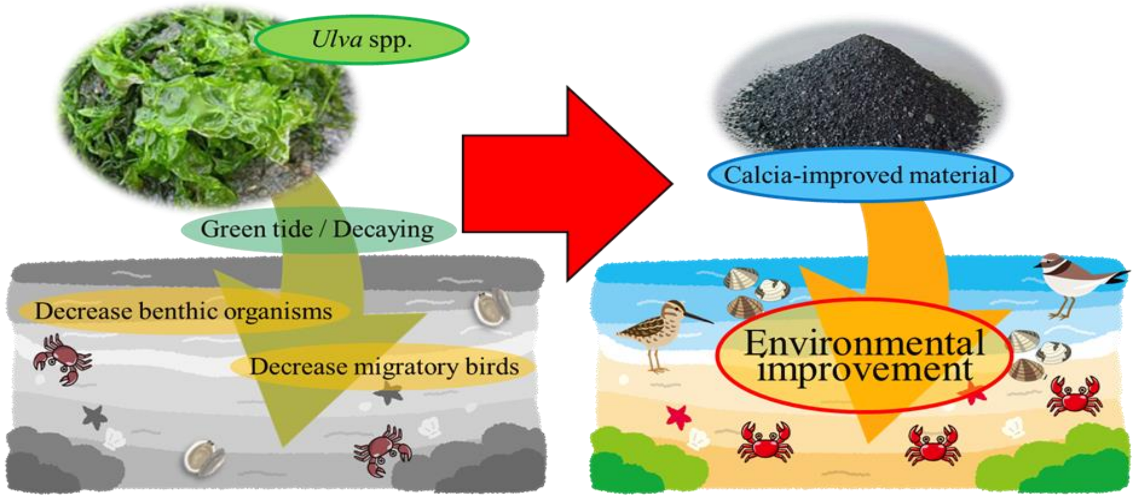

Fig.9 Conceptual diagram of the improvement of tidal flat environment through the use of Calcia-modified materials

nutrient removal through calcium–phosphate precipitation and the simultaneous release of organic substances associated with the Calcia-modified material. This suggests that excessively high mixing ratios may induce secondary organic loading despite improved nutrient removal.

4.2 Improvement Effect on Ecosystem Structure

Fig.7 illustrates temporal changes in plankton abundance in the microcosm systems. In the system receiving eluate filtered through Calcia-improved soil with a 20% mixing ratio, several zooplankton species (*Cyclidium glaucoma*, *Lecane* sp., *Philodina erythrophthalma*, and *Aeolosoma hemprichi*) and some phytoplankton species (e.g., *Tolypothrix* sp.) decreased markedly or became locally extinct at higher addition concentrations. However, partial recovery of certain species, particularly *Lecane* sp. and *Philodina erythrophthalma*, was observed over time. In contrast, in the system treated with eluate filtered through Calcia-improved soil with a 30% mixing ratio, several zooplankton species declined or disappeared, and no recovery was observed at the 40% and 80% addition levels. These results indicate that higher mixing ratios exert stronger and more persistent adverse effects on ecosystem structure. Based on the observed species responses and recovery patterns, a Calcia-modified material mixing ratio of 20% was evaluated as more suitable for mitigating green tide impacts while maintaining ecosystem resilience.

4.3 Improvement Effect on Ecosystem Function

Fig.8 shows changes in the P/R ratio as an indicator of ecosystem functional balance. In the systems receiving eluate filtered through Calcia-improved soil with a 20% mixing ratio, the P/R ratio converged to approximately 1 at most addition concentrations, indicating the maintenance of metabolic balance within the microcosm. In systems treated with eluate filtered through Calcia-improved soil with a 30% mixing ratio, the P/R ratio also converged to around 1 at most addition levels; however, at the 80% addition level, both production and respiration rates decreased markedly, indicating functional impairment. Table 2 summarizes the results of the branched-type ANOVA. Statistically significant effects were detected in all addition systems. Based on these results, the lowest-observed-effect concentration (LOEC) was identified, and the microcosm no-observed-effect concentration (m-NOEC) was conservatively defined as half of the LOEC. Consequently, the m-NOEC was determined to be 2.5%, and the corresponding toxicity unit (TU) was calculated as 40.

4.4 Steel Slag Utilization for Green Tide Influence Reduction

With respect to structural parameters (organism abundance), effects were observed at concentrations ≥40% in the 20% Calcia-improved soil treatment and ≥20% in the 30% treatment. Notably, all zooplankton species were strongly suppressed at the 40% and 80% addition levels in the 20% treatment system. For functional parameters (P/R ratio), large fluctuations were observed immediately after addition; however, values converged toward 1 over time in most systems, suggesting that ecosystem function can recover following moderate disturbance. An exception was observed in the 80% addition system using eluate filtered through Calcia-improved soil with a 30% mixing ratio, where sustained functional impairment occurred without recovery. The pronounced effects observed at high addition concentrations are considered to be largely attributable to elevated pH levels caused by the Calcia-improved soil prior to solidification. In addition, the pH of the test stock solution was not adjusted before use, which should be taken into account when interpreting the results. Because this study employed a flask-scale simulated ecosystem, the potential for overestimation of environmental impacts must be acknowledged. Previous studies have proposed an extrapolation (safety) factor of 200 when translating microcosm results to real environments. Applying this factor, Calcia-modified materials are unlikely to exert significant adverse effects under realistic environmental conditions. Overall, the results consistently indicate that a Calcia-modified material mixing ratio of approximately 20% is optimal for mitigating green tide impacts while minimizing ecological disturbance. Furthermore, the Calcia-improved soil used in this study was tested immediately after mixing; it is therefore expected that experiments using solidified material would yield different outcomes. Future studies should focus on optimizing application concentrations and examining the influence of *Ulva* spp. decomposition stages on eluate characteristics. The application of Calcia-improved soil as a sediment remediation material has the potential to improve tidal flat environments degraded by green tide events (Fig. 9).

5. CONCLUSION

In the microcosm systems receiving eluate filtered through Calcia-improved soil with a 20% mixing ratio, structural effects were observed only at the higher addition levels (40% and 80%). However, functional parameters remained largely stable, with the P/R ratio converging to approximately 1 at most addition concentrations, indicating maintenance of ecosystem metabolic balance. In contrast, in the systems treated with eluate filtered through Calcia-improved soil with a 30% mixing ratio, structural effects appeared at lower addition levels (≥20%). Although the P/R ratio converged to around 1 in most systems, a pronounced decline in both production and respiration was observed at the 80% addition level, indicating significant functional impairment. Based on branched-type ANOVA, the microcosm no-observed-effect concentration (m-NOEC) was determined to be 2.5%, corresponding to a toxicity unit (TU) of 40. These results indicate that a Calcia-modified material mixing ratio of approximately 20% provides an effective balance between green tide eluate mitigation and minimization of adverse ecological effects. It should be noted that the Calcia-improved soil used in this study was evaluated immediately after mixing. Therefore, the ecological effects of solidified Calcia-improved soil may differ from those observed here. Future studies should focus on refining optimal application concentrations and clarifying how changes in *Ulva* spp. decomposition stage influence eluate characteristics and ecological responses.

6. ACKNOWLEDGMENTS

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