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Brown Seaweeds (*Sargassum macrocarpum*) as Nitrate (NO3) Reducer

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ABSTRACT
This study entitled, BROWN SEAWEEDS (*Sargassum macrocarpum*) AS NITRATE (NO_3) reducer was conducted to find out if brown seaweeds can help lessen the nitrate content of contaminated water particularly water used in rice field irrigation. After the brown seaweeds were gathered, and the contaminated water from the river used in rice field irrigation was collected, treatments for experimentation were prepared. There were four treatments used: Treatments A, B, C, and D (control). One thousand (1000) mL of contaminated water was placed in each of the containers of all the treatments. After then 50 grams of brown seaweeds were placed in A, 100 g in B, 150 g in C and no seaweed for D. The treatments were observed for nine (9) days and its nitrate (NO_3) content tested every three days. The tests were done in the Water Laboratory of the National Power Corporation, Calaca, Batangas using the Spectrophotometer. Results showed that Treatment C with the greatest number of seaweeds eliminated more nitrate than the other treatments based on the statistical analysis of variance (ANOVA). It is therefore concluded that brown seaweeds (*Sargassum macrocarpum*) have the ability and the efficacy of reducing the nitrate content of contaminated water.

INTRODUCTION
Water is a source of life. Unknowingly, many of our individual actions in our homes and on the ground, water resources, affect the surface waters of rivers and lakes. The fishes and other wildlife that depend on these water bodies are critical in maintaining the delicate balance between healthy ecosystem and clean water. The quality of water starts with everyone. Every people's way of using water on land and in the water can affect our water quality. Many studies have been conducted to help lessen water contamination. One of these is the wastewater treatment.

Seaweeds refer to the large marine algae that grow almost exclusively in the shallow waters at the edge of the ocean. They provide home and food for many different sea animals and are directly valuable to man as food and industrial raw material. They are plenty and can be easily found at many coastal areas. Due to water pollution and contamination in our country, there is a need for the researchers to study organic materials that will help lessen this kind of problem and also help in wastewater treatment. For this reason, brown seaweeds (*Sargassum macrocarpum*) whose importance is ignored by many people were used to reduce the nitrate content of contaminated water.

MATERIALS AND METHODS
The materials used in this study were the following:

a. 300 g Brown Seaweeds (*Sargassum macrocarpum*)
b. 4000 mL Contaminated water
c. 4 wide mouthed bottles

d. Triple Beam Balance
e. Pipettes with aspirator
f. Spectrophotometer
g. Brucine alkaloid solution
h. Burettes
i. Reagent bottles

GENERAL TREATMENT/ PROCEDURE

Gathering of Materials

The three hundred grams (300 g) of brown seaweeds were gathered at Balayan Bay and 4000 mL contaminated water from the river that irrigates the rice field in San Juan, Balayan, Batangas were collected.

Measuring of Contaminated Water and Brown Seaweeds

The brown seaweeds were measured using triple beam balance and the researcher used beaker to measure the contaminated water.

Preparing the Treatments

Four treatments were used in this study as shown in the experimental design below:

Table 1. The Experimental Design.

Treatments	Amount of contaminated water (mL)	Number of brown seaweeds (g)	Storage Duration (day)		
			1st Test	2nd Test	3rd Test
A	1000	50	3	6	9
B	1000	100	3	6	9
C	1000	150	3	6	9
D (control)	1000	0	3	6	9

Testing of the water

Samples of the water in each of the Treatments were placed in separate reagents bottle for the testing of nitrate content at the Water Laboratory at the National Power Corporation, Calaca, Batangas. The calibration curve procedure was done by the researcher assisted by the NPC Chemists.

Using a pipette, two 5 mL portion was taken from the dilution and placed into two dried 50 mL beakers. One 5 mL portion served as blank. With the use of a burette, 0.2 mL Brucine alkaloid solution was added after which 10 mL of H_2SO_4 was added and was mixed thoroughly.

To the portion that served as blank a 10 mL of reagent water was added with the use of a pipette. It was then swirled to mix then cooled to room temperature. The solution was used to set the spectrophotometer to zero. After standing for 5 minutes, the absorbance of the Brucine treated portion was measured at 470nm wavelength using 20mm cell. The steps were repeated to measure the absorbance of each dilution. Finally, a calibration curve was made by plotting the nitrate concentration versus absorbance. The same procedures were followed for the succeeding tests.

Data Analysis

For the comparison of the Nitrate (NO_3) content from 1st, 3rd, 6th and 9th days, the researcher used the Analysis of Variance (ANOVA).

$$\sum X = \sum A + \sum B + \sum C + \sum D$$

$$\sum X^2 = \sum A^2 + \sum B^2 + \sum C^2 + \sum D^2$$

$$TSS = (\sum A^2 + \sum B^2 + \sum C^2 + \sum D^2) - (\sum X)^2/N$$

$$SSB = \frac{(A)^2}{N} + \frac{(B)^2}{N} + \frac{(C)^2}{N} + \frac{(D)^2}{N} - \frac{(\sum X)^2}{N}$$

$$SSW = TSS - SSB$$

Where: $\sum X$ =sum of the individual values per column

$\sum X^2$ = square of the sum of the individual values per column

TSS = total sum of squares

SSB = sum of squares between columns
SSW = sum of squares within columns
N = total sample size
n = size of the sample per column

RESULTS AND DISCUSSION

Findings

Table 1. The following tables show the results of the experiment.

Treatments	Amount of contaminated water (mL)	Number of brown seaweeds (g)	Storage Duration (day)	Nitrate (NO_3) content in day 1 (ppm)	Nitrate (NO_3) content in day3 (ppm)
A	1000	50	3	8.89	3.12
B	1000	100	3	8.89	2.14
C	1000	150	3	8.89	0.98
D (control)	1000	0	3	8.89	8.89

Table 2. Nitrate (NO_3) Content in the Third Day.

Treatments	Amount of contaminated water (mL)	Number of brown seaweeds (g)	Time Duration (days)	Nitrate (NO_3) content in day 1 (ppm)	Nitrate (NO_3) content present
A	1000	50	6	8.89	2.14
B	1000	100	6	8.89	1.16
C	1000	150	6	8.89	0.32
D (control)	1000	0	6	8.89	8.89

Table 3. Nitrate (NO_3) Content in the Sixth Day.

Treatments	Amount of contaminated water (mL)	Number of brown seaweeds (g)	Time Duration (days)	Nitrate (NO_3) content in day 1 (ppm)	Nitrate (NO_3) content present
A	1000	50	9	8.89	0
B	1000	100	9	8.89	-0.12
C	1000	150	9	8.89	-0.49
D (control)	1000	0	9	8.89	8.89

Table 4. Nitrate (NO3) Content in the Ninth Day.

Day	A	A^2	B	B^2	C	C^2	D	D^2
3	3.12	9.73	2.14	4.58	0.98	0.96	8.89	79.03
6	2.14	4.58	1.16	1.35	0.32	0.10	8.89	79.03
9	0	0	-0.12	-0.01	-0.49	-0.24	8.89	79.03
	$\sum A = 5.26$	$\sum A^2 = 14.31$	$\sum B = 3.18$	$\sum B^2 = 5.92$	$\sum C = 0.81$	$\sum C^2 = 0.82$	$\sum D = 26.67$	$\sum D^2 = 237.09$
	N = 9		df = 3 and 6		C.V. = 12.06	level of significance = 0.05		

Table 5. The Analysis of Variance (ANOVA) from day 3, 6 and 9.

SOURCE OF VARIATION	SUM OF SQUARES	DEGREE OF FREEDOM	MEAN SQUARE	F COMPUTED
Between Columns	106.55	4 - 1 = 3	MCB=106.55/3 = 35.51	Fc = MSB/MSW = 35.51/1.37Fc = 25.91
Within Columns	8.23	3(3-1) = 6	MSW = 8.23/ 6 = 1.37	

Analysis

After the thorough tests on the NO_3 content of the water being studied, the data were then tabulated. The NO_3 content of the water was taken in the first day of observation which was 8.89 parts per million (ppm) for all the treatments. In the third day, the NO_3 content was taken again, and Table 2 showed the result. Treatment C with 1000 ml of contaminated water and 150 g of brown seaweeds had the greatest amount of NO_3 reduced which was 0.98 ppm from the original 8.89 ppm, thus reducing 7.91 ppm. It was followed by treatment B w/ 100g. of seaweed from 8.89 ppm, the content became 2.14 with a reduction of 6.75 ppm. Tratment A with only 50g. of seaweeds had 3.12 ppm NO_3 content with a reduction of 5.77. The NO_3 content was not reduced in Treatment because being the control treatment it did not have seaweeds.

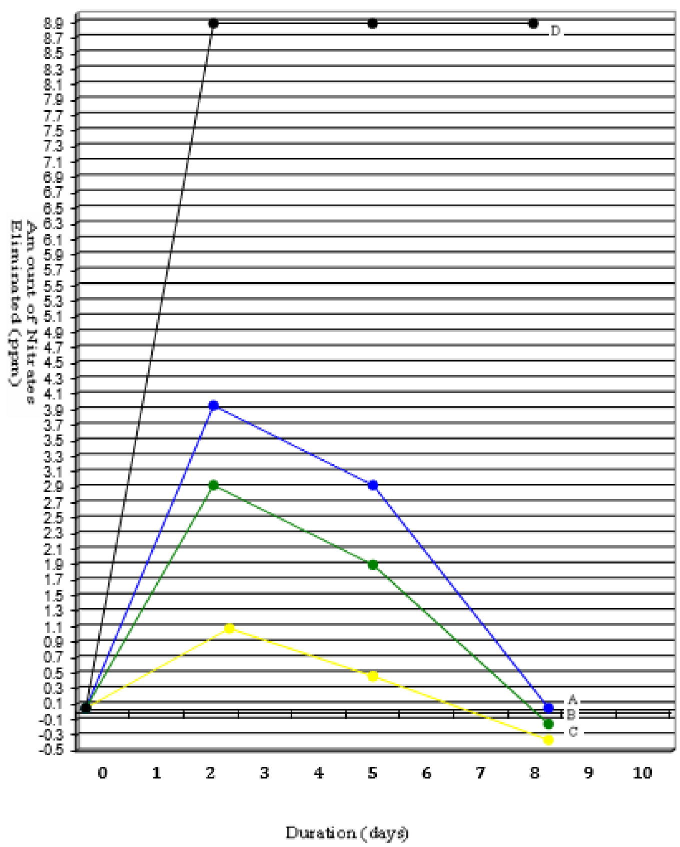
The Treatments were continuously stored and tested in the 6^{th} day, the result of which was shown in Table 3. It was Treatment C again which reduced the greatest amount of NO_3 which was 0.32 ppm or 8.57 ppm reduction, Treatment B followed which was 1.16 ppm or 7.73 ppm reduction, and Treatment A with 2.14 ppm or 6.75 ppm reduction. Treatment D being the control showed no reduction.

Table 4 shows the NO_3 content in the ninth day. This last test showed a tremendous fall in the NO_3 content wherein Treatment C had -0.12 ppm -8.77 ppm difference from the first day, Treatment B had -0.49 or -8.40 difference from the first day and Treatment A of -8.89 ppm, while Treatment D remained the same.

Table 5 shows the Analysis of Variance (ANOVA) from day 3, 6 and 9, the results were $\sum A= 5.26$, $\sum A^2 = 14.31$, $\sum B = 3.18$, $\sum B^2 = 5.92$, $\sum C = 0.81$, $\sum C^2 = 0.82$, $\sum D = 26.67$, $\sum D^2 = 237.09$. The Analysis showed that as the number of brown seaweeds increases and the longer the period of storage, the greater the amount of nitrate (NO_3) content reduced.

Table 6 shows the summary for the one-way analysis of variance. The source of variations between columns and within columns decreases from 10655 to 8.23 (sum of squares), the degree of freedom was from 3 to 6, the mean square was from 35.51 to 1.37, and the computed F was 25.91`, the reference value of F was 21.91, the F computed was greater than F tabular.

It can be noted from the Tables that the greater the number of seaweeds the greater the amount of NO_3 reduced. It can be further interpreted that the longer the period of storage the greater the amount of NO_3 content reduced. (Figure 1).



Legend:

Treatment A – blue   Treatment B – green

Treatment C – yellow   Treatment D – black

CONCLUSION

Based on the results and findings of the study, the following conclusions were drawn:

1. Brown seaweeds *(Sargassum macrocarpum)* are effective nitrate reducer.
2. There is a relationship between the efficacy of brown seaweeds as nitrate reducer and the period of treatment. The longer the storage duration the greater the amount of nitrate reduced from the contaminated water.
3. There is a significant relationship between the amount of nitrate reduced from contaminated water and the number of seaweeds in the treatments. The greater the number of seaweeds, the greater the amount of nitrate reduced.

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