

Redox Reactions in Soils Sequence of Redox Reactions in a Waterlogged Soil

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An apparatus is described which under laboratory conditions permits sampling of water and gases from a waterlogged soil throughout the experimental period. A cultivated soil supplied with 6% straw is waterlogged during 97 days and the sequence of some redox reactions is shown. After one day of waterlogging the redox potential declines and reaches negative values after six days. pH declines from 7.5 to 6.5 after 17 days of waterlogging and then slowly increases to about neutral again. NO_3^- is the first compound to be reduced and within two days all NO_3^- has disappeared. NO_2^- is only detected during the first two days. After NO_3^- has been reduced Mn^{2+} increases, followed by a large increase of Fe^{2+} . SO_4^{2-} has totally disappeared within the first three weeks of waterlogging. CO_2 is produced during the whole experimental period. A large production of CH_4 begins after three weeks of waterlogging. N_2O was not detected.

Introduction

When a soil is waterlogged drastic changes can occur. The solubility of some compounds increase, nutrients can be leached out and toxic compounds may be formed. These changes are caused by a decrease in redox potential. In an aerobic soil oxygen acts as electron acceptor, but when the soil is waterlogged the remaining oxygen is soon utilized (Takai et al. 1956). In this case other compounds become electron acceptors and are transformed into a more reduced

form, but the ability to accept electrons varies from compound to compound. Table 1 lists some typical redox reactions in soil systems and their redox potentials (Russell 1973).

Theoretically, the sequence of reduction of a submerged soil can be predicted by the laws of thermodynamics. The sequence would be the reduction of O_2 , NO_3^- , Mn^{4+} , Fe^{3+} , SO_4^{2-} , CO_2 and H^+ to their reduced counterparts H_2O , NH_4^+ , Mn^{2+} , Fe^{2+} , S^{2-} , CH_4 and H_2 (Ponnamperuma 1972).

In soils, reduced conditions can be the consequence of either biological or chemical reduction. Biological reduction is caused by microorganisms oxidizing organic material and transferring electrons to other chemical components of the soil. This is the most common type of reduction in submerged soils, but there is also evidence that chemical reduction may occur as well, especially in subsoils (Lind et al. 1976; Goshal et al. 1977).

This investigation is an attempt to clarify whether the theoretically known sequence of reduction also is applicable to natural waterlogged soils. In a laboratory experiment a cultivated soil supplied with organic material was submerged for 97 days. During this time some important chemical compounds in the soil were analysed and the sequence of reduction is discussed.

Table 1 – Redox potentials of typical soil systems (Russell 1973).

System	Redox potential in mV, 25°C At pH 7
$O_2 + 4H^+ + 4e^- = 2H_2O$	820
$NO_3^- + 2H^+ + 2e^- = NO_2^- + H_2O$	420
$MnO_2 + 4H^+ + 2e^- = Mn^{2+} + 2H_2O$	410
$Fe(OH)_3 + 3H^+ + e^- = Fe^{2+} + 3H_2O$	-180
$SO_4^{2-} + 10H^+ + 8e^- = H_2S + 4H_2O$	-220
$CO_2 + 8H^+ + 8e^- = CH_4 + 2H_2O$	-240
$2H^+ + 2e^- = H_2$	-413

Material and Methods

Equipment

A laboratory apparatus was constructed which permits sampling of water and gases from a waterlogged soil throughout the experimental period, see Fig. 1. Soil and water is incubated in a 2,000 ml flask sealed with a butylic rubber membrane. The gas produced is led through an outlet into a sealed smaller vessel containing

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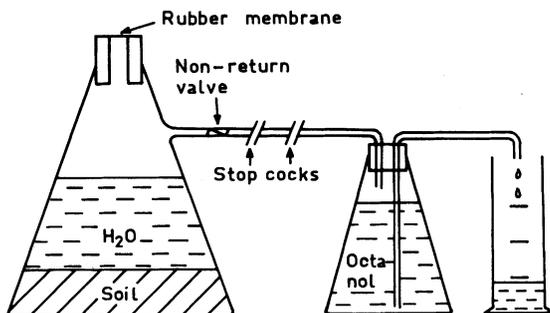


Fig. 1. Apparatus used in measuring the sequence of redox reactions in waterlogged soil.

octanol with 0.5% HCl. As the pressure increases in the octanol vessel due to the inflow of gas the corresponding outflow of octanol will be directly proportional to the amount of gas produced. Octanol was chosen as the solubility of most gases is very low in this alcohol.

Samples of water and atmosphere can be taken out with a syringe through the rubber membrane. When a water sample is taken out the ratio between soil and water is changed, but this does not influence the sequence of reduction. However, if a quantitative study of different compounds is made the changed ratio soil/water must be accounted for.

Procedure

The experiment was carried out in the laboratory at room temperature. The soil, a sedimental clay soil with 4.2% organic matter, which was taken from a cultivated field south of Uppsala, was partly air-dried until it could pass a 4 mm sieve. Finely ground wheat-straw with 46% carbon and 0.35% nitrogen was used as energy source for the microorganisms.

415 g dry weight (d.w.) of soil, 25 g (6%) of wheat-straw and 20 mg of NO₃-N/100 g d.w. soil in the form of KNO₃, was mixed and put into the flask. 1,500 ml of boiled tapwater saturated with N₂ was added. The flask was evacuated and filled with argon. This was repeated three times in order to get most of the oxygen out of the flask. The flask was connected to the smaller vessel containing octanol.

The flask was shaken for 30 minutes once a week. 12 water samples and 13 gas samples were taken out in an approximately exponential time sequence during the experimental period. 40 ml of water was taken out with a 100 ml syringe for analysis on each occasion. In order to maintain a constant pressure in the flask 40

ml of argon was injected. After 40 ml of water had been drawn into the syringe the volume was made up to 100 ml with argon in order to keep the contact with air-oxygen at a minimum. The electrodes for pH, redox potential and specific conductance were then inserted into the syringe. After the electrode measurements the 40 ml sample was centrifuged in a 40 ml plastic tube and then the different compounds were analysed. Except for NO_3^- and SO_4^{2-} , the analyses were made the same day as the sample was taken out of the flask. 100 μl of the gas samples were taken out through the rubber membrane and analysed immediately on the gas chromatograph. After 97 days of waterlogging the soil was taken out of the flask and analysed for $\text{NH}_4\text{-N}$ and elemental sulphur.

Analytical Methods

The redox potential (E_h) was measured with an Orion combination electrode, model 96-78. The reading was made 10 minutes after insertion. Ferrous iron (Fe^{2+}) was measured colorimetrically with the phenanthroline method (Charlot 1964). Manganese (Mn) was determined as water soluble manganese with atomic absorption spectrophotometry. As Mn^{2+} is the only water soluble form of manganese it may be considered acceptable to regard the water soluble fraction as reduced manganese. Nitrite-nitrogen ($\text{NO}_2\text{-N}$) was determined colorimetrically by the method of Griess-Ilosvay (Black et al. 1965). Nitrate-nitrogen ($\text{NO}_3\text{-N}$) was first reduced to $\text{NO}_2\text{-N}$ with a cadmium reductor and then determined as $\text{NO}_2\text{-N}$. Ammonium-nitrogen ($\text{NH}_4\text{-N}$) was extracted from the soil with 1.0 M KCl and determined colorimetrically with Nessler's reagent (Charlot 1964). Sulphate-sulphur ($\text{SO}_4\text{-S}$) was determined by titration with barium perchlorate using thordin as indicator (Fritz et al. 1955). Elemental sulphur (S^0) was extracted from the soil with hexane and acetone and analysed on a gas chromatograph with an electron capture detector. Carbon dioxide (CO_2), methane (CH_4) and nitrous oxide (N_2O) were analysed with a gas chromatographic separation method developed by Goshal et al. (1975).

Results and Discussion

The pH, pE and specific conductance are shown in Fig. 2a, and the relative concentrations of Mn^{2+} , Fe^{2+} and SO_4^{2-} in Fig. 2b. Fig. 2c shows the relative concentrations of NO_3^- and NO_2^- and the relative production of CO_2 and CH_4 . As only the sequence of reduction is of interest in the present context the concentrations of the compounds are illustrated as percentages of their maximum value obtained during the experimental period.

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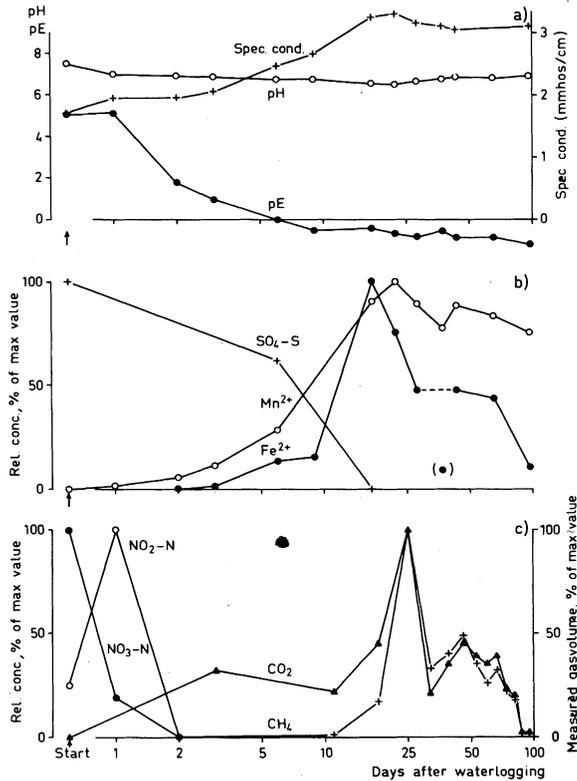


Fig. 2. Changes in a) pH, pE and specific conductance, b) SO₄-S, Mn²⁺ and Fe²⁺ and c) NO₃-N, NO₂-N, CO₂ and CH₄ as a result of waterlogging. All measurements, except gas-analysis, were made on a water sample. Concentrations are expressed as percentages of the maximum value for each compound during the experimental period.

Redox Potential

In Fig. 2a the redox potential is represented by its logarithmic function. This is obtained from the formula $pE = Eh/0.0591$ (Sillén 1964). pE is a measurement of the ability of a system to accept and donate electrons. On the first day pE remains at a relatively high level, but then it drops rapidly. After six days of waterlogging the redox potential is below 0, and it remains at a low level throughout the experiment. In this case the system is well supplied with organic material, which is used by microorganisms to obtain energy and as a result there is a large production of electrons. Under aerobic conditions oxygen would act as electron-acceptor, but when oxygen is not available the electrons are accepted by compounds in the sequence shown in Table 1, causing a gradual decrease in redox potential.

There are several possible explanations of the relatively high redox potential during the first day. There might, for instance, still be some oxygen left in the soil, which would then be utilized by the microorganisms. The surplus of NO_3^- could also prevent the decrease in redox potential, or there may be a lag-period before the microorganisms become active.

In this experiment the redox potential has been measured on a water sample. However, since Ponnampetuma et al. (1964) demonstrated that the redox potential is considerably higher in the soil solution than in the soil, the redox potential of the water sample should only be considered as a guideline in explaining the sequence of redox reactions.

pH

There were no great changes in pH during waterlogging for 97 days and it remained between 7.5 and 6.5. However, the changes that do occur are characteristic of waterlogged soils (Ponnampetuma 1972). During the first days pH decreases, probably due to the production of CO_2 . Furthermore, as all important redox reactions in soils involve the transfer of electrons and H^+ the redox reactions directly influence the concentration of H^+ ; a low redox potential generally results in a higher pH. This process counteracts the effect of dissolved CO_2 in water and in this case pH stabilises around neutral point.

Specific Conductance

Specific conductance is a measurement of the kind and total concentration of the ions in solution. There is a gradual increase in specific conductance during the first three weeks of waterlogging, which closely corresponds to the increasing amount of reduced iron and manganese. Other ions than those analysed, i.e. HCO_3^- , also influence the specific conductance, which is shown by only a small decrease in the conductance during the last two months, in spite of the large decrease in especially the concentration of Fe^{2+} .

Nitrate-Nitrogen

The first compound to be reduced is NO_3^- . Although the water contained 66 mg $\text{NO}_3^-/\text{N/l}$ when the experiment started, all of it had been transformed after two days. This shows how extremely sensitive NO_3^- is to reduced conditions. All NO_3^- need not have been lost from the soil as N_2 or N_2O . A minor fraction might have been reduced to NH_4^+ , which remains in the soil in an exchangeable form, fixed in clay minerals or immobilized by microorganisms.

Nitrite-Nitrogen

NO_2^- is an intermediate in the reduction of NO_3^- . In most cases NO_2^- is not a stable compound and does not accumulate in soil. The maximum value of NO_2^- ,

16 mg/l, was obtained after one day and on the second day all NO_2^- had disappeared. The first analysis was made three hours after start and already then some NO_3^- had been reduced to NO_2^- .

Ammonium-Nitrogen

When the soil was analysed after 97 days of waterlogging it contained 7 mg $\text{NH}_4\text{-N}/100$ g d.w. soil and the original soil contained 1 mg $\text{NH}_4\text{-N}/100$ g d.w. soil. Woldendorp (1965) and Nömmik (1956) have shown that only minor amounts of NO_3^- are reduced to NH_4^+ under anaerobic conditions and Ponnampereuma (1972) concludes that the accumulation of NH_4^+ in submerged soils is a result of mineralization of organic material. Thus, the increase of NH_4^+ after waterlogging in this experiment is probably mainly due to mineralization of organic nitrogen and not so much a result of nitrate reduction.

Manganous-Manganese

There is a small reduction of manganese compounds already after one day of waterlogging. When all NO_3^- is reduced the manganese reduction increases and reaches its maximum value, 23 mg $\text{Mn}^{2+}/1$ after about 22 days. During the final two months there was a small decrease in Mn^{2+} , which was probably due to precipitation of manganese as MnCO_3 (Ponnampereuma et al. 1969).

Ferrous-Iron

Although Fe^{2+} reaches its maximum value, 107 mg/l, about five days before Mn^{2+} , the reduction of iron starts later than the reduction of manganese. There are only small amounts of Fe^{2+} until after six days of waterlogging, by which time the redox potential has reached low values.

Iron and manganese do not exist as separate pure oxides in soil. Ferric oxides contain manganese compounds. This manganese is protected from reduction inside the ferric oxides and is not released until the ferric oxides are reduced (Turner et al. 1968). This explains why some manganese is reduced at the same time as iron.

It is surprising to find such a large decline in Fe^{2+} during the last two months of waterlogging. It may be due to precipitation of $\text{Fe}_3(\text{OH})_8$, $\text{Fe}_3\text{O}_4 \cdot n\text{H}_2\text{O}$ and FeS (Ponnampereuma 1972).

Sulphate-Sulphur

The water contained 18 mg/l of $\text{SO}_4\text{-S}$ when the first analysis was made after one day of waterlogging. Within 17 days all SO_4^{2-} had disappeared. Under strongly reduced conditions SO_4^{2-} can be transformed to elemental sulphur or sulphides, mainly FeS , SH^- and H_2S . The latter has a very characteristic smell, even in low concentrations but at no time during the experiment was such a smell noticeable.

The disappearance of SO_4^{2-} need not be due to respiratory SO_4^{2-} -reduction,

where microorganisms use SO_4^{2-} as an electron acceptor. Minor amounts of SO_4^{2-} are used by microorganisms to build up cell protein (Postgate 1965), but this decrease in SO_4^{2-} should not be seen as a consequence of reduced conditions. It is probable that the small amount of SO_4^{2-} present in this experiment was used for this purpose. In another experiment (to be published), where 320 ppm of $\text{SO}_4\text{-S}$ was added, it was clearly shown that the major part of SO_4^{2-} was reduced when most iron had been reduced to Fe^{2+} .

Elemental Sulphur

The original soil was free from elemental sulphur and when the soil was analysed after 97 days of waterlogging it contained 5 mg/100 g d.w. soil. The elemental sulphur detected does not necessarily come from reduced SO_4^{2-} but may also be a product of the dissimilation of organic compounds.

Carbon Dioxide

The method only accounts for CO_2 in the atmosphere. The total amount of CO_2 detected was 250 ml/100 g d.w. soil. CO_2 was produced during the whole period of waterlogging although it declined towards the end of the experiment. CO_2 production as well as reduced conditions in the soil are consequences of microbial activity and maximum CO_2 production also coincides with large amounts of reduced iron and manganese.

Methane

The reduction of organic compounds to CH_4 does not take place to any particular extent until after most manganese and iron have been reduced. This is well in accordance with the theoretical values of redox potentials. CH_4 is only produced during strongly reduced conditions and among the major compounds in soils it is only the reduction of H^+ to H_2 that occurs at a lower redox potential. There was a large increase in CH_4 production after three weeks of waterlogging. The total production of CH_4 during the experimental period was 360 ml/100 g d.w. soil.

The decline in production of CH_4 and CO_2 towards the end of the experiment is probably temporary, as only 6% of added carbon has been transformed into CH_4 and CO_2 .

Nitrous Oxide

N_2O was not detected in the atmosphere. This might have been due either to lack of production or, most probably, that the N_2O formed was further reduced to N_2 . Nömmik (1956) has shown that N_2O is an intermediate in the reduction of NO_3^- to N_2 . As N_2O has a high solubility in water the N_2O produced might have been further reduced to N_2 already in the water, which would explain why N_2O was not detected in the atmosphere.

Summary and Conclusions

The experiment demonstrated that the reduced conditions that develop in a waterlogged soil containing organic material lead to changes in the solubility and accessibility of different compounds. There are no great changes in pH and it remains between 7.5 and 6.5. Specific conductance reaches a maximum value of 3.3 mmhos/cm after three weeks of waterlogging and then there is a small decrease. NO_3^- is the first compound to be affected and within two days all NO_3^- has been reduced. NO_2^- is also detected only during the first two days of waterlogging. Manganese is the next compound to be reduced, but due to occurrence of manganese in ferric oxides, manganese is also liberated when ferric iron is reduced to Fe^{2+} . Large amounts of iron are reduced but after three weeks of waterlogging there is a decrease in the concentration of Fe^{2+} . In this experiment, where the soil contained only small amounts of SO_4^{2-} , this compound disappeared during the same period as manganese and iron were reduced. This could have been due to incorporation of sulphur into cell protein. Other experiments showed that when larger amounts of SO_4^{2-} are added to the soil, reduction of SO_4^{2-} takes place after the reduction of iron. CO_2 is produced during the whole experimental period. Production of CH_4 does not start until after two weeks of waterlogging. This sequence of redox reactions is in agreement with the theoretically expected sequence, although there is considerable overlapping in the various redox reactions.

In forthcoming papers the sequence of redox reactions in different soils and the influence of added organic material and inorganic compounds on some redox reactions will be discussed.

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