## A Study of the Relations Between Plant Growth and Combined Nitrogen in Winona Lake. $\dagger$

Thurman B. Rice.
Obyective. The purpose of the investigation as it was first conceived was to determine the effect of combined nitrogen content of the water upon the luxuriance of plant growth. As the work progressed, however, a considerable mass of data accumulated pertaining to the effect of the regetation upon the nitrogen content of the water, and so the scope of the work was broadened to include this phase of the subject.

Preliminary Work. Work was begun in July, 1915, by making an accurate map of the lake, and by making a close study of the entire littoral region in order to determine the most fruitful points of attack. The entire shoreliue was sounded at close intervals to a depth of five meters, this being found to be the limit of plant growth. The Lake was under constant observation from July, 1915, to September, 1916. During this time obserrations of some kind were taken nearly every day except while the lake was covered with ice.

Analyses. Analyses were begun in October and continued until August. In all, 135 analyses were made. About half of the analyses were complete for combined nitrogen, the others being for nitrates and nitrites only. Standard methods of water analysis were used (Dlason, '12 and Olsen, '08.)*

Samples were taken from clear unfiltered water just below the surface unless otherwise stated. The bottle used to carry samples were thoroughly cleaned and was sealed immediately after being filled. Analysis was begun as soon as the laboratory was rearhed, on the average about thirty minutes after the sample was taken.

A map of the lake with analysis stations is presented as Fig. 1.

Description of the Stations from which annlyses were made.
A Weedy, numerous conferwid algae, muddy sediment bottom.
B Weeds scanty, but dense close by, sediment bottom. 4m deep.
C Clear water, near center of the lake, deepest place, 21 m deep.
D Weedy, many algae, muddy sediment bottom.
E and G Very weedy, water much contaminated. 1 m deep.
$\dagger$ Contribution of the Zoology Deparment of Indiana University No. 156 . The problem was suggested by Dr. Will scott under whose direction the work was carried out.
*Mason, Examination of Water, 1912: Wiley and Sons.
Olsen, Quant. Chem. Anal. 190s, Van Nostrand. An error of one decimal place in the computation of nitrites as given in the 1908 edition (corrected in 1915 edition) was detected and computations were made accordingly:


I Few weeds at mouth but the creek was choked witl weeds, the discharge of the creek is small. No sewers empty into the creek.

K The main inlet of the lake. Water usually clear but much contaminated by discharge from septic tanks.

M Weedy, many algae and seed plants. 1 m deep.
N No weeds at mouth but the canal was choked with them. Flow very scanty.

O Second largest inlet. Water clear but carrying some silt.
P Dense Potamogeton field. $3-4 \mathrm{~m}$ deep.
Q Dense Potamogeton field. 2-3m deep.
T Extremely dense vegetation. Water Shallow.
S Peaty bottom. Described in detail below.
R Dense field of Potamogeton. 2-3 m deep.
U Weeds sparce. Sewers empty not far away.
V Weeds very dense, especially filamentous algae.

## Part 1. The Effect of the Vegetation Upon the Nitrogen Content.

## A In the Pelagic Region.

Description of Analysis Station. At the deepest place in the lake (21M) a barrel was anchored, and from this place the samples were taken. The position of this station is indicated by the letter C upon the map.

Discussion of the graphs.* The analyses taken in the late autumn showed a low nitrogen content due to the fact that the plants growing in the lake had assimilated a large amount of nitrogen during the summer previous. The following March showed a rise of everything except the nitrite which was practically absent. From Marsh until May the four nitrogen compoundsnitrates, nitrites, free ammonia, and albuminoid estimated as ammoniaincreased, reaching a maximum in late May.
(1) Nitrogen increased during winter and early spring due to the surplus of affluents and deray over effluent and fixation by plants.
(2) Nitrogen fell during the time the plant growth was rapidly increasing and profuse (after about Juve 10-15.)
(3) Storms mixed the lake by bringing into the body of the lake the more concentrated water of the bays. Note data taken just after the lake was rough; June 21, 29, July 13, 22.

[^0]

Fig.-2

(4) Note the practical absence of nitrate at the end of winter, and also note the general shape of the nitrite curve.
(5) Nitrate gradually rose, and then rapidly fell after the plants began to increase.

The reason for the increase during the winter is of course the fact that the factors tending to increase the content; i. e. the nitrogen content of the in ets, the release of nitrogen to simpler forms by decay, and the nitrogen compounds washed from the air by rain and snow, over-balanced the factors tending to reduce the content, such as loss from the outlet and nitrogen assimilation by plants. The very low nitrite content at the end of winter indicates that the disposal of this form over-balanced its production, at a time when pro duction was slow due to the fact that the cold weather inhibited bacterial action. When the warm weather of spring came, the bacterial action was resumed and the nitrites were again produced faster than they were used.

About the first of June, the phanerogams began to increase rapidly and by the end of July had reached the maximum. This growth although limited to the littoral region clearly affected the pelagic region as is shown by the fact that the results obtained from the open lake were comparable to those obtained where plants grew, except of course the less striking results were found in the open lake, since it acted as a reservoir. It was noticed particularly that the nitrate decreased and came to practically equilibrium at a low level at the time that plant growth was at its maximum.

The Effect of Stratification. Vertical series were run from Station C, the deepest place in the lake. It was found that the lower part of the lake contained more n trogen than the upper. This general fact was noted by Birge and Juday, 1911* in investigations on Lakes Garvin and Mendota, but in other respects my results differed from theirs, due perhaps to differences in the lakes.

The graph showing the nitrogen content at different levels (Fig. 4) does not represent a single series, but is the average of two or three analyses for each depth.

This graph offers a partial explanation of the rise of nitrogen content during the winter, since the water mixes from top to bottom during that time due to the holothermous condition, and the water of the bottom being more concentrated causes the surface content to rise. This is insufficient, however, to account for all of the increase since the winter and spring content at the surface is much higher than that at the bottom later in the season at least. Before the spring statification began, the content must have been practically uniform from top to bottom, and the fall to the conditions found when the vertical series was run was probably due to the settling of the albuminoid, and the utilization of nitrates by plants, at least these two were the com-

[^1]pounds which showed the greater part of the decrease．It was also true that the eontont of the surface of the pelagice region rontinned to rise in the spring after the stratification had begun（See Figs． 2 and 3）．So it would seem that winter mixing is not the only，and perhaps not the main factor in rausing the increased nitrogen concentration．A considerable part of the abbuminoid increase in winter and spring is donbtless dne to the sediment of the hottom being stirred up，the lake being much rongher in spring than in summer．

Interesting questions outside the soope of this paper are suggested by the relation of the various compounds to the thermocline（Fig 4）．It may he said


The doted lite marked $T$＇$T$＇is the thermoeline．
onespareremoscots．ジ F 。
here，howerer，that shortage of oxyern near the bothom probahly aceomes for the behaivor of the nitrate and nitrite．Determinations of dissolved oxygen made at rarions times by Dr．Will scott show an arerage of about 1．5）ee per liter for the water of the hottom of the lake during the summer．

13．Littoral，Regons Wruere I＇lant（irowth was Open Enough to Allow Free Circtlation of Curbexts Set fp by the Wind．

As might be experted these regions showed close similarity to the pelagie region．＇The water mixed freely with that of the open lake，and local varia－ tions were slight．

A region of this nature was that along the shore of the island made by the canal. This region was close to the town and received a considerable amount of sewage, but supported only a moderate plant growth. The region gave upon analysis a content quite close to that of the open lake, as can be seen by comparison of stations $C$ and U in Figs. 1,5. Station U had a sparse growth of various Potamogetons. Its location is shown on the map.


Fig. 5-Map af Stations in South Bay.
Five stations in South Bay were intermediate between this condition and the next, and it was found that the contents of the five stations were nearly identical at any given date, although the stations were very diverse as to plant growth.

The map (Fig. 5) shows the relations of the stations which are also described in the list giving the characteristics of the stations. The general region is indicated on the map of the lake (Fig. 1) by the letter S.

There was a distinct and fairly constant relation between the stations as shown by the analyses but no opportunity was afforded for an accumulation of any form of nitrogen due to the fact that the currents kept the region mixed. See figure $\mathbf{1 4}$ for graphs for these stations.


Fig. 2- Stallion 13. (see Table + for Datat
The significanfe of the corves is dise ussed in the text.
The graphs shown in this figure are very typical of the conditions existingin regions of moderate density near the shore.
C. Littoral Regions Where Phanerogams Grew so Dense that the Circulation of the Wind Driven Currents was Inhibited or Wholly Prevented.
(a) Regions of Dense Growth.

Stations A, B, R, P and Q answered to this description and from them the following data were taken. They are readily located on the map (Fig. 1).

Nitrate Behavior. In these stations nitrate fell very rapidly during the last of June and the first few days of July. All stations agreed in this respect, as is shown by Fig. 7, which also shows the five graphs from the South Bay stations. After July 5, the nitrate content seemed to have come to a state of equilibrium and was produced and used at about the same rate. The explanation of the nitrate curve is rather simple. The bacteria increased very rapidly during the warm days of spring and produced a great surplus of nitrate before the plants got fairly started. Then the plants being supplied with abundance of plant food and bright sunshine grew apare and succeeded in cutting down the surplus of nitrate and early in July established nitrate equilibrium at a rather low level.

Nitrite Behavior. The nitrite content fell gradually until about July 5, remained low until July 19 after which it gradually rose until the last analyses were made (Aug. 3). The explanation of the first part of the nitrite curve is no doubt similar to that for the nitrate of the same time. Then during the following period of low content, photosynthesis was extremely active due to the bright sun and warm weather.

This resulted in a supersaturation of free oxygen in the water, as was evidenced by the fact that bubbles of oxygen were seen over the surface of the plants. The then existing eonditions would facilitate the passage of nitrites into nitrates as will be further discussed under the conditions existing in regions of extremely dense plant growth.

Following this period the reproductive processes predominated over the vegetative and hence the amount of free oxygen produced by photosynthesis was reduced and the amount used for respiration was increased with the end result that the amount of free oxygen in the water considerably lessened. At this time the nitrites began to increase due to the fact that less was being converted into nitrate. This result was noted in all of the stations considered in this division, and was also noted in the five stations in South Bay and in the lake body itself indieating that the process was general rather than local.

Confirmatory evidence of the above explanation is found in the fact that during the latter part of July and the whole of August, the vegetation was much paler than earlier in the season, and the plants were mainly engaged in producing flowers and fruit, rather than vegetative growth. Katabolie processes using free oxygen probably predominated over anabolic processes produeing free oxygen.


Fig. 7 -Graphs for Nine Stations superimposed for comparative purposes.
The extreme similarity of the stations is striking, and is indicative of a general procees not dependent upon location factors

The curves no dombt womd have been still more have been made on the same days, but this was impossible

## (b) Regions of Extremely Dense Vegetation.

In the two regions of this description the regetation was so extremely dense that a boat could be pushed through only with great difficulty. Great stagnation existed in both places. The water in station $T$ at the place where the samples were taken was . 3 M deep. The station was well protected as its location on the map will show. The water in the other station of this description, the canal, was about 1 M deep.

The plant growth consisted mainly of various pondweeds, Potamogetons; waterpest, Elodea canadensis; duckweed, Spriodela polyrhi:a; eel grass, Vallisneria spiralis; Naias flexilis; filamentous and confervoid algae, and phytoplanketon. The algae predominated in the canal while the phanerogams constituted the main bulk of the plants in station $T$.

Nitrate Beharior. In each case the stations showed a rapid fall in nitrate and then remained low, in fact extremely low, throughout the remainder of the season. The reason for the fall in content is apparent in the light of the great drain that such a dense vegetation would make upon the nitrate in solution. In this connection, it should be remembered that the water could circulate very little due to the resistance offered by the mat of plants. The water in this station was also quite shallow, as before stated, and hence could hold no great store.

Nitrite Behavior. The behavior of the nitrite in these stations is peculiar but very significant. As has been stated before, the nitrite was very low at the end of winter as the ice was going off. It then gradually rose until April 20 at which time it began to show a rapid rise, due no doubt to the fact that while at that time the higher plants has been unable to get a start, the bacteria on the other hand had flourished inordinately. Toward the latter part of June the phanerogams began to flourish and much free oxygen was prodnced as a by-product of photosynthesis. The effect of this was to oxidize the nitrite, and hence the precipitous fall of nitrite from June 24 to July 12.

The conditions in these regions remained more uniform than in regions of less plant growth, due I think, to the fact that large amounts of algae and phytoplaneton were found in these regions and this tended to keep the vegetative processes in the ascendency, which was not the case in the regions of less density. The temperature of the water was high, due to the fact that there was little interchange with other regions, and to the fact that the water was shallow $\left(100^{\circ} \mathrm{F}\right.$ was frequently reached and temperatures as high as $106^{\circ} \mathrm{F}$ were observed). The presence of so much free oxygen, a high temperature, a high bacterial count, and a nitrate content which remains low, due to the constant drain upon it, results in the oxidation of the nitrites almost as soon as they are formed.

Two Points of Interest outside the scope of this paper to be noted at this point are:

| (1) |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| 1 |  |  | ct |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| 1 |  |  | duto |  |  |  |  |  |  |  |  |  |  |  |  |  |  | 1 |  |  |  |  |
|  |  |  |  |  |  |  |  |  |  |  |  |  |  | - |  |  |  |  |  |  |  |  |
| 1 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| 1 |  |  |  |  | 4 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| $\xrightarrow{1}$ |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| +1 |  | -- |  | $\square$ | - 3 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
|  | 1 | - |  | - | - 3 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
|  |  |  |  |  | 0 |  |  |  |  |  | 1 | 1 |  |  |  |  |  |  |  |  |  |  |
|  | 1 |  |  | - | - |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
|  | 1 |  |  | $\square$ | 3 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
|  | 1 |  | Ste | tion | 0 | 0 |  | - |  | $+$ |  |  |  |  | 5 |  |  |  |  |  | - |  |
|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
|  | -1 | - - | -- |  | 5. | $L^{2}$ |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
|  | 1 |  |  |  | 0 | 0 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  | , |
|  | $\cdots$ |  |  |  | 工9 | 0 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
|  |  |  |  |  | \% 2 | 5 |  |  |  |  |  |  |  |  |  |  |  |  |  |  | 11 |  |
|  |  |  |  |  | I | 0 |  |  |  |  |  |  |  |  |  |  |  |  |  |  | 1 |  |
|  |  | 1 |  |  | 5 | $\checkmark$ |  |  |  |  |  |  |  |  |  | - |  |  |  |  | 11 |  |
|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
|  |  | 1 | + |  | $5 y$ |  |  |  |  |  |  |  |  |  |  |  |  |  |  | Y |  |  |
|  |  | 1 | 1 | $\square$ | 10 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
|  | 11 | $\underline{-1}$ |  | - | $\longrightarrow 1$ | 1 |  | $\square$ |  |  |  |  |  |  |  |  |  |  |  |  | + |  |
|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
|  |  |  |  |  |  |  |  |  |  |  | 1 |  |  |  |  |  |  |  |  | , |  |  |
|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  | опмеL |  |  | socier |  |
| - |  |  |  |  |  |  |  |  | is |  |  |  |  |  |  |  |  |  |  |  |  |  |
| ${ }^{8}$ |  |  |  |  |  |  |  | ツ | - |  |  |  |  |  |  |  |  |  |  |  |  | m' |
| 5 |  | Juls |  |  |  |  |  |  | 3. |  |  |  |  | Jul |  |  |  |  |  |  |  | Ang. |

Fig. 8--(See Table 4 for Data)

[^2]First, in regions of very dense or even of fairly dense vegetation where great contamination exists, a chemical determination of nitrates or nitrites as an indicator of pollution in making a sanitary water analysis is absolutely worthless in itself.


Fig, 9 -See Table 7 for Data
(1) The nitrite and nitrate curves are extremely regular.
(2) Compare with Station $T$ of Plate 5. These two are the only regions of extremely dense vegetation.
(3) Note the effect of the windy weather of April 17. At this time the turbid, very much contaminated water of Entrance Bay was brought in.
(4) Absence of data covering hiatus of 60 days is very unfortunate.

Second, we have a possible explanation of the fact that a diurnal variation of the free oxygen dissolved in the water of densely vegetated regions has not been found to be as great or as constant as was predicted on theoretical grounds. It would seem that during active photosynthesis the water would contain much more free oxygen than in the early morning after several hours of respiration by plants and animals in the water. But in case the free oxygen combined to a considerable extent with the nitrites to produce nitrates as seems indicated by the results obtained in these observations, such a large variation would be prevented.

Concerning the Ammonia Content. Not much account was taken of the ammonia content, either as free ammonia or as albuminoid since no great effect of the profuse plant growth could be noted upon it. Perhaps very active photosynthesis would indirectly hasten its oxidation to simpler forms due to the free oxygen produced. It seems clear from the results obtained that the ammonia acts merely as a reservoir from which nitrites and later nitrates are derived. It should also be remembered that aside from the ammonia in solution there is always the large amount in the ooze at the bottom. This would serve as a storehouse for the production of all of the compounds under discussion, and would be extremely difficult of estimation. There is no doubt that the bacterial action which results in higher oxidized forms would be more active in the ooze than in the water. It was noticed that the ammonia analyses showed great variation, and judging from the fact that large amounts were found after a rough period and smaller amounts after a calm, there seems to be no doubt that the variation is due to the water being more or less stirred up at one time than another. It was impossible from the number of analyses taken to arrive at any definite eonclusions concerning the ammonia, and sinee it acts merely as a storehomse, at all times well supplied it seems quite unessential that further attention should be given to it in this part of the paper.

Part II. The Effect of the Nitrogen Content Upon Plant Growth.
Attention is called to the map of Winona Lake (Fig. 10) in which the regions of plant growth are stippled in proportion to the degree of density. Special care was taken to get the correct proportions but in spite of this the shading must be regarded as only approximate. Several conditions contributed to the difficulty of representing on paper the amount of vegetation. The following complicating factors may be mentioned; scasonal variations; the impossibility of making aceurate quantitative measurements of plant growth per unit area; the great diversity of the growth as to species; the variations in level at which the plants were found; and finally the difficulty in judging the degree of shading required to represent a given rondition.

It is at once apparent from a study of the map that the regions of densest plant growth are contiguous to the town of Winona Lake which is situate upon and drains into the north two-thirds of the east shore of the lake.

A statement of the drainage conditions of this town will be instructive at this place. Practically every house in the town has connection to the sewer system. These pipes pass to a large number of septic tanks which in turn discharge into the lake within the limits of the line on the map. A very large tank discharges into Pocanhontas Creek and its intermittent discharge is


Fig. 10-Map showing distribution of Plant Crowth.
responsible for the great variation in the analyses of the samples taken from the station near the mouth of the creek. (Fig. 11) Relatively few tanks discharge along the shore of the island made by the canal. During the summer the town of Winona Lake has a large population, and the sewage is very
considerable in amount. The regions near the town showed a higher nitrogen content as a look at the data will show. The ease with which the albuminoid was broken up by the permanganatr-alkali solution used in the analyses strongly indicated that the greater part of the albuminoid was of animal rather than vegetable origin. The broad conclusion can hardly be escaped; the large amount of available plant food resulting from proximity to the town is largely responsible for the very abundant plant growth; i. e. the determining factor is the chemical composition, particulaly the nitrogen content of the water. Speeific statements are not so apparent.


Fig. 11.- Pocahontas Creek, the main inlet of the Lake. (Sce Table 8.)
(1) Note the extreme fluctuation during March. 'This was due to the intermiternt diseharge of a large septic tank up thr course of the ereek, and also to the alternate freezes and thaws, bus influeneing the drainage of surfare water from a momber of farm lots and pig sties above.
(2) Total nitrogen was high.

Note first that in all stations whether of dense or very dense plant growth the nitrate was the only one of the four compounds under consideration that was consistently affected. In every graph the nitrate line fell coincident with abundant plant growth. This was to be expeeced since the nitrate is the form in whieh the nitrogen is assimilated by the plant. Other forms were merely feeders of nitrogen to the nitrate form.

But note the extremely small amount of nitrate required to support a rapidly growing and later very dense vegetation as shown by the following analyses:

| No. | Station | Parts per million of |  |  |
| :---: | :---: | :---: | :---: | :---: |
|  |  | $\mathrm{NO}_{3}$ | $\mathrm{NO}_{2}$ | Sum |
| 80 | T | .025* | . 006 * | 031 |
| 122 | T* | .0275* | .006* | 0235 |
| 131 | ${ }^{T}$ | . 02 * | .0015* | 0215 |
| 86 | F | . $022^{*}$ | .03* |  |
| 119 | F | . 01 * | .011* | 021 |

Note also that the next lower form of combined nitrogen was likewise extremely low. Yet these stations contained the very densest regetation to be found in the entire lake. Analyses at different depths show that the nitrogen content was practically the same from top to bottom in these stations. Hence samples taken from near the surface were trustworthy. The following two vertical series show this clearly.

| No. | Station | Depth | Parts per million of |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: |
|  |  |  | $\mathrm{NO}_{3}$ | $\mathrm{NO}_{2}$ | Sum |
| 98 | R | Surface | . $24^{*}$ | 055* | 295 |
| 99 | R | 15M deep | . 40 * | 09* | 49 |
| 100 | R | 1.0M deep | . 40 * | 085* | . 485 |
| 101 | R | 1.5M deep | . 32 * | .075* | . 41 |
| 102 | R | 1.8M (bottom) | . $29^{*}$ | .075* | 355 |
| 119 | F | Surface | .0125* | .007* | 0195 |
| 118 | F | . 4 M deep | .01* | .011* | . 021 |
| 120 | F | .8入I (bottom) | .02* | .012* | . 032 |

From the fact that the regions of greatest plant growth are the regions of lowest content of available nitrogen it would seem that nitrogen content of itself can not be a determining factor of great importance. This rather radical statement is to be tempered somewhat in consideration of the fact that most of the increase of plant growth was made before the nitrate went so low. A look at the data will show, however, that some increase was made after July 1, when nitrate was .09* parts per million and a

[^3]great increase was made after June 26 when the nitrate was . $20^{*}$ parts per million. At both of these times, however, the nitrite was very high and no doubt nitrate was being formed very rapidly by conversion of nitrites into nitrates. In fact the data indicate such a process most definitely. In one case I fonnd (Station T; July 12, Fig. 8) a rapid increase of plants while both nitrates and nitrites were low (Nitrate .025* and Nitrite . 006 parts per million.) If this last case could be considered representative it would mean that preformed nitrate or nitrite content is not a determining factor in plant growth, but one analysis, checked though it was, is too slender a basis for so broad a statement. It does, however, serve to emphasize the probability that the nitrogen supply in so far as it is a determinant acts not as a preformed, static condition, but as a dynamic process whereby the complex molecules of albuminoid are converted to constantly simpler and higher oxidized forms available for plant assimilation.

It might he thought that plants absorb considerable nitrate from the sediment of the bottom. Sereral considerations make this improbable:
(1) Although no analyses of the sediment were made, it is unlikely it rontained a very large amount of nitrate. The analyses taken of samples rery near the hottom show exell a lower eoneentration at that plaee. Then too, diffusion would tend to remove the soluble form from the sediment.
(2) The lower part of the stem of plants like I'otamogetons and Elodea, which were most abundant has very little plysiologieal function, but serves merely as a holdfast.
(3) It is diffocult to undorstand how a plant stem of small diameter would be able to fransport a highly soluble and readily dialyzed substances through a spare filled with water in which a low tension of the same substance exists, without losing it to the surrounding medinm.
(4) In these same regions of densest growth floating plants were especially abundant (Algat and Spirodela predominating). These plants of course had no riered eonneretion with the bottom.

After eonsideration of the above facts it serms rerg unlikely that the content of the sediment has any appreceiable effere upon the plant growth except as ft affeets the composition of the water about the plants.

Summary: The following facts are indieated by the data:
(1) Tho growth of plants greatly redueres the amounts of nitrates and nitrites in regions where eurrents are inhibited, and to a less extent in all regions of the lake. This reduction bereme marked in this lake ahout the first of July.
(2) The contents of all the compounds undre consideration rose during the winter with the exception of the nitrite which fell almost to zero.
(3) Nitrate and nitrite rose very rapidly in the months of April and May and first few days in June. This was no doubt due to the bacteria getting started before the plants and so building up a surplus of these forms.
(4) In regions of extremely dense vegetation the nitrate and nitrite were reduced to an extremely low figure after the latter part of July.
(5) The nitrate supply for the growing plants need not be preformed, provided there is a supply of the more complex compounds upon which bacteria may work and so produce the simpler forms required for plant assimilation.
(6) The albuminoid and ammonia content of the water serves as a storehouse from which the simpler forms are made by oxidation processes. The sediment of the bottom serves in the same way. Stirring of the water produces great variations in the content of the albuminoid and ammonia.
(7) Plants may flourish in water containing an extremely small amount of nitrate and nitrite, provided the conditions for producting these forms are present.
(8) Sewage discharge into the lake was favorable to plant growth. It is likely that the availability of the nitrogen in this discharge was the main determining factor.

## Appendix.

The following pages contain a number of graphs which are of interest only to those who are especially interested in the subject. The data from which all of the graphs in the paper were made are included in this portion.

For the sake of brevity various arbitrary symbols were used in making the graphs and in recording the data. The following key will render them intelligible.

Condition of the Sky-
c-clear.
d-dark, cloudy.
Condition of the surface of the Lake-
m -calm, smooth, mirror.
t-ripples.
r-rough.
Temperature conditions-
h-hot.
w-warm.
n-moderate.
x -cool.
z-cold.
Amount of Vegetation-
vv-extremely dense.
v -dense.
a-abundant.
s-sparce.
o-none.

When the above symbols are enclosed in parenthesis () they indicate that the conditions represented existed just previous to the time of taking the sample. In case a nmmber is enclosed in the parenthesis it means that the represented conditions have existed for that many days.

Illustration of the use of the symbols:
Cmh(10) vy means dear, calm, hot, at the time and for the ten days just preceding, very dense vagetation.

Ctn(dxr)o means rlear, ripples, moderate temperature, at the time sample was taken and that the period preceding was dark, cool, and the lake rough; no vegetation found in that particular station.

The following arbitrary signs are used in recording the data also.

* after an analysis means it was ehereked for accuracy.
??? means that an analysis was doubtful and hence rejected.
-. - means that no attrmpt was made to determine the amount of the particular compound in question.

The nitrite for station F is very similar to that for station T (Fig. S.)
Ako as in Fig. 13, the region of greatest plant growth was the region of lowest nitraterontent.

Vegetation was rapidly inderesing during the time covered.
(1) Note the great difference in uitrite at different dates in the case of station T .
(2) In rach rase the region of greatest plant growth is the region of lowest nitraterontent.
(3) Nitate estimate in analysis 10 ) is probably an error, being too high. Analysis 109 was one of the very few of the last one hundred analyses which was uncheceked for aceorarar.
(4) The amount of vergetation increases rapidly from Junc e2s to July 26.
(5) In the following three graphs the horizontal spaces have no time significance. 'The gromping is entirely arbitrary and merely a mater of convenience.
(1) Note that altho the graph for nitrite is nearly straight, in every rase save that for July it deseribes a very much flatemed out letter M. This seems more than adededen, and considering the slight differences is surprising. Note too that an error of . Ots parts per million wond be all that was necessary to spoil the figure even in that (ase (.July 5 ).
(2) Note that the nitrites gradually aseend in relation to the nitrates.
(3) 'The peoints indieated in (1) and (2) indicate that a definite relation exists betweren the stations and that these redations remained quito constant evell whild the group relations changed as time progressed.


Fig. 12. North Biology Bay.
(Data for Station C given in Table 1; for Station F in Table 7; and for Stations U, V, and Q in Table 5.)


Fig. 13.-South Biology Bay


Fig. 14 .


Fig. 15.-South Bay (See Table 3.)


Fig. 16.
Entrance Bay-(See Table 8.)
The total nitrogen content greally increased due to the large inerease of albuminoid over winter.

TABLE 1.-(See Figs. 2 and 3.)
Samples taken from the lake body at the deepest place.
Data represented in Figures 2 and 3.

| No. | Sta. | Date | Remarks | $\mathrm{NO}_{2}$ | $\mathrm{NO}_{2}$ | Sum. | Frea <br> $\mathrm{NH}_{3}$ | $\begin{aligned} & \mathrm{Alb} \text {. } \\ & \mathrm{NH}_{3} \end{aligned}$ | $\begin{aligned} & \text { Total } \\ & \mathrm{NH}_{3} \end{aligned}$ | Total N (combined) |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| 5 | ${ }^{1}$ | 10-15 | cin(wr)o | 72 | 066 | 786 | ??? | ??? | 59 | 1.376 |
| 7 | C | 110-18 | clo | 72 | 07 | 7!) | 076 | (in6 | 752 | 1.542 |
| 2.5 | C | 3-25 | rx-some ice | 120 | 0014 | 1204 | 01 | 1.76 | 1.77 | 2.974 |
| 28 | C | $+1$ | r(t) | 1.01 | $03 \%$ | 1.976 | 034 | 1.3!6 | 143 | 2506 |
| 34 | ( | 422 | ( x r $(\mathrm{r}) \mathrm{O}$ | 2.31 | 064 | 2.56 | 10* | 987* | 2007 | 4571 |
| H | ( | 619 | 0 | 1.35) | . $10^{*}$ | 1.65 | ??? | ??? | 5.54 | . 204 |
| 17 | ( | 6-2 ${ }^{\text {¢ }}$ | $r(\mathrm{r}) \mathrm{O}$ | 1.90** | 16.5 | 2.06 .3 |  |  | 1194 | 3259 |
| 60 | ${ }^{\prime}$ | (5-2) | (mblu)o | $160{ }^{*}$ | $00^{*}$ | 1.67 |  |  |  |  |
| 62 | (' | 7-1 | (tmbil)o | $2.010^{*}$ | $0 \times 3$ | 2.08.5 |  |  |  |  |
| 73 | 1 | 7-8 | (eth 0 | $51^{\circ}$ | $09^{*}$ | (ii) |  |  |  |  |
| \$2 | 1 | 7-12 | cther 0 ) | 55* | $04^{*}$ | (i2 |  |  |  |  |
| $x$ | (' | 7-13 | cmh(11) | 15* | (14.5) | 515 |  |  |  |  |
| $8!$ | ' | 7-1.5 | cther)o | 3.)* | $17{ }^{*}$ | 12 |  |  |  | - |
| 105 | C | 7-22 | cris(3) 0 | $10^{*}$ | (19.9** | 194 | 115 | 1.60 | $174 \%$ | 2239 |
| 111 | (' | - 26 | cmhl 10 | $36{ }^{*}$ | 096; | 154; |  |  |  |  |
| 113 | C | 7-27 | cmil(2)o | ご | 072* | . 352 |  |  |  |  |
| 129 | (' | -1 | r(r)o | $2{ }^{1}$ | $16^{*}$ | 40 |  |  |  |  |
| 133 | C | 43 | crh o | $32 *$ | $102^{*}$ | . 34 |  |  |  |  |

Table 2. (See Fig. 4.)
Data from which Vertical Series Graphs were made.

| No. | Date | $\begin{aligned} & \text { Depth } \\ & \text { in Meters } \end{aligned}$ | $\mathrm{NO}_{3}$ | $\mathrm{NO}_{2}$ | Total Nitrogen |
| :---: | :---: | :---: | :---: | :---: | :---: |
| 89 | 7-15 | Surace | 35* | (1)** | ?? ${ }^{\text {? }}$ |
| 105 | 722 | surface. | 40* | .094* | 2. 239 |
| 129 | $8-1$ | surface. | 24* | 16* | 1.485 |
| 133 | $\mathrm{S}-3$ | surface. | $32^{*}$ | . 18* |  |
|  | Average | Surface. | 33 | 126 | 1.85 |
| 90 | $7-15$ | 4 | 51** | 07* | ? $?$ ? |
| 106 | 7-22 | 4 | 32* | 10.5* | 1.022 |
| 134 | $8-3$ | 4 | $28^{*}$ | 11* | 131 |
|  | Average | 4 | .37 | . 95 | 16 |
| 91 | 6-15 | 10 | 1.30* | 008 | ? ?? |
| 107 | 7-22 | 10 | 1.20* | (122* | 2.147 |
| 135 | 8-3 | 10 | 1.32* | $02 *$ | 2.16 |
|  | Average | 10 | 1.27 | 017 | 2.15 |
| 92 | 7-15 | 14.5 | 1. $00^{*}$ | 014* | 1549 |
| 104 | 7-21 | 14.5 | 1.36* | 025* | 2.015 |
|  | A verage | 14.5 | 1.18 | 0195 | 1.932 |
| 103 | 7-21 | 21 | 44 | 14* | 1. 695 |
| 128 | S-1 | 21 | 60* | $10^{*}$ | 2.105 |
|  | A verage | 21 | $53 *$ | 12 | $184!$ |

TABLE 3.
Samples taken from South Bay.
Data represented in Figures 14 and 15.

| No. | Sta. | Date | Remarks | $\mathrm{OO}_{3}$ | NO. | sum. | Free $\mathrm{NH}_{3}$ | $\begin{aligned} & \mathrm{Alb}_{3} \\ & \mathrm{NH}_{3} \end{aligned}$ | Total $\mathrm{NH}_{3}$ | Total N (combined) |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| 48 | S3 | 6-23 | dnm(3) ${ }_{0}$ | 1.80* | 08* | 1.88 | 036 | 549 | 584 | 2.464 |
| 52 | S1 | 6-27 | emw (3) a | 1.80* | $06{ }^{*}$ | 1. $86{ }^{*}$ |  |  |  |  |
| 53 | S2 | 6-27 | cmw (3)a | 2.80* | $05^{*}$ | 2.85 |  |  |  |  |
| $5 \cdot$ | S 3 | $6-27$ | cmw(3)0 | $2.30{ }^{*}$ | (8* | 2.38 |  |  |  |  |
| 55 | S4 | 6-27 | cinw(3)s | 1.40* | $10^{*}$ | 1.50 |  |  |  |  |
| 56 | $N 5$ | $6-27$ | $\operatorname{cmw}(3): \downarrow$ | 1.60** | $10^{*}$ | 170 |  |  |  |  |
| 67 | S1 | 7-5 | cmh(3) a | . 51 * | $029{ }^{4}$ | 53:! |  |  |  |  |
| 6is | S 2 | 7-5 | $\operatorname{mmb}(3) \times$ | . $40{ }^{*}$ | $0^{1013}{ }^{\text {a }}$ | 41:3 |  |  |  |  |
| 6!) | 43 | 75 | cmh(3) 0 | . 56 * | $0.51 *$ | (il) 1 |  |  |  |  |
| 70 | S4 | 7-5 | (mh(3)s | .355* | 0.51 * | 106 |  |  |  |  |
| 71 | 85 | 7-5 | (mbh(3): | . 56 * | $01.5 *$ | 1005 |  |  |  |  |
| 72 | S3 | 7-6 | cmintlo | . $55 *$ | 15:5* | 705 | 10 | 298 | 398 | 1103 |
| 74 | S1 | 7-11 | cth(m9) a | 40* | (0.)* | 4.5 |  |  |  |  |
| 75 | N2 | 7-11 | cth(m) ${ }^{\text {a }}$ | 16** | 06 s * | 524 |  |  |  |  |
| 76 | S3 | 7-11 | (th(m!) 0 | 15* | 116.3 * | 513 |  |  |  |  |
| 77 | S4 | 7-11 | cth(m! ${ }^{\text {a }}$ s | 58* | $075{ }^{*}$ | (i.) 5 |  |  |  |  |
| 78 | K5 | 7-11 | cth(m! ${ }^{\text {a }}$ | . $8.1{ }^{*}$ | $10 i^{*}$ | is |  |  |  |  |
| 93 | 81 | 7-1s | cth 1 la | $2 \cdot 1 *$ | $1176{ }^{*}$ | 316 |  |  |  |  |
| 94 | S2 | 7-15 | chlda | .32** | 09\%* | 11 |  |  |  |  |
| 95 | S3 | 715 | cthe 110 | $31^{*}$ | 0 S 5 | 125 |  |  |  |  |
| 96 | St | 7 18 | cth(1) s | $2 \mathrm{~S}^{*}$ | 0991* | 371 |  |  |  |  |
| 97 | 85 | 7-18 | cth(4):a | . $36{ }^{*}$ | .091** | 451 |  |  |  |  |
| 123 | S1 | 7-29 | $\operatorname{cmh}(1){ }^{\text {a }}$ | . 36 * | 111* | 50.1 |  |  |  |  |
| 124 | S2 | 7-29 | $\operatorname{cmb}(4) a$ | $20{ }^{*}$ | $16^{*}$ | $36{ }^{*}$ |  |  |  |  |
| 125 | S3 | 7-29) | $\operatorname{mmh}(1) 0$ | . $32 *$ | $1 \mathrm{i}^{*}$ | 18 |  |  |  |  |
| 126 | St | 7-29 | $\operatorname{cmh}(4) \mathrm{s}$ | .32* | 20* | .52 |  |  |  |  |
| 127 | 85 | 7-29 | $\operatorname{cmh}(1) a$ | 32* | $152^{*}$ | 472 |  |  |  |  |

TABLE 4.-(See Figures 6, 78 and 13.)
Samples taken in South Biology Bay.

| No. | Sta. | Date | Remarks | $\mathrm{NO}_{3}$ | $\mathrm{NO}_{2}$ | Sum. | Free $\mathrm{NH}_{3}$ | $\begin{aligned} & \mathrm{Alb.} \\ & \mathrm{NH}_{3} \end{aligned}$ | $\begin{aligned} & \text { Total } \\ & \mathrm{NH}_{3} \end{aligned}$ | Total N (comnined) |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| 3 | B | 10-13 | $\mathrm{cm}(\mathrm{r}) \mathrm{s}$ | . 56 | . 034 | . 594 | 18 | 8 | 86 | 1. 454 |
| 4 | B | 10-14 | $\mathrm{r}(\mathrm{cm}) \mathrm{s}$ | 64 | . 036 | . 676 |  |  |  |  |
| 9 | B | 10-21 | $\mathrm{m}(\mathrm{m}) \mathrm{s}$ | . 72 | 04 | 76 | 09 | 712 | 802 | 1.562 |
| 23 | B | 3-20 | ice on lake body | 80 | 024 | . 824 | 05 | 65 | 70 | 1.524 |
| 36 | B | 4-15 | cr(r)o | 1.44 | 05 | 1.49 | 04 | . 585 | 625 | 2.115 |
| 45 | P | 6-20 | cm a | 75 | 05 | . 80 | 068 | . 97 | 1.038 | 1.838 |
| 57 | B | 6-28 | cmh(3)s | 1.10* | 144* | 1.244 |  |  |  |  |
| 58 | T | 6-28 | $\operatorname{cmh}(3)_{V}$ | .15* | . 40 * | . 55 |  |  |  |  |
| 59 | R | 6-28 | cmh(3)a | 1.70* | 18* | 1.88 |  |  |  |  |
| 79 | B | 7-12 | $\mathrm{cmh}(10) \mathrm{s}$ | .22* | $08^{*}$ | . 30 |  |  |  |  |
| 80 | T | 7-12 | cmh(10) vv | .025* | $06^{*}$ | . 031 |  |  |  |  |
| 81 | R | 7-12 | cmh(10)a | . 30 * | 055* | . 355 |  |  |  |  |
| 98 | R | 7-19 | cmh(6)a | $24^{*}$ | 055* | 295 |  |  |  |  |
| 108 | B | 7-26 | cmh(1)s | $46^{*}$ | .094* | . 554 |  |  |  |  |
| 109 | T | 7-26 | $\operatorname{cmh}(1) \mathrm{vv}$ | 35 (?) | .004* | 354 |  |  |  |  |
| 110 | R | 7-26 | $(\mathrm{mh}(1) \mathrm{a}$ | 36* | . $10^{*}$ | . 46 |  |  |  |  |
| 122 | T | 7-28 | cmh(3)vv | 0275* | $006 *$ | 0235 |  |  |  |  |
| 130 | B | 8-3 | chr $s$ | $24^{*}$ | $20^{*}$ | 44 |  |  |  |  |
| 131 | T | 8-3 | chr vv | 02* | .0015* | 0215 |  |  |  |  |
| 132 | R | 8-3 | chr a | $32^{*}$ | . 18 * | . 50 |  |  |  |  |

Table 5.-(Sce Fig. 12.)
Samples taken in North Biology Bay

| No. | Sta. | Datc | Remarks | $\mathrm{NO}_{3}$ | $\mathrm{NO}_{2}$ | Sum. |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| 63 | U | 7-1 | $\operatorname{cmh}(1) \mathrm{s}$ | 1.30* | ??? | ??? |
| 65 | V | 7-1 | $\operatorname{cmh}(1) \mathrm{v}$ | . $70^{*}$ | . $10^{*}$ | 80 |
| 66 | Q | 7-1 | $\operatorname{cmh}(1) \mathrm{a}$ | 175* | . 075 | 250 |
| 85 | U | 7-3 | $\mathrm{cmh}(11) \mathrm{s}$ | 50* | . 07 | 57 |
| 87 | V | 7-13 | $\operatorname{cmh}(11) \mathrm{V}$ | 055* | 056* | 111 |
| 88 | Q | 7-13 | cmh(11)a | 45* | . 034* | . 484 |
| 114 | $\mathbf{U}^{+}$ | 7-27 | $\operatorname{cmh}(2) s$ | 32* | . $09 *$ | 41 |
| 116 | V | 7-27 | $\operatorname{cmh}(2) \mathrm{V}$ | .18* | 65* | 245 |
| 117 | Q | 7-27 | $\operatorname{cmh}(2) \mathrm{a}$ | $28^{*}$ | 06* | . 34 |

TABLE 6.-(See Fig. 16.)
Samples taken in Entrance Bay.

| No. | Sta. | Date | Remarks | $\mathrm{NO}_{3}$ | $\mathrm{NO}_{2}$ | Sum. | Free $\mathrm{NH}_{3}$ | $\begin{aligned} & \mathrm{Alb} . \\ & \mathrm{NH}_{3} \end{aligned}$ | $\begin{aligned} & \text { Total } \\ & \mathrm{NH}_{3} \end{aligned}$ | Total N (Combined) |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| 1 | A | 10-19 | ems | 1.12 | 172 | 1292 | 64 | 102 | 1.66 | 2.952 |
| 2 | A | 10-12 | $\mathrm{cm}(\mathrm{r}) \mathrm{s}$ | 88 | 108 | 988 | 48 | 78 | 1.26 | 2.268 |
| 6 | A | 10-16 | em(r)s | 96 | 072 | 1032 | 104 | 584 | 688 | 1.73 |
| 14 | H | 11-2 | xr (cmw) s | 144 | 048 | 11.88 | 052 | 68 | 732 | 2.22 |
| 30 | A | 4-10 | rp | 1.00 | . 016 | 1016 | 09 | 314 | 323 | 4246 |
| 37 | A | 4-21 | dxfo | 2.00 | . 06 | 2.06 | 86 | 2.60 | 2.686 | 4.746 |

TABLE 7.-(See Figure 9.)
Samples taken from the Canal between 11 th and 12 th Streets.

| 10 | F | 10-22 | cmw (7) vv | 0.5 | 016 | 046 | 0.5 | 994 | 1.041 | 111 |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| 11 | G | 10-23 | (mw(S)Vv | 09 | 02: | 11:3 | 106 | 70 | . 806 | . 918 |
| 12 | ( 1 | 10-25 | cmw(10) wv | 0s | 013 | 093 | 1096 | $7 \mathrm{S2}$ | 578 | 971 |
| 13 | F | 10-2s | comw(13)wv | 0 S | . 016 | 96 | 0.2 | 1. 024 | 1075 | 1.172 |
| 26 | M | 3-27 | $\mathbf{r}(\mathbf{r})_{0}$ | 1.20 | 036 | 1236 | 17 | 794 | 1.261 | 251 |
| 32 | V' | $+17$ | $\mathbf{r}(\mathrm{r}) 0$ | $3.15 *$ | 0.5 | 353 | ??? | ??? | 219 | 572 |
| 31 | (i) | 1-19 | cmo | 160 | 0.96 | 16.56 | ??? | ??? | . 95 | 2.1606 |
| 3.5 | F | 1-20 | c 0 | 1.36 | 06 | 142 | ??* | ??? | 1.106 | 2526 |
| 41 | Gi | 1-25 | dx $(1 \times x) 0$ | ??? | ??" | ??? | 16 | 492 | .66 |  |
| 49 | (i) | (i-24 | wm(3) s | $80^{*}$ | $10^{* *}$ | 180 | (0) | 1.432 | 1482 | 3282 |
| 50 | (i) | (i-26 | cm a | $20^{*}$ | 4! ${ }^{*}$ | 69) | 51 | . 518 | 102 S | 1.718 |
| 64 | 1 | 7-1 | cmh(1)v | 09* | $254^{-7}$ | 3.17 |  |  |  |  |
| 86 | F | 7-13 | (mhltily | 012* | $03^{*}$ | 05 |  |  |  |  |
| 119 | F | 7-24 | cmh(3)vv | 01* | $011 *$ | 021 |  | ... . |  |  |

TABLE 8.-(Sce Figure 11.)
Samples taken from Porahontas Creek. (Main Inlet.)


Compare with sample taken at mouth of Sugar C'reek.

| 42 | 0 | $4-26$ | $\mathrm{dx}(\mathrm{dx}) 0$ | .48 | 03 | .51 | .004 | .40 | .404 | .914 |
| :--- | :--- | :--- | :--- | :--- | :--- | :--- | :--- | :--- | :--- | :--- |


[^0]:    *In all of the graphs showing a time element the horizontal spaces represent days unless the graph is interrupted. The date and the number of the analysis are shown just below the base line. The amounts of the different compounds are represented by the vertical spaces. These values are uniform throughout. In the cases of nitrate, total ammonia and total nitrogen .1 part of nitrogen per million or .1 milligram of nitrogen per liter is taken as the unit. In case of nitrite, however, . 01 parts of nitrogen per million is taken as the unit. This was done in order to throw the nitrite grapli within comparable distance to the other graphs. This change in scale must be kept in mind.

[^1]:    *Birge and Juday, Wisconsin Geological and Natural History Survey, 1911.

[^2]:    For comparative purposes an extremely dense station ( T ) is set beside a moderately dense station (station R), It is quite likely that the analysis for Station T taken July 26 is too high. The analysis was not elieeked, whieh is unfortunate, Compare station $T$ with Canal station in Fig. 9 . Compare station $R$ with Fig. 6 and 7
    The curves for Station $T$ and $R$ are very typieal for thokind of regions they represent. Note that in the less dense region the nitrates and nitrites do not fall so low and that there is a distinet rise in the nitrite after the period of maximum vegetative growth; i.e. when the plants were ehiefly engaged in producing flowers and fruit.

[^3]:    *After an analysis indicates that the analysis was carefully checked.
    8432-23

