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MEASUREMENT OF THE REDUCING ABILITY OF NATURAL WATERS AND SEDIMENTS: A SIMPLE LIMNOLOGICAL METHOD

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The first redox measurements were carried out in limnology by KARSINKIN and KUZNETSOV (1932), and eversince this first attempt redox potential came into the foreground of interest (KUZNETSOV, 1935; PEARSAL and MORTIMER, 1939; HUTCHINSON et al. 1939; DEEVEY, 1941; ALLGEIER et al. 1941). The classical work of MORTIMER (1941—1942) once again called the attention of researchers to the redox processes (ZOBELL, 1946; HAYES et al. 1958; GORHAM, 1958) instead of pondering upon certain material migrations occurring on the borderline of mud and water, and to some relationships existing between the former and certain redox changes.

At this time, it has been pointed out by HAYES and his collaborators (1958) that in carrying out redox measurements unexpected difficulties may arise, quite recently STUMM (1967) passed severe criticism as to the practicability of directly measured redox potential in natural, mixed systems. Contrary to this, at the same time, BORCHARD (1967) and WAGNER (1967) state that the directly measured redox potential within a natural, mixed system yields valuable and practicable information. RABOTNAVA (1957) gives a very detailed analysis on the properties of the redox potential of biological objects, and establishes that it differs in many respects from the properties of the conception used in chemical sciences. WHITFIELD (1969) considers the problems arising in connection with redox measurements from the point of view of a limnologist, and in order to describe the distribution of reduced and oxidized sediments with good efficiency he uses the following "operational parameter" E_h.

Consequently, it is reasonable and important at the same time to know and carry out measurements as regards to the momentary redox state of waters and sediments from the point of view of limnology. This is supported by the ever increasing number of measurements carried out far and wide (DRABKOVA, 1966; ROMANENKO, 1966; MIHAYLENKO, 1967; PATRICK and TURNER, 1968; KJENSMO, 1968; WHITFIELD, 1969).

However, besides measuring the momentary redox state, we think that to measure the intensity of redox possesses occurring in natural waters and sediments are also deserve attention. In order to study the redox processes occurring in the Hungarian lakes with a great stretch of water and in shallow water the measurement of the reducing ability of water and mud is especially suitable, where owing to continuous atmospheric oxygen supply the momentary redox measurements are insufficient to survey the quantitative relationships of the process. We have found no reference in the literature as to the measurement and to the potential reduction ability of natural waters and sediments, thus, its order of magnitude may only be calculated from the degree of oxygen consumption. Accordingly, we have elaborated for a direct measurement a simple new method, which is based on the redox changes of a closed system containing natural substrate.

Description of method

The oxygen has a very important role in establishing the redox state of natural waters and sediments as it generally has in the redox processes. The reducing ability of the examined water and sediment was measured during longer or shorter periods of incubation time depending on the sample by excluding the atmospheric oxygen supply and terminating the process of photosyntesis.

The graduated vessel (*Fig. 1*), a 250 ml glass container of 6.5 cm diametre fitted with a ground glass or rubber stopper. The measuring and reference electrodes built into the stopper were immersed throughout the analysis into the liquid to be measured. By completely excluding oxygen diffusion we could not solve the problem of coupling the reference electrode's agar bridged or its simplified variety in the measuring space (Kovács and Matkovics, 1954). We incubated our samples at 25 °C, at the dark parallels the measuring vessel has been covered with aluminium sheet or with thick black paper. The light parallel has been illuminated by 5000 Lux. The measuring vessel was filled excluding all bubbles and the samples were saturated with oxygen by bubbling through them air at 25 °C before filling up.

Throughout our investigations we used electrodes manufactured by Radelkis (Electrochemical Instruments, Budapest). The measuring electrode is a smooth platinum sheet with a surface area of 2×0.5 mm^2. It has been washed before application in cronic-sulphuric acid many times, followed by a careful rinsing in distilled water. The measuring electrodes were calibrate by ZoBell's solution (1964). Our reference electrode was a saturated calomel electrode. The measurements were carried out in a Beckman GS-type pH

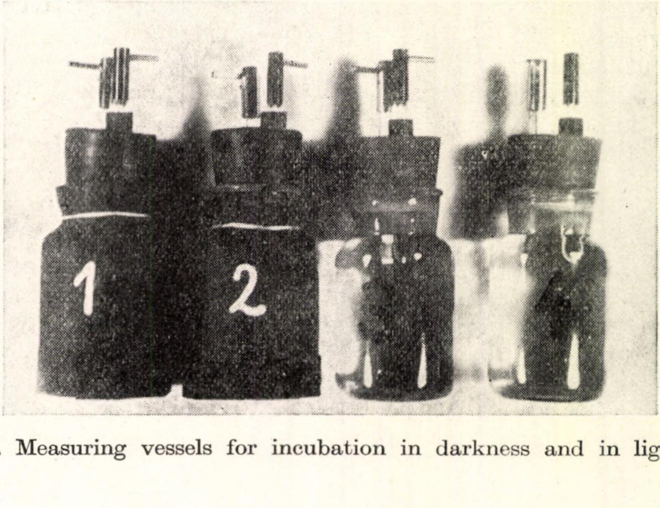

Fig. 1. Measuring vessels for incubation in darkness and in light

measurer and to the values thus obtained we added 250 mV and so the final values were given in E_h. According to the purpose of the analysis we carried out 1—6 measurements a day. When the measurement closely followed the setting up of the investigation quite frequently the significant drift greatly hindered read off, which, however, after a period of 120 min, but mostly after 20 min, ceased.

Working with closed systems the repeated measurements could be reproduced with an exactness of ± 10 mV.

Our investigations were carried out with sample waters and sediments from Lake Balaton and from the Inner Lake of Tihany. The water samples were taken by the help of FRANCEV's sampler (KUZNETSOV, 1962) from a depth of 50 cm disregarding vertical sampling, while the mud samples were taken partly by using the Ekman-Birge dredge, and partly by a mud borer. To determine the reducing ability of the sediment we placed some 50 g moist mud into the measuring vessel then at 25 °C oxygen-saturated lake water was layered on it. Then by differential filtration using Soviet and Oxoid filters (pore size: 100 μ, 6 μ, 0.5 μ) we were able measure separately the role of zoo-, phyto- and bacterioplanktons in the reducing ability of the lake.

The oxygen concentration during the investigation was determined by the Winkler method. The saprophytic microorganisms were counted on the sodium-caseinate agar for this medium proved to the most efficient in the case of water from Lake Balaton (OLÁH and VÁSÁRHELYI, 1970). The total quantity of microbial plankton was determined by RAMUZOV's direct method (1932) and likewise was the quantity of phytoplankton determined by the help of a membrane filter.

When the lake water was incubated in light the redox potential within the measuring vessel remained unchanged for quite a long period of time, or it increased *(Fig. 2)*. Even during a 42-day incubation, this was the longest, no decrease in redox potential was observed. Long lasting incubations generally brought about an increase in the redox potential of the sample, especially when the water sample was taken near the substrate. When the same samples were incubated in darkness — i.e. excluding photosynthesis — depending on the origin of the sample, after a longer or shorter period of time the redox potential decreased. This phenomenon may be called the darkness-induced reducing ability of the sample. The main characteristics of the curve obtained during incubation in darkness *(Fig. 2)*: time requirement until the decrease in redox potential (1); length of duration of the decrease itself (2); and the redox potential value characteristic for the state of equilibrium (3).

By excluding the continuous oxygen supply in the measuring vessel gradually results in the total consumption of oxygen *(Fig. 3)* which in turn set off further processes causing an even more pronounced decrease in redox potential set in (1), consequently depends primarily on the intensity of oxygen consuming processes. The significant decrease in redox potential coincides with the complete disappearance of oxygen (HUTCHINSON, 1957), thus, we obtain data as to the rate of oxygen consumption by observing the darkness-induced reducing ability, and furthermore, we may obtain information on the biochemical oxygen demand (BOD) of natural waters and sediments. By knowing the oxygen content of an oxygen saturated water at a given temperature and the volume of the measuring vessel we can calculate the quantity of the oxygen used up during a given period of time.

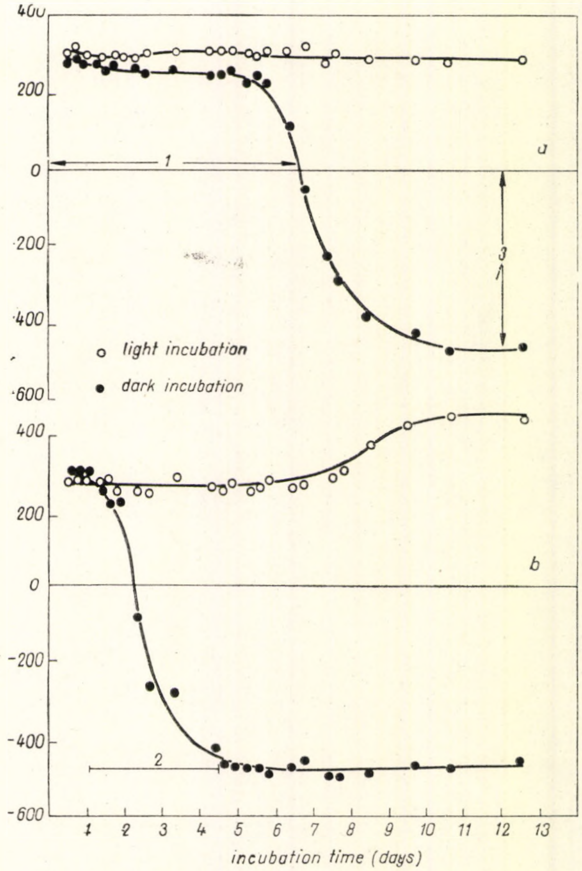

Fig. 2. Changes in redox potential during incubation in darkness and in light (a) surface water, (b) bottom water from the Inner Lake of Tihany

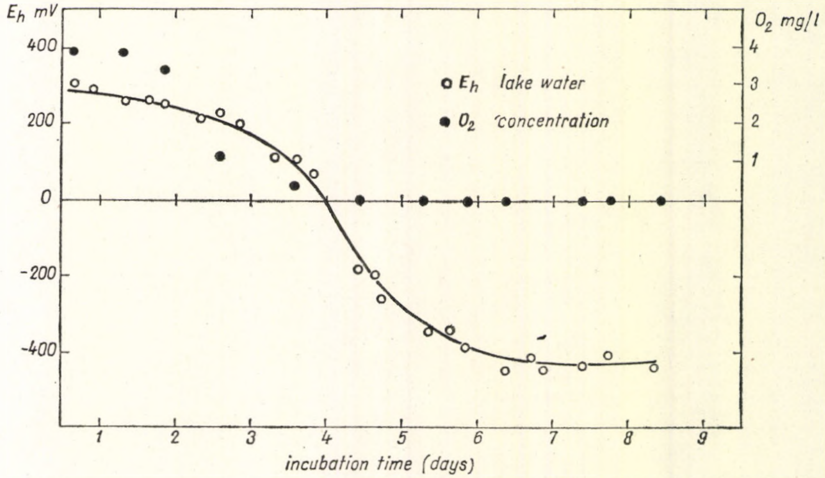

Fig. 3. Relationship between redox potential and oxygen concentration in the water of the Inner Lake of Tihany

The redox potential, however, independently from oxygen concentration, sufferes further changes under the influence of several other factors *(Fig. 3)*. From this fact it follows that by simply measuring the concentration of oxygen we do not get a picture true to reality as regards actual redox state. In the oxygen-free period according to the state of equilibrium the redox potential (3 at any rate depends on the special biological and chemical composition of the sample. The length of the decreasing part (2) on the curve, on the other hand, depends additionally on the change in oxygen concentration and on the chemical and biological composition of the sample.

Some typical experiments with the method and the interpretation of results

On the 23rd May, 1969 we measured the reducing ability in the water full of reed fragments in a reedery in Lake Balaton in front of our Research Institute. A decrease was observed in the redox potential in the lotic zone the open water after the 8th day, while this decrease occurred in the littoral, lenitic zone separated from the open water already on the 2nd day. Here, the

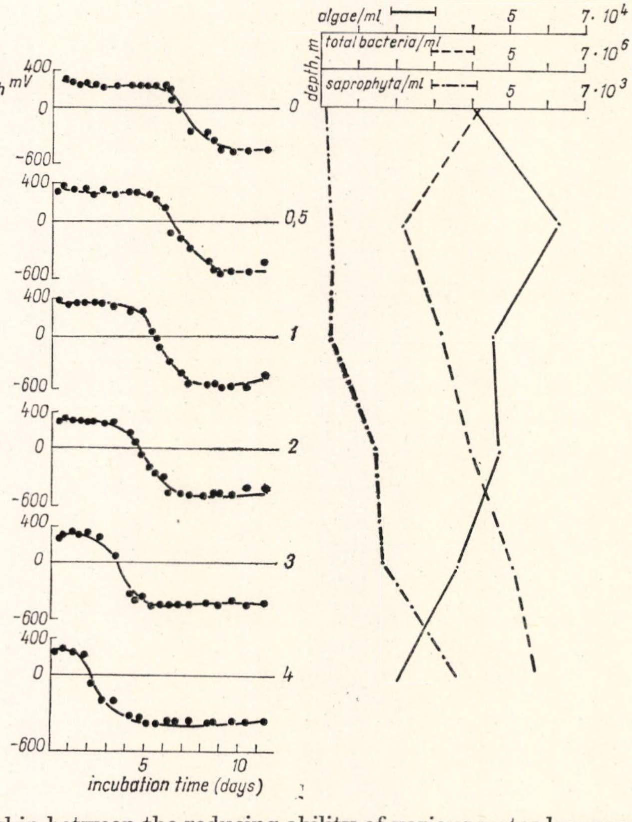

Fig. 4. Relationship between the reducing ability of various water layers and the vertical distribution of plankton in the Inner Lake of Tihany

value for the state of equilibrium was 300 mV, while for the lotic zone this value was only 450 mV. We found no significant difference in the reducing ability between the vertical samples originating from within the reedery and from the open water. A decrease in the redox potential was observed in the reedery on the 6th day, while in the open water on the 18th day.

On the 12th June, 1969, the reducing ability of the water in the strongly eutrophic Inner Lake of Tihany significantly differed verically, too *(Fig. 4)*. Redox potential decrease was measured in the bottom water on the 3rd day, while in the surface water on the 8th day. The time required for the decrease in redox potential from the surface water proceeding downward is gradually shortening by 1 day per metre. Parallel with this, the total quantity of microbial plankton gradually increases towards the bottom from $2 \cdot 10^6$/ml to $6 \cdot 10^6$/ml. The number of saprophytic organisms increased with a similar tendency from 200/ml to 3800/ml. On the contrary, the quantity of phytoplankton decreased toward the bottom. In the surface water the reducing ability is well-night the same down to a depth of some 50 cm, it is probable that the sudden decrease in the microbial plankton at a depth of 50 cm is compensatedby the maximum number of phytoplankton existing here.

The darkness-induced reducin ability of the water from the Inner Lake of Tihany displayed marked changes according to seasons. The biggest was (4 days) on the 30th July and 3rd September, while the smallest was (9 days) on the 4th December (see *Fig. 5)*.

The vertical examination of the Inner Lake of Tihany proves that the reducing ability of water and sediment is primarily determined by the quantity and quality of living organisms. By differential filtering the effects of individual

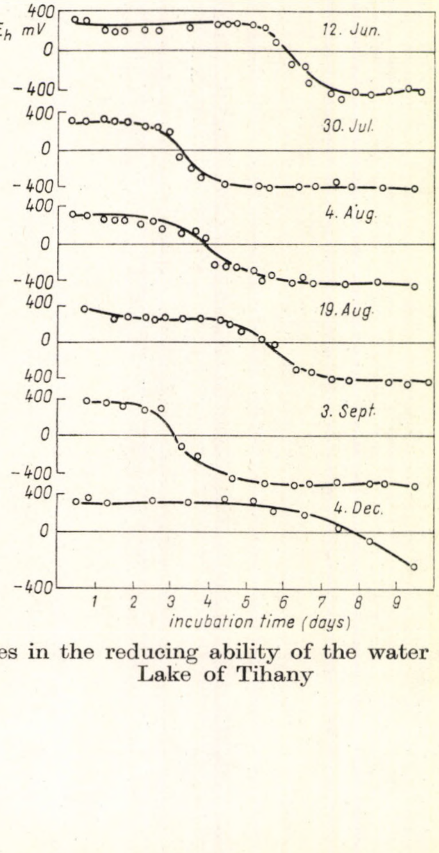

Fig. 5. Seasonal changes in the reducing ability of the water deriving from the Inner Lake of Tihany

components may well be separated. Significant difference was observed between the reducing ability of the filtered and unfiltered water of the Inner Lake of Tihany and that of Lake Balaton. The 6 μ filtering *(Fig. 6)* of the water of Lake Balaton shifted the decrease of the redox potential by a day and a half, and the so obtained state of equilibrium hardly showed any difference from

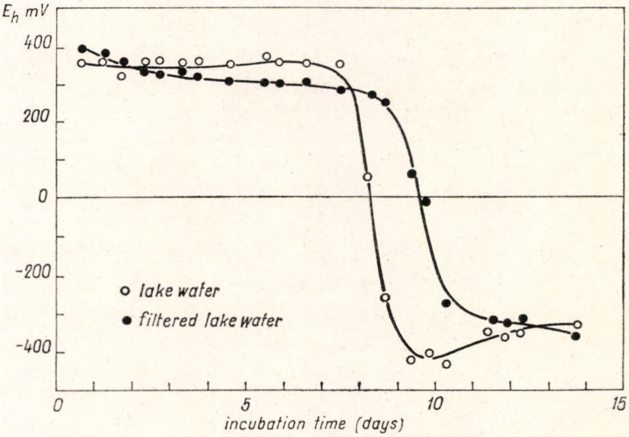

Fig. 6. Reducing ability of filtered and unfiltered water of Lake Balaton

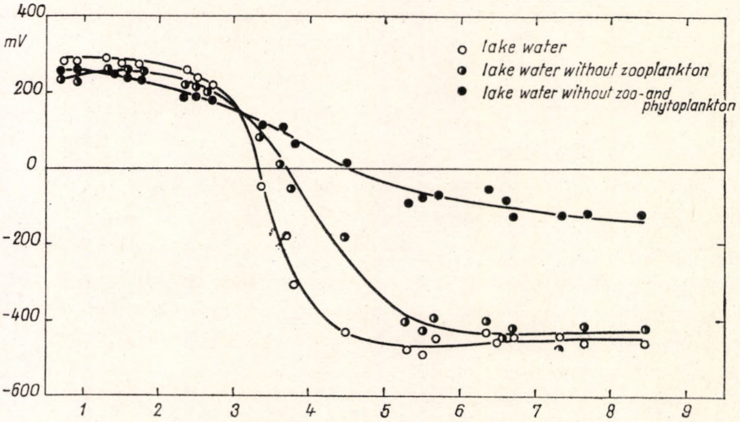

Fig. 7. Reducing ability of filtered and unfiltered water of the Inner Lake of Tihany

that of the unfiltered water. Examining the same in the Inner Lake of Tihany on the 30th July, 1969, the 6 μ filtering *(Fig. 7)* shidted the decrease of the redox potential by three days, on the other hand, the so acquired state of equilibrium was 300 mV more positive than that of the unfiltered water. The separation of zooplankton shifted the decrease of redox potential only by on day, and the so gained state of equilibrium hardly differed from the previous state. Consequently, in the case of the Inner Lake of Tihany the phytoplankton play an important role in the formation of redox potential corresponding to the oxygenfree equilibrial state.

On the 16th April, 1969, we measured the reducing ability of sediments coming from different depths from the reedery of Lake Balaton in front of our Research Institute. The best reducing ability was displayed by the upper, active layer containing the highest number of bacteria. The decrease in redox potential occurred already two days after incubation. A longer period of time is needed for the decrease in redox potential in deeper lying layers. This, however, does not increase parallel with depth. For example, the decrease requires three days in a layer lying at 12—15 cm deep, while this decrease is attained

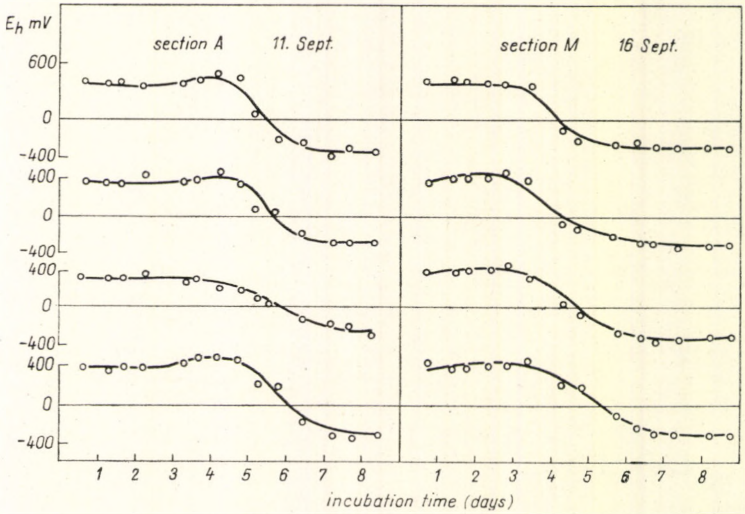

Fig. 8. Reducing ability of mud examined at 4 sites each of sections "A" and "M" (Lake Balaton)

only after six days at a depth of 9—12 cm. This indicates, that the reducing ability of the sediment in a reedery has a definite stratification. The reducing ability of the various layers increases parallel with the quantity of the particulate organic material. Thus, layers displaying a very strong reducing ability are at the same time the accumulational zones of reed detritus.

The reducing ability of the sediment is dependent to a great extent on the origin and age of the detritus. The reducing ability of the mature detritus (Rodina, 1964) of a sediment in a reedery, is greater than that of open-water sediment; at the same time, the reducing ability of young detritus originating from a reedery (Rodina, 1964) is smaller still than that of open-water sediment.

We analyzed the reducing ability of 5 standard sections of Lake Balaton (Tamás, 1967) on the 11th April, 1969 but found no great divergencies. A decrease in redox potential occurred the quickest in the sediment deriving from section M (Keszthely-Bay). On the 11th September, 1969, we took 4 samples each from sections M and A and measured the darkness-induced reducing ability of the sediment *(Fig. 8)*. The similarity in the measured reducing ability of sites lying closely to one another within one profile confirm the exactness of the method in investigating sediments. The time requirement for the decrease of redox potential in section M was 5 days, while the same for section A was 7 days. Which prove the great reducing abilit yof the sediment deriving from Keszthely-Bay (section M).

The darkness-induced reducing ability of natural waters and sediments may be used in studying the processes of mineralization and by their help we are able to measure the influence of various materials exerted on redox potential. Under aerobe condition the mineralization of organic materials is proportional with oxygen consumption. From the quantity of consumed oxygen, and from the rate of consumption we may conclude as to the intensity of mineralization. On the other hand, under anaerobe condition the mineralization products exerting effect on the redox potential is dominant.

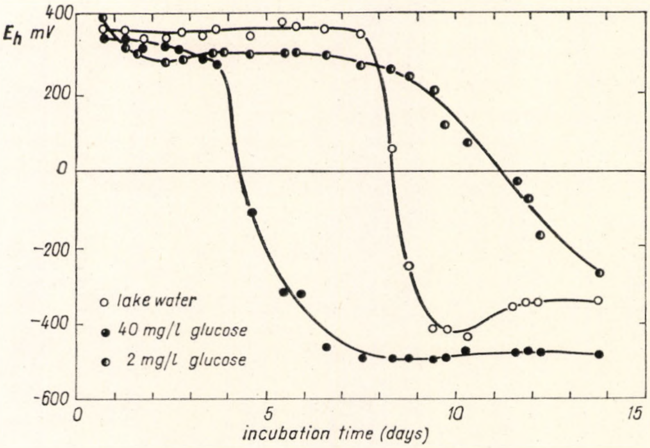

Fig. 9. Influence of glucose on the reducing ability of water deriving from Lake Balaton

When giving to the water of Lake Balaton a 40 mg/l end-concentration glucose solution *(Fig. 9)*, we find that the length of time required for a decrease in redox potential is 4 days shorter. Using a 250 ml measuring flask at 25 °C with a 100% saturation the consumation time of 2 mg oxygen decreases to its half. Carrying out the same experiment with the water of the Inner Lake of Tihany, whose time requirement for a decrease in redox potential is shorter than that of Lake Balaton, under similar conditions with a 40 mg/l end-concentration glucose solution the length of time required for a decrease in redox potential is only one day shorter. Thus, by adding glucose the required time for a decrease in redox potential for the water of Lake Balaton and the Inner Lake of Tihany becomes balanced. On the effect of glucose the redox potential corresponding to the equilibrial state both in the cases of Lake Balaton and in the Inner Lake of Tihany, decreases by nearly 100 mV. On the 19th August, 1969 the lake water of the Inner Lake of Tihany free of phyto- and zooplankton the time required for a decrease in redox potential lengthened by 2 days to the unfiltered water. Comparing it to the one measured on the 30th July the redox potential corresponding to the state of equilibrium it became more positive only by 50 mV. This clearly indicates that even within a season in a lake the role of individual components change in the formation of the potential reducing ability. When we added to the filtered lake water of the Inner Lake of Tihany a 40 mg/l end-concentration glucose solution we obtained a result very similar to that of the unfiltered water. Consequently, at the time of examination the role of bacteria was decisive in the process.

It was interesting to note, that giving to the water of Lake Balaton and to the Inner Lake of Tihany a 2 mg/l end-concentration glucose solution caused a 4 and 1 day shift in the decrease of redox potential. When the same concen-

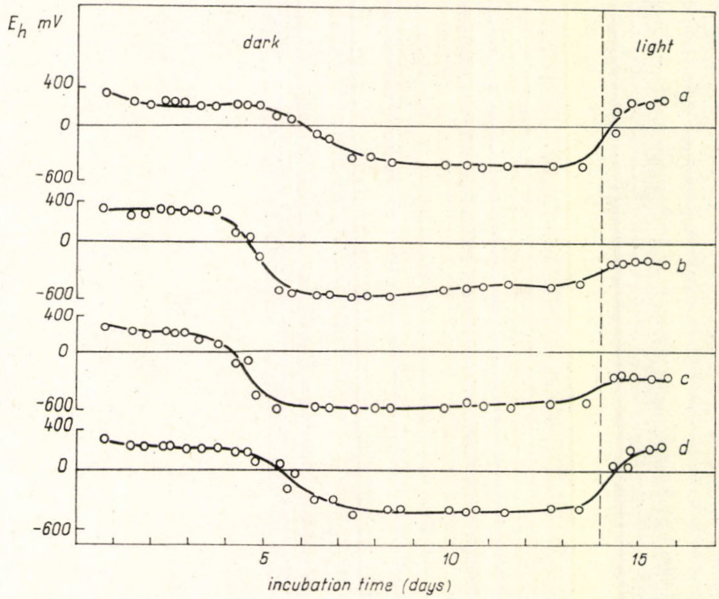

Fig. 10. Influence of glucose and darkness, then of light incubation on the reducing ability of water deriving from the Inner Lake of Tihany.
(a) lake water + 2 mg/l glucose; (b) lake water + 20 mg/l glucose; (c) lake water + 40 mg/l glucose; (d) lake water

tration was given to the water filtered through a 6 μ filter deriving from the Inner Lake of Tihany the length of time required to cause a decrease in redox potential, doubled. Comparing the redox potential corresponding to the state of equi ibrium to the control a 200 mV higher positivity was measured, while the same compared to water to which a 40 mg/l end-concentration glucose solution was added this value reached 400 mV. So far we have no explanation to the phenomena accompanying the addition of glucose with low concentration.

If the sample gaining the equilibrial state characteristic for oxygen-free condition, is placed in light *(Fig. 10)* its redox potential attains a value of the corresponding initial state. In the case of samples filtered through 6 μ sieve — without phytoplankton — reoxidation, naturally, cannot be effected. It is interesting, that after adding a 20 and a 40 mg/l end-concentration glucose solution disregarding a slight increase in redox potential the sample is not reoxidized. On the other hand, using a low concentration of glucose solution the process of reoxidation passes freely.

Summary

In order to measure the potential reducing ability of natural waters and sediments we employed a simple, direct method, which is based on the changes in redox potential of closed systems containing natural substrate. The measuring vessel is a 250 ml graduated glass container fitted with a ground glass stopper or made of rubber into which reference and measuring electrodes are built. By discontinuing the atmospheric oxygen supply, and by excluding photosynthesis because of incubation in darkness, the redox potential decreases in the measuring vessel containing natural substrate. Information received during measurement: 1. time requirement for redox potential decrease; 2. length of decrease; 3. redox potential value characterizing the state of equilibrium.

Some typical experiments exemplifying the applicability of the method brought the following results:

1. The supertrophic water of the Inner Lake of Tihany displays a greater reducing ability than that of Lake Balaton, and further, this reducing ability shows significant changes as regards seasons.
2. In the reedery of Lake Balaton the reducing ability of the water in the lotic zone is smaller than in the lenitic zone, and in both cases the reducing ability is higher than that of the open water.
3. The reducing ability of the water deriving from Lake Balaton does not change vertically, at the same time, in the Inner Lake of Tihany the reducing ability towards the bottom increases. The growth showed a direct relation to the quantitative distribution of the total and saprophytic microbial plankton.
4. The influence of bacterio-, phyto- and zooplankton on the reducing ability of the water of Lake Balaton and the Inner Lake of Tihany is different.
5. The sediment in the reedery of Lake Balaton with regard to reducing ability shows a stratification. The highest reducing ability is displayed by the upper, active layer, which contains the highest percentage of bacteria.
6. The reducing ability of the young detritus is smaller than that of the mature detritus.
7. The reducing ability of the sediment in section M in Lake Balaton is greater than in section A.
8. By measuring reducing ability we may also obtain data as to the intensity of mineralizational processes.
9. If the sample gaining the equilibrial state characteristic for oxygen-free condition, is placed in light, its redox potential attains a value of the corresponding initial state.

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TERMÉSZETES VIZEK ÉS ÜLEDÉKEK REDUKÁLÓ KÉPESSÉGÉNEK MÉRÉSE: EGY EGYSZERŰ LIMNOLÓGIAI MÓDSZER

Oláh János

Összefoglalás

A természetes vizek és üledékek potenciális redukáló képességének közvetlen mérésére egy új, egyszerű módszert alkalmaztunk, amely a természetes szubsztrátumot tartalmazó zárt rendszer változásain alapszik. A mérőedény egy 250 ml-es üvegedény, melynek becsiszolt üveg vagy gumi dugójába mérő és referenciaelektródák vannak beépítve. A levegőből az oxigénutánpótlás megszűnése és a sötét inkubálás folyamán a fotoszintézis kizárása a természetes szubsztrátumot tartalmazó mérőtérbe a redoxpotenciál csökkenéséhez vezet. A mérés során kapott információk: 1. a redoxpotenciál csökkenéséhez szükséges idő; 2. a csökkenési szakasz nagysága; 3. a beálló egyensúlyi helyzetet jellemző redoxpotenciál érték.

A módszer felhasználási lehetőségeit bemutató néhány típuskísérletből a következők állapíthatók meg:

1. A szupereutróf Belső-tó vize a Balatonénál nagyobb redukáló képességgel rendelkezik, és a redukáló képesség szezonálisan jelentős mértékben változik.
2. A Balaton nádasában a víz redukáló képessége a lotikus zónában kisebb, mint a lenitikus zónában, és mindkét helyen nagyobb a nyíltvízben mért redukáló képességnél.
3. A Balaton vizének redukáló képessége vertikálisan nem változik, ugyanakkor a Belsőtóban a fenék felé a redukáló képesség növekedett. A növekedés a teljes és szaprofita mikrobiális plankton mennyiségének eloszlásával egyenes összefüggést mutatott.
4. A Balaton és Belső-tó vizének redukáló képességében a bakterio-, fito- és zooplankton hatása eltérő.
5. A Balaton nádasüledéke redukáló képességét tekintve határozott rétegezettséget mutat. Legnagyobb redukáló képességgel a felső legtöbb baktériumot tartalmazó, aktív réteg rendelkezik.
6. A fiatal detritusz redukáló képessége kisebb, mint az idős detrituszé.
7. A Balaton M szelvényén az üledék redukáló képessége nagyobb, mint az A szelvényen.
8. A redukáló képesség mérésével a mineralizációs folyamatok intenzitásáról is adatok nyerhetők.
9. Az oxigén nélküli redox egyensúlyra beállt mintát fényre helyezve a redoxpotenciál közel a kiindulási értékre áll vissza.

ИЗМЕРЕНИЕ РЕДИЦИРЮЩТЕЙ СПОСОБНОСТИ ПРИРОДАХ ВОД И ОСАДКОВ: ПРОСТОЙ ЛИМНОЛОГИЧЕСКИЙ МЕТОД

Я. Олах

Для измерения потенциальной редицируюшей способности природных вод и осадков разработан простой прямой метод, основанный на изменениях окислительно-восстановительного потенциала в замкнутых системах, содержащих природный субстрат. Измерительным сосудом служит градуированный стеклянный контейнер на 250 мл, снабженный нижним стеклянным краном или резиновой пробкой, в которую вмонтированы измеряющий и референтивный электроды. Исключением доступа атмосферентивный электроды. Исключением доступа атмосферногокислорода и исключением фотосинтеза (инкубация в темноте) достигается снижение окислительно-восстанавительного потенциала. В ходе измерения можно получить сведения о: 1. времени, необходимом для снижения потенциала; 2. размере снижения; 3. значениепотенциала после установления равновесия. В нескольких типичных экспериментах, дающих представление о возможностях метода, получены следующие результаты:

1. Супертрофическая вода Внутреннего Озера (Тихаиь) проявляет более высокую редуцирующую способность, чем вода Балатона, и ёё способность проявляет значительные сезонные изменения.

2. В тростинковых зарослях Балатона редуцирующая способность воды из лотической зоны меньше, чем способность воды открытой части озера.

3. В озере Балатон вода не проявляет вертикальной изменчивости по своей редуцирующей способности, тогда как во Внутреннем Озере (Тихань) способность увеличивается от поверхности к дну. Этот рост прямо соответствует количественному распределению общего сапрофитного микропланктона.

4. Влияние бактерио-, фито- и зоопланктона на редицирующая способность воды двух упомянутых озер различно.

5. Тростниковые осадки Балатона по своей редуцирующей способности стратифицированы. Наивышей способностью обладает самый верхний, активный слой, содержащий больше всего бактерий.

6. Редуцирующая спосбность молодого детрита меньше чем зрелого.

7. Редуцирующая способность осадков озера Балатон больше в разрезе М, чем в разрезе А.

8. Посредством измерения редуцирующей способности можно также получать сведения об интенсивности минерализационных процессов.

9. Если образец, дотигший состояния равновесия в отсутствие доступа кислорода, поместить на свет, его окислительно-восстановительный потенциал сдвигается к исходному значению.