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**INVESTIGATION OF THE RECOVERY OF SEAWEED AT BARREN GROUND
BY DEPOSITION ON BOTTOM OF DAM RESERVOIR IN JAPAN ^(*)**

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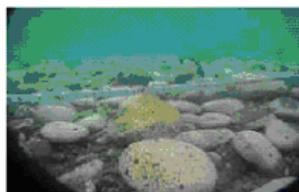
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JAPAN

1. INTRODUCTION

Iron (Fe) is an essential micronutrient for algal growth [1], [2]. Macroalgae need high concentrations of iron as well as nitrogen and phosphorus [3]. Fe is an essential micronutrient for phytoplankton growth. Since Fe has been recognized as a key element in ocean biogeochemical cycles, many studies have increased our understanding of the iron cycle in seawater [4]. The increase of the area of barren ground lead to a significant reduction in fisheries production and biodiversity. Several reasons for barren grounds have been proposed, for instance, elevated seawater temperatures [5], grazing by herbivorous animals [6], and a shortage of dissolved iron [7], [8]. Sadakata et al. demonstrated that such iron -humate (Fe-HS) were effective in recovering the vegetation on the areas of barren ground in the sea of Japan (Fig. 1).

^(*) *Étude de réintroduction des algues sur sol nu par déposition au fond d'un réservoir artificiel au Japon*



Before fertilizing (May, 2004)



Fertilization of HS and iron-containing waste (May, 2004)



Growth of the sea tangle at Shaguma coast (June, 2006)



Growth of the sea tangle at Shaguma coast (June, 2007)

Fig. 1

Demonstration at Shaguma coast in Hokkaido, Japan
Démonstration sur la côte de Shaguma à Hokkaido, Japon

On the bottom of dam reservoir, fallen leaves and timbers are input into the lake from the upper reaches of the river, and deposition of such organic materials can result in producing sediments including humic substances (HS). This leads to one of reasons for the reduction of active storage capacity of the dam reservoir. However, HS that binds to Iron(II) (Fe(II)) can form stable Fe-HS, and those may be deposited into the bottom of dam reservoirs. Surveys of potentiality for depositing Fe-HS in the bottom of dam reservoirs and development of technologies for supplying Fe-HS to rivers and coast are important from the ecological point of view. Applications of Fe-HS depositing on the bottom of dam reservoirs to their supply in rivers and coastal areas may be a method for supplying the nutrients that can mimic natural environments, and this can be utilized to recover and increase resources of fishery. In addition, fixation of CO₂ by the growth of seaweeds can contribute to the suppression of global warming as well as increase of life-time for dams. To evaluate the potentiality for applying the sediments including Fe-HS on the bottom of dam reservoirs, we analyzed the contents of Fe-HS in the sludge.

2. INVESTIGATIONS OF IRON-HUMATES ON THE BOTTOM OF DAM RESERVOIRS

2.1. INVESTIGATIONS OF IRON-HUMATE AT YONEOKA RESERVOIR

2.1.1. *Sampling and Pretreatment*

In Japan, the area of barren ground has been increased year by year. Red line in Fig. 2 indicates the area of barren ground. (Fig. 2). In the present study, sediments were sampled using a grove sampler in Yoneoka reservoir in Okushiri island and Ainumanai Dam in Hokkaido district (Fig. 2). Samples were stocked in freezer at -10°C and freeze-dried. The dry samples were pulverized using a pestle and motor, and filtered through a stainless-steel sieve (0.22 mm). Particles less than 0.22 mm in size were tested.

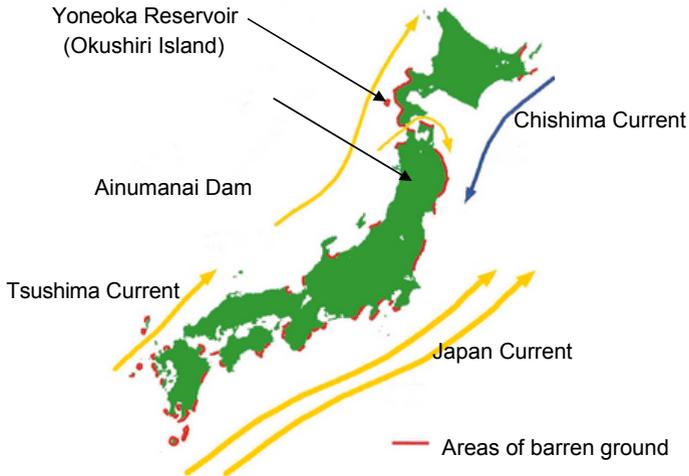


Fig. 2

Sampling stations at Yoneoka reservoir and Ainumanai Dam in Japan
Stations d'échantillonnage au réservoir d'Yoneoka et au barrage d'Ainumanai au Japon

2.1.2. *Analyses of Samples*

HS were extracted and analyzed by a method according to the International Humic Substances Society [9]. The iron species in samples were analyzed using a sequential extraction method by Yuan et al. [10]. Fig. 3 shows the scheme for the sequential extraction of the sample, and the fraction of C corresponds to Fe-HS.

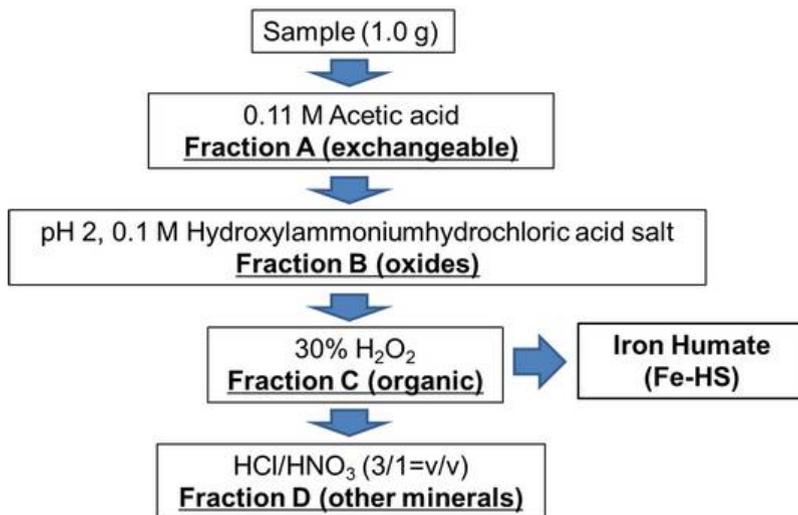


Fig. 3

Scheme for sequential extraction method
Schéma pour une méthode d'extraction séquentielle

2.1.3. *Contents of HS in Samples*

The results of the speciation analyses of Fe are summarized in Table 1. Although fraction D was the largest in all samples, rates of Fraction C corresponding to Fe-HS were in the range of 2.72–5.94 %. In this study, samples collected at two sites (St. 1 and 2) in Yoneoka reservoir in Japan was used in test area at barren ground area in Okushiri Island. Rate of Fraction C in Yoneoka reservoir was higher than that in Ainumanai Dam in Hokkaido (Table 1).

Table 1
Rate of Fe species to total Fe in samples collected at two sites (St. 1 and 2) in
Yoneoka reservoir and Ainumanai Dam in Japan

Samples	[wt%]			
	Fraction A	Fraction B	Fraction C	Fraction D
St. 1	0.20	2.69	5.94	38.99
St. 2	0.27	1.81	3.22	29.99
Reference 1 (Ainumanai Dam)	0.20	2.84	2.72	45.88

3. FIELD EXPERIMENT

3.1. EXPERIMENTAL SITES

The field experiments performed on the Nagahama coast in Okushiri-cho, Hokkaido, Japan. Okushiri Island is an island in Hokkaido, Japan. It has an area of 142.97 km². The area of barren ground spread in Mottate area of weastern coast and Nagahama area of eastern coast of Okushiri Island (Fig. 4). Experimental test by using iron humate elution unit (Fe-HS) made with steel making slag has been performed at Mottate area since 2006 and dam iron humate elution unit (Fe-HS) (Fig.1) made with deposition on bottom of dam reservoir has been performed at Nagahama area in Okushiri Island since 2009. In this paper, the experimental results on Nagahama area are discussed. In 2009, dam iron humate elution unit (Unit A), dam humic substance unit(Unit B), and iron humate elution unit (Unit C) was buried at the coast in Site A, B, and C, respectively (Table 2). The weight of one columnar unit (width: 0.48m, length: 1.0m) is about 100 kg and 135 units were buried in three ditch rows (45m long) parallel to the shoreline under 1.2 m of sea level at each site. Unit A consisted of a mixture of deposition on bottom of dam reservoir and slag with a volume ratio of 1:1. Unit B contains only deposition on bottom of dam reservoir. Unit C consisted of a mixture of slag and compost with a volume ratio of 1:1. Site E is control site (no elution unit) (Fig.4). We analyzed the iron humate, phosphoric acid, and silicic acid concentration in seawater at each site and discussed the relationship with the growth of seaweed. The growth of each seaweed community was evaluated using the quadrat methods. The growth areas of seaweed species growing at distance of 0, 5, 10, 20, and 30 m offshore were measured once a year for two years. Sea water sampling (2 L) at about 10m from seashore in all experimental sites was performed in July, October, and December in 2010 and May, July, September, and December in 2011 in three replication.

In addition, during from 2006 to 2008, the sampling and measurement of deposition on bottom of dam reservoir was carried out at Ainumanai dam (Hokkaido Electric Power Co., inc.) that was established in 1930 at Hokkaido (Fig. 1).

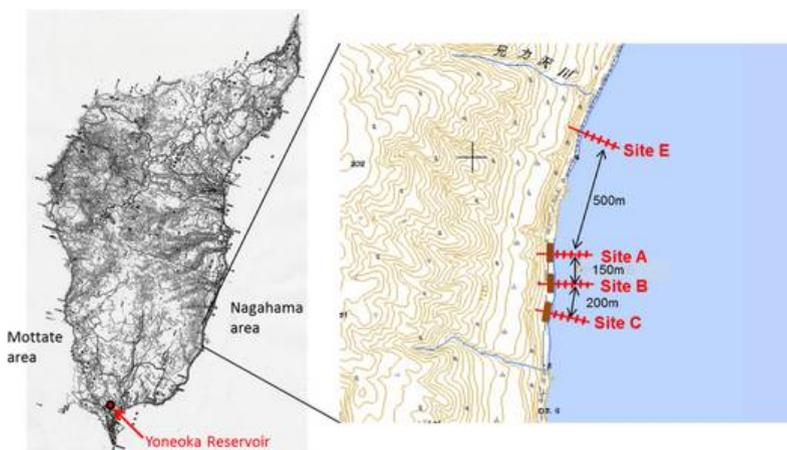


Fig. 4

Experimental site in Okushiri Island (Nagahama area)
Site expérimental dans l'île d'Okushiri (région de Nagahama)

Table 2

Experimental sites in Nagahama area of Okushiri Island and units in each site

Site	Unit (contains)
A	Unit A (Dam + Slag)
B	Unit B (Dam)
C	Unit C (Compost + Slag)
E	-

3.2. PRODUCTION OF ELUTION UNITS FOR THE DAM IRON HUMATE

The palm tube used for the elution unit has the slowest speed of degeneration among natural fibers (5-7 years in soil, 10 years or more in seawater). It excels in durability, is environmentally friendly, has a comparatively slow speed of water permeability, and is a substance in association with which the elution of Fe(II) is gradual. The basic objective had been to create an elution unit (Fig. 5) by

filling the palm tube with a mixture of iron contained substances and dam humic substances. Because the elution unit was made of palm husk fiber, whose degeneration speed is the slowest among natural fibers, the elution of Fe(II) was facilitated while maintaining the iron contained substances over a long period of five years or more. The purpose of mixing-in the iron contained substances (steel slag) was to neutralize the acidulous dam humic substance by making steel alkalescency slag, which works as a retarder in delaying the elution speed of high-density humis acid iron.

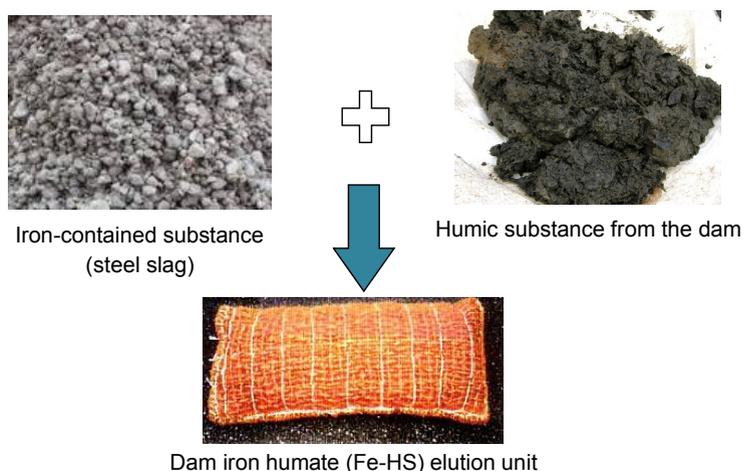


Fig. 5
Unité d'éluion d'humate de fer du barrage (Fe-HS)
(Fe-HS)

3.3. ANALYTICAL METHOD FOR IRON HUMATE IN SEAWATER

DAX-8 (a poly(methylmethacrylate) resin under $75 \mu\text{m}$ in diameter washed ultrasonically in methanol, hydrochloric acid and water for 20 minutes. The resin was immersed in an indium solution ($10 \mu\text{g In ml}^{-1}$, pH 5) overnight to eliminate cation-exchange sites. This resin (2.0 g) was packed into two columns (100 mm X 50 mm i.d.), respectively. Seawater and river water sample were filtered through $0.45 \mu\text{m}$ Nuclepore membrane filters to remove suspended particles. A 2.0 L aliquot of the sample was acidified with 20ml formaldehyde in sampling and the sample passed through the indium-treated DAX-8 column at a flow rate of about 1.5 ml/min to collect humic complexes. The resin at each column

after infiltration was added to 10mL of 4.0 molL⁻¹ nitric acid and was stirred adequately and washed ultrasonically for 20 minutes. The solution was filtered by disk filter (diameter: 0.20 μ m) and analyzed by Inductively Coupled Plasma - Atomic Emission Spectroscopy (ICP-AES) (SPS7700, Seiko Instruments Inc.).

In addition, the phosphate concentration by the molybdenum blue method and the concentration of silica by a molybdenum yellow method were analyzed.

4. RESULTS AND DISCUSSION

4.1. SEAWEED GROWTH AT EXPERIMENTAL SITES

Dam iron humate elution unit (Unit A), dam humic substance unit (Unit B), and iron humate elution unit (Unit C) was buried at the coast in Site A, B, and C (Table 2) and Site E was control site. The growth of each seaweed community was evaluated using the quadrat methods. The growth areas of seaweed species growing at distance of 0, 5, 10, 20, and 30 m offshore were measured once a year for two years.

Figs. 6, 7, 8 and 9 show the seaweed growth at distance of 0, 5, 10, 20, and 30 m offshore in Site E, A, B, and C, respectively. Tables 3, 4, 5, and 6 show the growth area of seaweed at distance of 0, 5, 10, 20, and 30 m offshore in Site E, A, B, and C, respectively. The growth area of seaweed at seashore from 0m to 10m in Site A, B, C, and E in 2010 and 2011 was shown in Fig. 10.

The growth area of seaweed at Site A and B in 2010 was larger than that at Site E (Figs. 6, 7 and 8, Tables 4 and 5). In 2011, the growth area of seaweed at Site B and C was larger than that at Site E (Figs. 8 and 9, Tables 5 and 6, Fig. 10). The 2nd year's seaweed growth at experimental site by using Unit C was higher than 1st year's growth in 2007 at Okushiri Island. Therefore, we could confirm the same tendency in seaweed growth. The growth effect of seaweed in Site B was highest among four sites (Site A, B, C, and E) (Fig. 10).

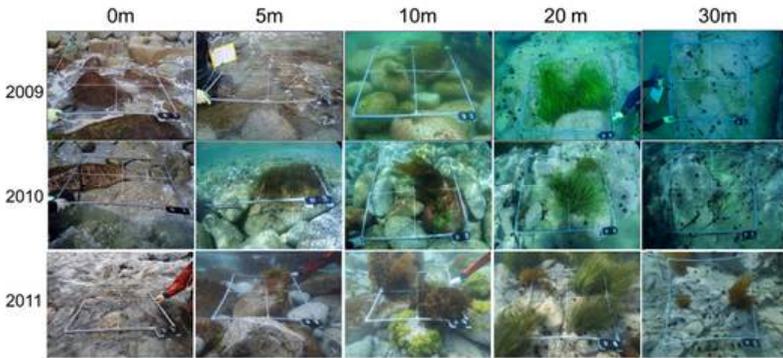


Fig. 6
Seaweed growth at seashore in Site E
Croissance des algues sur le rivage du site E

Table 3
Growth area of seaweed at seashore in Site E

Year/Distance	[%]				
	0m	5m	10m	20m	30m
2009	35	10	33	25	0
2010	10	28	38	20	0
2011	33	35	60	45	15

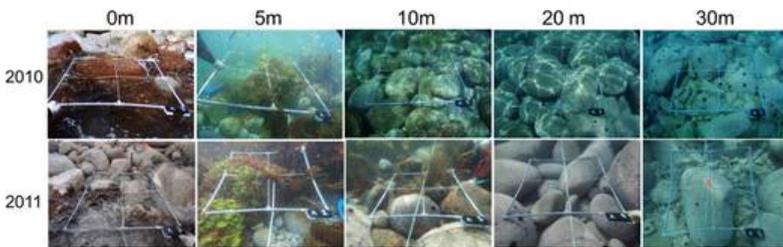


Fig. 7
Seaweed growth at seashore in Site A
Croissance des algues sur le rivage du site A

Table 4
Growth area of seaweed at seashore in Site A

Year/Distance	[%]				
	0m	5m	10m	20m	30m
2010	100	53	0	0	0
2011	23	53	26	0	0

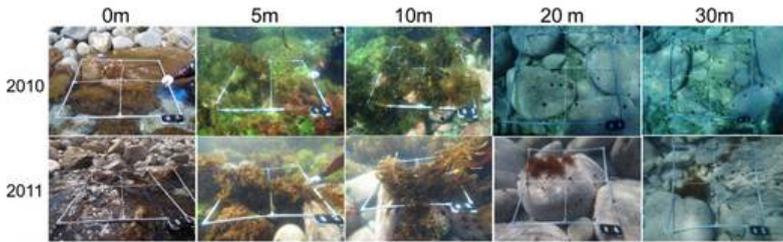


Fig. 8
Seaweed growth at seashore in Site B
Croissance des algues sur le rivage du site B

Table 5
Growth area of seaweed at seashore in Site B

Year/Distance	[%]				
	0m	5m	10m	20m	30m
2010	86	80	89	0	0
2011	60	65	60	20	10

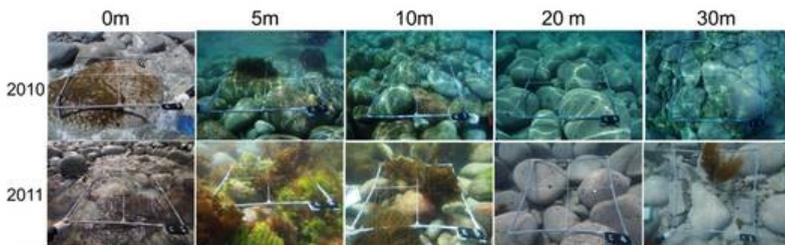


Fig. 9
Seaweed growth at seashore in Site C
Croissance des algues sur le rivage du site C

Table 6
Growth area of seaweed at seashore in Site C

Year/Distance	[%]				
	0 m	5 m	10 m	20 m	30 m
2010	35	23	0	0	0
2011	25	80	50	0	16

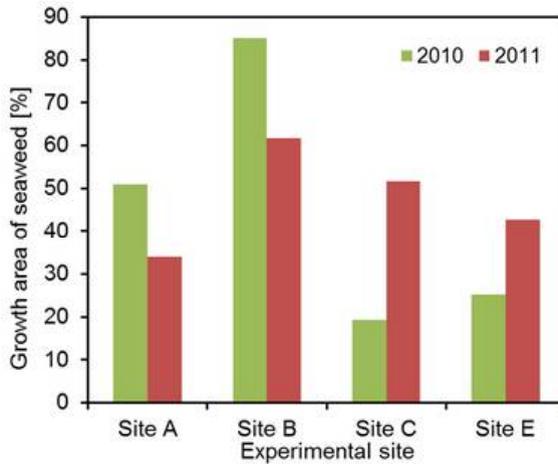


Fig. 10

Growth area of seaweed at all sites (2010 and 2011)

Surface de croissance des algues sur tous les sites (2010 et 2011)

4.2. IRON HUMATE, PHOSPHATE, AND SILICATE CONCENTRATIONS IN SEAWATER

The iron humate, phosphoric acid, and silicic acid concentration in seawater at all sites (A, B, C, and E) were analyzed in 2010 and 2011. Fig. 11 indicates the change in concentration of iron humate in all experimental sites in 2010 and 2011. The concentration of iron humate at all sites ranges from 3.8 nM to 18.1 nM (Fig. 11). The change of iron humate concentration from July in 2010 to December in 2011 at four experimental sites took the almost the same tendency of increase and decrease. The concentration of Fe-HS in December decreased in May (Fig. 11). The period is the growth term of seaweed and this decrease has the possibility of absorption of Fe-HS to seaweed. Therefore, the tendency seems to have the relationship with the life history of seaweed.

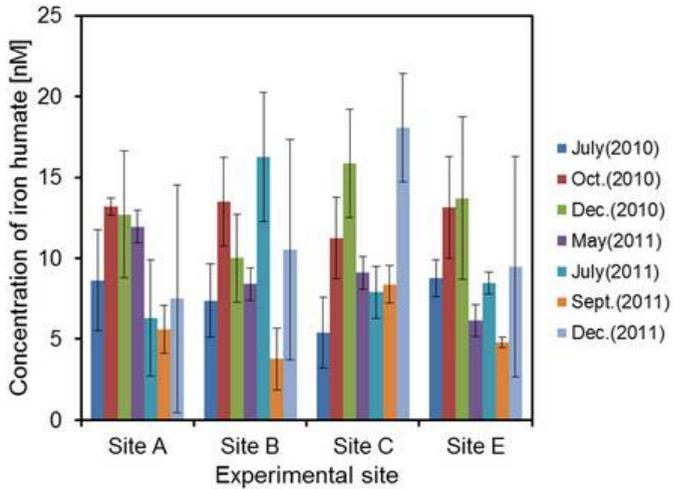


Fig. 11

Change in concentration of iron humate in all experimental sites
Evolution de concentration d'humate du fer sur tous les sites expérimentaux

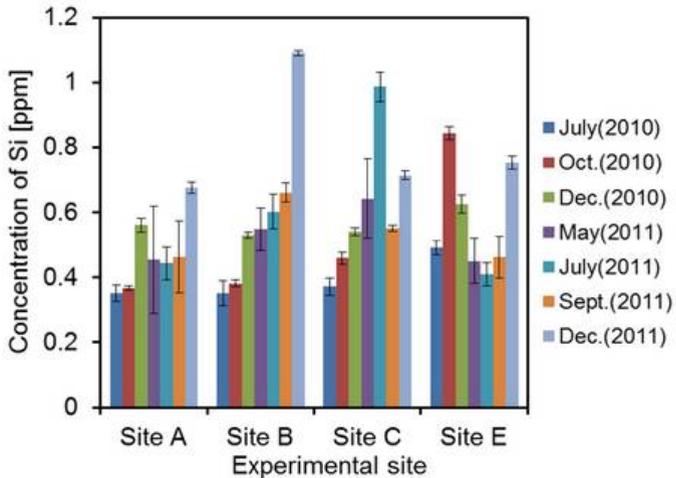


Fig. 12

Change in concentration of Si in all experimental sites
Évolution de concentration de Si sur tous les sites expérimentaux

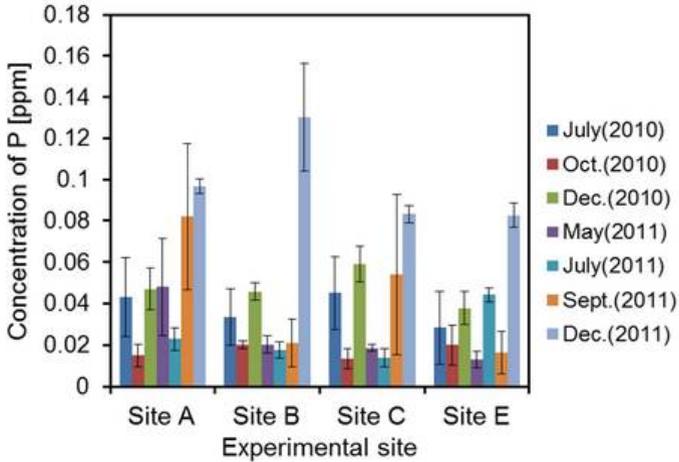


Fig. 13

Change in concentration of P in all experimental sites

Évolution de concentration de P sur tous les sites expérimentaux

Si concentration at Site A, B, and C increased with time processing (Fig. 12). In addition, the concentration of Si in this experimental site was mainly low Si concentrations from about 0.1 ppm to 0.5 ppm [11]. Phosphate concentrations in experimental sites took the value under 0.09 ppm (Fig. 13). Therefore, Si concentration is suitable for fishery in Japan. Error bars indicates the standard deviation of data ($n \geq 3$) in Figs. 11, 12, and 13.

The correlation coefficients (r) and levels of significance (P value $< 0.05^*$, 0.01^{**}) were calculated among growth area of seaweed in each distance, iron humate, Si, and P concentration in all sampling time in Site A, B, and C. Fig. 14 shows the relationship between concentration of iron humate in May and growth area of seaweed in 10 m offshore at all experimental sites. The growth area of seaweed increased with the decrease of the concentration of iron humate ($r = -0.722^{**}$) (Fig. 14). This result has the possibility of the usage of iron humate during the growth of seaweed. In addition, the growth area of seaweed in 5 m offshore increased with the decrease of the concentration of iron humate in May ($r = -0.518^*$) P in July ($r = -0.481^*$) and the increase of Si in May ($r = 0.566^*$) and July ($r = 0.504^{**}$). Therefore, iron humate, Si, P concentrations has the relationship with the growth of seaweed.

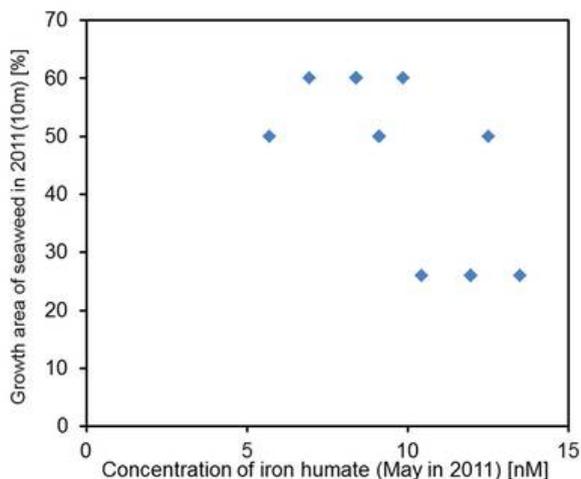


Fig. 14

Relationship between concentration of iron humate and growth area of seaweed
Rapport entre la concentration d'humate du fer et la surface de croissance des algues

5. CONCLUSION

The speciation analyses of Fe in Yoneoka reservoir and Ainumanai Dam in Japan was performed. As a result, rates of Fraction C corresponding to Fe-HS were in the range of 2.72–5.94 %.

Dam iron humate elution unit (Unit A), dam humic substance unit (Unit B), and iron humate elution unit (Unit C) was effective for the growth of seagrass experimental sites at barren ground and the growth effect of seaweed in Site B (Unit B) was highest among four sites.

The change of iron humate concentration from July in 2010 to December in 2011 at four experimental sites took the almost the same tendency of increase and decrease. Si concentration at Site A, B, and C increased with time processing and was mainly low Si concentrations. In addition, phosphate concentration is suitable for fishery in Japan.

From relationships between concentration of iron humate, Si, and P and growth area of seaweed at all experimental sites, the concentration of iron humate in May was considered to be effective for the growth of seaweed.

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SUMMARY

The increase of barren ground lead to a significant reduction in fisheries production and biodiversity in Japan. Iron is an essential micronutrient for algal growth. Macroalgae need high concentrations of iron as well as nitrogen and phosphorus. The speciation analyses of iron in Yoneoka reservoir and Ainumanai Dam in Japan was performed. As a result, rates of Fraction C corresponding to Fe-HS were in the range of 2.72-5.94 %. Dam iron humate elution unit (Unit A), dam humic substance unit (Unit B), and iron humate elution unit (Unit C) was effective for the growth of seagrass experimental sites at barren ground in Japan. And the growth effect of seaweed in Site B (Unit B) was highest among four sites. The change of iron humate concentration from July in 2010 to December in 2011 at four experimental sites took the almost the same tendency of increase and decrease. Si concentration at Site A, B, and C increased with time processing and was mainly low Si concentrations. In addition, phosphate concentration is suitable for fishery in Japan. From relationships between concentration of iron humate, Si, and P and growth area of seaweed at all experimental sites, the concentration of iron humate in May was considered to be effective for the growth of seaweed. Conclusively, we could propose the new barren ground reclamation method using deposition on bottom of dam reservoir in Japan.

RÉSUMÉ

L'augmentation des sols nus entraîne une réduction importante de la production halieutique et de la biodiversité au Japon. Le fer est un micro-nutriment essentiel pour la croissance des algues. Les macroalgues ont besoin d'une forte concentration de fer, d'azote et de phosphore. Des analyses de fer ont été effectuées dans le réservoir de Yoneoka et le barrage d' Ainumanai. Les résultats montrent que la fraction C correspondante au Fe-HS se trouvaient dans la plage de 2,72-5,94 %. Une unité d'éluion d'humate de fer (A), une unité de substance humique (B) et une unité d'éluion d'humate de fer (C) ont été efficace pour la

croissance des algues sur les sites expérimentaux, sur sol nu au Japon. Parmi les quatre sites, la croissance maximale a été observée sur le site B (unité B). L'évolution de la concentration d'humate de fer entre juillet 2010 et décembre 2011 sur les quatre sites expérimentaux ont suivi les mêmes tendances d'augmentation et de réduction. La concentration en Si sur les sites A, B et C a augmenté avec le temps, tout en restant relativement faible. Par ailleurs, la concentration en phosphate est adaptée à la pêche au Japon. En considérant les concentrations d'humate de fer, de Si et de P et la surface de croissance des algues sur tous les sites expérimentaux, c'est la concentration d'humate de fer en mai qui a été déterminée comme la plus efficace. En conclusion, nous proposons une nouvelle méthode de culture des sols nus par déposition sur le fond des réservoirs au Japon.