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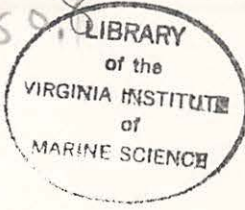
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THE EFFECT OF SEWAGE EFFLUENTS AND
THEIR CONSTITUENTS UPON THE VEGETATIVE GROWTH
OF ULVA LACTUCA (LINNAEUS) 1753
(SEA-LETTUCE)

Second and Final Progress Report
Submitted to
Hampton Roads Sanitation District Commission

by
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July 1975

INTRODUCTION

This report is a continuation of the one submitted in March 1974 and reports on the effect of additional sewage samples on the growth rate of Ulva lactuca (Linnaeus). The protocol for these experiments follows exactly that as presented in detail in the March 1974 report; the details of this protocol will not be again presented here. The reader is referred to the first report for the conditions under which these experiments were conducted.

RESULTS

Table IX presents the data obtained from the experiment on the effect of 1% and 10% unchlorinated, secondary effluents from the Chesapeake and Elizabeth River plant on the growth rate of U. lactuca. It appears that 1% of this type of sewage effluent has little influence on the growth of U. lactuca over that of the control while additions at the 10% level have a somewhat more stimulatory effect.

Table X presents the data obtained from the experiment on the effect of 1% and 10% unchlorinated, secondary effluents from the James River plant on the growth rate of U. lactuca. From this data it is evident that 1% addition of this sewage effluent produces a good stimulatory effect while at the 10% level there seems to be an inhibitory effect on the growth rate over that of the control. This is probably a reflection of a toxic sub-

stance in the sewage effluent at the time it was collected at the sewage treatment plant. At the lower concentration of 1% sewage effluent, it should be pointed out that while there is a stimulatory effect, this effect might have been much greater if the inhibitory substance evidenced at the 10% level had not been present.

Table XI presents the results of the experiments on the effect of chemically treated (with CaO) sewage from the Lamberth's Point plant on the growth of U. lactuca. Sewage effluents treated with both 100 mg/l and 250 mg/l of CaO stimulated greatly the growth rate of U. lactuca. In those experiments where the CaO was used at the rate of 100 mg/l, the 10% sewage effluent showed somewhat better growth than at the 1% level. But at both the 1% and 10% levels, the 100 mg/l of CaO treatment showed better growth than was realized when the CaO was used at 250 mg/l.

With the CaO addition at the 250 mg/l level stimulatory growth was realized at both 1% and 10% levels, but the stimulatory effects were not as great as at the 100 mg/l level. A decrease in the stimulatory effect was noted when, at the 250 mg/l level, the sewage effluent was added to the 10% level. This decrease at the 10% level, as opposed to that growth rate realized at the 1% level, can probably be attributed to a precipitation of a necessary macro or micro growth substance or factor, but not necessarily to inhibitor or toxic substances. This is supported by the fact that at 100 mg/l of CaO added at 10% concentration

-3-

had no such inhibitory effect (contrast in Table XI, S-35 vs S-37).

Table XII presents the data obtained from the experiment on the effect of 1% and 10% concentration of final clarified sewage effluents from the Williamsburg and Richmond plants on the growth of U. lactuca.

In the first report, data was presented which indicated that the final clarified sewage effluent from the Williamsburg plant showed a slightly inhibitory effect on the growth of U. lactuca.

Since the effect of additional sewage effluent as reported in this final report as represented by Table IX, X, XI showed, at least at the lower concentrations, a stimulatory effect on the growth of U. lactuca, it was decided to repeat the experiments of the Williamsburg sewage treatment plant and to test another one from a different source, i.e. the Richmond sewage treatment plant.

From Table XII, it is evident that the final clarified sewage from the Williamsburg sewage treatment plant permitted, at the 1% additional level, little growth over that of the control and at the 10% level was indeed inhibitory and this agrees essentially with the data as presented in the first report.

The effect of sewage effluent from the Richmond sewage treatment plant is somewhat different than that of the Williamsburg sewage treatment plant. At the 1% level there was a significant stimulatory effect while at the 10% level the sewage effluent was significantly inhibitory on the growth rate of U. lactuca.

Tables XIII - XV present data on various nitrogen sources on the growth of U. lactuca. In all cases, the nitrogen source was added at the rate to give an equivalent nitrogen concentration equal to 0.20 g/l of NaNO_3 (or some fraction thereof), a concentration of nitrogen commonly used in enrichments of seawater when employed for the cultivation of marine algae. Common types of both inorganic and organic nitrogen sources were used, e.g. ammonium, nitrate, nitrite, urea, and glycine.

In these experiments with nitrogen it was necessary to assure that all other growth substances and factors, including micronutrients, vitamins, and macronutrients, i.e. phosphorus, were in sufficient supply to assure that only the effect of the nitrogen was measured and that an inhibitory or non-stimulatory effect was not caused by the absence of some essential macronutrients, micronutrients, or vitamin.

Thus, these types of substances were added to raw, filtered seawater prior to the addition of the nitrogen source being tested. These additions per liter are:

1 ml of 1% Na_2HPO_4

1 ml of micronutrients

1 ml of Guillard's 3-vitamin mix

Please see the March 1974 report for the composition and concentration of the micronutrients and vitamins.

A control (S-28 of Table XIII) was also run with seawater.

Seawater enriched, but with no nitrogen (S-25, S-28 of Table XIII, S-38 of Table XIV, and S-49 of Table XV), showed, in most cases, better growth than when nitrogen was added (a single exception being 0.007 g of urea as per S-39 of Table XIV) and also better growth than did the control with no additions whatever (S-28 of Table XIII).

It was shown (Table XIII) that ammonium ion has an inhibitory effect on the growth rate of U. lactuca over that of the controls (S-16, S-28, S-25) and over nitrate ion (S-18, S-26) with the greatest inhibitory effect at the higher concentration (0.12 ug/l of NH_4Cl).

Nitrate ion produces better growth than does the ammonium ion, but at the lower concentration almost double the growth rate is realized. Both controls (S-25 and S-16), containing all enrichments except a nitrogen source, yield growth rates twice to triple respectively over that of the seawater control (S-28) containing no addition of macronutrients, micronutrients, and vitamins.

Table XIV shows that urea is a better nitrogen source than is the nitrite ion for U. lactuca; the nitrite ion showing a inhibitory effect over the control. The inhibitory effect was, however, not as great as was the case with the ammonium ion (contrast S-17, S-27 of Table XIII with S-41, S-42 of Table XIV).

For both urea and nitrate, the better growth rates occurred at the lower concentrations of nitrogen (S-39 vs. S-40 and S-41 vs. S-42).

Table XV demonstrates that glycine is a poor nitrogen source for U. lactuca but does do better at the higher concentration. However, both concentrations are inhibitory acting on the growth rate of U. lactuca over that realized in the control.

From Tables XIII - XV, it appears that urea is a better nitrogen source than is nitrate, nitrite, ammonium, or glycine.

Because it was not physically possible, given our available personnel and laboratory facilities, to run these nitrogen-source experiments simultaneously under a single control and with a single uniform batch of seawater, it probably has little meaning to rank these nitrogen sources as to their stimulatory effects on U. lactuca, so the following listing in increasing inhibitory order should be viewed as only tentative:

- 1) Urea
- 2) Nitrate ion
- 3) Glycine
- 4) Nitrite ion
- 5) Ammonium ion

Tables XIII - XV give us some insight into some of the difficulties encountered when using natural seawaters as a basis for nutritional experiments. If we contrast S-25, S-26 of Table XVII, S-38 of Table XIV and S-49 of Table XV, the controls containing all the enrichment except nitrogen, we will see S-16 and S-49 produced exceptional growth while S-25 and S-38 produced considerable less growth. The difference between these four controls resides in that they are derived

from different batches of seawater (from Wachapreague) and hence, probably contain varying amounts of "naturally occurring", though unknown, micronutrients, macronutrients, vitamins and other growth factors and substances. Similar discrepancies can be also found if one compares the controls for the experiments on the effect of various sewage effluents.

Regretfully, it has not been possible to follow through with experiments on the effect of different phosphorus sources on the growth rate of U. lactuca. In the experiment with nitrogen sources (Tables XIII - XV) inorganic phosphorus as Na_2HPO was utilized along with vitamins and micronutrients. Exceptional growth rates were realized (even in the absence of nitrogen) in some of these controls, e.g. S-16 of Table XIII and S-49 of Table XV. However, it is not possible to attribute all of this growth to phosphorus alone as there were also added the micronutrients and vitamins.

CONCLUSION

- 1) Different sewage effluents have a different effect on the growth rate of U. lactuca.
- 2) The final clarified sewage effluent from the Williamsburg sewage treatment plant was inhibitory, or produced negligible growth, at the two concentrations tested (1% and 10%) and on the two occasions tested. Similar effluents from the Richmond sewage treatment plant produced good growth at 1% concentration while

at the 10% concentration a definite inhibitory effect was realized.

- 3) Unchlorinated secondary effluent from the Chesapeake Elizabeth River plant showed only a little stimulatory effect at the two concentrations while similar effluents from the James River plant produced good growth at the 1% level and was inhibitory at the 10% level.
- 4) Chemically treated sewage from the Lambert's Point plant showed a marked stimulatory effect on the growth rate of U. lactuca except at the 10% level for the 250 mg/l of CaO treated sewage. It could be that this high level of CaO (250 mg/l) has precipitated on some essential nutrient, growth factor, or growth substance.
- 5) Different nitrogen sources affect the growth of U. lactuca to different degrees. Urea and nitrate ion at the equivalent of 0.02 mg/l of NaNO_2 appear to promote growth the best while ammonium ion and nitrite ion are definitely inhibitory when compared with the growth of the control. Glycine was inhibitory when compared with its control but significant growth was still realized when glycine was utilized and the higher level of 0.176 g/l.
- 6) However, it should be emphasized here that better growth was obtained when the seawater was enriched with vitamins, phosphorus, and trace elements, but containing no nitrogen.
- 7) The exceeding luxuriant growth in the absence of additional nitrogen above that found naturally occurring in seawater could be attributed to phosphorus, but since vitamins and trace elements

were also added, no definitive statement can be made at this time.

- 8) Preliminary work on artificial seawater also showed that organic phosphorus e.g. sodium glycerol phosphates, produced a good growth of Ulva, but again, this approach had to be discontinued because of the exhaustion of funds. Because the artificial seawater work is incomplete, this will not be reported here.
- 9) There appears to be some discrepancy between the growth of the Ulva in different batches of seawater which had been collected at different times. This implies very strongly that these are present, in naturally occurring seawater, substances which affect the growth of U. lactuca and which vary with the seasons (or the tide).
- 10) The only way to avoid these types of discrepancies is to utilize an artificial seawater as the basis of these types of experiments. The disadvantage to this, of course, is the number of man hours required to prepare the artificial seawater and the cost of chemicals. However, this should be seriously considered before future work is undertaken.

Table IX

Effect of 1% and 10% Unchlorinated Secondary Effluents
from Chesapeake Elizabeth River Plant on the Growth Rate of Ulva lactuca

Sewage Effluent	Growth of 3 x 1 cm Inocula After Exactly Two Weeks										Average Size in 2 Weeks	Total Size in cm ²	Actual Increase	Percent Increase
	Flask													
	<u>1</u>	<u>2</u>	<u>3</u>	<u>4</u>	<u>5</u>	<u>6</u>	<u>7</u>	<u>8</u>	<u>9</u>	<u>10</u>				
S-13	4.0	5.5	5.4	5.5	5.0	5.2	5.8	4.9	5.5	5.3	5.2	9.03	6.03	201.0
	X	X	X	X	X	X	X	X	X	X	X			
	1.5	1.6	1.6	1.7	1.7	1.6	2.3	1.5	1.9	1.8	1.7			
S-14	4.7	5.5	3.9	4.8	3.8	4.9	5.1	6.2	6.1	5.9	5.1	9.38	6.38	212.7
	X	X	X	X	X	X	X	X	X	X	X			
	1.9	2.2	1.2	1.5	1.2	1.8	2.3	1.8	2.0	2.1	1.8			
S-15	5.1	5.3	6.4	5.1	5.5	8.7	7.1	6.3	6.1	7.3	6.3	14.88	11.88	396.0
	X	X	X	X	X	X	X	X	X	X	X			
	2.0	1.4	2.3	2.0	2.3	3.4	2.7	2.3	2.1	2.4	2.3			

S-13 = 75% Seawater

S-14 = 1% Sewage in 75% Seawater

S-15 = 10% Sewage in 75% Seawater

Table IX

Table X

Effect of 1% and 10% Unchlorinated Secondary Effluents from
James River Plant on the Growth Rate of Ulva lactuca

Sewage Effluent	Growth of 3 x 1 cm Inocula After Exactly Two Weeks										Average Size in 2 Weeks	Total Size in cm ²	Actual Increase	Percent Increase
	Flask													
	<u>1</u>	<u>2</u>	<u>3</u>	<u>4</u>	<u>5</u>	<u>6</u>	<u>7</u>	<u>8</u>	<u>9</u>	<u>10</u>				
S-19	6.6	5.9	7.0	6.1	6.4	6.3	6.9	6.5	6.9	6.5	6.5	13.60	10.60	353.3
	X	X	X	X	X	X	X	X	X	X	X			
	2.1	2.3	2.1	2.2	2.0	1.7	1.9	2.2	2.2	2.2	2.1			
S-20	7.3	7.0	7.0	6.8	7.2	6.6	6.6	8.2	6.9	6.8	7.0	17.70	14.70	490.0
	X	X	X	X	X	X	X	X	X	X	X			
	2.3	2.5	2.8	2.3	2.5	2.5	2.6	2.7	2.5	2.5	2.5			
S-21	6.5	6.4	6.2	5.3	6.8	5.9	5.5	7.4	5.7	6.2	6.2	12.42	9.42	314.0
	X	X	X	X	X	X	X	X	X	X	X			
	2.0	1.8	2.3	2.4	2.5	2.1	1.6	2.4	1.5	2.0	2.1			

S-19 = 75% Seawater

S-20 = 1% Sewage in 75% Seawater

S-21 = 10% Sewage in 75% Seawater

Table X

Table XI

Effect of Chemically Treated Sewage from the Lambert's
Point Plant on the Growth Rate of Ulva lactuca

Sewage Effluent	Growth of 3 x 1 cm Inocula After Exactly Two Weeks										Average Size in 2 Weeks	Total Size in cm ²	Actual Increase	Percent Increase
	Flask													
	<u>1</u>	<u>2</u>	<u>3</u>	<u>4</u>	<u>5</u>	<u>6</u>	<u>7</u>	<u>8</u>	<u>9</u>	<u>10</u>				
S-33	4.9	5.0	5.4	4.2	5.3	4.1	4.2	4.5	4.7	4.1	5.64	7.49	4.49	149.9
	X	X	X	X	X	X	X	X	X	X	X			
	1.7	1.8	1.8	1.2	1.7	1.3	1.7	1.8	1.7	1.3	1.60			
S-34	7.7	7.0	7.2	8.5	8.0	7.3	7.5	6.7	7.2	7.0	7.41	19.0	16.0	533.3
	X	X	X	X	X	X	X	X	X	X	X			
	2.2	2.6	2.7	2.8	3.0	2.4	2.4	2.3	2.9	2.2	2.55			
S-35	8.0	8.5	8.2	7.0	7.5	8.5	7.5	8.6	9.1	8.7	8.16	21.7	18.7	623.3
	X	X	X	X	X	X	X	X	X	X	X			
	3.0	3.1	2.9	2.1	2.4	2.3	3.4	2.4	2.4	2.6	2.66			
S-36	8.8	7.5	8.8	7.0	6.0	7.9	7.5	7.8	9.1	7.1	7.75	21.0	18.0	600.0
	X	X	X	X	X	X	X	X	X	X	X			
	2.7	3.0	3.4	2.1	1.7	3.1	2.6	2.7	2.4	3.1	2.68			
S-37	5.5	6.5	6.3	6.6	7.0	5.0	6.5	6.2	6.8	6.9	6.33	13.6	10.6	353.3
	X	X	X	X	X	X	X	X	X	X	X			
	2.3	2.2	2.0	2.1	2.5	1.8	2.4	1.9	2.0	2.2	2.14			

S-33 = 75% Seawater

S-34 = 1% Sewage Treated with 100mg/l of CaO in 75% Seawater

S-35 = 10% Sewage Treated with 100mg/l of CaO in 75% Seawater

S-36 = 1% Sewage Treated with 250mg/l of CaO in 75% Seawater

S-37 = 10% Sewage Treated with 250mg/l of CaO in 75% Seawater

Table XI

Table XII
 The Effect of 1% and 10% Concentrations of Final Clarified Sewage
 Effluent from Williamsburg and Richmond Plants on Growth Rate of Ulva lactuca

Media	Growth of 3 x 1 cm Inocula After Exactly Two Weeks										Average Size in 2 weeks	Total Size in cm ²	Actual Increase	Percent Increase
	Flask													
	<u>1</u>	<u>2</u>	<u>3</u>	<u>4</u>	<u>5</u>	<u>6</u>	<u>7</u>	<u>8</u>	<u>9</u>	<u>10</u>				
S-43*	6.3	5.8	6.2	5.9	6.1	6.3	5.7	5.6	4.7	5.9	5.85	11.03	8.03	268%
	X	X	X	X	X	X	X	X	X	X	X			
S-44	2.1	2.0	1.8	1.8	2.0	1.9	1.9	1.7	1.7	1.9	1.88	12.26	9.26	309%
	6.3	7.3	5.9	6.0	5.9	5.7	7.0	5.8	5.8	5.6	6.43			
S-45	X	X	X	X	X	X	X	X	X	X	X	15.4	12.4	413%
	2.1	2.3	2.0	1.8	2.0	1.8	2.1	1.9	2.0	1.9	1.99			
S-46**	8.4	6.3	7.0	8.1	6.2	7.1	7.2	7.6	6.5	6.5	7.09	14.03	11.03	368%
	X	X	X	X	X	X	X	X	X	X	X			
S-47	2.5	2.3	2.5	2.5	2.1	2.0	2.2	3.2	2.2	2.2	2.37	13.72	10.72	357%
	6.5	5.9	6.7	5.9	6.5	6.7	6.8	7.0	5.9	6.6	6.4			
S-48	X	X	X	X	X	X	X	X	X	X	X	10.90	7.90	263%
	2.0	2.0	2.3	2.0	2.3	2.3	2.3	2.2	2.1	2.2	2.2			
S-48	6.8	6.8	6.8	6.4	5.6	6.5	6.5	6.5	5.9	5.7	6.4	10.90	7.90	263%
	X	X	X	X	X	X	X	X	X	X	X			
S-48	2.1	2.4	2.0	2.2	1.8	2.4	2.0	2.7	2.2	1.7	2.2	10.90	7.90	263%
	5.5	5.3	6.0	5.6	6.0	5.9	5.8	5.7	5.0	5.8	5.7			
S-48	X	X	X	X	X	X	X	X	X	X	X	10.90	7.90	263%
	1.8	1.9	2.0	1.7	2.1	2.2	2.2	1.8	1.7	1.8	1.9			

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Table XII

Table XII

The Effect of 1% and 10% Concentrations of Final Clarified Sewage
Effluent from Williamsburg and Richmond Plants on Growth Rate of Ulva lactuca

S-43 = 75% Seawater

S-44 = 1% Williamsburg Sewage in 75% Seawater

S-45 = 1% Richmond Sewage in 75% Seawater

S-46 = 75% Seawater

S-47 = 10% Williamsburg Sewage in 75% Seawater

S-48 = 10% Richmond Sewage in 75% Seawater

* Control for S-44 and S-45

** Control for S-47 and S-48

Table XII

Table XIII
Effect of Ammonium and Nitrate
on Growth Rate of Ulva lactuca

Media	Growth of 3 x 1 cm Inocula After Exactly Two Weeks										Average Size in 2 weeks	Total Size in cm ²	Actual Increase	Percent Increase
	Flask													
	<u>1</u>	<u>2</u>	<u>3</u>	<u>4</u>	<u>5</u>	<u>6</u>	<u>7</u>	<u>8</u>	<u>9</u>	<u>10</u>				
S-28*	7.6	7.7	6.6	6.7	5.9	6.7	6.3	6.3	6.6	7.2	6.7	16.60	13.60	453.3
	X	X	X	X	X	X	X	X	X	X	X			
	3.2	2.3	2.2	2.5	2.4	2.5	2.4	2.2	2.5	2.3	2.45			
S-25*	10.3	8.9	8.7	9.0	8.9	8.4	10.1	9.5	10.6	7.9	9.23	31.80	28.80	960.0
	X	X	X	X	X	X	X	X	X	X	X			
	3.3	3.6	3.2	3.5	3.6	3.4	3.6	3.4	3.2	3.7	3.45			
S-16**	12.3	12.9	12.3	11.4	11.1	11.7	9.4	9.4	11.7	11.1	11.3	43.40	40.40	1343.3
	X	X	X	X	X	X	X	X	X	X	X			
	4.2	3.8	3.7	4.5	3.8	3.9	3.8	3.5	3.5	3.5	3.8			
S-17	3.7	4.0	4.2	3.7	3.5	4.0	3.7	3.9	3.9	3.6	3.8	4.87	1.87	62.3
	X	X	X	X	X	X	X	X	X	X	X			
	1.3	1.6	1.4	1.1	1.0	1.1	1.2	1.4	1.4	1.2	1.3			
S-27	4.6	4.4	4.9	4.6	5.2	5.4	4.6	4.4	4.6	4.9	4.76	7.89	4.89	163.0
	X	X	X	X	X	X	X	X	X	X	X			
	1.4	1.9	1.5	1.6	2.0	1.9	1.8	1.5	1.3	1.6	1.65			
S-18	6.5	6.1	7.3	6.6	6.5	6.1	5.2	6.5	6.7	6.0	6.4	14.45	11.45	348.3
	X	X	X	X	X	X	X	X	X	X	X			
	2.2	2.3	2.4	2.2	1.8	2.2	2.1	2.3	2.0	2.0	2.2			

Table XIII

(continued on next page)

Table XIII
Effect of Ammonium and Nitrate
on Growth Rate of Ulva lactuca

Media	Growth of 3 x 1 cm Inocula After Exactly Two Weeks										Average Size in 2 Weeks	Total Size in cm ²	Actual Increase	Percent Increase
	<u>1</u>	<u>2</u>	<u>3</u>	<u>4</u>	<u>5</u>	<u>6</u>	<u>7</u>	<u>8</u>	<u>9</u>	<u>10</u>				
S-26	10.1	7.8	7.8	7.8	7.9	7.0	9.0	9.2	10.2	7.4	8.42	25.00	23.00	766.7
	X	X	X	X	X	X	X	X	X	X	X			
	3.5	2.3	3.5	2.4	2.9	2.2	3.2	3.4	3.1	2.8	2.93			

S-16 = 75% Seawater, enriched, but no nitrogen

S-17 = 75% Seawater, enriched, with the Nitrogen as 0.124g/1 of NH₄Cl

S-18 = 75% Seawater, enriched, with the Nitrogen as 0.20g/1 of NaNO₃

S-28 = 75% Seawater, unenriched

S-25 = 75% Seawater, enriched, but no Nitrogen

S-26 = 75% Seawater, enriched, with the Nitrogen as 0.020g/1 of NaNO₃

S-27 = 75% Seawater, enriched, with the Nitrogen as 0.0124g/1 of NH₄Cl

* S-28 and S-25 control for Nos. 26 and 27

** S-16 control for Nos. 17 and 18

Table XIII

Table XIV
Effect of Urea and Nitrite
Upon the Growth Rate of Ulva lactuca

Media	Growth of 3 x 1 cm Inocula After Exactly Two Weeks										Average Size in 2 Weeks	Total Size in cm ²	Actual Increase	Percent Increase
	<u>1</u>	<u>2</u>	<u>3</u>	<u>4</u>	<u>5</u>	<u>6</u>	<u>7</u>	<u>8</u>	<u>9</u>	<u>10</u>				
S-38	10.6	8.5	8.6	7.1	10.1	8.5	8.2	9.2	7.4	10.2	8.84	26.94	23.94	798.0
	X	X	X	X	X	X	X	X	X	X	X			
S-39	3.3	3.3	3.0	2.9	2.9	2.6	2.7	4.0	2.3	3.2	3.02	32.45	29.45	981.7
	9.8	10.2	10.6	11.1	13.0	9.1	8.6	8.2	7.0	6.0	9.36			
S-40	X	X	X	X	X	X	X	X	X	X	X	26.1	23.1	770.0
	4.0	3.9	3.4	3.6	3.9	3.1	3.0	3.0	2.6	2.5	3.40			
S-41	9.9	8.7	7.5	10.4	10.2	8.4	8.4	5.4	7.2	6.7	8.28	12.14	9.14	304.7
	X	X	X	X	X	X	X	X	X	X	X			
S-42	3.2	3.2	2.7	4.0	3.6	3.4	3.5	1.1	2.7	2.7	3.01	6.14	3.14	104.7
	5.6	6.5	6.5	6.0	8.0	7.5	7.1	6.0	6.7	5.0	6.29			
S-42	X	X	X	X	X	X	X	X	X	X	X	1.92	1.92	1.92
	1.9	2.2	1.5	2.0	2.1	2.2	2.0	1.8	1.8	1.7	1.92			
S-42	4.4	4.2	4.1	4.9	3.6	4.8	4.4	4.3	4.3	4.8	4.38	6.14	3.14	104.7
	X	X	X	X	X	X	X	X	X	X	X			
S-42	1.4	1.3	1.1	1.6	1.2	1.7	1.4	1.4	1.3	1.5	1.39	6.14	3.14	104.7
	1.4	1.3	1.1	1.6	1.2	1.7	1.4	1.4	1.3	1.5	1.39			

S-38 = 75% Seawater, enriched, but no Nitrogen

S-39 = 75% Seawater, enriched, with the Nitrogen as 0.007g/l of Urea

S-40 = 75% Seawater, enriched, with the Nitrogen as 0.07g/l of Urea

S-41 = 75% Seawater, enriched, with the Nitrogen as 0.016g/l of Na₂NO₂

S-42 = 75% Seawater, enriched, with the Nitrogen as 0.167 g/l of Na₂NO₂

Table XIV

Table XV
 Effect of Glycine on
 Growth of Ulva lactuca

Media	Growth of 3 x 1 cm Inocula After Exactly Two Weeks										Average Size in 2 Weeks	Total Size in cm ²	Actual Increase	Percent Increase
	Flask													
	<u>1</u>	<u>2</u>	<u>3</u>	<u>4</u>	<u>5</u>	<u>6</u>	<u>7</u>	<u>8</u>	<u>9</u>	<u>10</u>				
S-49	11.2	10.4	10.5	9.3	10.5	10.7	11.1	10.3	11.0	10.7	10.61	39.75	36.75	1225%
	X	X	X	X	X	X	X	X	X	X	X			
S-50	4.2	3.8	3.5	3.7	3.6	3.9	3.7	3.4	3.8	3.8	3.74	22.02	19.02	634%
	6.7	6.8	10.5	10.5	7.0	6.0	9.3	7.5	8.0	4.5	7.68			
S-51	X	X	X	X	X	X	X	X	X	X	2.69	6.10	3.10	103%
	2.0	3.2	3.7	4.1	2.4	1.5	3.5	2.6	2.2	1.7	4.30			
S-51	4.0	4.5	4.2	4.6	4.2	4.2	4.6	4.5	4.2	4.0	4.30	6.10	3.10	103%
	X	X	X	X	X	X	X	X	X	X	X			
	1.3	1.6	1.7	1.4	1.4	1.5	1.3	1.6	1.3	1.3	1.44			

S-49 = 75% Seawater, enriched, but no Nitrogen
 S-50 = 75% Seawater, enriched with the Nitrogen as .176g/l Glycine
 S-51 = 75% Seawater, enriched with the Nitrogen as .0176g/l Glycine

Table XV

Table XVI
Chemical Analyses, p. 1

Sample	DKN (mg/1)	NH ₃ (mg/1)	DP (ugAtP/1)	NO ₂ (ugAt ^N /1)	NO ₃ (ugAt ^N /1)	O-PO ₄ (mgAt ^P /1)
S 13	Unable to run	23.05	0.83	0.28	0.98	0.51
S 14	Unable to run	24.39	2.73	0.29	0.77	2.44
S 15	Unable to run	9.51	25.90	0.33	0.77	23.22
S 16	5.15	0.44	20.00	3.29	6.71	No Sample
S 17	35.28	39.20	19.39	2.38	6.42	72.60
S 18	4.70	0.05	20.56	0.34	2222.97	80.97
S 19	0.67	0.01	1.26	0.27	Unable to run	0.18
S 20	0.73	0.19	3.32	0.28	0.73	1.92
S 21	2.30	2.41	12.20	0.26	0.91	21.78
S 22	0.78	.005	3.35	0.27	0.88	1.23
S 23	2.35	2.44	11.20	0.26	1.10	25.41
S 24	0.56	.005	1.36	0.26	0.68	0.44
S 25	5.04	0.31	38.60	0.18	3.66	39.93
S 26	4.98	0.23	35.80	0.23	18.27	50.82
S 27	33.21	28.99	27.39	0.26	73.65	29.04
S 28	0.45	0.03	16.83	0.13	Too Low	0.47
S 29	5.00	0.26	18.84	0.41	5.50	0.87
S 30	1.09	0.25	27.10	0.22	18.27	43.56
S 31	4.00	31.40	21.54	0.24	51.93	21.78
S 32	0.17	0.02	0.34	0.14	0.17	0.47
S 33	0.16	0.02	0.68	0.19	0.10	0.87
S 34	0.35	0.22	1.19	0.18	0.21	1.45
S 35	2.11	2.80	19.43	0.70	1.88	18.15
S 36	0.36	0.18	0.75	0.22	0.15	0.44
S 37	1.89	2.32	4.68	0.24	0.68	6.12

Table XVI

(continued on next page)

Table XVI
 Chemical Analyses, cont'd.

Sample	DKN (mg/1)	NH ₃ (mg/1)	DP (ugAt ^P /1)	NO ₂ (ugAt /1)	NO ₃ (ugAt ^N /1)	O-PO ₄ (mgAt ^P /1)
S 38	1.91	0.02	10.31	0.16	4.17	29.04
S 39	10.78	0.53	15.38	0.33	3.19	21.78
S 40	34.44	0.07	22.65	0.22	3.43	29.04

Please see pp. 3, 4, and 5 of this table for definition of Sample Numbers

Table XVI

Table XVI
Chemical Analyses, p. 2

Sample	DKN (mg/1)	NH ₃ (mg/1)	DP (ugAt ^P /1)	NO ₂ (ugAt ^N /1)	NO ₃ (ugAt ^N /1)	O-PO ₄ (mgAt ^P /1)
S 41	6.27	0.02	23.97	182.70	56.53	25.41
S 42	4.37	.005	20.12	20.11	2.14	21.78
S 43	0.56	.005	1.02	4.69	2.49	1.20
S 44	0.73	.005	0.91	1.06	3.16	0.94
S 45	0.73	0.07	2.72	1.49	8.89	2.14
S 46	0.21	0.04	1.08	1.07	0.44	0.37
S 47	0.42	0.05	27.66	1.04	30.84	10.64
S 48	0.50	0.23	24.92	4.16	316.52	15.13
S 49	5.47	0.32	32.14	0.87	7.01	4.45
S 50	5.35	0.31	30.32	0.46	7.44	4.45
S 51	24.60	15.90	26.76	3.50	25.01	24.38

DKN = Dissolved Kjeldahl nitrogen

NH₃ = Ammonia

DP = Dissolved phosphorus

NO₂ = Nitrite

NO₃ = Nitrite

O-PO₄ = Ortho-phosphate

Please see pp. 3, 4, and 5 of this table for definitions of Sample Numbers

(continued on next page)

Table XVI

Table XVI
Chemical Analyses, p. 3
(Definition of Sample Numbers)

S 13	75% seawater, unenriched, (Control for S-14 and S-15).
S 14	1% sewage,, (Chesapeake Elizabeth River Plant), in 75% seawater.
S 15	10% Sewage, (Chesapeake Elizabeth River Plant), in 75% seawater.
S 16	75% seawater, enriched, but no nitrogen (Control for S-17 and S-18).
S 17	75% seawater, enriched, with the nitrogen as 0.124 g/l of NH_4Cl .
S 18	75% seawater, enriched, with the nitrogen as 0.20 g/l of NaNO_3 .
S 19	75% seawater, (Control for S-20 and S-21).
S 20	1% sewage, (James River Plant), in 75% seawater.
S 21	10% sewage, (James River Plant), in 75% seawater.
S 22	duplicate analyses on S-20.
S 23	duplicate analyses on S-21.
S 24	duplicate analyses on S-19.
S 25	75 % seawater, enriched, but no nitrogen (partial control for S-26 and S-27).
S 26	75% seawater, enriched, with the nitrogen as 0.020 g/l of NaNO_3 .
S 27	75% seawater, enriched, with the nitrogen as 0.0124 g/l of NH_4Cl .
S 28	75% seawater, unenriched, (Control for S-25, S-26 and S-27).

(continued on next page)

Table XVI

Table XVI

Chemical Analyses, p. 4
(Definition of Sample Numbers)

S 29	duplicate analyses of S-25.
S 30	duplicate analyses of S-26.
S 31	duplicate analyses of S-27.
S 32	duplicate analyses of S-28.
S 33	75% Seawater, (Control for S-34, S-35, S-36 and S-37).
S 34	1% sewage, (Lambert's Point Plant), treated with 100 mg/l of CaO and in 75% seawater.
S 35	10% sewage, (Lambert's Point Plant), treated with 100 mg/l of CaO and in 75% seawater.
S 36	1% sewage, (Lambert's Point Plant), treated with 250 mg/l of CaO and in 75% seawater.
S 37	10% sewage, (Lambert's Point Plant), treated with 250 mg/l of CaO and in 75% seawater.
S 38	75% seawater, enriched, but no nitrogen (Control for S-39, S-40 and S-42).
S 39	75% seawater, enriched, with the nitrogen as 0.007 g/l of urea.
S 40	75% seawater, enriched, with the nitrogen as 0.007 g/l of urea.
S 41	75% seawater, enriched, with the nitrogen as 0.016 g/l of NaNO ₂ .
S 42	75% seawater, enriched, with the nitrogen as 0.167 g/l of NaNO ₂ .
S 43	75% seawater, (Control for S-44 and S-45).

(continued on next page)

Table XVI

Table XVI

Chemical Analyses, p. 5
(Definition of Sample Numbers)

S 44	1% sewage, (Williamsburg Plant), in 75% seawater.
S 45	1% sewage, (Richmond Plant), in 75% seawater.
S 46	75% seawater, unenriched, (Control for S-47 and S-48).
S 47	10% sewage, (Williamsburg Plant), in 75% seawater.
S 48	10% sewage, (Richmond Plant), in 75% seawater.
S 49	75% seawater, enriched, but no nitrogen.
S 50	75% seawater, enriched, with the nitrogen as 0.176 g/l of glycine.
S 51	75% seawater, enriched, with the nitrogen as 0.017 g/l of glycine.

Table XVI

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