NITROGEN TRANSFORMATIONS AND REDOX POTENTIAL CHANGES IN IRRIGATED ORGANIC SOIL

Teresa Włodarczyk, Urszula Kotowska

EDITED BY
TERESA WŁODARCZYK, URSZULA KOTOWSKA
GRZEGORZ JÓZEFACIUK, RYSZARD T. WALCZAK

Centre of Excellence for Applied
Physics in Sustainable Agriculture
AGROPHYSICS

Institute of Agrophysics
Polish Academy of Sciences

EU 5th Framework Program
QLAM-2001-00428

Centre of Excellence for Applied
Physics in Sustainable Agriculture
AGROPHYSICS
ul. Doświadczalna 4,
20-290 Lublin, Poland

Institute of Agrophysics
of the Polish Academy of Sciences
ul. Doświadczalna 4,
20-290 Lublin, Poland

EU 5th Framework Program
QLAM-2001-00428

NITROGEN TRANSFORMATIONS AND REDOX POTENTIAL CHANGES IN IRRIGATED ORGANIC SOIL

Teresa Włodarczyk, Urszula Kotowska

EDITORS:
Teresa Włodarczyk
Urszula Kotowska
Grzegorz Józefaciuk
Ryszard T. Walczak

Lublin 2005

ISBN 83-89969-00-9

Part of the work was performed under contracted research project
No. PBZ 31-03

Edition: 180 copies

Project of cover: Grzegorz Józefaciuk
Computer edition: Dorota Matyka-Sarzyńska

Printed by: ALF-GRAF, ul. Kościuszki 4, 20-006 Lublin, Poland

CONTENTS:

1. INTRODUCTION

Soil as a natural body occupies a specific place among the elements of geographical environment, which sustains all animated nature. It is a place of accumulated mineral elements and mediator of their absorption by plants. This mediation is a result of the soil agrophysical status, which determines the availability of water, oxygen and nutrients to plants. Air conditions influence the chemical status of macro- and microelements and decide the degree of their availability to plants and their migration down the soil profile. This indirect effects of soil aeration are characterized by the redox potential.

The objective of the study was to: 1) investigate nitrogen transformation and redox potential changes in organic soil covered with different plants and irrigated with municipal waste waters; 2) verify the possibility of using such organic soil and plants as a method of waste water treatment under the conditions of the „Hajdów" experimental object.

2. WASTE WATERS

Water plays a very special role in the mutually related processes that take place in ecosystems, constituting a fundamental, necessary for their functioning, abiotic element of the environment. At the same time it is a highly valuable and renewable raw material, whose resources are variable in time, that performs multiple and fundamental functions in economic activity.

These extraordinary functions of water mean that not only is it absolutely necessary to protect it from contamination, but also to observe the requirements of rational and economy oriented management of its resources. The qualitative and quantitative protection of water resources is therefore an integral element of environmental protection.

The problem of contamination of water is a global one, as most of the major rivers flow through the territories of many countries.

The gigantic influence of human activity on river ecosystems accounting for the construction of an enormous number of dams and reservoirs, changing of hydrological regime of water bodies; pollution of rivers and lakes from point and diffusion sources, as well as resulting from trans-boundary transfers of pollutants with air currents; direct withdrawal of water from water bodies for irrigation, water supply of industry and population. All of these influence the water, thermal, hydrochemical and ice regimes and, also, the flow of sediments leading to partial or total changing of flora and fauna of river ecosystems. The violations reached their apogee at the end of the 20th century (*Fashchevsky and Fashchevskaya, 2003*).

A serious threat to the aquatic environment is presented by unpurified industrial wastes. Poland contributes to a significant extent to the pollution of the Baltic with nitrogen compounds and with other macro- and micro-elements. Lublin also has its participation in the load of pollutants introduced into the surface waters and into the Baltic (*Report WIOS, 1997*).

Poland's entry into the structures of the European Union imposes on us an obligation to drain liquid wastes to waters and to the ground in accordance with EU Standards, i.e. in the case of nitrogen 15 $g \cdot N \cdot m^{-3}$, while the equivalent Polish standard currently in force provides for a limit of 30 $g \cdot N \cdot m^{-3}$.

Liquid wastes drainage into the ground is the oldest form of their disposal. It is the most natural method, as the substances return to where they have been produced and, moreover, this closes the cycle of the circulation of mater. The development of technology and civilization, however, disrupted that circulation and its recreation is difficult and in many places even impossible.

The choice of a suitable receptacle for liquid wastes depends on the characteristics of the area and on the amount and quality of wastes to be disposed of.

Municipal sewage drainage to the ground and its agricultural utilization provides an alternative solution to the problem of protection of water reservoirs.

3. NITROGEN AND ITS IMPORTANCE

Together with carbon, oxygen and hydrogen, nitrogen is one of the four most common elements in living cells and an essential constituent of proteins and nucleic acids, the two groups of substances, which can be said to support life. Yet the element is not particularly common on Earth, with the exception of the atmosphere which contains almost 80% nitrogen. The estimated 11 000 to 14 000 teragrams (10^{12}) of nitrogen contained in living biomass (mainly terrestrial plants) is equivalent to about three parts per million of the atmospheric nitrogen. Other important nitrogen pools are soil organic matter, rocks (in fact the largest single pool), sediments, coal deposits, organic matter in ocean water, and nitrate in ocean water. The next most common gaseous form of nitrogen in the atmosphere after molecular nitrogen is dinitrogen oxide (*Tamm, 1991*).

The N atom exists in different oxidation and physical states. Shifts between them are commonly mediated by soil organisms. The ease with which shifts occur in the oxidation states results in the formation of different inorganic forms that are readily lost from the ecosystem. The NO_3^- form is readily soluble in water and thus subject to leaching and water transport. The NH_4^+-NH_3 forms are subject to volatilisation and fixation both by clays and by soil organic mater (SOM). Nitrogen shortages, therefore, often limit plant productivity. Also, both the gaseous and

the soluble phases of this nutrient lead to environmental pollution (*Paul and Clark 1996*).

The size of pools does not indicate anything about the dynamics of annual global fluxes of nitrogen between the more important pools.

3.1. Nitrogen in soil

As in plants, nitrogen in soil occurs both in organic and inorganic forms. Organic nitrogen is in reduced form, some of it as amide nitrogen, relatively easily available to decomposer organisms unless protected mechanically or chemically. Another part of soil organic nitrogen occurs as a constituent of large and often resistant molecules with nitrogen in heterocyclic aromatic rings (*Tamm, 1991*).

Inorganic nitrogen is usually fully reduced, ammonium, or fully oxidized, nitrate. Intermediary oxidation stages also exist but do not accumulate in measurable amounts, except for nitrite under special circumstances. There are transfers not only between the various soil nitrogen pools, but also between the soil pools and gaseous phase, where nitrogen compounds at different oxidation levels also occur (NH_3, N_2, N_2O, NO) (*Tamm, 1991*).

Only a small part of nitrogen store in the soil is available to plant roots at any given moment. Most is in organic form, usually in large molecules insoluble in water. Organic nitrogen in natural ecosystems originates from dead organisms, plants, and microorganisms. Much of the nitrogen in fresh litter is still in protein form or in decomposition products of proteins, i.e., peptides and amino acids. These substances are attractive substrates for microorganisms, which often can be used as a source of carbon as well as of nitrogen. Their residence time in the soil is short, unless association with less attractive substances in, e.g., cell walls protects them mechanically or chemically (*Tamm, 1991*).

The decomposition of litter does not mean that litter nitrogen is immediately transferred to inorganic nitrogen or transformed into the limited number of low-molecular organic compounds in which it may be available to plant roots and mycorrhizal fungi. Microorganisms do the chemical degradation of the litter, and even if they may produce extracellular enzymes, most take the nitrogen up themselves. The rate at which the microbial nitrogen is transferred to the available pool depends on the C/N ratio of the substrate and on the death rate the microorganisms (*Tamm, 1991*).

As far as nitrogen is concerned, the end product of the decomposition process as such is ammonium ions. Ammonium ions in water solutions are in equilibrium with undissociated ammonia molecules, but the amounts of ammonia are negligible until pH rises above seven. In such cases some ammonia may well be emitted

to the atmosphere. In dense vegetation, e.g., under a forest canopy, much of that ammonia may be reabsorbed by the foliage and thus retained within the ecosystem (*Tamm, 1991*).

The normal case, however, is that most of the ammonium liberated stays in the ecosystem, although rapidly removed from the soil solution along one of the following pathways: 1) uptake by plant roots (directly or via mycorrhizal hyphae), 2) uptake by microorganisms, 3) adsorption on the surface of soil colloids (in clay-rich soils partly followed by ammonium fixation in the lattice of certain clay minerals), and 4) chemical binding to organic substances. Any ammonium ions left in the soil solution may leave the soil with percolating water, but this is seldom an important pathway in natural ecosystems (*Tamm, 1991*).

Adsorption of ammonium ions to soil colloids is a removal from the pool of dissolved nutrients, but does not make them unavailable for plants; when roots or mycorrhizal hyphae deplete the soil solution of ammonium ions, such adsorbed ions go into solution again according to well-known chemical principles. However, ion transport by diffusion is a slow process. So unless there is a mass flow of soil water, roots and hyphae have to grow close to the sites of adsorption. The energy cost for uptake from a soil increases in comparison with that from a nutrient solution. Lattice-fixed ammonium ions can also be redissolved, but this is a slow process of limited ecological importance under normal conditions and time perspectives (seasons, years, even decades) (*Tamm, 1991*).

4. NITROGEN TRANSFORMATION

Nitrogen, like other elements, is subject to cyclic circulation in nature. In the course of a full year, nitrogen fluxes and losses occur in the soil, accompanied by numerous and complex transformations, such as: ammonification, nitrification assimilatory and dissimilatory nitrate reduction.

4.1. Mineralization of nitrogen (Ammonification)

The three biological forms of N proteins are microbial cell wall constituents such as chitin and peptidoglycans, and the nucleic acids. Protein is a basic constituent of all life forms. During decomposition, it is hydrolysed to peptides by proteinases and peptidases. The proteinases are classified as to whether they attack peptide linkages between specific amino acids. The reaction mechanism is the reverse of that used in the formation of peptide bonds. The N group receives a proton (H^+), and the C atom of the linkage receives an OH^- during the nucleophilic displacement reaction (*Paul and Clark 1996*).

Mineralization of organic N refers to the degradation of proteins, amino sugars, and nucleic acids to NH_4^+, the mineral form. When deamination occurs, removal of NH_4^+ is most often carried out by enzymes such as glutamate dehydrogenase, which requires the coenzyme nicotine adenine dinucleotide (NADH) as acceptor of the reducing equivalents (*Paul and Clark 1996*).

Whether NH_4^+ is immobilized or accumulates in the soil depends on the microorgamism's requirement of N for growth. The C:N ratio of microorganisms is not constant. Fungi can have wide C:N ratios; their C contents are quite constant at approximately 45% C. With N contents of 3 to 10%, their C:N ratios range from 15:1 to 4.5:1. Bacteria have N in their cytoplasm and in the peptidoglycan of their cell walls: C:N ratios usually are in the range of 3:1 to 5:1 (*Paul and Clark 1996*).

4.2. Nitrification

Nitrification is an aerobic process, performed both by autotrophs and heterotrophs in soils.

Autotrophic nitrification is defined as the biological oxidation of NH_4^+ to NO_2^- and NO_3^- in a two step reaction as presented in the following equations where *Nitrosomonas* performs the first energy yielding reaction:

$$NH4+ + 1.5\ O2 \rightarrow NO2- + 2H+ + H2O + energy$$

and *Nitrobacter* the second energy yielding reaction:

$$NO_2^- + 0.5\ O_2 \rightarrow NO_3^- + energy$$

The chemoautotrophic nitrifiers are generally aerobes that derive their C largely from CO_2 or carbonates but NH_4^+ can originate from mineralization of soil organic material by other organisms or from fertilizer. All organisms in this family are capable of obtaining all their energy requirements for growth from oxidation of either ammonium or nitrite (*Belser 1979*).

The bacteria are classified based on whether they oxidize NH_4^+ to NO_2^- (*Nitroso-*) or NO_2^- to NO_3^- (*Nitro-*). In most habitats they are closely associated and NO_2^- rarely accumulates (*Paul and Clark 1996*).

Nitrification has been typically associated with chemoautotrophic bacteria, although it is now recognized that heterotrophic nitrification occurs in some soils too acid for known autotrophic nitrifiers, or lacking them for other reasons, and can be of significance especially in forest soils. It has been shown that nitrate formation may continue in the presence of inhibitors known to stop autotrophic nitrification (*Kreitinger et al. 1985*). This indicates the occurrence of so-called heterotrophic nitrification, mediated by certain fungi (*Focht and Verstraete 1977*) or by methylotroph bac-

teria (*Verstraete 1981*). It is clear that heterotrophic nitrifiers form nitrate at a much slower rate than autotrophic nitrifiers (with the same biomass). However, a slow rate may be compensated for by a high biomass (*Tamm, 1991*).

Heterotrophic organisms use organic substances as both a carbon and an energy source. They can obtain part of energy from oxidation of NH_4^+ or organic nitrogen compounds. Fungi are apparently the most important of these. Different pathways have been postulated, but their role in fungal metabolism is largely unknown (*Killman 1986*):

$$\text{inorganic: } NH_4^+ \rightarrow NH_2OH \rightarrow NOH \rightarrow NO_2^- \rightarrow NO_3^-$$

$$\text{organic: } RNH_2 \rightarrow RNHOH \rightarrow RNO \rightarrow RNO_2 \rightarrow NO_3^-$$

The rate of nitrification in a soil is affected directly and indirectly by many factors, such as temperature, moisture, C/N ratio, occurrence of inhibitors of the process itself, or of organic matter decomposition. Yet a prime prerequisite for nitrification is access to ammonium ions in the soil or, for some heterotrophic nitrifiers, easily available amino compounds. It was mentioned earlier that plant roots promptly absorb ammonium ions (as well as nitrate ions), while many microorganisms prefer the ammonium form. Some fungi cannot even use nitrate nitrogen. Concentration of ammonium ions high enough to support an active population of bacteria using oxidation of ammonium to nitrite as their sole source of energy (e.g., the genus *Nitrosomonas*) only occurs when the competition for nitrogen is low or moderate, i.e., when ammonia influx to the soil compartment (by ammonification or as input from outside) temporarily or permanently exceeds biological uptake (*Tamm, 1991*).

The heterogeneity of a soil means that there may be a large variation in many soil properties, including acidity, between microsites. pH is an important controlling factor, not only for the occurrence of nitrification, but also for any by-products that may be formed. As *Nitrobacter* seems to require somewhat higher pH than *Nitrosomonas*, some accumulation of nitrite may occur under certain circumstances. Gaseous products may also be formed, at different rates under different conditions (*Tamm, 1991*).

It remains to be stated that nitrification is an acidifying process. Under undisturbed conditions, when the nitrate formed is rapidly taken up by roots and reduced back to ammonium and other reduced forms, there is no net acidification (*Tamm, 1991*).

Nitrite accumulates only under conditions where *Nitrobacter* appears to be inhibited while *Nitrosomonas* is not. Typically these conditions are high pH (7.5) and very cold temperatures (*Smith and Chalk, 1980; Bouwman, 1990b*).

Although nitrification is understood to be an aerobic process, there is strong evidence that it can also occur under anaerobic conditions. Nitrifying bacteria have been shown to produce NO and N_2O, the intermediates in autotrophic nitrification showing the possible sites for gaseous losses during this process (*Paul and Clark 1996*).

Nitrification is often considered to be the dominant source of N_2O in "aerobic" soils (*Bremner and Blackmer, 1978; Sahrawat and Keeney, 1986*).

4.3. Assimilatory reduction of nitrate

Soil fixed nitrogen resources may be conserved through both assimilatory and dissimilatory nitrate reductive processes, or they are reduced by dissimilatory reduction. Assimilatory and dissimilatory nitrate reduction both involve the transfer of electrons to nitrogen compounds, but they differ in the ultimate fate of the reduced nitrogen atom.

In the absence of NH_4^+ and organic-N and under conditions where only NO_3^- is available, bacteria, fungi, yeast and algae have first to reduce the NO_3^- (*Freney et al., 1979*). This process is less O_2 sensitive than denitrification and therefore would be expected to occur under aerobic conditions (Payne, 1981; Mosier et al., 1983). The aerobic assimilation of nitrate or assimilatory nitrate reduction is the process of NO_3^--N incorporation into biomass (*Mosier et al., 1983*). Some microorganisms reduce NO_3^- to NH_4^+. They use the N in the production of biomass (assimilatory reduction), but the process can also serve other purposes (dissimilatory reduction) e.g. as a source of energy or for detoxification of NO_2^-. N_2O can escape during these processes (*Scott Smith and Zimmerman 1981; Kaplan and Wofsy 1985; Cole 1988; Tiedje 1988*).

$$NO_3^- \rightarrow NO_2^- \rightarrow [H_2N_2O_2] \rightarrow [NH_2OH] \rightarrow NH_3$$
$$\downarrow$$
$$N_2O$$

In nitrate assimilation, the first step is the reduction to nitrite, which is accomplished by the enzyme nitrate reductase. Subsequently, the nitrite is reduced to hydroxylamine by the enzyme nitrite reductase to finally be reduced to ammonia (*Payne 1973*). The net reaction is shown in the following equation:
where N_2O rather than N_2 may be produced as a by-product from the indicated intermediate (hyponitrite) (*Freney et al., 1979*). The reaction shown is essentially the same as that which occurs during NO_3^- reduction to NH_4^+ and involves the

same precursor of N_2O again, probably hyponitrite (*Freney et al., 1979; Mosier et al., 1983*). This pathway as a nitrous oxide source seems to be significant from studies on forest soils where fungal activity is important.

Some of the studied nitrate reductases show the existence of an active form and an inactive form that depends on the oxireduction conditions of the environment (*Stouthamer 1976*). Under reducing conditions, the enzyme is converted into the inactive form. The regulation of the synthesis of the enzyme varies in different species, being constitutive in several species and repressible in others. In *Rhizobium japonicum,* for instance, the assimilatory enzyme is induced in aerobiosis and in the presence of nitrate; meanwhile in anaerobiosis, a dissimilatory nitrate reductase is induced (*Daniel and Grey 1976*). Both enzymes have different molecular weights and different sensitivity to inhibitors (*Stouthamer 1976*).

4.4. Dissimilatory reduction of nitrate

Dissimilatory reduction is the process through which some microorganisms use the energy generated by the electron transport from an organic or inorganic source to nitrate or to a more reduced nitrogen oxide. This metabolic reduction uses cytochromes mostly as electron donors and occurs with a liberation of dinitrogen as the final product. However, some bacteria lack N_2O reductase, and so produce this gas as a terminal product, or lack nitrite reductase, yielding nitrite as an end product (*Ingraham, 1981*).

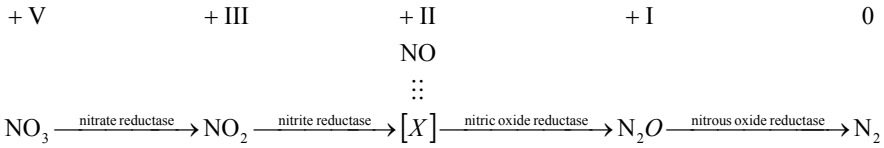
When the dissimilative reduction produces the gaseous dinitrogen or nitrous oxide compounds, the process is termed **denitrification**. However, since reduction, through the metabolic pathway of cytochromes, in some case results in the production of ammonia or nitrite, some authors prefer the more general name of **nitrate respiration** for the process. In other cases, the metabolic pathways do not involve membrane-bound enzymes, cytochromes, or electron transport phosphorylations, and the main product is ammonia. This process is called **fermentative nitrate reduction**. (*Fenchel and Blackburn, 1997*).

In contrast to assimilatory reduction (nitrogenous compound is incorporated into cellular biomass) for dissimilatory nitrate reduction, the nitrogenous compounds accept electrons in support of cellular respiration. The final products, dinitrogen, nitrous oxide, or ammonium, are released from the cell and accumulate in the environment in concentrations far beyond that necessary for biomass synthesis. Three commonly evaluated microbial processes are classed under the title of dissimilatory nitrate reduction. These processes can be distinguished by their respective products: a) nitrite, b) ammonium, and c) nitrous oxide and dinitrogen – denitrification.

Biological denitrification is the last step in the N-cycle, where N is returned to the atmospheric pool of N_2. It is an anaerobic process (*Granli and Bøkman, 1994*).

Biological denitrification is a respiratory process in which N-oxides (electron acceptors) are enzymatically reduced under anaerobic conditions to nitrous oxide and dinitrogen for ATP production by organisms that normally use O2 for respiration. Most denitrifying organisms are heterotrophic. However, heterotrophic denitrification is one of the most important processes as a source for N2O. Nitrous oxides are well documented gaseous products of the heterotrophic denitrifiers (Abou-Seada and Ottow 1985; Myrold and Tiedje 1985; Benckiser and Simarmata 1994)

The process of denitrification (including rhizobial denitrification) can be presented as follows (*Firestone and Davidson, 1989*):



Nitric oxide (NO) is believed to be either a true intermediate or rapid exchange with an unidentified intermediate (X).

Many microorganisms can use NO_3^- as their primary electron acceptor for obtaining energy from organic compounds when low O_2 availability restricts their metabolism (*Granli and Bøckman 1994*):

$$5(CH_2O) + 4NO_3^- + 4H^+ \rightarrow 5CO_2 + 7H_2O + 2N_2 + \text{energy}$$

Some microorganisms can obtain energy by using NO_3^- for oxidation of inorganic compounds, e.g. S^{2-}, Fe^{2+} (autotrophic denitrification). This occurs where NO_3^- diffuses into zones rich in FeS, e.g. sediments in shallow waters (*Golterman 1991*).

The majority of soil bacteria seem able to denitrify (*Umarov 1990*). The complete reduction of nitrate proceeds via nitrite, nitric oxide, and nitrous oxide, but not all denitrifiers can carry out the complete reduction from nitrate to N_2. Denitrifying bacteria exhibit a variety of incomplete reduction pathways. The enzymes most commonly missing are nitrate reductase or nitrous oxide reductase; some bacteria produce only N_2, while others give a mixture of N_2O and N_2, and some only N_2O (*Stouthamer 1988, Robertson and Kuenen, 1991*).

The **nitrate reductase** of the dissimilatory reduction is a molybdo-iron sulfide protein, but different from the assimilatory enzyme (*Ruiz-Harrera and DeMoss 1969, Ruiz-Harrera et al. 1969*). Nitrtate reductase has been found to be a membrane-bound enzyme except in *Spirillum iteronii* where it is found as a soluble enzyme (*Gauthier et al. 1970*).

The nitrite reductase is the key enzyme that drives the NO_2^- ion toward the synthesis of the gases and NO in contrast with the more economic pathway of ammonia synthesis.

The nitrous oxide reductase is possibly a Cu protein and closes up the recycle of nitrogen by releasing dinitrogen back to the atmosphere (*Knowles, 1982*). Thus, the function of this enzyme is essential and prevents N_2O from being released into the atmosphere, avoiding the photochemical production of NO; this gas is supposed to be responsible for destroying the atmospheric ozone (*Delwiche and Bryan; 1976*).

Some denitrifiers lack the ability to catalyze the last step from N_2O to N_2 (*Tiedje, 1988*).

There has been some doubt if NO is a true intermediate or a by-product (*Amundson and Davidson 1990*) in the process, but a bacterial nitric oxide reductase has recently been characterized: *Pseudomonas stutzeri* loses the ability to denitrify if the genes for this enzyme are blocked (*Braun and Zumft 1991*).

That N_2O is an obligatory intermediate in denitrification is widely accepted (*Payne 1981; Zumft and Kroneck 1990*).

N_2O is reduced to N_2 by the labile enzyme nitrous oxide reductase (*Stouthamer 1988*). The reduction can also be carried out by the even more labile enzyme nitrogenase (the enzyme that reduce N_2 to NH_3).

Apart from free living denitrifiers such as *Pseudomonas ssp., Rhizobium ssp*. which live in a symbiotic relationship with leguminous plants, have the ability to denitrify. This later process is referred to as rhizobial denitrification (*O'Hara and Daniel, 1985*).

The denitrification process may be performed by N_2-fixers, specifically by *Azospirillum*, and by *Rhodopseudomonas* (*Aleem 1985, Casella et al. 1984*). These species are capable of using nitrtate as an electron acceptor, an alternative to oxygen, for generating ATP for nitrogenase activity. Studies with stable isotopes showed that *Rhodopseudomonas spheroides*, strain IL-106, did not directly assimilate nitrate into cell nitrogen, but rather denitrified nitrate to dinitrogen gas which was reutilized via nitrogenase as a source of ammonia for its assimilation (*Nicholas 1985*). *Rhizobium japomicum* and cowpea strains exhibit substantial rates of denitrification as either free-living or bacteroid cells. *R. triflii, R. leguminosarum* and *R. hedysarum* were able to use nitrate as an electron acceptor, liberating N_2O gas. This liberation was inhibited in the absence of nitrate by aerobiosis or when rich media were used. Similar studies were carried out with nodulated plants, with the aforementioned fast-growing rhizobia, showing that *Rhizobium* is an active denitrifier in symbiosis as well as in the free- living state (*Casella et al. 1984*). Denitrification is usually thought of as a bacterial process, but *Shoun et al.* (1992) reported that many fungi are capable of evolving N_2O under anaerobic conditions.

Some researchers have suggested that soil microbial population dynamics may be a more important factor than soil physical and soil chemical factors in explaining the characteristics of nitrous oxide production from soil (*Abou Seada and Ottow, 1985; Schmidt et al., 1988; Granli and Bøckmen, 1994*).

The influence of aeration on N_2O emission is complex and dependent on interacting factors. N_2O production and emission is usually greatest when the average soil conditions are such that both aerobic and anaerobic sites are abundant. This has been found in several laboratory studies (*Focht, 1974*).

5. SOME PHYSICAL AND CHEMICAL FACTORS INFLUENCING MICROBIOLOGICAL PROCESSES IN SOILS AND SEDIMENTS

5.1. Aeration and moisture status

The intensity of biological processes of oxygen absorption and carbon dioxide emission in the soil environment as well as the physical processes of gas exchange between the soil and the atmosphere determine the state of soil aeration. The movement of gases in the soil directly depends on the diffusion coefficient, determined by the number, tortuosity and continuity of the pores filled with air, and indirectly on the bulk density (porosity) of the soil, the distribution function of the soil pores, and the amount of soil watering (*Witkowska-Walczak et al., 2002; . Sławiński et al., 2000; Stepniewska, 1988; Gliński and Stępniewski, 1985; Hillel, 1998; Gliński et al., 2000a,b; Stepniewska, et al., 2000; Stępniewski et al., 2000*).

Soil is heterogeneous and commonly has both aerobic and anaerobic sites. The oxygen status in soil, which is inversely proportional to the amount of moisture held there, appears in many studies to be one of the key factors influencing its aeration status. As the free oxygen in soil is depleted, a number of predictable changes in microbial activity occur. When the soil oxygen tension has been reduced to less than 1 percent (v/v), the microbial population appears to shift from being predominantly aerobic to anaerobic.

With the development of reducing atmosphere, growth yields decline because the energy yielded per mole of fixed carbon oxidized anaerobically is far less than that produced from aerobic respiration. The inverse relationship between the rate of denitrification and O_2 concentration has been demonstrated in many studies (*Focht, 1974; Betlach and Tiedje, 1981; Burton and Beauchamp, 1985*).

Similar results were obtained by *Parkin and Tiedje* (1984). Denitrification rates in their soil cores remained low, less than 2% of anaerobic rate, as long as O_2 concentration in the gas was greater than 3%. At lower O_2 concentrations the

rates increased, and rapidly approached anaerobic rates when the O_2 concentration decreased below 0.5%.

The inverse relationship between denitrification rate and O_2 concentration is more pronounced at high (34.5°C), rather than at low (19°C), temperature (*Focht and Verstraete, 1977*)

In aerobic soils denitrification can occur in anaerobic microsites such as in the center of aggregates (*Parkin, 1987; Horn, 1994*) or in areas of localized high oxygen consumption ("hot spots") which can be associated with the breakdown of particulate organic material (*Parkin, 1987*). Furthermore, some groups of denitrifiers are able to use simultaneously both oxygen and nitrate or nitrite as electron acceptor. Therefore, denitrification by those organisms can occur under aerobic conditions. **"Aerobic denitrification"** can occur in the presence of significant amounts of oxygen. Those denitrifiers are able to simultaneously utilize oxygen and nitrate or nitrite, even when the dissolved oxygen concentration approaches air saturation. An explanation for the usage of both acceptors might be the presence a rate-limiting step in the transfer of electrons from its substrate to oxygen. The provision of a second electron acceptor, in this case nitrate, would allow it to use an additional branch in the electron transport chain (*Robertson and Kuenen, 1990; Robertson and Kuenen, 1991; Zumft and Kroneck, 1990*). In anaerobic respirometry experiments, it was observed that aerobically grown *Thiobacillus pantotropha* began to denitrify immediately when it was supplied with substrate and nitrate. Similarly grown cultures of the other strains required 2 to 4h to induce their denitrifying enzymes (*Robertson and Kuenen, 1984b*). Oxygen and nitrate electrodes were used to monitor the activity of these cultures, and simultaneous nitrate and oxygen removal in *T. pantotropha* suspension was clearly observed (Robertson et al., 1986). When grown in batch cultures with acetate as the substrate, *T. pantotropha* cultures provided with both oxygen (at a dissolved oxygen concentration of 80% air saturation) and nitrate grew more rapidly than similar cultures which had only one electron acceptor (*Robertson and Kuenen, 1984b*).

The presence of anaerobic microsites, particularly within heavy textured clay soils, where gaseous diffusion is slowed or restricted is observed. Nitrous oxide emissions are often high from these soils, especially those with a large proportion of anaerobic microsites (*Burford et al., 1981; McKenney et al., 1980a*). Such microsites exist where root or soil respiration rates exceed the capacity of the soil to allow adequate gaseous diffusion to or from the microsites.

The role of O_2 diffusion in soil for denitrification was described in the model of *Smith* (1980). This model calculates concentrations in soil and describes how O_2 diffuses down the profile and into aggregates, and the fraction of the soil volume that is anaerobic. The diffusion of O_2 into aggregates rather than down the soil profile appears to be the main rate-determining step for denitrification in this

model. Diffusion of NO_3^- from aerobic to anaerobic sites with subsequent reduction in the latter may also occur.

In aerobic soil, denitrification and autotrophic nitrification, each with its associated N_2O production, may occur simultaneously at spatially distinct microsites (*Bouwman, 1990b*). Highest N_2O fluxes are expected under microaerophilic conditions in soil where N_2O reduction to N_2 during denitrification is inhibited by O_2 gas and where nitrifiers are sufficiently limited in O_2 gas supply to also form N_2O (*Klemedtsson et al., 1988b*).

After a heavy rainfall, with the presence of nitrate and suitable carbon sources, significant losses of fixed nitrogen from soil can result from the induction of denitrifiers. In soils and waste water, even if well aerated, anaerobic energy-conserving processes can occur inside aggregates and sewage flocculates in the sequence NO_3^-, MnO_2 and Fe_2O_3 respiration followed by SO_4^{2-} and CO_2 reduction (*Ottow and Glathe 1973*).

Soil water content is a major factor determining the rate of denitrification (*Grundman and Rolston 1987; Myrold 1988*). Highest emissions are often correlated with very wet soil conditions (*Dowdell and Smith, 1974; Benckiser et al. 1986; Mosier et al. 1986; Anderson and Levine, 1987; Murakami and Kumazowa 1987; Mancino et al. 1988; Malhi et al. 1990; K.A. Smith and Arah 1990; Parsons et al. 1991; Groffman and Tiedje 1991; Weier et al. 1993*). Such findings reflect the fact that denitrification is an anaerobic process. Increasing denitrification rate with increasing soil water content seems most marked above about 60% WFPS (water-filled pore space) (*Terry et al. 1981b; Linn and Doran 1984; Aulakh et al. 1984a; Mulvaney and Kurtz 1984; Heinemeyer et al. 1988; Vinther 1984; Nugroho and Kuwatsuka 1992a*).

Denitrification may cease if the soil remains wet for some time, and higher denitrification rates are observed where soils are going through wetting/drying cycles than where soil water content is constantly high (*Mulvaney and Kurtz 1984*). *Groffman and Tiedje* (1988) showed that the rate of denitrification did not depend on water content in a simple manner. They dried intact soil cores and found that denitrification rates decreased markedly when water content declined from flooding to field capacity. With further drying the decline was less rapid. However, when water content was increased from dry conditions, the sharpest increase in rate of denitrification occurred at low water content. Others also found that denitrification rates depend on history of the sample (*Galsworthy and Burford 1978; Letey et al. 1980a*).

The amount of nitrous oxide emitted via denitrification is related to the factors which influence the enzyme production for the several steps in the denitrification sequence. Low pH, high nitrate concentration, low moisture and low availability of

oxidisable organic material all tend to increase the nitrous oxide fraction in the denitrification products (*Arah and Smith, 1990b*). At saturated moisture conditions or under strictly anaerobic conditions (e.g. poorly drained soils and in sediments) N_2-production is favored as the principal gaseous product (*Mulvaney and Kurtz, 1984; Davidson et al., 1986*). With an increase in aeration to an air-filled porosity of about 10%, denitrification and hence the overall gas production (N_2 plus N_2O) declines but the mole fraction of N_2O tends to increase (*Letey et al. 1980b*).

Many studies showed that the reduction of N_2O to N_2 is more prone to inhibition by O_2 than reduction of NO_3^- to N_2O, thus the N_2O/N_2 ratio decreases with decreasing O_2 concentration. Thus, the presence of O_2 reduces the activity and delays the synthesis of nitrous oxide reductase relative to nitrate reductase and nitrite reductase, so that the N_2O/N_2 ratio increases with increasing O_2 concentration (*Focht 1974; Smirnov et al. 1979; Firestone et al. 1980; Betlach and Tiedje 1981; Smith C.J. et al. 1983; Erich and Bekerie 1984; Tiedje 1988; Bonin et al. 1989; Masscheleyn et al. 1993*). The N_2O/N_2 ratio usually decreases with increasing soil water content and tends to be high when the denitrification rate is low (*Murakami and Kumazowa 1987; Rolston et al. 1978; Rolston et al. 1982; Terry et al. 1981b; Aulakh et al. 1984b; Schuster and Conrad 1992; Weier et al. 1993*).

With increasing water content mineralization rate increases and nitrification increasingly produces N_2O. Also, denitrification becomes significant with a high N_2O/N_2 ratio as O_2 diffusion becomes impeded. At high soil water content gas diffusion is severely hindered, denitrification proceeds increasingly towards N_2 and N_2O emission declines. Thus, soil water content where both denitrification and nitrification can proceed will generally give the maximum emission of N_2O. The range of soil water content is normally 45 to 75% WFPS (*Granli and Bøckman 1994*), though *Klemedtsson et al.* (1988b) and *Hansen et al*. (1993) have indicated a higher level. The maximum N_2O emission for denitrifiers or nitrifiers is normally close to FC (field capacity) (*Parton et al. 1988; Klemedtsson et al. 1988b; Schuster and Conrad 1992; Davidson 1992*). Most authors find a strong and positive correlation between N_2O emission and soil water content when either denitrification (*Heinemeyer et al. 1988; Davidson 1992*) or nitrification (*Klemedtsson et al. 1988b; Hutchinson and Brams 1992; Davidson et al. 1993*) is the main N_2O generating process.

The relationship between soil moisture content and N_2O emission rate is also often seen in field studies as an association between corresponding values of N_2O emission and water content obtained over a period of time, e.g season or year, and over a wide range of water content levels (*Folorunso and Rolston 1985; Duxbury and McConnaughey 1986; Parton et al. 1988; Skiba et al. 1992*). This relationship is illustrated by *Mosier et al.* (1981) who found N_2O emission from a native short grass steppe during a summer sampling period to be positively correlated with soil

water content in the upper 5 cm. Emissions were some 10-fold higher at 18 vol-% (36% WFPS) than at 10 vol-% (20% WFPS). *Conrad et al.* (1983) made similar observations at water contents of 10 to 20 weight-%. Maximal N_2O fluxes from soils are reported shortly after irrigation or rainfall (*Conrad et al. 1983; Cates and Keeney 1987b; Hao et al. 1988; Hansen et al. 1993*).

Davidson et al. (1993) studied N_2O emission in a dry tropical forest. Emissions were higher in the wet season than in the dry season, but addition of water to dry soil caused rapid formation of NH_4^+ from mineralization and large pulses of N_2O emission.

Waterlogged conditions are mostly undesirable in agriculture, except for paddy rice. These fields usually emit only small amounts of N_2O while flooded (*Buresh and Austin, 1988*). *Mosier and Hutchinsen* (1981) reported that an irrigated field of maize lost 59% of the seasons loss of N_2O during the week following the first irrigation, when restricted O_2 diffusion favoured denitrification.

The high rates of denitrification that occur when soils pass through wetting/drying cycles also show up as high N_2O emissions (*Patten et al. 1980; Davidson 1992*). When a soil is wetted sufficiently by rain or irrigation water to cause anoxic conditions and to initiate denitrification, N_2O will be produced more rapidly than it is reduced. If the soil dries within 24 to 72 hours, insufficient time will have elapsed for the development of nitrous oxide reductase, thereby preventing N_2O reduction to N_2 (*Letey et al. 1980b; Cates & Keeney 1987b*)

Several workers observed highest nitrous oxide fluxes from soil during fluctuating moisture conditions compared to either continuously well-aerated or continuous anaerobic conditions (*Firestone and Tiedje, 1979; Smith and Tiedje, 1979*).

Firestone and Tiedje (1979) showed that after the onset of anaerobiosis essentially three time periods could be distinguished based upon the response of the native microbial population. In the period from 16 to 33 hours following anaerobiosis, 40 to 90% of the gaseous denitrification product is evolved as N_2O. Initially, NO_3-reductase production is stimulated and enzyme is produced more rapidly than N_2O-reductase. Thus, N_2O accumulates and can be released into the atmosphere. The moisture conditions which seem to favour N_2O production are, therefore, alternating wetting and drying cycles during which both autotrophic nitrification and denitrification are active but where there is not enough time for substantial levels of N_2O-reducatse to form. The large pulses of N_2O which typically follow rainfall or irrigation may exceed background levels by up to 3 orders of magnitude, especially after long periods of dryness (*Conrad et al., 1983; Sherlock and Goh, 1983*).

During fluctuating soil moisture conditions, drying and rewetting cycles may enhance the availability of soil organic matter and this will also favour denitrifica-

tion. Drying causes shrinkage and disruption of soil aggregates and exposes organic matter not previously accessible to microbial attack. In addition, death of part of the microbial biomass during drying releases additional available carbon. As a result, upon rewetting there is a characteristic flush of soil microbial activity (*Patten et al., 1980*).

5.2. Nitrate concentration

Nitrous oxide emission following nitrogenous fertilizer applications to soil tends to follow a similar pattern irrespective of the type or form of fertilizer used (*Sahrawat and Keeney, 1986*). Emission are characterized by a 'large' efflux of N_2O following fertilizer application at rates which may be between 1 and 3 orders of magnitude higher than baseline levels. This period typically continues for 5 to 8 weeks following the fertilizer application and is followed by declining rates of emission which gradually approach baseline levels. The dynamics of these post-fertilization events parallel the presence of free NH_4^+ and NO_3^- within the soil which is directly accessible to the soil microorganisms. Some researches have found nitrous oxide emissions from soil were significantly higher after amendments with nitrifiable-N sources compared to nitrite fertilizer application. This emphasizes that nitrification is an important process for nitrous oxide production under field conditions (*Breitenbeck et al., 1980; Conrad et al., 1983*). Indeed, comparisons of different fertilizer forms show, for the same application rate, that nitrous oxide emission is highest after application of anhydrous ammonia (*Breitenbeck and Bremner, 1986*).

Total denitrification fluxes (N_2O plus N_2) are directly proportional to soil NO_3^- concentrations when the other important component, a readily metabolizable organic substrate, is also present and non rate-limiting. When a lack of metabolizable organic matter limits potential denitrification, N_2 plus N_2O fluxes do not increase with increasing NO_{3-} concentration (*Sahrawat and Keeney, 1986*).

Large emission of N_2O requires that soil inorganic N and organic C supply is adequate (*Mosier and Hutchinsen 1981; Ryden 1983; Ryden and Lund 1980b; Freney et al. 1985; Li et al. 1992a,b*).

Freney et al. (1985) found that emissions increased by 1 to 2 orders of magnitude following heavy irrigation of a field cropped with sunflower and fertilized with urea. Most of the urea had been converted to NO_3^- at the time of the emission measurements.

High emissions associated with rainfall/irrigation are favoured when fertilizer is applied simultaneously with, or soon before, the event (*Mosier and Hutchinson 1981; Webster and Dowdell 1982; Hutchinson and Brams 1992*)

It is well established that an increase in soil or sediment NO_{3-} concentration leads to an increase in the N_2O:N_2 ratio in the product gases. This is attributed to the inhibition of N_2O reductase by NO_{3-} (*Blackmer and Bremner, 1978a; Firestone and Tiedje, 1979; Terry and Tate, 1980; Zumft and Kroneck, 1990*) and, as noted earlier, this effect is further enhanced at low pH.

5.3. Temperature

Water temperature effects all vital processes of organisms and takes one of the main places among abiotic factors of the environment. Temperature is one of the most important ecological factors influencing physical, chemical, biochemical and biological processes which define the ecosystem conditions: possibility of life of various species, self purification, etc. Temperature effects are vivid not only on the living organisms directly but also indirectly with the solubility of oxygen and carbon dioxide, salts, density and viscosity, etc. In some cases these effects can be enhanced or diminished depending on the trophic links and differences in the development of algae, invertebrates and fish. The effects of thermal regime on ichthyofauna development have been given a thorough study. While temperature rises, the toxic effects of toxins, oxygen demand, food requirements increase (*Fashchevsky and Fashchevskaya, 2003*).

A very important factor controlling soil denitrification is temperature. It is not known how climate change would affect the mineralization or the storage of organic carbon in soils (*Van de Geijn and Van Veen, 1993; Post et al., 1982*). Despite the widespread belief that higher global temperatures will increase the rates of microbial decomposition in soils, there are few data that document the magnitude or the duration of this effect in different soil horizons and specific ecosystems (*Kirschbaum, 1995*). Only a few attempts have been to isolate the relationship between temperature and soil respiration (Winkler et al., 1996). Microbial activity varies with temperature. The rate of biological process is negligible below a threshold, but increases rapidly as this is exceeded (*Granli and Bøckman 1994*).

Low rates of denitrification have been reported at temperatures as low as –2^{o}C (*Dorland and Beauchamp, 1991*) *and –4^{o}C* (*Malhi et al. 1990*), but higher temperatures, > 5^{o}C, are usually required for a significant denitrification rate (*Benckiser et al., 1986*). Detailed studies indicate that the nitrous oxide flux follows closely the diurnal temperature pattern from the soil depth where nitrous oxide is produced (*Denmead et al., 1979; Conrad et al., 1983; Goodroad and Keeney, 1984c; Slemr et al., 1984; Mosier 1989*). This diurnal characteristic can be attributed to the changing activation energy of nitrous oxide producing bacteria with temperature, and by temperature induced solubility changes of nitrous oxide in

soil water (*Blackmer et al., 1982; Conrad et al., 1983*). In addition, correlating the nitrous oxide flux with temperature measurements from various soil depths can provide an indication of the site of nitrous oxide production (*Conrad at al., 1983*). Microbial activity, denitrification and nitrification rate, all increase with temperature. The denitrification product ratio (N_2O/N_2) falls with increasing temperature, while that from nitrification (N_2O/NO_3^-) tends to rise. The combined effect is that N_2O emission rates increase with temperature (*Granli, T. and Bøckman, 1994*).

The dependence of decomposition rates on temperature is usually described by the Q_{10}-function, an expansion of the Arrhenius function (*Jørgensen, 1994*). Ecosystem process models which attempt to model the seasonal cycle of terrestrial gas exchange have traditionally used a Q_{10} value (the factor by which the activity is increased when the temperature increases by 10°C) near 2 (*Raich et al., 1991*). A Q_{10} expression is used for soil temperature response function regulating all biological process. A Q_{10} of about 2 is common for biochemical reactions. A Q_{10} greatly different from 2 indicates that other factors than temperature also affect the reaction rates. Such factors can be: physical (e.g. changes in gas solubility in water), biological (changes in microbial populations), both (e.g. increasing temperatures further O_2 consumption, thus extending anaerobic zones (*Smith and Dowdell, 1974*).

Generally, lower temperatures (in particular below 15° C) decrease microbiological activity, increase nitrous oxide solubility and slow down gaseous diffusion, thereby leading to lower nitrous oxide emission (*Goodroad and Keeney, 1984c*). In addition, the overall gaseous N production under denitrification declines with declining temperature. However, the same amount of nitrous oxide may still be emitted due to an increase in the N_2O/N_2 ratio which tends to occur from 25 to < 15° C (*Keeney et al., 1979; Bailey, 1976; Nõmmik, 1956*). This can lead, in temperate climatic zones, to significant proportions of annual N_2O emissions occurring in late autumn and early spring since soils during these periods tend to have low oxygen levels due to water saturation (*Fillery, 1983*). Considerable N_2O emissions also occur from frozen soils at the spring thaw and can constitute as much as 30 - 40 % from the total N_2O produced over the year (*Goodroad and Keeney, 1984a*). Possible explanation for this occurrence include the release of dissolved nitrous oxide production in subsurface soils due to denitrification, nitrification and chemo-denitrification which is subsequently released during the thaw (*Goodroad and Keeney, 1984a; Cates and Keeney, 1987b*). *Włodarczyk et al.,* (2001) studied the influence of temperature on the rate of denitrification and respiration of brown (B) and chernozem (C) soils from A horizon in the laboratory experiment. Their study showed an essential influence of temperature and kind of soil on the rate of denitrification and respiration of soils in the range of 4ºC to 20ºC. The average maximum cumulative efflux of N_2O was 1.7 and 1.2

times higher in the range of 4ºC to 10ºC and from 10ºC to 20ºC, respectively. In the case of respiration of soils, four times higher rate of CO_2 emission in the range of higher temperature (10 – 20ºC) and about twice higher in the lower one (4 – 10ºC) were found. The Q_{10} for the rate of denitrification was 8.4 and 4.2 in the range of temperature of 4 – 14ºC and 10 – 20ºC, respectively, while the Q_{10} for the rate of respiration was 4.2 and 4.5, respectively. An essential difference was found in the Q_{10} value between the chernozem and the brown soils. Generally, the influence of temperature (in the range from 4 – 20ºC) on denitrification process was found to be stronger than on the respiration one of the investigated soils.

6. REDOX POTENTIAL

Oxygen is only sparingly soluble in water and diffuses about 10^4 times more slowly in water than in air. Many organisms inhabiting lakes and flooded soils must survive with relatively low concentrations of oxygen. In some cases, heterotrophic respiration may totally deplete oxygen in wetland environments. For instance, within a few millimetres of depth, the environment of wetland sediments often changes, from one where aerobic metabolism of organic matter (i.e., via the Krebs cycle) is possible, to one where various forms of anaerobic metabolism are required (*Schlesinger, 1997*).

Nutrient cycling in lakes and freshwater wetlands is controlled by reduction potential, informally known as *redox,* and by microbial transformations of nutrient elements that occur under conditions in which O_2 is not always abundant. For example, the availability of phosphorus in lakes differs strongly between the surface waters, which are more or less saturated with atmospheric O_2, and deeper waters in which O_2 may be depleted. Anaerobic microbial processes – denitrification, sulphate reduction, and methanogenesis – are responsible for the release of N_2, H_2S, and CH_4 from wetland sediments. Other anaerobic microbial processes are coupled to changes in the oxidation state of iron and manganese in wetland soils. Anaerobic decomposition is often incomplete, so many wetlands store significant amounts of organic carbon - net ecosystem production – in their sediments (*Schlesinger 1977*). Wet soils contain about 1/3 of all the organic matter stored in soils of the world (*Eswaran et al. 1995*). Vast deposits of coal represent the net ecosystem production of swamps during the Carboniferous Period (*Berner 1984*).

Mathews and Fung (1987) estimate that 3.6% of the world's land area is wetland, but the present area of wetlands has been significantly reduced by human activities during the last 100 years. The unique environment of wetlands and their role in chemical transformations mean that their importance to global biogeochemistry is much greater than their proportional surface area on Earth would

suggest. Recent environmental legislation recognizes the critical importance of wetlands as wildlife habitat and as an arena for biogeochemistry.

6.1. Redox potential – the basics

Just as pH expresses the concentration of H^+ in solution, redox potential is used by chemists to express the tendency of an environment to receive or supply electrons. Oxic environments are said to have a high redox potential because O_2 is available as an electron acceptor. For instance, iron (Fe) oxidizes when it shares the electrons of its outer shell with O_2 to become Fe_2O_3 (rust):

$$4Fe + 3O2 \rightarrow 2Fe2O3 \quad (1)$$

Heterotrophic organisms in oxic environments capitalize on the use of O_2 as a powerful electron acceptor. Electrons are derived from the metabolism of reduced organic compounds that are obtained from the environment and oxidized to CO_2. In eukaryotic cells aerobic respiration occurs in the mitochondria. Every four electrons that flow across an internal membrane of the mitochondria combine with one O_2 and four H^+ to form two molecules of water – with the internal membrane system allowing an especially efficient capture of energy for biochemistry.

The oxidation state of the environment, or redox potential, is determined by the particular suite of chemical species that described a hypothetical situation in which two containers hold iron chloride in different oxidation states Fe^{2+} and Fe^{3+}. The containers are connected by a wire which passes through a voltmeter and ends in inert platinum electrodes that are placed in each solution. A salt bridge allows Cl^- to diffuse between the containers so as to maintain a neutral charge. One might expect that electrons would flow from left to right until an equilibrium was established:

$$Fe^{2+} - e^- \leftrightarrow Fe^{3+} \quad (2)$$

The voltmeter could then be used to measure the current passing between the containers. For this simple system, we would say that the container on the right is an oxidizing environment, because it draws electrons from Fe^{2+}, the more reduced or electron-rich species on the left.

If the container on the right also contains O_2, a greater voltage would be measured by the voltmeter. When oxygen is present, it acts as a powerful electron acceptor, and Fe will precipitate (as Fe^{3+}) in soils and sediments, viz.,

$$4Fe^{3+} + 3O_2 + 12e- \rightarrow 2Fe_2O_3. \quad (3)$$

Thus, Fe^{3+} can accept electrons from more reduced substances, such as Fe^{2+}, but not from O_2, which is more strongly oxidizing. In the absence of strongly oxidizing substances, Fe^{2+} persists in the environment.

Of course, natural environments are not isolated into separate containers, nor do they contain such a simple mixture of constituents. In practice, we measure the redox potential of a natural environment by expressing the disequilibrium of its suite of constituents relative to a standard electrode which contains H_2 gas overlying a solution of known H^+ concentration. We connect the environment to the standard electrode using an inert platinum electrode, which takes on the potential of the environment, without altering the tendency for electrons to move among chemical constituents. When a voltmeter is placed in this circuit, the redox potential (E_h) is measured as the voltage required to prevent the interconversion of H^+ and H_2 at the standard electrode. In practice, a standard hydrogen electrode is difficult to maintain in the field, so investigators often use other reference electrodes that are calibrated against a hydrogen electrode (*Bricker 1982, Faulkner et al. 1989*).

When O_2 is present, it accepts electrons at the platinum electrode:

$$O_2 + 4e^- + 4H^+ \rightarrow 2H_2O \quad (4)$$

The electrons are generated at the hydrogen electrode,

$$2H_2 \leftrightarrow 4H^+ + 4e^-, \quad (5)$$

and the voltmeter records a high voltage or redox potential (e.g., ca. 1100 mV at pH 2.0). Because Eq. 4 is more likely to proceed to the right under acid conditions, a higher redox potential will be found at lower pH, assuming that all other factors are the same. As oxygen is depleted, other constituents, such as Fe^{3+}, may accept electrons, following Eq. 2, but a lower voltage will be recorded. An equimolar solution of Fe^{3+} and Fe^{2+} will have a redox potential of +770 mV relative to the standard electrode, when its pH is 2.0.

The pH of the environment affects the redox potential established by Fe^{3+} and other species. In an anoxic environment at pH 5.0, an equilibrium between Fe^{2+} and Fe^{3+} is found at a redox potential of about +400 mV, with the underlying reaction being

$$Fe^{2+} + 3H_2O \leftrightarrow Fe(OH)_3 + 3H^+ + e^- \quad (6)$$

The equation is more likely to proceed to the right at higher pH, so Fe^{3+} will prevail in neutral and alkaline environments, while Fe^{2+} is most likely to persist in the acid, anoxic waters of peat bogs. Thus, oxidation proceeds more readily, and at lower redox potentials, in neutral or alkaline environments, and various forms

of anaerobic metabolism, such as denitrification, are more likely to occur in acid environments (*Weier and Gilliam 1986*).

Much of the recent work expresses redox in units of pe, which is derived from the equilibrium constant of the oxidation–reduction reaction. For any reaction,

$$\text{oxidized species} + e^- + H^+ \leftrightarrow \text{reduced species}, \tag{7}$$

and the equilibrium constant, K, is determined by

$$\log K = \log(\text{reduced}) - \log(\text{oxidized}) - \log(e^-) - \log(H^+) \tag{8}$$

If we assume the concentration of oxidized and reduced species are equal, then

$$pe + pH = \log K. \tag{9}$$

Here, pe is the negative logarithm of the electron activity ($-\log(e^-)$), and it expresses the energy of electrons in the system (*Bartlett 1986*). Because the sum of pe and pH is constant, if one goes up, the other must decline. When a given reaction occurs at lower pH, it will occur at higher redox potential, expressed as pe. Measurements of redox potential that are expressed as voltage, E_h, can be converted to pe following

$$pe = \frac{E_h}{(RT/F)2.3}, \tag{10}$$

where R is the universal gas constant (1.987 cal $mole^{-1}K^{-1}$), F is Faraday's constant (23.06 kcal V^{-1} $mole^{-1}$), T is temperature in Kelvin, and 2.3 is a constant to convert natural to base-10 logarithms.

Redox potential is also described by Nernst's equation:

$$E = E_0 + \frac{RT}{nF} \ln \frac{[Ox] \cdot [H^+]^m}{[\mathrm{Re}\, d]} \tag{11}$$

where: E_o – standard redox potential, R – universal gas constant, T – temperature in Kelvin, F – Faraday's constant, (Ox) – concentration of oxidized form, (Red) – concentration of reduced form, (H^+) – concentration of hydrogen ions, n – number of exchangeable electrons, m –number of hydrogen ions taking part in the reaction.

Environmental chemists use E_h-pH or pe-pH diagrams to predict the oxidation state of various constituents in natural environments. All diagrams are bounded by two lines. If redox potentials were ever to fall above the upper line, even water would be oxidized. Although the photolysis of water occurs during photosynthesis and by exposure of water vapour to ultraviolet light in the upper atmosphere, we do not normally find such strongly oxidizing conditions in the natural waters at the surface of the Earth. An environment dominated by Cl_2 would be more oxidizing than water, so

as long as the Earth has contained abundant liquid water, any Cl_2 from volcanic emissions has been dissolved in ocean waters as Cl^- (*Bohn et al. 1985*):

$$2Cl2 + 4e- + 4H+ \rightarrow 4HCl \quad (12)$$

$$2H_2O \rightarrow 4H^+ + 4e^- + O_2. \quad (13)$$

Similarly, any conditions above the lower line allow the reaction

$$H_2 + 2OH^- \rightarrow 2H_2O + 2e^-, \quad (14)$$

but the reverse of this reaction – the reduction of water – is also rarely seen in the natural environment. Elemental Na reduces water,

$$2Na + 2H_2O \rightarrow 2Na^+ + 2OH^- + H_2, \quad (15)$$

which is why sodium exists in ionic form at the surface of the Earth (*Bohn et al. 1985*). These boundary conditions vary with pH; E_h decreases by 59 mV with each unit of pH increase, reflecting that oxidation requires a higher redox potential under acid conditions.

Important to biogeochemistry, E_h determines what modes of microbial activity are possible in a given environment.

In most cases, organic matter contributes a large amount of "reducing power" that lowers the redox potential in flooded soils and sediments (*Bartlett 1986*). High concentrations of Fe^{2+} will be found in flooded, low-redox environments, where impeded decomposition leaves undecomposed organic matter in the soil and humic substances impart acidity to the soil solution. Where organic matter is sparse, iron may persist in its oxidized form (Fe^{3+}) even when the soils are flooded (*Couto et al. 1985*). The tendency for iron to precipitate in oxidized form at high redox potential or high pH underlies the use of aeration and liming as techniques for ameliorating lakes that are affected by acid mine drainage.

Soils and sediments that resist changes in their redox potential are said to be highly poised. Conceptually, poise is to redox potential as buffer capacity is to pH (*Bartlett, 1986*). As long as soils are exposed to the atmosphere, they will appear to be highly poised, since O_2 will maintain a high redox potential under nearly all conditions. However, in the absence of O_2, these soils may show a rapid decline in redox potential as various weakly oxidizing constituents (e.g., NO_3^-, Mn^{4+}, Fe^{3+}, and SO_4^{2-}) are reduced. Redox potential will fall less rapidly – there is more poise – when concentrations of Mn^{4+} and Fe^{3+} are high (*Lovley and Phillips 1988b, Achtnich et al. 1995*).

6.2. Redox reactions in natural environments

Few oxic environments have redox potentials of less than +600 mV. A progressive decrease in redox potential occurs when soils are flooded. Redox potential drops as heterotrophic respiration of organic carbon depletes the soil of O_2 (*Callebaut et al. 1982, Megonigal et al. 1993*). The diffusion of oxygen in flooded soils and sediments is so slow that redox potentials also decline rapidly with increasing depth in wetland soils (*Stolzy et al. 1981*). Where organic carbon is abundant, a strong gradient of redox potential may develop in sediments over a depth as short as 2 mm (*Howeler and Bouldin 1971, Sweerts et al. 1991*). The high spatial and temporal variation of redox potential in wetlands accounts for much of the total range of redox potential that has been reported for the surface of the Earth.

The results of many studies suggest that a particular sequence of reactions is expected as progressively lower redox potentials are achieved (Table 1; *Ponnamperuma 1972, Patrick and Jugsujinda 1992, Achtnich et al. 1995, Peters and Conrad 1996*). After O_2 is depleted by aerobic respiration, denitrification begins when the redox falls to +747 mV (at pH 7.0). Denitrifying bacteria use nitrate as an alternative electron acceptor during the oxidation of organic matter. When nitrate is depleted, reduction of Mn^{4+} begins below a redox of +526 mV, followed by reduction of Fe^{3+} at E_h < -47 mV (*Lovley 1995*). Certain types of bacteria (e.g., *Shewanella putrefaciens*) can couple the reduction of Mn and Fe directly to the oxidation of simple organic substances (*Lovley and Phillips 1988a, Nealson and Myers 1992, Caccavo et al. 1992*), but usually these reactions are catalyzed by a suite of coexisting bacteria – with some species using fermentation to obtain metabolic energy, while others oxidize the hydrogen, using Mn^{4+} and Fe^{3+}as electron acceptors (*Lovley and Phillips 1989*), e.g.,

$$C_6H_{12}O_6 \rightarrow CH_3COOH + CH_3CH_2COOH + CO_2 + H_2 \quad (16)$$

$$2Fe^{3+} + H_2 \rightarrow 2Fe^{2+} + 2H^+. \quad (17)$$

In many cases there appears to be some overlap between the zone of denitrification and the zone of Mn reduction in sediments (*Klinkhammer 1980, Kerner 1993*), and most of the microbes in this zone are facultative anaerobes that can tolerate periods of aerobic conditions. There is little overlap between the zone of Mn reduction and that of Fe reduction, because soil bacteria show an enzymatic preference for Mn^{4+}, and Fe^{3+} reduction will not begin until Mn^{4+} is depleted (*Lovley and Phillips 1988b*).

Below the zone of Mn^{4+} reduction, most redox reactions are performed by obligate anaerobes. Our earlier emphasis on the redox state of iron reflects the widespread use of Fe as an index of the transition from oxidizing to strongly reducing conditions.

Iron is a convenient indicator in the field, because oxidized iron is easily recognized in soils by its red color, known as *chroma*, whereas reduced iron is greyish (*Evans and Franzmeier 1988*). Soil layers with reduced iron are called gley.

Obligate anaerobes such as *Clostridium* use the energy derived from fermentation/Fe^{3+} reduction to engage in nitrogen fixation (*Ottow 1971*). Such nitrogen fixation is probably essential to augment the meagre supplies of nitrogen that result from anaerobic mineralization. Below the depth of iron reduction, the redox potential progressively drops to -221 mV, where sulfate reduction commences, and to –244 mV, where methanogenesis occurs (*Lovley and Phillips 1987*). These reactions are performed by obligate anaerobic bacteria, some of which also engage in nitrogen fixation (*Postgate et al. 1988*). A few, highly reducing (E_h < -700 mV at pH 7.0) environments appear to allow the production of phosphine gas (PH_3) by microbial reduction of PO_4^{3-} (*Gassmann and Glindemann 1993, Dévai et al. 1988*).

Table 1. Thermodynamic sequence for reduction of inorganic substances by hydrogen (pH 7, 25°C)

Reaction	E_h (V)	ΔG^b
Reduction of O_2		
$O_2 + 4H^+ + 4e^- \leftrightarrow 2H_2O$	0.812	-29.9
Reduction of NO_3^-		
$NO_3^- + 2H^+ + 2e^- \leftrightarrow NO_2^- + H_2O$	0.747	-28.4
Reduction of Mn^{4+} to Mn^{2+}		
$MnO_2 + 4H^+ + 2e^- \leftrightarrow Mn^{2+} + 2H_2O$	0.526	-23.3
Reduction of Fe^{3+} to Fe^{2+}		
$Fe(OH)_3 + 3H^+ + e^- \leftrightarrow Fe^{2+} + 3H_2O$	-0.047	-10.1
Reduction of SO_4^{2-} to H_2S		
$SO_4^{2-} + 10H^+ + 8e^- \leftrightarrow H_2S + 4H_2O$	-0.221	-5.9
Reduction of CO_2 to CH_4		
$CO_2 + 8H^+ + 8e^- \leftrightarrow CH_4 + 2H_2O$	-0.244	-5.6

[a] Calculated from *Stumm and Morgan* (1981, p.459)

[b] Kcal $mole^{-1}$ per e^-, assuming coupling to the oxidation reaction

¼ CH_2O + ¼ H_2O → ¼ CO_2 + H^+ + e^- and $\Delta G = -RT\ln(K)$

The environment of flooded soils and sediments exists as a dynamic equilibrium that is maintained by the availability of oxygen at the surface and buried organic carbon as a source of reducing power at depth. A declining yield of metabolic energy determines the order of the anaerobic microbial processes (*Stumm and Morgan 1981; Patrick and Jugsujida 1992*). Table 1 shows that the free energy of reaction (ΔG) is greatest for aerobic respiration (-29.9) and least for methanogenesis (-5.6), and at any redox potential the microbial population conducting metabolism with the greatest energy yield will usually out-compete the rest (*Lovley and Klug 1986, Achtnich et al. 1995*). Note that the potential energy

available to aerobic heterotrophs is only slightly greater than that from denitrifiers which often coexist in upland soils (*Carter et al. 1995*). The low energy yield of reactions that occur at lower redox potential accounts for the inefficiency of anaerobes and the preservation of organic carbon in sediments (*Gale and Gilmour 1988, Albers et al. 1995*)

If the surface of a wetland soil is exposed to the air, as might occur with seasonal fluctuations of the water table, the position of each redox reaction will shift downward in the profile (*Megonigal et al. 1993*). Products of previous reduction reactions become substrates for oxidizing bacteria. For example, the total rate of denitrification is enhanced when seasonal periods of aerobic conditions stimulate the mineralization and nitrification of organic nitrogen, which makes NO_3^- more available for denitrifiers when the water level later rises (*Reddy and Patrick 1975, 1976*). In continuously flooded soils, nitrate must diffuse downward from aerobic layers supporting nitrification to anaerobic layers supporting denitrification (*Patrick and Tusneem 1972*). Simultaneously, reduced substances diffuse up from anoxic sediment layers and are reoxidized at the surface (*Sweerts et al. 1991*). Metabolism can combine unexpected couples of elements in redox reactions; *Straub et al*. (1996) found that microbial reduction of nitrate can be coupled to the oxidation of Fe^{2+} in anaerobic sediments.

In studies of soil material, redox potential is used as an index of oxidation, particularly useful in the range of low oxygen concentrations. All nitrogen transformations are accompanied with changes in the redox potential of the soil. The measured value of Eh in soils is the resultant of all redox couples occurring in the soil, creating the so-called mixed potential (*Bohn, 1971; Gliński and Stępniewski, 1984; Stępniewska, 1988; Gliński and Stępniewski, 1985*). The value of this index in the soil depends to a decisive extent on:

- The amount and quality of organic matter – source of electrons
- Oxidized inorganic compounds – electron acceptor
- Activity of microbes enzymatically catalyzing the processes of oxidation and reduction
- Physical conditions of the soil; the most important among those are the water-air relations in the soil and temperature.

As mentioned above, the value of Eh depends, among others, on organic matter, and the rate of the processes of reduction and changes in Eh and pH increases with growing content of organic substance (*Januszek, 1978; Gliński and Stępniewski, 1985*).

The current value of the redox potential provides information on the rate of ongoing processes of oxidation and reduction.

Intense periodic irrigation or flooding of soil causes intensification of redox processes. The intensity of the processes can be estimated on the basis of changes in and current value of the redox potential which is one of the more important indexes used for the description of the status of soil oxygenation (*Yu Tian Ren, 1985; Stępniewska, 1988; Stępniewska et al., 1997*).

In the course of drying, as the former oxygen conditions are restored, the value of redox potential returns to the level from before the flooding (*Gliński and Stępniewski, 1984; Rowell, 1988; Stępniewska, 1988*).

The knowledge of the dynamics of redox changes in soils subjected to irrigation may be helpful for the estimation of the availability of various forms of nitrogen compounds (*Stępniewska, 1988; Włodarczyk, 2000*).

7. WATER POLLUTION

There exists a need, both from the ecological and the sanitary points of view, of reducing the content of nitrogen compounds in waters.

In the face of the existing water deficit, actions are necessary, aimed at the protection of surface and underground water reserves from their contamination with insufficiently purified waste waters.

It is well known that modern treatment plants can remove approximately 90-95% of organic matter and 10-40% of inorganic matter from waste waters. In order to ensure the water quality standards in the existing water bodies, there is a necessity for multiple dilution of wastewaters discharged from treatment plants (by 10-50 times). Efforts to enhance additional treatment to 98-99% lead to increased costs of treatment as much as 2-10 times, and electrical power consumption. However, in some cases the nature itself aids man in maintaining favourable ecological situation by the self purification process in rivers and lakes. One of the most important physical factors of self purification is the hydrodynamic one involving dilution, solution and mixing of pollutants (*Fashchevsky and Fashchevskaya, 2003*).

7.1. Protection of waters from pollution

Not less an important task than admissible regulation and withdrawal of flow is the task of assessment of admissible discharges of wastewaters as from point and non-point sources pollution into water bodies with taking into account the processes of accumulation, bioconcentration (with trophic chains) and self purification of pollutants in river ecosystems (*Fashchevsky and Fashchevskaya, 2003*).

Self purification is effected by many processes occurring in rivers and lakes: 1) Resulting from the biochemical conversion in the water body depth, in suspended loads and in bottom deposits, 2) Resulting from the chemical oxidation by dissolved oxygen and photochemical oxidation, 3) Resulting from the physical and chemical processes such as sorption and desorption, settling, coagulation, etc., 4) Resulting from the biological effects on pollutants through trophic chains (bacterial, animal and vegetation population in water). The intensity of each process is characterized by its self purification coefficient depending on many factors: current velocity, water and air temperature, solar radiation and oxygen content, micro-organisms and invertebrates, aquatic and sub-aquatic vegetation. Apart from the dilution effect, which is conventionally considered to be a self purification factor, biological and physicochemical factors are of great significance in the self purification processes. The self purification rate is dependent on dissolved oxygen content, water and air temperature, ultra-violet radiation, velocities, and sorption and desorption, cationic exchange, microorganisms, and so on. The oxidation process has the greatest effect on organic matter. The kinetics and mechanism of the oxidation reaction of organic compounds in rivers and lakes are determined by the structure of the substances and environmental conditions: pH value, water temperature, ultra-violet radiation, etc. In the case of complex organic compounds in wastewater, two simultaneous processes occur: complex molecule destruction into simpler ones and, vice versa, making up of complex molecules from simpler ones. Organic matter disintegration occurs mainly under the influence of microorganisms. At first, the carbon skeleton of organic compounds undergoes transformation (CO_2 and H_2O) and later nitrogen is oxidized to nitrates and nitrites. It is known that the quantity of organic matter in water, which is capable of oxidation by microflora to carbon dioxide, and water is estimated by complete biochemical oxygen demand (BOD_{com}). The total organic matter content is determined by chemical oxygen demand (COD); for this purpose organic compounds are destructed by complex oxidants (potassium dichromate, potassium iodate, etc.) in acid media at high temperature. Restore-oxidation processes play an important role in self purification of water bodies and formation of some hydroxidants of metals (iron, manganese, etc.). However, the decisive role in self purification of polluted water bodies by metals (copper, zinc, nickel, cobalt, etc.) belongs to sorption processes. Besides, sorption has a great effect on the concentration of organic matter i. e. anion active and non-inorganic (*Fashchevsky and Fashchevskaya, 2003*).

The self purification process of rivers polluted with suspended matters is much slower than in rivers with dissolved pollutants. The character of self purification of water sources from suspended particles depends on the ratio of mineral and organic parts of suspensions. The self purification of water bodies from or-

ganic pollution accumulated in bottom sediments is a very complex process of biochemical oxidation by way of direct oxidation in the contact of solid particles with dissolved oxygen and non-aerobic (without access of oxygen) decomposition of matter (*Fashchevsky and Fashchevskaya, 2003*).

The oxidation process of organic suspension occurs in surface layers, contacting with water, which contains dissolved oxygen, while non-aerobic decomposition of organic substances prevail in deep layers. In addition to the chemical factors of water pollution (by industrial and agricultural discharges), bacterial pollution also contributes to changing the ecological situation of rivers and lakes. As a result, pathogenic bacteria and viruses cause such dangerous diseases as cholera, dysentery, typhus and others. Bacterial self purification of water bodies is determined by dying of microorganisms due to the effects of physical, chemical and biological factors. It should be kept in mind that each type of bacteria is characterized by its own rate of death. According to Fashchevsky (1996), on plain rivers bacteria decrease by over 90% after 60-70 hours, while bacterial pollution is removed in 90-95% at a 5-10 times faster rate in mountain rivers. Thus, in choosing a water intake location, it is necessary to consider the self purification capacity of water sources (*Fashchevsky and Fashchevskaya, 2003*).

8. SOIL POLLUTION

Soil is the fundamental acceptor of wastes generated in nature. Wastes purification utilizing the soil and plants is the result of complex processes - chemical, physical, physicochemical, biochemical – that take place in the aeration and saturation zone. The system constitutes a universal laboratory of matter transformations and energy flux. Soil is the habitat or living environment of underground parts of plants, of bacteria, fungi, and of fauna. As a result of changing environmental conditions, dynamic diurnal and seasonal changes take place in the soil – changes in the biological composition, in the gas composition of the soil air, in the intensity of biochemical, chemical and physical processes, in the content of biogenic substances.

Agricultural chemicals have significantly increased the production and protection of food, feed, and fiber worldwide. However, the fact that pesticide and fertilizer residues can leach from farms and pollute the surface and ground water resources is of great concern. Often the problem arises because excessive loads of contaminants cause microbial bioremediation (biodegradation, biotransformation, or biodetoxification) to become electron donor– or acceptor–limited. In such cases, addition of a suitable electron donor or acceptor is a viable option for efficient bioremediation. Amendment of soil with the soluble or readily available

form of limiting nutrients has been successfully used for several in situ soil decontamination endeavors, especially dealing with organic pollutants (*Al-Hadhrami et al., 1997; Fava and Gioia, 1998*). If such a strategy is applied for remediation of pesticides, herbicides, and fertilizers from agricultural soils, it is of utmost importance to evaluate first its effects on the soil properties that are important to later agricultural use and sustainable development (*Banerji et al., 1997; Haynes and Naidu, 1998; Sort and Alcaniz, 1999a,b*). Fertilizer nitrate that remains in the unsaturated zone at the end of the farming season is the most common contaminant of subsurface ground water (*Zhou et al., 1997*). Several studies have shown that addition of readily available organic carbon has the potential of controlling nitrate contamination of soil and ground water (*Firestone, 1982; Knowles, 1982; Hasselblad and Hallin, 1998; Verstraete and Philips, 1998*). The readily available organic carbon serves as an electron donor, and creates a reduced environment that stimulates denitrification.

The reduced soil environment is more pronounced below the water table, where oxygen supply is low and biological oxygen demand is high (*Buol et al., 1989*), thus enhancing degradation of fertilizer nitrate by indigenous denitrifying bacteria (*Knowles, 1982; Korom, 1992*). However, a reduced environment also causes reduction of redoximorphic compounds, such as iron (Fe) and manganese (Mn) hydroxides (*Cate, 1964; Lidster and Ford, 1981*). Iron compounds are reduced to the ferrous form, which is known to be highly mobile. The ferrous form then is lost from the system if there is a net downward or upward and outward movement of ground water. If the water is then removed from the soil, precipitation or deposition of the dissolved compounds will not occur in the profile. However, if the water table is raised, the reduced mobile forms of Fe and Mn are transported upward in the soil and this movement of Fe and Mn may result in the formation of redoximorphic features in the soil profile (*Anonymous, 1992*). During subsequent desaturation, the reoxidation and precipitation of these compounds in the soil profile results in the development of Fe and Mn coatings on mineral surfaces, and soft masses or hard concretions or nodules are formed. Furthermore, if the reduced Fe remains in the system in the presence of a relatively high level of OM, it can react to form sulfides and related compounds (*Bloomfield, 1952; Jeffery, 1960; Ford, 1971, 1974, 1975*). In this case, sub irrigation water containing a high amount of dissolved organic carbon might lead to mobilization, transportation, and precipitation of Fe and Mn compounds, consequently affecting the agricultural quality of the soil.

8.1. Wastewater purification by soil and plants

The climatic conditions of Poland are conducive to the utilization of wastewaters in agriculture as one of the available purification options. It should be kept in mind that in the course of developing such a utilization project, such meteorological factors as air temperature, precipitations, wind directions and velocity should be taken into consideration.

Weakly loamy sands are among the soils that are the most suitable for wastewater purification. Investment and development costs related to such fields are low, as such soils do not require drainage. Fields located on such soils and irrigated with wastewaters reach full operational efficiency within years from the start of the irrigations.

Light loamy sands have relatively high permeability and are characterized by sorptive capability. They do not require draining, can take high doses of wastewater, can be irrigated the year round and used for growing a variety of crops.

Strongly loamy sands have to be drained when under a heavy load of wastewaters. It can be stated, generally, that the costs of wastewater purification on loamy soils are much higher than on sandy soils where optimum irrigation is harder to apply (*Baran and Turski, 1999*).

Organogenic soils - that cannot be drained - are not suitable for wastewater irrigation, but with appropriate lowering of the ground water table and application of correct techniques of irrigation can be used for the purpose. Worthy of recommendation is the application of wastewater irrigation on much soils and dried peats. Experiments conducted on peat soils showed very good results of wastewater purification and good yields in meadow crop production (Brandyk, 1978).

For decades now, studies have been conducted on the elimination of biogenic compounds, nitrogen included. An important area of the studies is concerned with hydro botanical purification plants that utilize the soil and plants and the activity of a specific type of microflora inhabiting the soil and plant rots (*Błażejewski, 1993; Kalisz, 1993; Kowalik and Obarska-Pempkowiak, 1997; Kowalik and Lewis, 1995; Białkowicz, 1995; Kadlec,1987; Obarska-Pempkowiak, 1991; Reed and Brown, 1992; Richardson* and *Davies, 1987; Wojciechowski, 1995*).

Methods used so far for environmental purification are very costly. They require sophisticated equipment and are often difficult to implement, especially when dealing with relatively slight pollution spread over large areas. Also, their implementation destroys all life activity in the environments purified (*Baker et al., 1995; Cunningham et al., 1995; Salt et al., 1995; Boyajian and Sumner, 1997; Ensley et al., 1997*). Therefore, there is ongoing search for new solutions, more effective and more economical. One of those, and stirring up increasing in-

terest and growing expectations, may be the utilization of plants for the purification of the environment in a process called the phytoremediation. In most general terms, the concept covers all measures consisting in the utilization of plants for the degradation, extraction or stabilization of contaminants (*Cunningham et al., 1996*).

Among other things, plants can extract from the soil, from waters and from the atmosphere organic contaminants, heavy metals, radioactive substances, or excess of salt. With respect to the type of contamination and the manner of its detoxification, several methods of phytoremediation can be distinguished– phytodegradation, volatilization, phytostabilisation, rhizofiltration and phytoextraction. Rhizofiltration can be used for the purification of surface and ground waters, industrial wastewaters, as well as municipal and agricultural wastewaters.

For the treatment of liquid wastes generated by farms, more and more often small waste treatment units are set up, based on cultures of selected species of willow (*Perttu and Kowalik, 1997; Punshon and Dickinson, 1997*), poplars (*Dix et al., 1997*), reeds, cattails, bulrush and other wetland plants and rooted aquatic plants (*Dunbabin and Bowmer, 1992; Kondzielski et al., 1996*). The attractiveness of utilization of willows and poplars (both the species belong to the family *Salicaceae*) as biological filters follows, among other things, from their fast rate of growth, high index of transpiration, capacity to uptake and biodegrade organic contaminants, but also to accumulate heavy metals contained in waste waters. Moreover, those plants are used neither for food nor for fodder, and their wood – used as fuel – can provide a cheap source of energy (*Dix et al, 1997; Perttu and Kowalik, 1997*).

In Poland, the requirements concerning waste waters drained to the ground and to waters are regulated by the Decree of the Minister of Environmental and Natural Resource Protection and of Forestry, dated 5th November, 1991.

The highest permissible values of nitrogen compound indexes are as follows:

- Ammonia nitrogen N-NH_4^+ - 6 mg·dm^{-3}
- Nitrate nitrogen N-NO_3^- - 30 mg·dm^{-3}
- General nitrogen - 30 mg·dm^{-3}

In Poland, root-type waste treatment units began to develop at the end of the seventies of the last century. Soil-plant waste-water treatment units could achieve a high level of importance for the sanitation of rural and recreational areas. In Poland there are about 40 sewage treatment units of this type, while in the United States there are about 150 root-reed waste-water treatment units alone (*Reed and Brown, 1992; Kowalik and Obarska-Pempkowiak, 1997*).

In treatment units of this type, the purification of waste waters is the result of physicochemical processes taking place in the substrate, of the activity of plants, and of the activity of the specific microflora inhabiting the soil and the plant roots (*Kadlec, 1987*). The efficiency of waste water treatment in root-type treatment

units depends on the kind of plants, on the engineering solutions applied, and on the climatic conditions (*Kalisz, 1993; Błażejewski, 1993*).

The basic factors that affect the efficiency of biogene elimination from waste waters include: hydraulic loads, depth of flux zone, time of retention, burden of biogenic compound charges, type of soil, type of plants, methods of treatment unit operation. In the course of studies on natural wastewater treatment plants, the parameter that is most frequently determined is the efficiency of constituent elimination from the water waters treated (*Richardson and Davies, 1987; Reed and Brown, 1992*).

In the period of 1997-1999, at the Institute of Agrophysics in Lublinie, a study was conducted on the processes taking place under the conditions of high-rate irrigation of soil and plants with treated municipal waste waters from the Hajdów treatment plant.

In available literature concerning the utilization of soil and plants in the process of elimination of biogenes from waste waters, there is a notable deficit of data describing the processes taking place in soil solutions at various depths in the soil profile, in conjunction with changes in the redox potential, inherently related to those processes.

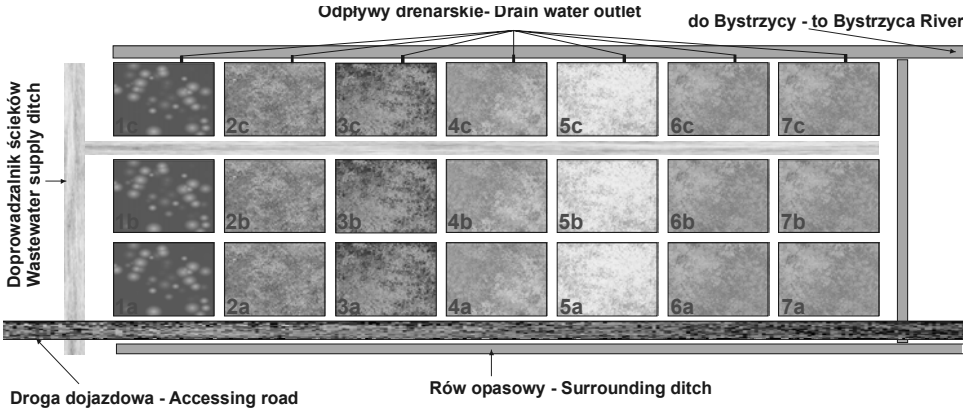
9. OBJECT OF THE STUDY

The study was conducted on an experimental object localized in the valley of the Bystrzyca river on peat-muck and mineral-muck soils. The object was irrigated with municipal waste waters from the city of Lublin, treated at the Hajdów treatment plant. Drainage of the irrigation waste water from individual plots of the object was by means of a system of drains discharging to a drainage ditch. The experiment involved several plant species.

9.1. Description of the object

The object covers the area of approximately 8 hectares. The whole area was divided into seven fields, and each of those into three plots - A, B and C. Irrigation was applied only to plots B (single dosage of waste water, optimum for a given plant) and C (double the optimum dosage), while plots A, not irrigated with waste water, provided the control. All the plots were separated from one another with small dykes. To preclude water infiltration from the sedimentation lagoons of the Hajdów treatment plant and from the Bystrzyca river, the whole object was protected with a belt ditch on two sides – south-west and south-east. On the north-eastern side of the object, the drainage ditch collecting water from the individual

fields doubled as the belt ditch as well. Drainage was applied over the whole area of the object that performed the dewatering function. Drainage waters from the ditch were then directed on to the Bystrzyca river. To the north-west of the object there was a feeder ditch carrying waste waters, so field 1 was exposed to the risk of infiltration from the feeder and of influx of ram ground waters from nearby hills. Waste water used for the irrigation was supplied via a pipeline to the feeder, thence through an inlet to the irrigation furrow running in the dyke system, and then through outlets to the field plots B and C. A schematic diagram of the object is presented below.



Schema 1. Schematic diagram of the object

9.2. Soil conditions

In 1995, in the territory of the Hajdów object, in each of the three plots of the experimental field basic soil pit was made, down to the depth of 1.5 m. Each soil profile was located in plot centre. Samples for detail analyses were taken from the horizons of 0-20 cm, 20-40 cm, 40-60 cm and 60-80 cm.

The soils of the area under study are representative of the division: hydrogenic soils, type: much and moorsh soils, subtype: peat-moorsh and mineral-moorsh soils. In the western part of the experimental field there are soils classified as type: much soils, subtype: peat-muck soils, with organic matter content of 32-36%. In the eastern part of the field, closer to the bed of the Bystrzyca river, there are soils classified as type: moorsh soils, subtype: mineral-moorsh soils with organic matter content of 13.3-17.2%. The layer of peat overlies sand as the mineral substrate. Both soil subtypes were developed from low sedge peat (*Final Report Ref. PBZ-31-03,1998*).

The density of the soil studied was low and characteristic of muck and peat soils with low content of mineral components. The variability of this feature within the experimental field was considerable, the values of density varying from 0.19 to 0.94 $Mg \cdot m^{-3}$.

In most of the soil profiles studied, the total porosity of the soil – as a feature closely related to soil density – fell within the range of high values, in extreme cases exceeding the level of 90.0 $cm^3 \cdot 100\ cm^{-3}$ (*Final Report Ref. PBZ-31-03,1998*).

The values of current soil moisture content obtained at the time sampling can be interpreted as a condition close to the field water capacity; the level of ground water table varied from 45 to 70 cm. Therefore, the conditions prevailing in the soil were conducive to capillary uptake and permitted for water deficit to be compensated for from the ground water table. In this situation, the current soil moisture content was dependent on the level of the ground water table (*Final Report Ref. PBZ-31-03,1998*).

9.3. Vegetation

On the drained plots of the experimental object, cultures of the following plant species were set up:

Field 1 – Poplar (*Popolus nigra and Populus alba*)
Field 2 – Basket willow (*Salix americana*)
Field 3 – Maize (z*ea mays*)
Field 4 – Hemp (*Cannabis sativa*)
Field 5 – Spring rape (*Brassica napus ssp. oleifera*)
Field 6 – Grass mix I
Field 7 – Grass mix II

Components of grass mix I: meadow grass, reed canary grass, reed fescue, meadow fescue, smooth-stalked meadow grass, white bent grass, smooth-stalked bog grass.

Components of grass mix II: meadow grass, meadow fescue, common cocksfoot, timothy grass, smooth-stalked meadow grass, smooth-stalked bog grass, ryegrass.

Discussed here are the results for fields 2 – basket willow and 6 – grass mix II, obtained over the period of 1997-1999, and additionally the results for the field of rape from 1997.

9.4. Characterization of the „Hajdów" waste water treatment plant

The receiving body of water for treated waste water from the „Hajdów" treatment plant is the river Bystrzyca, the second largest tributary of the river Wieprz. The water quality class, from the riverhead to the outlet of the treated

waste water was determined as class II, and below the outlet as class III. The „Hajdów" waste water treatment plant is located in the eastern part of Lublin and treats all municipal and industrial waste waters from the towns of Lublin and Świdnik (but not from WSK PZL-Świdnik). Both the towns have separate sewage systems. Rainwater is drained through the storm sewage system directly to the receiving body of water. The „Hajdów" plant is a mechanical-biological waste water treatment plant. Waste water is treated in successive objects of the technological cycle and, once fully treated, routed directly to the river Bystrzyca.

9.4.1. Chemical characteristics of waste water

In the course of the study, a check was performed to test the quality of treated municipal waste water used in the field experiment. Treated waste water from the „Hajdów" plant, used for irrigation in the experiment, was characterized by low variability of physicochemical parameters (Tab. 2).

Table 2. Physicochemical parameters of treated waste water (Final Report Ref. PBZ-31-03,1998)

Parameter	Unit	Range of values
pH	–	6,47-8,41
ChZT	g $O_2 \cdot m^{-3}$	30,1-56,3
BZT_5	g $O_2 \cdot m^{-3}$	8,3-22,6
$N\text{-}NH_4$	g $N \cdot m^{-3}$	1,1-7,1
$N\text{-}NO_3$	g $N \cdot m^{-3}$	20,2-38,4
N-tot	g $N \cdot m^{-3}$	22,3-43,6
$P\text{-}PO_4$	g $P \cdot m^{-3}$	3,1-6,8
P-tot	g $P \cdot m^{-3}$	3,7-7,0
Na^+	g $Na \cdot m^{-3}$	24,3-69,4
K^+	g $K \cdot m^{-3}$	11,8-27,7
Ca^{2+}	g $Ca \cdot m^{-3}$	59,7-95,2
Mg^{2+}	g $Mg \cdot m^{-3}$	12,6-19,7
SO_4^{2-}	g $SO_4 \cdot m^{-3}$	43,6-116,3
Cl^-	g $Cl \cdot m^{-3}$	67,8-121,6
Zn	mg $Zn \cdot m^{-3}$	18-800
Cu	mg $Cu \cdot m^{-3}$	6-198
Pb	mg $Pb \cdot m^{-3}$	7-96

The parameters of nitrogen and phosphorus concentration in the treated wastewater, important for the process of irrigation, occurred at levels acceptable

under the Water Law Permit issued for the „Hajdów" treatment plant, under which the plant can dump waste water to the river Bystrzyca.

The treated waste water occasionally showed slightly exceeded levels of eutrophic substances, especially total nitrogen, but was fully acceptable for soil irrigation within the confines of the experiment.

For the irrigation of particular plants, the treated waste water was applied at suitable doses:

Field 2 – willow – full single dose - 900 (mm), double dose - 1800 (mm), number of doses – 12,

Field 5 – rape – full single dose - 400 (mm), double dose - 800 (mm), number of doses –10,

Field 6 – grass mix – full single dose - 600 (mm), double dose - 1200 (mm), number of doses –10.

10. METHODS

Chemical analyses of $N\text{-}NO_3^-$, $N\text{-}NO_2^-$, $N\text{-}NH_4^+$ and determinations of redox potential values (Eh) were performed on the following experimental material: soil, irrigation waste water, soil solutions, drainage waters.

10.1. Sampling

Soil solutions were taken from the particular depths (10, 30, 50, 70, 100 cm) by means of ceramic filters installed within the soil profile of each sampling pit. To establish the initial status, analyses of the concentration of mineral forms of nitrogen were made prior to the irrigations, and then, after the sowing/planting, in accordance with the irrigations cycle. Samples taken were transported to the laboratory and stored as per the requirements of the relevant Polish Standards.

For redox potential measurements, three platinum electrodes were permanently fixed at depths of 10, 30, 50, 70, 100 cm in each of the permanent soil pits.

Eluates of drainage waters were taken from the individual outlets. The drainage system covered the whole area of fields from 1 to 7. Two types of drainage outlets were made – one for stepped collectors on the slope, and another for drain pipes with direct discharge to the drainage ditch. In plots B drainage wells were placed, collecting water from plots A and B. A single type of drainage well was adopted for the whole object - type S-1 with a diameter of 100 cm and a height of 200 cm.

10.2. Redox potential measurement

Redox potential measurements were made using a calomel electrode as the reference electrode with constant non-zero potential, well tested and accurately calibrated with relation to the standard hydrogen electrode (*Malicki and Walczak, 1983*). Platinum electrodes were first carefully calibrated in the Michealis buffer (solution with constant redox potential of 176 mV). In the object under study, redox measurements were taken using an Orion Ioanalyzer 404 portable apparatus. Due to temperature variations during the measurements, reference electrode potential corrections were applied, assuming the values of 254, 251, 247 and 244 mV for temperatures of 10, 15, 20 and 25^{o}C, respectively.

10.3. Determination of mineral nitrogen forms

Determination of particular nitrogen forms in the material studied was performed with the help of the FIA-Star 5010 flow-through spectrophotometric analyser made by Foss Tecator:

Determination of N-NO_3^- was made by passing the sample tested through a column filled with copper plated cadmium to reduce nitrates(V) to nitrates(III). Next, sulfanilamide was added to create diazo bonds. Diazo compounds, reacting with N-(1-naphthyl)-ethylenediamine hydrochloride, yield diazo red whose absorbance was measured at wavelength of 540 nm.

Determination of N-NH_4^+ consists in converting ammonium ions into gaseous ammonia. This is done by inserting the sample into the flow of 0.5 molar solution of NaOH. The emitted gaseous ammonia diffuses through the membrane to the indicator flow which is a weak organic acid. A well dissociated salt is formed, whose concentration is proportional to the concentration of ammonium ions in the sample tested. The change in colour is measured at wavelength of 590 nm.

11. RESULTS AND DISCUSSION

11.1. Wastewater from the „Hajdów" treatment plant

Treated wastewater from the „Hajdów" treatment plant, used to irrigate willow, rape and grass mix, among others, are characterized by low variability of basic physicochemical parameters.

Concentrations of nitrogen forms, especially of ammonium and nitrate(V) nitrogen, showed strong differentiation. The concentration of N-NH_4^+ varies within the range of 1.1-7.1 g·m^{-3}, while that of N-NO_3^- varies from 20.2 to 38.4 g·m^{-3} (Tab. 2).

Treated wastewater can supply biogenic substances in amounts equivalent to high-rate fertilization of the soil. The application of the optimum irrigation dose of 600 mm supplied at least 180 kg $N \cdot ha^{-1}$to the soil. In the field plots irrigated with the double dose the available amounts of fertilizer substances were twice as high (*Final Report Ref. PBZ-31-03, 1998*).

11.2. The experimental object prior to experiment setting-up

To characterize the initial status of the experimental object in the aspect of the parameters under analysis and the status of oxidation that were to be studied under flooding conditions, measurements of the redox potential in the soil profile were taken at depths of 10, 30, 50, 70 and 100 cm and analysis was made of concentration of nitrogen ammonium form and nitrates(V) in soil solutions taken from the same depths.

11.2.1. Transformations of the ammonium form

Analysis of average concentration of $N\text{-}NH_4^+$ for the whole analytical period (from April to October) showed significant differences between the upper horizons in the soil profile (10, 30 cm) and the lower ones, i.e. 50-100 cm (Fig. 1). Distinctly lower average concentrations of ammonium ions were found at depths of 10 and 30 cm (0.48 and 0.40 $g \cdot m^{-3}$), which could have been related with the reaction of oxidation of NH_4^+ to nitrate(V) ion in the process of nitrification. Higher concentrations of $N\text{-}NO_3^-$ at depths of 10 and 30 cm seem to support that suggestion (Section 10.2.2).

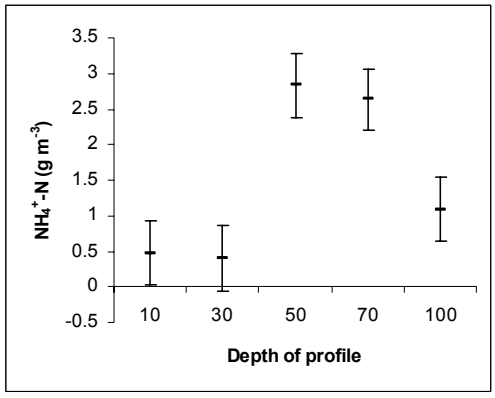


Fig. 1. Average $N\text{-}NH_4^+$ ($g\ m^{-3}$) concentrations in soil solution from spring to autumn 1996 period as a function of soil depth. Bars indicate 95% coincidence intervals

Increase in the concentration of $N\text{-}NH_4^+$ at depths of 50 and 70 cm, where the average values were 2.83 and 2.63 $g \cdot m^{-3}$, respectively, could have been caused by anaerobic dissimilative reduction of NO_3^- where the final product was $N\text{-}NH_4^+$, by retardation of the process of nitrification, and by domination of processes of organic matter mineralization over processes of immobilization of NH_4^+ by microorganisms, whose number – as a rule – drops with increasing depth into the soil profile. When the main product of nitrate reduction is ammonia, the process is called the fermentative reduction of nitrates (*Fenchel and Blackburn, 1997*). The process of dissimilative reduction of NH_4^+ can take place under the same conditions as denitrification proper, and then it becomes a competitor in the utilization of NO_3^- (*Tiedje, 1981*). Generally, it can be stated that the rate of denitrification increases upon the addition of nitrates (*Ambus and Lowrance,1991; Colbourn and Harper, 1987; Robertson et al.,1987; Ryden, 1983; Samson et al.1990; Vinther, 1984*). The lower concentration of ammonium ions at the depth of 100 cm may, as in the case of the depths of 50 and 70 cm, be related to the low biological activity of that area within the soil, as well as to the effect of dilution in ground water.

11.2.2. Transformations of nitrates(V)

At the depths of 50, 70 and 100 cm the soil was characterized by significantly lower concentrations of $N\text{-}NO_3^-$, of 3.7; 5.5 and 1.9 $g \cdot cm^{-3}$, respectively (Fig. 2).

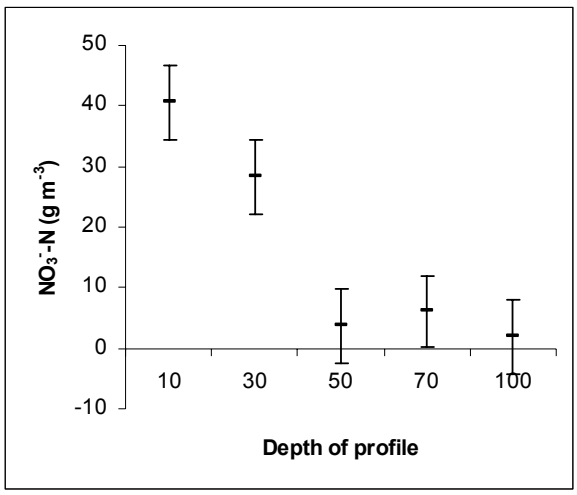


Fig. 2. Average $N\text{-}NO_3^-$ ($g\ m^{-3}$) concentrations in soil solution from spring to autumn 1996 period as a function of soil depth. Bars indicate 95% coincidence intervals

Analysis of variance of the concentration of nitrates(V) in the soil profile showed significant differences between the upper horizons of the profile (10 and 30 cm) and the soil layers at depths from 50 to 100 cm (Fig. 2). The highest concentrations of nitrates(V) were found at the depth of 10 cm (average value of 40.6 $g \cdot cm^{-3}$), somewhat lower at 30 cm (average value of 28.3 $g \cdot cm^{-3}$), i,e, in the zone of best oxidation of the soil and high microbial activity, including that of nitrifiers. The considerable drop in the concentration of $N\text{-}NO_3^-$ at the depths of 50, 70 and 100 cm, to the values of 3.70, 6.07 and 1.93 $g \cdot cm^{-3}$, respectively, could have been related with the dissimilative reduction of nitrates(V) with the involvement of enzymes of denitrification path, when under the conditions of low oxygen concentration there occurs an activation of reductases of the dissimilative path that use NO_3^- as electron acceptors alternative with relation to O_2. In the view of many authors, microbes can use NO_3^- as the principal electron acceptor for obtaining energy from organic compounds when low content of O_2 retards their metabolism; moreover, certain denitrifiers may simultaneously use oxygen and nitrates(V) or nitrates(III) as electron acceptors (oxic denitrification) (*Robertson and Kuenen, 1990; , Zumft and Kuenen, 1990; Robertson and Kuenen, 1991; Granli and Bøckman, 1994; Włodarczyk, 2000*).

11.2.3. Process of nitrogen mineralization

Figure 3 illustrates the process of nitrogen mineralization expressed as the ratio between the average concentration of nitrate(V) ion to ammonium ion. The process was the most intense in the surface horizons of the soil profile, down to 30 cm, with best soil oxidation, where the process of nitrification of ammonium ion was the most intensive. Somewhat higher mineralization of N took place at the depth of 30 cm, which could have been related to slightly higher soil moisture content compared to the surface horizon of soil without vegetation.

Lower biological activity at the three depths from 50 to 100 cm and poorer aeration conditions at the depths of 70 and 100 cm were reflected in much narrower NO_3^-/NH_4^+ ratio as compared to the depths of 10 and 30 cm. This probably resulted from distinctly restricted nitrification and continuous ammonification and was accompanied with increasing concentration of $N\text{-}NH_4^+$ and a decrease of $N\text{-}NO_3^-$, which clearly indicates inhibition of the process of nitrification with ongoing process of decomposition of organic matter in the process of ammonification. Insufficient oxygen diffusion below 50 cm, caused e.g. by higher moisture content, could result in inhibition of nitrification which is a distinctly oxic process. This hypothesis finds supporting evidence in the decrease of the value of redox potential that will be discussed in detail in section 10.5.1). Low value of redox

potential can suggest the beginning of the process of denitrification of NO_3^- ion to N_2O and N_2.

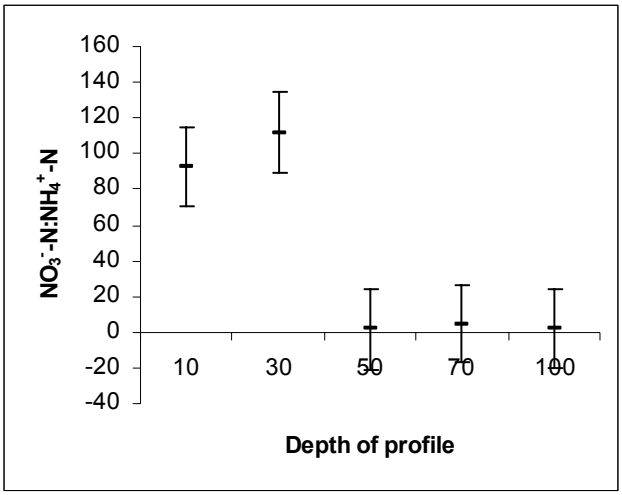


Fig. 3. Average ratios of initial N-NO3– to N-NH4+ concentrations in soil solution of profile at various depths (10, 30, 50, 70 and 100 cm) from spring to autumn 1996 period

11.3. Nitrogen transformations in fields irrigated with wastewater after 2nd stage of treatment

In 1996, following engineering work involved in the adaptation of the object for the experiment, there was a notable decrease in the water filtration index of the soil. Deterioration of the filtration properties of soil affected virtually the whole experimental object, with the exception of soil in the 20-40 cm horizon in plots 5B, 5C and 7A, where an improvement in that respect was observed. The very strong drop in water permeability and its high statistical variation were probably determined by changes in the structure and the physical condition of the soil. There occurred shifting and mixing of the soil material, as well as its considerably drying and oxidation; the natural structure of the soil was destroyed, and the system of soil capillaries was sheared and shifted (*Final Report Ref. PBZ-31-03, 1998*).

Comparative analysis of ammonium form concentration in the soil prior to the setting-up of the experiment and during the first year of the study (Tab. 3 and 4) showed that it dropped by half in 1997 when the average concentration of $N\text{-}NH_4^+$ for the whole profile from three control plots of the field was 0.65 g $N \cdot m^{-3}$, while the initial average concentration of the analysed ion in 1996 equalled 1.43 g $N \cdot m^{-3}$.

Table 3. Average concentrations of nitrogen forms and values of Eh in 1996 in the soil profile at particular depths.

Depth (cm)	$N\text{-}NH_4$ (g m^{-3})	$N\text{-}NO_3$ (g m^{-3})	$N\text{-}NO_3/N\text{-}NH_4$	Eh (mV)
10	0,48	40,59	92,73	418
30	0,40	28,30	111,79	454
50	2,83	3,70	1,48	480
70	2,79	5,51	3,50	222
100	1,09	1,93	1,91	60
Average	1,43	16,0	42,28	327

In 1997 a notable decrease of native nitrates (V) could be observed as compared to 1996 (Tab. 3 and 4) when their average concentration in the profile exceeded 16 g $N \cdot m^{-3}$, while in the first year of the experiment the average concentration of $N\text{-}NO_3^-$ in the control plots (A) for the three plants under analysis was approximately 4.5 g $N \cdot m^{-3}$. Probably, the change in the filtration properties of the soil and in its physical condition resulted in the decrease in the concentration of nitrate(V) form.

Detailed analysis of the transformations of all nitrogen forms was made on the basis of their concentrations for all irrigations made in 1997, after 3 hours, and 2 and 7 days from the application of wastewater on the fields with willow, rape and grass mix, as an effect of their influence on the soil environment.

In parallel to the determination of concentrations of various forms of nitrogen in the soil solution at various depths in the soil profile, analysis was made of the concentration of the ions analysed in drainage waters. The objective of that work was to determine the applicability of the soil and the plants as an additional filter for wastewater purification, and to monitor changes in the concentration of N compounds in time.

Table 4. Average concentrations of nitrogen forms and values of Eh in control plots (A) for particular plants in the first year of the study

	Depth (cm)	$N\text{-}NH_4$ (g m^{-3})	$N\text{-}NO_3$ (g m^{-3})	$N\text{-}NO_3/N\text{-}NH_4$	Eh (mV)
1	2	3	4	5	6
	10	1,89	4,56	2,41	440
	30	0,96	4,05	4,22	460
	50	1,34	6,69	4,99	490
Willow	70	1,26	6,29	4,99	160
	100	0,31	3,15	10,16	-170
	Average	1,15	4,94	5,35	276
	Drainage waters	0,58	2,51	4,33	-

Table 4. Continued

1	2	3	4	5	6
	10	0,18	7,11	39,5	414
	30	0,22	4,04	18,36	396
	50	0,61	3,26	5,34	397
Rape	70	0,31	3,15	10,16	97
	100	0,12	3,01	25,08	96
	Average	0,28	4,11	19,68	280
	Drainage waters	0,41	2,78	6,78	-
	10	0,24	4,9	20,41	491
	30	0,96	7,98	8,31	474
	50	0,88	3,46	3,93	420
Grass mix	70	0,31	2,95	9,51	54
	100	0,16	2,49	15,56	-86
	Average	0,51	4,35	11,54	270
	Drainage waters	0,41	2,33	5,68	-

Additionally, a statistical analysis was made, in which the results from three years of the experiments were used as the basis for the identification of general characteristics and trends in nitrogen transformations and redox potential in organic soil subjected to periodic flooding and drying.

11.3.1. Nitrogen transformations in the field with willow (Field 2)

11.3.1.1. Transformations of ammonium form

1997 year

Average concentration of ammonium nitrogen in the first year of the experiment in the control plot (A) varied from 1.89 to 0,31 g $N \cdot m^{-3}$ at depths of 10 and 100 cm, respectively (Tab. 4), usually decreasing with successive depths down the soil profile. Relatively low concentration of $N\text{-}NH_4^+$ was found at the depth of 30 cm, likely related to the structure of the root system of willow and to more intensive uptake of ammonium nitrogen in that part of the soil profile.

Analysis of ammonium nitrogen concentration in samples of soil solution taken 3 hours after flooding the fields with treated wastewater (Fig. 4) showed a higher value at the depth of 50 cm as compared to 10 and 30 cm. In plot (C) (treated with double dose of wastewater) the concentration of ammonium nitrogen at the depth of 10 cm increased about 4-fold as compared to plots (B). The initial concentration of the ammonium form measured 3 hours after the application of wastewater at the depth of 10 cm varied from 1.23 (plot B) to 4.67 $g \cdot m^{-3}$ (plot C) with average concentration of $N\text{-}NH_4^+$ in the wastewater being ~ 4 $g \cdot m^{-3}$.

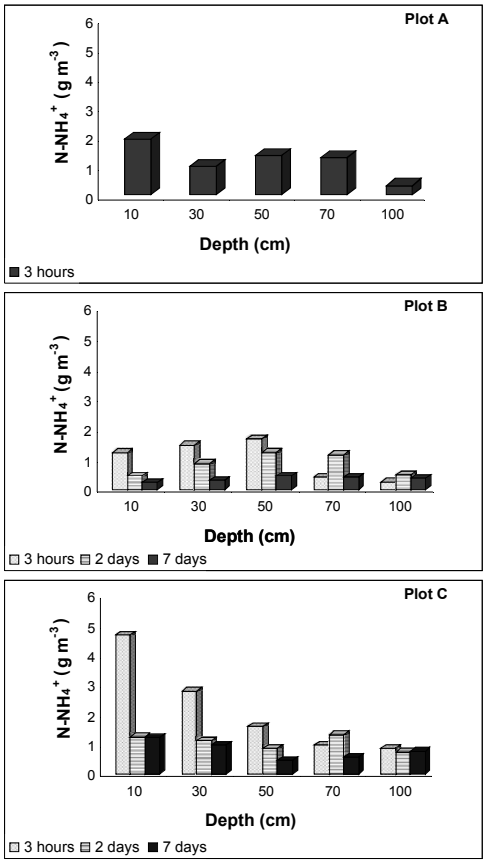


Fig. 4. $N\text{-}NH_4^+$ concentrations (g m^{-3}) in soil solution during 7 days after flooding under willow (field 2) in a control plot A and as dependent on the wastewater dose (single B and double C)

In the field irrigated with the single dose of wastewater, observations after 2 and 7 days showed an untypical distribution of concentration of $N\text{-}NH_4^+$ down to the depth of 50 cm, manifest by an increase caused, perhaps, by increased biological sorption and – partly – by physical sorption in the upper part of the soil profile. A decrease of $N\text{-}NH_4^+$ concentration occurred at the depths of 70 and 100 cm, which could be related to the effect of wastewater dilution with ground waters.

A clear tendency of ammonium nitrogen content to decrease down the soil profile was observed 3 days after the application of wastewater irrigation in plot (C). After the second day from the moment of flooding the differences in concentration between particular depths in the profile were only slight.

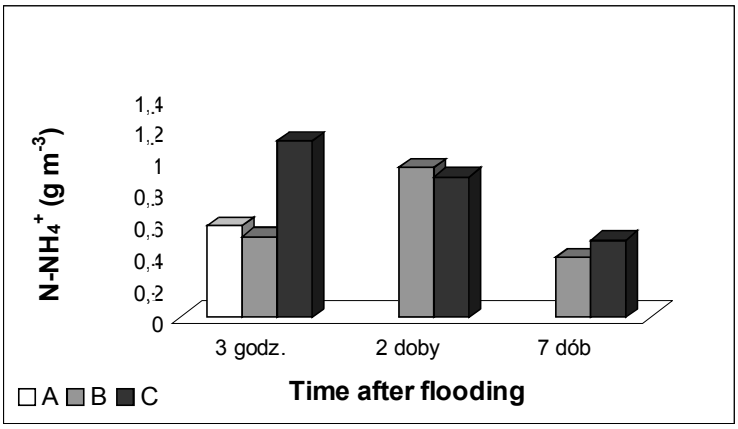


Fig. 5. N-NH4+ concentrations (g m–3) in drainage water during 7 days after flooding under willow (field 2) in a control plot A and as dependent on the wastewater dose (single B and double C)

Figure 5 presents the concentrations of NH_4^+ ion in drainage waters with relation to the time of application and the dosage used. In the case of the field with willow, both the dosage of wastewater and the time of application had an effect on the concentration of ammonium ion in the drainage waters. The highest concentration of $N\text{-}NH_4^+$ in the field with single irrigation dose was observed on day two, while in the case of double dosage already after 3 hours from the moment of application, which means that the time of wastewater filtration in the case of the field with willow is inversely proportional to the dosage. After 7 days from application, the concentration of $N\text{-}NH_4^+$ distinctly drops as compared to day two.

In the case of plot (B), concentration of ammonium ion exceeds the background value only on day two from application of irrigation, while in plot (C) it drops below the value in the control only on day seven. It seems that in the case of the analysed ion the single dose of wastewater does not affect the water environment too drastically. With relation to the amount introduced with the wastewater, the concentration of NH_4^+ decreases from 3 to 10-fold.

1997-1999 years

Figures 6a, b, c, d, e present analysis of variance of the concentration of $N\text{-}NH_4^+$ in field 2 (willow) on the basis of results obtained in the years 1997-1999.

Figure 6 a presents average concentration of ammonium ion with relation to the irrigation dosage. As a result of 3 years of application of wastewater irrigation, in the field with willow an approximately two-fold drop was observed in the concentration of ammonium ion as compared to the control. This indicates a nota-

ble cumulation of NH_4^+ ion in non-irrigated soil, while its concentration in plots B and C remains at a level close to the concentration of N-NH_4^+ introduced from wastewater (~ 4 g N·m^{-3}) as a resultant of N transformations under the conditions of variable availability of oxygen.

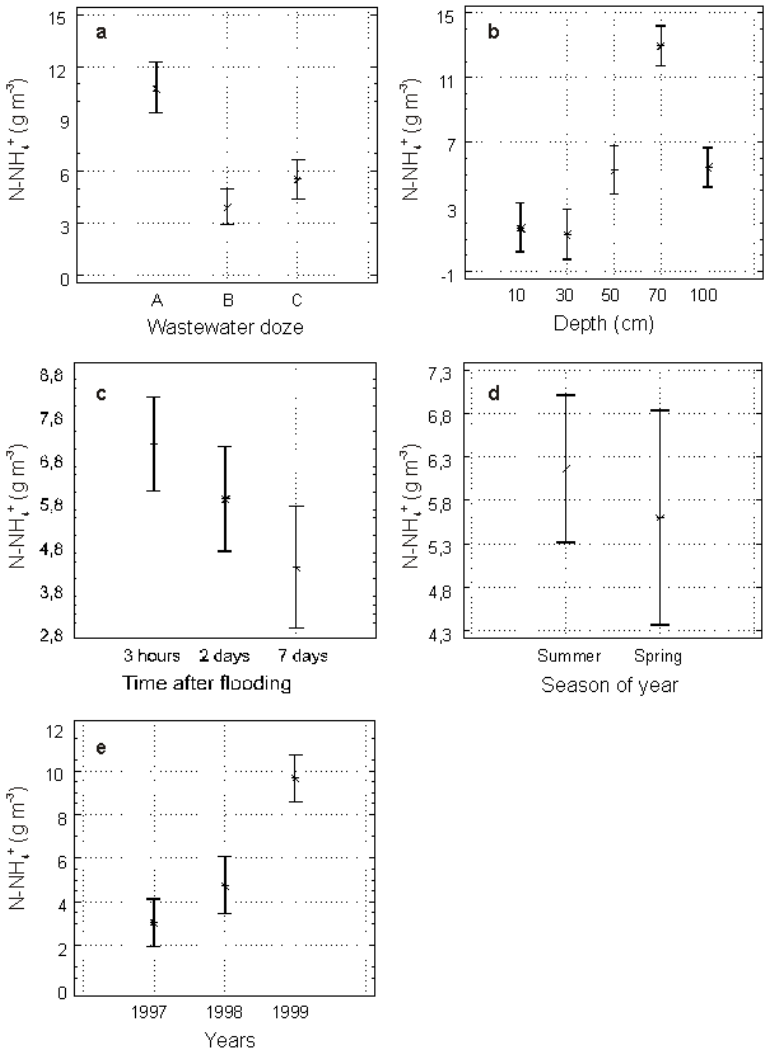


Fig. 6. Average N-NH_4^+ (g m^{-3}) concentration in soil solution under willow (field 2) as dependent on wastewater dose (a), soil depth (b), time after flooding (c), season of the year (d), and years of experiment (e). Bars indicate 95% coincidence intervals

Figure 6 b presents average concentration of $N\text{-}NH_4^+$ with relation to depth, where, unfortunately, from 50 cm an increase in the concentration of ammonium ion is observed, similar to the concentration of the analysed ion brought in with the wastewater. The results obtained indicate that in the field with willow ammonium ion is subject to the most intensive transformations related to biochemical processes and to its uptake by plants down to the depth of 30 cm.

Figure 6c presents average concentration of $N\text{-}NH_4^+$ in the function of time of filtration with a clear downward trend towards the level of concentration in the wastewater, though the trend is not statistically significant. This fact is evidence for ammonium ion utilization by plants and microbes, and for its facility of entering into biochemical reactions.

Figure 6d presents concentration of $N\text{-}NH_4^+$ with relation to the season of the year, i.e. conditions related to temperature, moisture, plant development phase, biological activity of the soil. A certain slight cumulation of ammonium ion was observed in the summer season, indicating a slowing-down of biochemical processes involving $N\text{-}NH_4^+$.

Figure 6e presents the effect of time on the concentration of ammonium ion. Data shown indicate a clear tendency for the concentration of $N\text{-}NH_4^+$ to increase in annual periods, especially between 1998 and 1999 when the concentration of ammonium ion increased over two-fold, mainly as a result of wastewater application.

11.3.1.2.Transformations of nitrate(V) form

1997 year

Figure 7 presents concentrations of nitrates(V) in the control plot (A) and in plots (B and C) in the period from 3 hours till 7 days after the application of irrigation under willow. A slight accumulation of nitrates(V) in soil solution from the control plot was observed at the depths of 50 and 70 cm, which may be related to lower biological sorption of that part of the soil. Average concentrations of $N\text{-}NO_3^-$ in the soil profile are approximately 5 $g \cdot m^{-3}$, with the highest concentration recorded at the depth of 50 cm, equal to 6.69 $g \cdot m^{-3}$ (Tab. 4).

The introduction of wastewater distinctly modifies the chemical conditions of the habitat of plants and microbes. Average concentration of nitrate(V) form introduced to the soil with the wastewater was about 29 $g \cdot m^{-3}$.

Concentrations of $N\text{-}NO_3^-$ in soil solution similar to those in the wastewater were found, three hours after application, in plot (B) at the depth of 10 cm, and somewhat lower in plot (C). With increasing depth and the passage of time from wastewater application the concentration of nitrates(V) decreases.

Figure 8 presents the dynamics of transformation of various forms of nitrogen, expressed by the NO_3^-/NH_4^+ ratio.

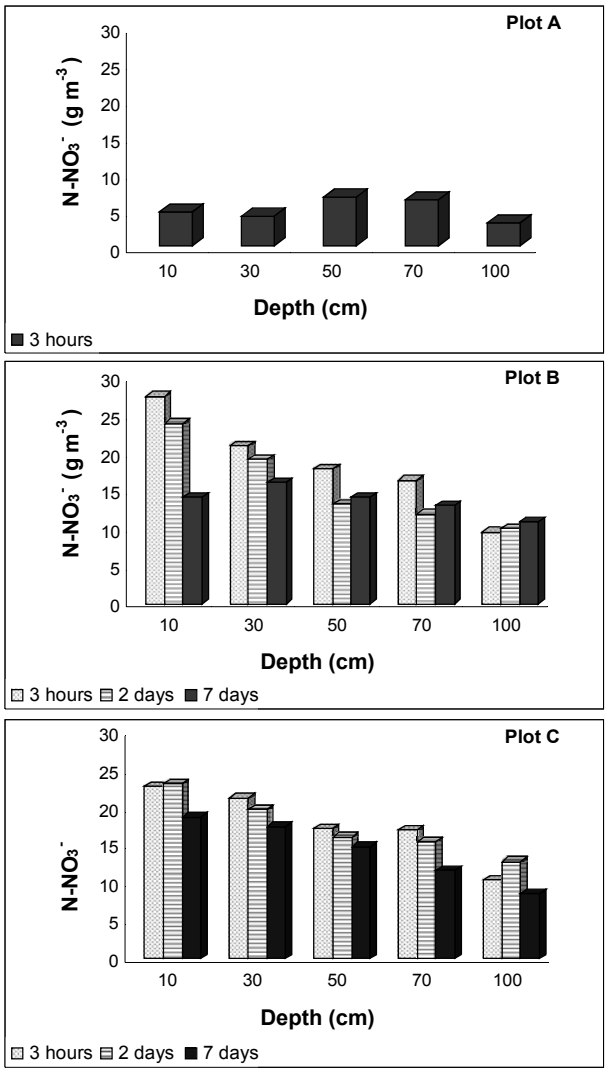


Fig. 7. $N\text{-}NO_3^-$ concentrations (g m^{-3}) in soil solution during 7 days after flooding under willow (field 2) in a control plot A and as dependent on the wastewater dose (single B and double C) and soil depth

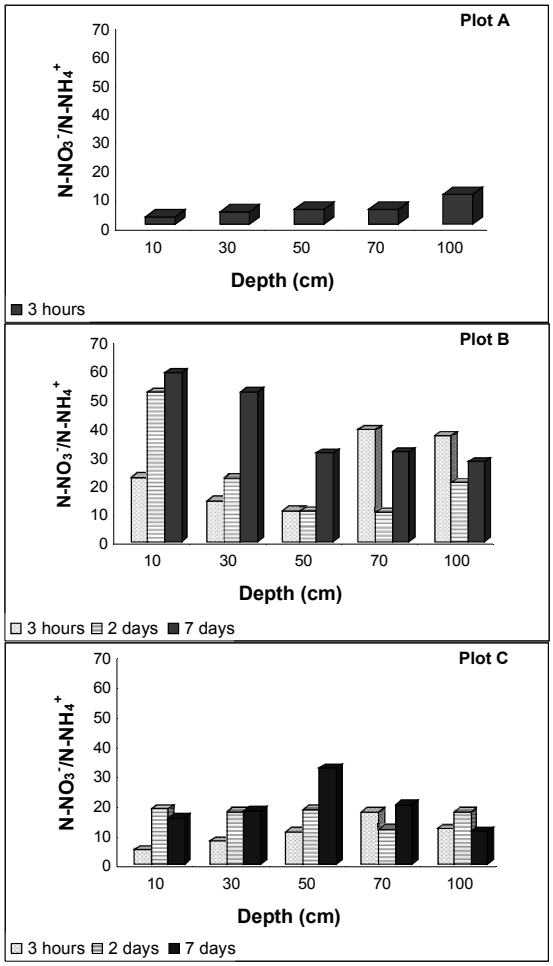


Fig. 8. Ratios of $N\text{-}NO_3^-$ to $N\text{-}NH_4^{+-}$ concentrations (g m^{-3}) in soil solution during 7 days after flooding under willow (field 2) in a control plot A and as dependent on the wastewater dose (single B and double C) and soil depth

Under the conditions of the control plot of the field, the NO_3^-/NH_4^+ ratio is relatively low and its maximum value exceeds 10 only by a small margin. The slightly higher value of the NO_3^-/NH_4^+ ratio at the depth of 100 cm may result not so much form the intensity of biological processes as from physical sorption of NH_4^+ on soil colloids of the mineral part of the soil, clearly observable in that part of the profile as opposed to the organic soil dominant in the upper horizons of the profile.

The strongest intensity of nitrogen transformations was observed in plot (B), with a notable slowing-down of the processes of nitrogen mineralization in plot (C). The double dose of wastewater causes unfavourable changes to the aeration conditions in the soil, and those have a special importance in the process of nitrification. This is clearly reflected in the greater dynamics of N transformations in plot (B). With the passage of time, the column of wastewater traversing the soil profile causes reoxidation of the soil, a process that is manifested in amplification of processes of oxidation of NH_4^+ to NO_3^-, especially intensive at the depth of 10 cm after days 2 and 7. At the other depths this phenomenon can be observed after 7 days. Similar tendencies were also observed in plot (C), though they were expressed by a clearly narrower NO_3^-/NH_4^+ ratio.

Figure 9 illustrates the concentration of $N\text{-}NO_3^-$ in drainage waters with relation to the time of filtration and irrigation dosage in the field with willow. The highest concentrations of nitrates(V) in drainage waters were found after 3 hours from wastewater application, which provides supporting evidence for the mobility of the ion in the soil profile. On subsequent days of analysis, the concentration of the ion decreased slightly. The dosage of wastewater had practically no greater influence on the concentration of $N\text{-}NO_3^-$ in drainage waters.

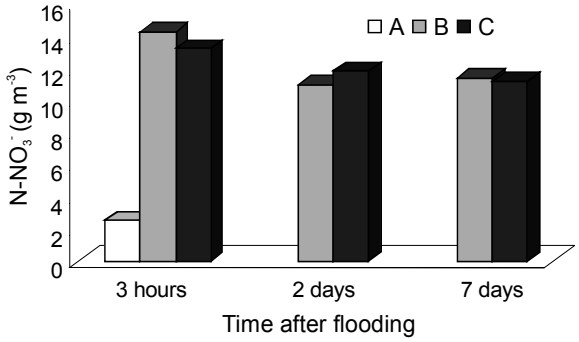


Fig. 9. $N\text{-}NO_3^-$ concentrations (g m^{-3}) in drainage water during 7 days after flooding under willow (field 2) in a control plot A and as dependent on the wastewater dose (single B and double C)

The high concentration of nitrates(V), several times higher than in the control object (over 4-fold), persists for a period of 7 days from the moment of wastewater application. However, it never exceeded the value of 15 g $N \cdot m^{-3}$ i.e. the limit value accepted in the European Union. With relation to the amount of

N introduced from the wastewater, the concentration of $N\text{-}NO_3^-$ decreased on average by a factor of 2 to 2.5.

1997-1999 years

Figures 10a, b, c, d, e present analysis of variance of the concentration of $N\text{-}NO_3^-$ in field 2 (willow) on the basis of results obtained in the years 1997-1999.

Data in figure 10 a show that the average concentration of nitrate (V) ion, much more mobile than the NH_4^+ ion, increased in plots (B and C) with relation to plot (A) due to the application of wastewater. It should also be mentioned that there was a slight increase in the concentration of $N\text{-}NO_3^-$ ion also in the control plot, as compared to the 1st year of the experiment (1997), from 4.94 g $N \cdot m^{-3}$ (Tab. 4) to 6.27 g $N \cdot m^{-3}$ – the average value of nitrates(V) concentration for three years of the experiment. This could have been caused, among other things, by an improvement of the physical properties of the soil with respect to 1997 when the deteriorating effect of engineering work on $N\text{-}NO_3^-$ transformations could be the strongest. The average maximum concentration of nitrate ion observed in plot (C) – 10,16 g $N \cdot m^{-3}$, was lower by almost 20 g $N \cdot m^{-3}$ than the load of nitrates(V) introduced with the wastewater (about 29 g $N \cdot m^{-3}$), which shows that the ion was subject to strong transformations under the conditions of the field with willow.

Figure 10 b shows a clear decreasing tendency of the concentration of nitrate(V) ion with increasing depth. This relation supports earlier suggestions that higher concentration of $N\text{-}NO_3^-$ in the surface horizon of the soil may be strongly affected by the process of nitrification whose intensity decreases with increasing depth, which – in the case of field 2 – entails simultaneous decrease in the concentration of the NH_4^+ ion (Fig. 6b) at 30 cm. The lower concentration of nitrate(V) ion below the depth of 70 cm, in spite of its high mobility, may be related to the effect of dilution by ground waters.

Analysis of the average concentration of nitrate ion with relation to the duration of wastewater effect (Fig. 10c) shows a decrease in the concentration of $N\text{-}NO_3^-$ between days 0 and 2, and a significant increase up to day 7 of the experiment. Probably, the increase is related to an improvement in the air-water relations in the soil and intensification of the process of nitrification.

Figure 10d presents average concentration of $N\text{-}NO_3^-$ in the function of the season of the year which, when considered against the concentration of $N\text{-}NH_4^+$ (Fig. 6 d), shows that the higher concentration of nitrates(V) in spring could be related with an intensification of the process of nitrification of ammonium ion in that period.

Figure 10 e indicates a distinct accumulation of $N\text{-}NO_3^-$ ion in the soil solution in 1998, which may suggest an effect of climatic conditions in comparison to 1997 and 1999.

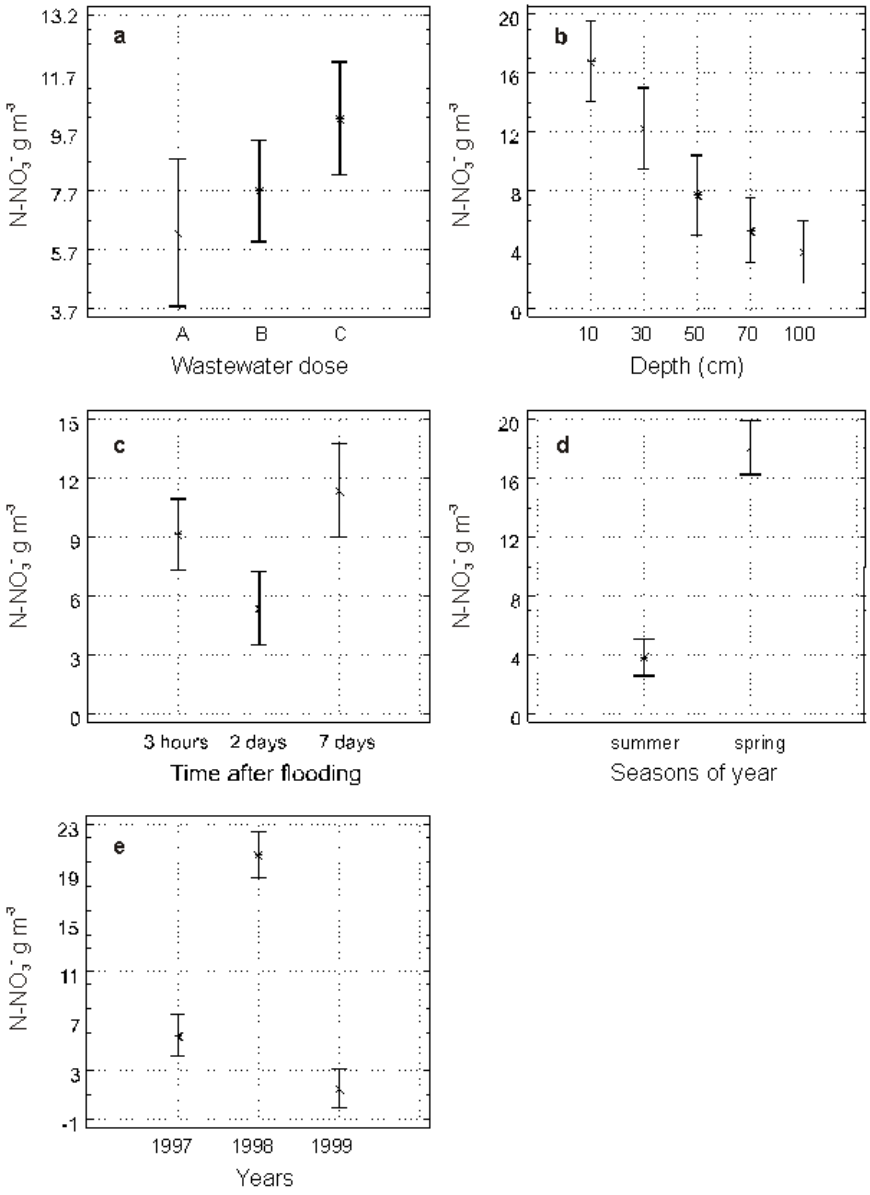


Fig. 10. Average $N\text{-}NO_3^-$ ($g \cdot m^{-3}$) concentration in soil solution under willow (field 2) as dependent on wastewater dose (a), soil depth (b), time after flooding (c), season of the year (d), and years of experiment (e). Bars indicate 95% coincidence intervals

11.3.2. Nitrogen transformations in the field with rape (Field 5)

11.3.2.1. Transformations of the ammonium form

1997 year

In the first year of the experiment, average concentration of ammonium nitrogen in the field with rape not irrigated with wastewater was low and only slightly exceeded the value of 0.61 g $N \cdot m^3$ at the depth of 50 cm, decreasing to 0.12 g $N \cdot m^3$ at the depth of 100 cm (Tab. 4).

Analysis of $N\text{-}NH_4^+$ concentration in soil solution after 3 hours from wastewater application showed the highest value at the depth of 10 cm in plots (B) and (C) alike (Fig. 11).

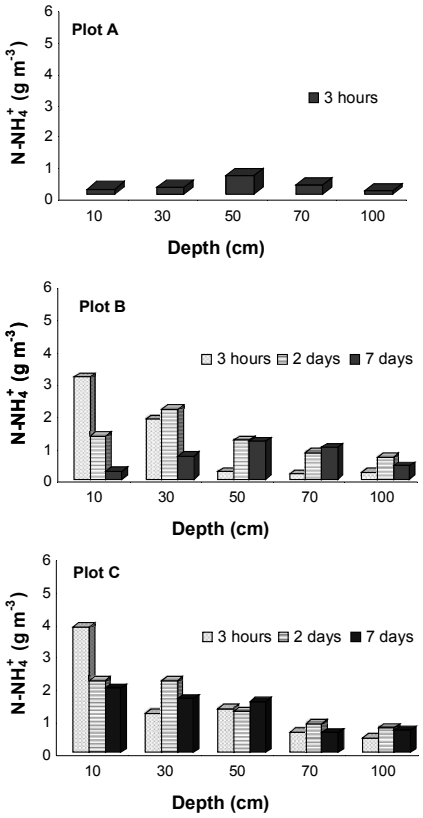


Fig. 11. $N\text{-}NH_4^+$ concentrations (g m^{-3}) in soil solution during 7 days after flooding under rape (field 5) in a control plot A and as dependent on the wastewater dose (single B and double C)

A slightly higher concentration of ammonium form at the beginning was found in plot (C). In the other horizons of the soil profile the concentration of N-NH_4^+ decreased with depth. The initial concentration of ammonium form in plots (B and C) varied from 3.16 to 3.87 g $N \cdot m^{-3}$, respectively.

On the remaining analytical days, the dynamics of N-NH_4^+ concentration in plot (B) was clearly influenced by the time necessary for the wastewater to penetrate down the profile. Systematic decrease in the concentration of ammonium form was observed only at the depth of 10 cm. In all other cases, on day two from irrigation there was an increase in the concentration as compared to the initial day. With the double dose of wastewater, where the process of filtration could take place within a shorter time, the phenomenon was observed only down to the depth of 30 cm.

Figure 12 presents the concentration of N-NH_4^+ ion in drainage waters with relation to the dosage of wastewater and to the duration of its effect. In the case of the field with rape, there was a notable effect of the dosage of wastewater on the concentration of ammonium ion in drainage waters. With the single dose of wastewater, the concentration of the ion analysed increased up to day 7, which was probably related to the filtration properties of the soil. With the double dose, the concentration of N-NH_4^+ reached its maximum already on day two. The amount of N-NH_4^+ with relation to the native ammonium form increases more than two-fold, and the period of 7 days is insufficient for the concentration to return to the initial level. However, the concentration of N-NH_4^+ drops by a factor of 4 to 5 with relation to the amount introduced with the wastewater.

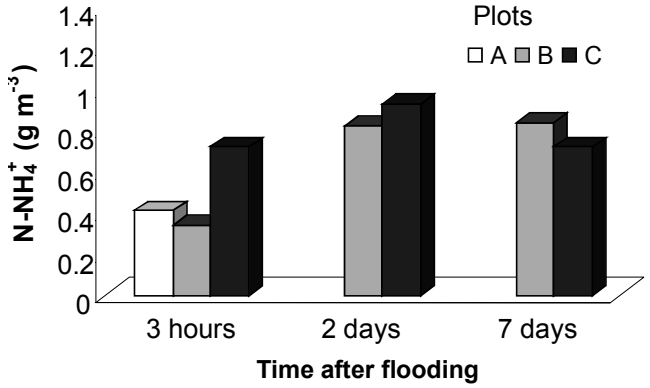


Fig. 12. N-NH_4^+ concentrations (g m^{-3}) in drainage water during 7 days after flooding under rape (field 5) in a control plot A and as dependent on the wastewater dose (single B and double C)

11.3.2.2. Transformations of nitrate(V) form

Figure 13 illustrates the distribution of $N\text{-}NO_3^-$ concentration within the whole soil profile with relation to the irrigation dosage applied and to the days of irrigation. The highest concentration of nitrates(V) in the control plot was found at the depth of 10 cm (7.11 g $N \cdot m^{-3}$), and the lowest at the depth of 100 cm (3.01 g $N \cdot m^{-3}$), with a clear tendency to decrease with depth (Tab. 4).

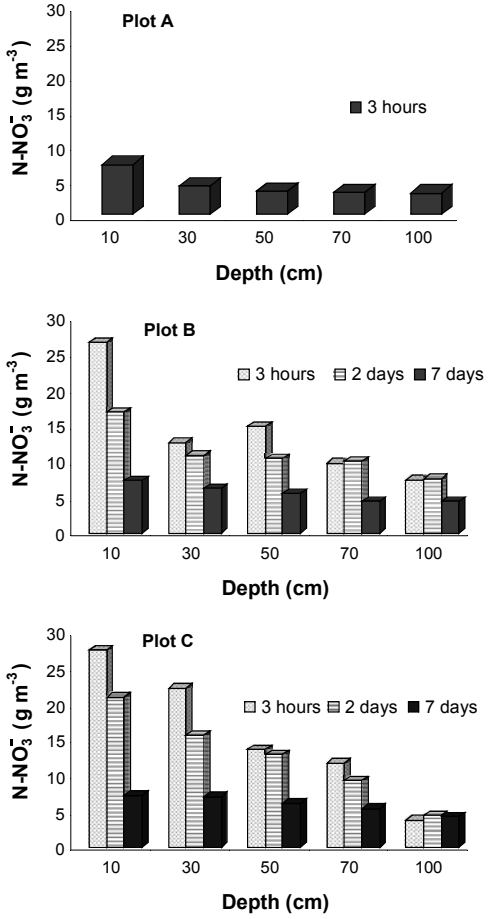


Fig. 13. $N\text{-}NO_3^-$ concentrations (g m^{-3}) in soil solution during 7 days after flooding under rape (field 5) in a control plot A and as dependent on the wastewater dose (single B and double C)

The addition of wastewater to the soil in the field with rape had a similar effect on the dynamic of nitrate(V) nitrogen transformations. The initial concentration of $N\text{-}NO_3^-$ for both irrigated plots was about 27 g $N \cdot m^{-3}$ and decreased with time and with depth, with a slightly higher dynamics of changes in plot (B) (Fig. 13).

Figure 14 presents the concentration of $N\text{-}NO_3^-$ in drainage waters from the field with rape, with relation to the irrigation dosage applied and to the day of irrigation. The mobile nitrate(V) ions reached their maximum concentration in the drainage waters after three hours from wastewater application. Their concentration decreased with time and on day 7 reached a value equal to less than half of the initial concentration. Analysis of the data shows that the concentration of the ion in question in the drainage waters increased maximally by almost a factor of 5 (2 days after irrigation), on day 7, however, reaching a concentration less than double that of the control. No clear effect was observed on the irrigation dosage on the concentration of nitrate(V) ion in the drainage waters. Considering the average concentration of $N\text{-}NO_3^-$ in the wastewater, its content in the drainage water decreased by a factor of from 2 to 5.

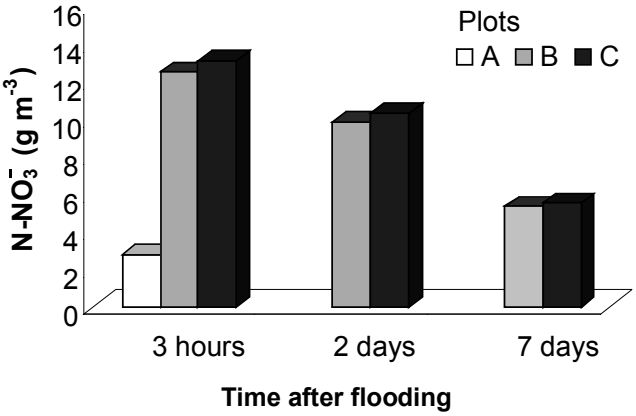


Fig. 14. $N\text{-}NO_3^-$ concentrations (g m^{-3}) in drainage water during 7 days after flooding under rape (field 5) in a control plot A and as dependent on the wastewater dose (single B and double C)

The dynamics and direction of nitrogen transformations are presented in figure 15. The value of the NO_3^-/NH_4^+ ratio in the control plot decreases down to the depth of 50 cm, which may be related to changes in the aeration conditions in the soil and to the slower oxidation of the ammonium form to NO_3^- in the process of nitrification. The high ratio of NO_3^-/NH_4^+ in the soil layer under analysis (down to 30 cm) may result from easier biological sorption of ammonium rather than ni-

trate form, as in the uptake of the latter additional energy is necessary for the reduction of the nitrate ion to the reduced form.

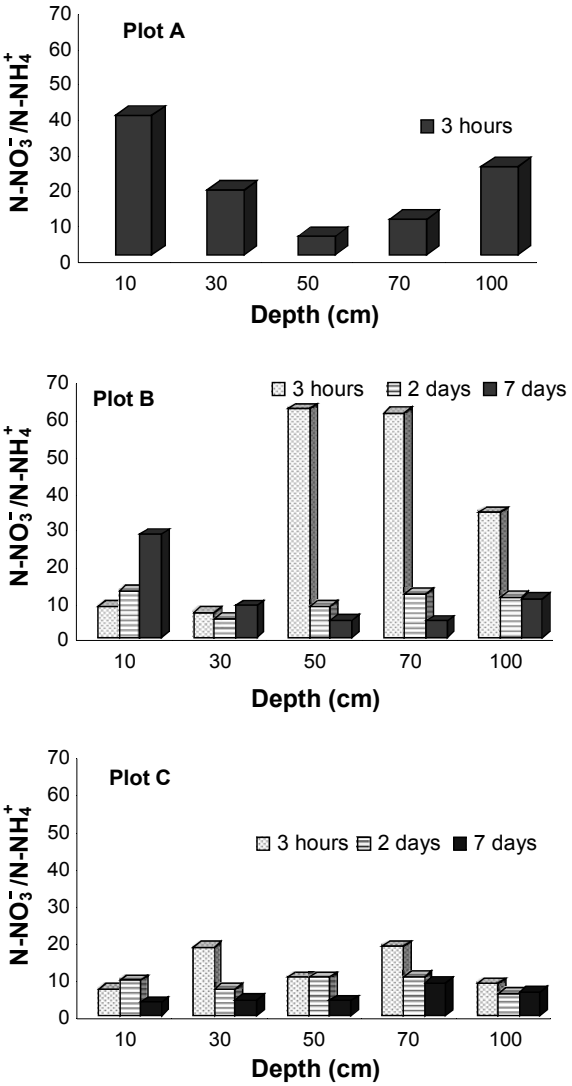


Fig. 15. Ratios of $N\text{-}NO_3^-$ to $N\text{-}NH_4^+$ concentrations (g m^{-3}) in soil solution during 7 days after flooding under rape (field 5) in a control plot A and as dependent on the wastewater dose (single B and double C)

Therefore, on the one hand, good oxidation is conducive to ammonium ion nitrification, and on the other facilitates its utilization by plants and microbes for the formation of proteins. But the increase of the ratio from the depth of 70 cm is

probably related to increased sorption of NH_4^+ on soil colloids and to lower microbial activity (weaker proteolysis and ammonification), and to the lower mobility of ammonium ion in the soil profile with precipitation waters as opposed to the nitrate(V) ion, characterized by very high mobility.

Introduction into the soil environment of excessive amounts of water in the form of wastewater radically modifies the habitat of plants and microbes. Much greater disproportions in favour of nitrate(V) ion were noted in plot (B) as compared to plot (C) where the ratio was more balanced irrespective of depth or the factor of time. Increasing ratio ofNO_3^-/NH_4^+ with the passage of time in plot (B) down to the depth of 10 cm indicates an improvement in oxidation conditions with lowering wastewater table in the soil profile. This type of a tendency is observable only to a slight degree at the depth of 30 cm. The drastic drop in the content of the ammonium form to the depth of 50 cm (13-fold as compared to 10 cm) and only two-fold decrease in the content of the nitrate(V) form get reflected in the very high value of the NO_3^-/NH_4^+ ratio. A similar phenomenon, though on a lesser scale, was also observed in the case of the field with willow. Poor oxidation conditions suggest that the high value of the ratio cannot result from ammonium form transformations in the process of nitrification, but rather from the different susceptibility to sorption and the different mobility of the ions under analysis. The ratio narrows noticeably on day two from the irrigation with wastewater, and assumes a value close to the average value of the NO_3^-/NH_4^+ ratio in the wastewater (7,25). This suggests that there occurred the process of saturation of the sorptive complex with NH_4^+ ion. With the double dosage of wastewater (plot C), the process can proceed much faster, which is expressed by a higher ratio between the oxidized form of nitrogen to the reduced form.

11.3.3. Nitrogen transformations in the field with grass mix I (Field 6)

11.3.3.1. Transformations of the ammonium form

1997 year

The highest average concentration of ammonium form in the first year of the experiment on the control plot was found at the depth of 30 cm (0.96 g $N \cdot m^{-3}$), and the lowest at 100 cm (0.16 g $N \cdot m^{-3}$) (Tab. 4). Distinctly lower concentration of ammonium ion in the surface part of the soil profile (10 cm) could be related to more intensive uptake of ammonium N by the root system of the grasses (Fig. 16).

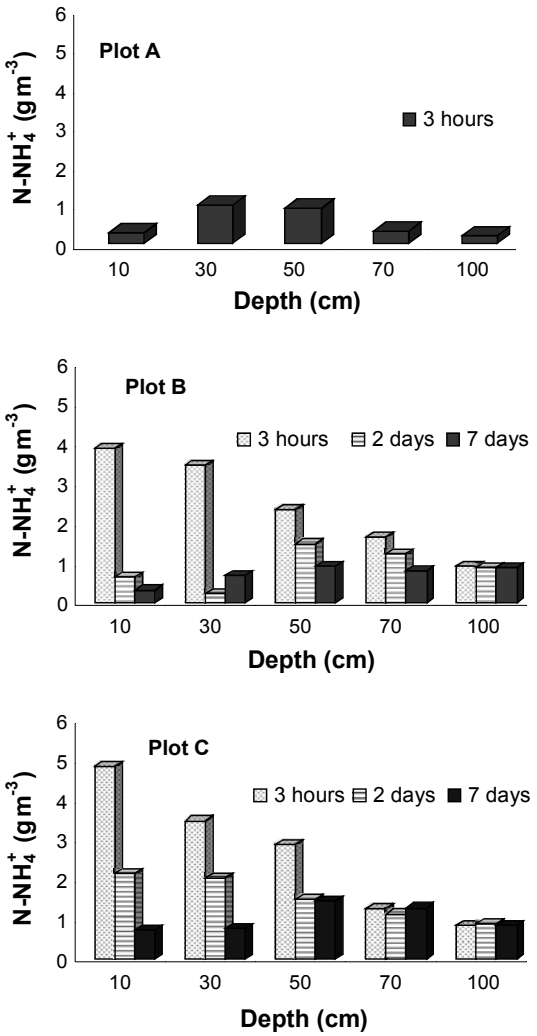


Fig. 16. N-NH_4^+ concentrations (g m^{-3}) in soil solution during 7 days after flooding under grass mixture (field 6) in a control plot A and as dependent on the wastewater dose (single B and double C)

Wastewater irrigation in the single and double doses has its reflection in the content of the ion analysed in soil solutions taken from plots (B and C). The addition of wastewater caused an increase in the concentration of ammonium ion to 3,87 and 4,82 g $N \cdot m^{-3}$, respectively for plots (B and C) for the depth of 10 cm, i.e. almost four- and five-fold as much as the native concentration of ammonium form in the control plot. A somewhat lower increase was observed at depths below 10 cm in the soil profile. In the initial stage of wastewater effect on the soil and the

grasses only a slightly higher concentration of NH_4^+ was observed at the depth of 10 cm; at the other depths the concentration of ammonium ion was very much similar in both plots. Considerably greater differences in the concentration of the ion under analysis were observed on the second day from flooding, especially at depths down to 30 cm.

This supports the earlier supposition that the decrease in the concentration of ammonium ion at the lower dose of wastewater results, among other things, from the process of nitrification, its fixing in the sorptive complex of the soil, and immobilisation by plants and microbes.

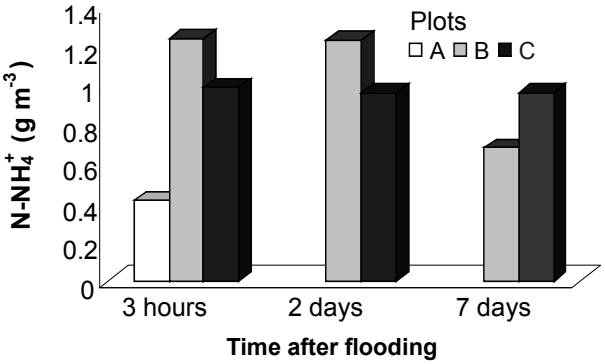


Fig. 17. N-NH_4^+ concentrations (g m^{-3}) in drainage water during 7 days after flooding under grass mixture (field 6) in a control plot A and as dependent on the wastewater dose (single B and double C)

Concentration of ammonium ion in drainage waters under grass mix is presented in figure 17 with relation to wastewater dosage. A higher concentration of the NH_4^+ ion was noted in plot (B), where it remained on a fairly stable level from the 3rd hour after irrigation till the second day. On day 7 the concentration of N-NH_4^+ dropped nearly by half. This fact may be related to the distinctly higher index of filtration of plot (B) as compared to that of plot (C). In the case of the latter, the concentration of ammonium ion in the drainage waters remained stable on a relatively high level for a period of seven days. The higher level of the NH_4^+ ion in the drainage waters with relation to the control varied within the range from 3-fold to 1.5-fold. However, taking into consideration the amount of N-NH_4^+ ions introduced with the wastewater, their concentration in the drainage waters dropped from 3- to 6-fold.

1997-1999 years

Figure 18a illustrates the effect of wastewater dosage applied – single and double – on the concentration of the N-NH_4^+ ion in the soil solution. Analysis of variance did

not show any significant effect of wastewater dosage on the concentration of ammonium ion, though an increase in the concentration was observed, proportional to the wastewater charge. With relation to the initial condition of 1997 (Tab. 4), the average concentration of $N\text{-}NH_4^+$ in the spectrum of the three years of the experiment increased two- to three-fold in plots A and C, respectively.

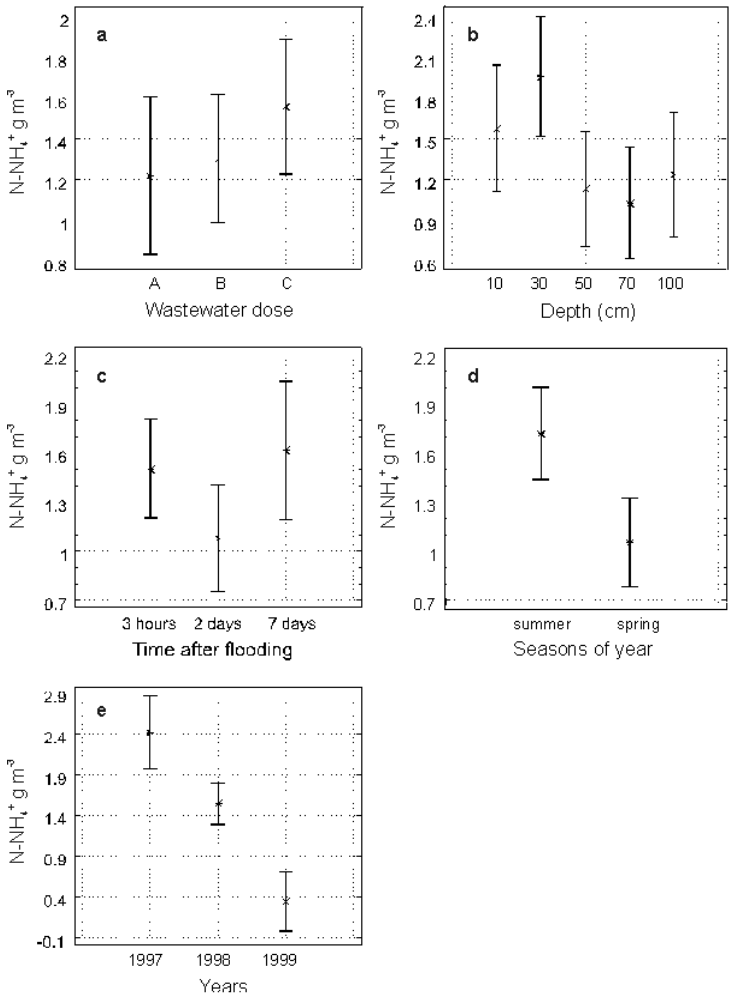


Fig. 18. Average $N\text{-}NH_4^+$ (g m^{-3}) concentration in soil solution under grass mixture (field 6) as dependent on wastewater dose (a), soil depth (b), time after flooding (c), season of the year (d), and years of experiment (e). Bars indicate 95% coincidence intervals

Figure 18b presents average concentration of the $N\text{-}NH_4^+$ ion in the function of depth. Statistical analysis did not reveal any significant differences in the con-

centration of ammonium ion, but an increase in the concentration could be observed down to the depth of 30 cm. At all the depths, cumulation of $N\text{-}NH_4^+$ was observed with relation to the initial level (1997), the highest at the depths of 100 and 10 cm, from 7- to 8-fold, respectively.

Diurnal average concentration of ammonium ion under the grass mix is presented in figure 18c. Analysis of variance did not show any significant differences between the particular analytical days, though on day two of wastewater effect a decrease in the concentration was noted. On analytical day seven, the concentration of $N\text{-}NH_4^+$ increased a little above the initial level, which could be related to favourable conditions for organic matter mineralization under the conditions of field 6.

The effect of the season of the year on the average concentration of NH_4^+ ion is presented in figure 18d. Like in the case of the field under willow, a significantly greater concentration of the analysed ion was observed in the summer, as compared to the spring. It is to be supposed that under the conditions of the experiment the process of mineralization of the ammonium form was more intensive in spring than in the summer.

Figure 18e presents the average concentration of ammonium ion in the function of time. Analysis of variance showed a significant drop in the concentration of $N\text{-}NH_4^+$ in the course of three years of wastewater application, from the level of 2.41 (1997) to 0.35 g $N \cdot m^{-3}$ (1999), a value that is also lower than the initial concentration (Tab. 4).

11.3.3.2. Transformations of nitrate(V) form

1997 year

Figure 19 illustrates the distribution of nitrate(V) form concentration within the whole soil profile with relation to wastewater dosage. The native content of nitrates(V) varied from 2.49 to 7.98 g $N \cdot m^{-3}$, respectively for the depths of 100 and 30 cm (Tab. 4). Somewhat lower concentration of $N\text{-}NO_3^-$ occurred at the depth of 10 cm (like in the case of the ammonium form), which should be ascribed to intensive development of the root system of the grasses in that part of the soil profile, especially in the first year of cultivation.

The application of wastewater caused a similar effect on the dynamics of transformations of nitrate(V) nitrogen irrespective of the dosage, with the exception of its initial concentration at the depth of 10 cm (Fig. 19). Comparison of $N\text{-}NO_3^-$ concentration within the whole period of wastewater effect on the soil and plants, as well as in the soil profile between single and double dosage, suggests that in the case of the grass mix the amount of nitrogen(V) introduced does not play any significant role in its transformations.

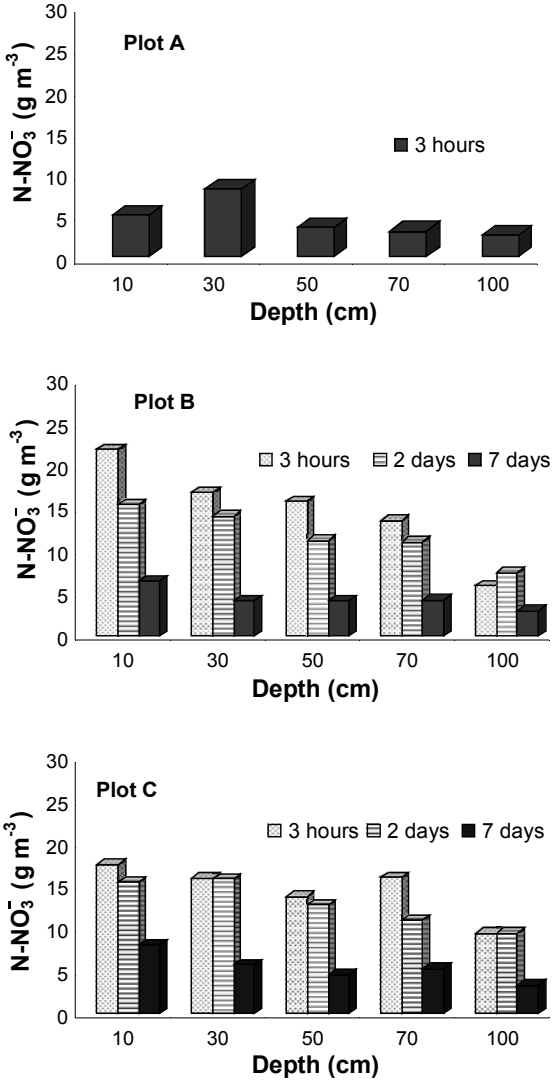


Fig. 19. N-NO_3^-concentrations (g m^{-3}) in soil solution during 7 days after flooding under grass mixture (field 6) in a control plot A and as dependent on the wastewater dose (single B and double C)

Figure 20 presents the ratio of concentration of N-NO_3^- to N-NH_4^+ in the field with the grass mix. A relatively strong dynamics of ammonium nitrogen transformations was characteristic of the control field, where the ratio analysed decreased with depth down to 50 cm, which could be related to a decrease in the intensity of

the process of nitrification with decreasing soil oxidation. The reversed trend – increase of the ratio down to 100 cm – could be due to similar causes as in the case of the field with rape (Section 10.3.2). In the initial stage of its presence in the soil, the application of wastewater results in a narrowing of the values of the ratio, frequently below the average value for wastewater (~7). The strongest dynamics of mineralization of nitrogen was observed in plot (B), down to the depth of 30 cm, especially on the second day from application. At the other depths in plot (B) the intensity of nitrogen transformations was low. It appears that, except for the depths down to 30 cm in plot (B), the application of wastewater strongly inhibits nitrogen transformations as compared to the control plot.

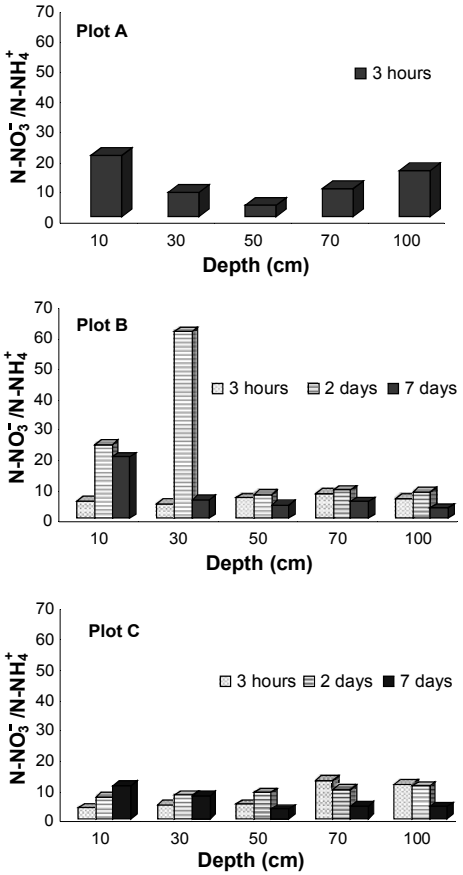


Fig. 20. Ratios of $N\text{-}NO_3^-$ to $N\text{-}NH_4^+$ concentrations (g m^{-3}) in soil solution during 7 days after flooding under grass mixture (field 6) in a control plot A and as dependent on the wastewater dose (single B and double C)

Figure 21 presents the concentration of $N\text{-}NO_3^-$ in the drainage waters with relation to wastewater dosage. The mobility of nitrates(V) caused that already after 3 hours from the moment of wastewater application their maximum concentration was observed, proportionally to the amount of wastewater applied. On day seven of the experiment, the concentration of $N\text{-}NO_3^-$ dropped three-fold, and taking into consideration the amount of nitrates introduced with the wastewater, the concentration of $N\text{-}NO_3^-$ decreased by a factor of six.

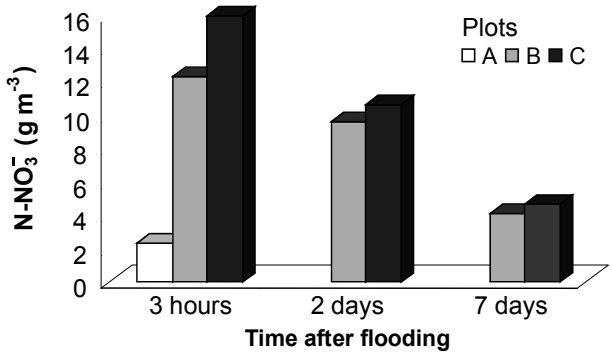


Fig. 21. $N\text{-}NO_3^-$ concentrations (g m^{-3}) in drainage water during 7 days after flooding under grass mixture (field 6) in a control plot A and as dependent on the wastewater dose (single B and double C)

1997-1999 years

Figure 22a illustrates average concentrations of nitrate(V) ion with relation to wastewater dosage, where an increase of concentration is observed, proportional to the wastewater dosage, though statistically insignificant. Average concentrations of nitrates(V) in field plots with single and double doses of wastewater varied from 19.8 to 23,2 g $N \cdot m^{-3}$, respectively. Noteworthy, however, is the high average concentration of the analysed $N\text{-}NO_3^-$ ion in the control plot, where its concentration (17,9 g $N \cdot m^{-3}$) exceeds almost four-fold the initial value from the beginning of the experiment. This indicates a high contribution of the plants and microbes in N transformation in the field in question.

Figure 22b illustrates the distribution of average concentration of nitrates(V) in the soil profile.

The concentration decreases with depth, indicating significant differences between the surface horizon of the soil and the deeper horizons. From 50 cm, the differences do not exceed 3 g $N \cdot m^{-3}$. The high concentration of nitrates(V) at the depth of 10 cm, higher than the average concentration of $N\text{-}NO_3^-$ in wastewater (~29 g $N \cdot m^{-3}$), suggests a high nitrification activity of the field under the grass mix.

Figure 22c presents the concentration of nitrate(V) ion in the function of time expressed in days of irrigation, where its significant effect is observed on the second day since the application of wastewater. Increase above 30 g $N \cdot m^{-3}$ indicates intensified process of nitrification on the second day from fields irrigation with wastewater. The abrupt drop of $N\text{-}NO_3^-$ concentration observed on day 7 could be due both to dissimilative and to assimilative reduction, depending on the status of soil aeration. *Bouwman* (1990b) stated that there occurs NO_3^- diffusion from oxic to anoxic areas, followed by its reduction. Therefore, in oxidized soils, there are simultaneous denitrification and autotrophic nitrification processes, combined with the production of N_2O in various micro-spaces.

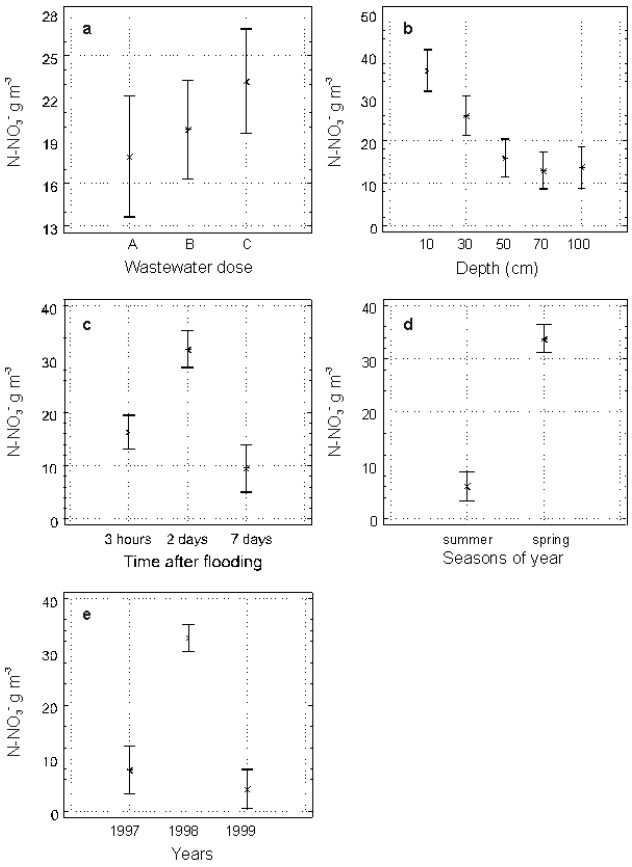


Fig. 22. Average $N\text{-}NO_3^-$ (g m^{-3}) concentration in soil solution under grass mixture (field 6) as dependent on wastewater dose (a), soil depth (b), time after flooding (c), season of the year (d), and years of experiment (e). Bars indicate 95% coincidence intervals

Figure 22d presents the concentration of nitrate(V) ion in the function of the season of the year. The high concentration of the NO_3^- ion in spring in combination with the low concentration of the NH_4^+ ion (Fig. 18d) seems to support the hypothesis that under the conditions of field 6 under the grass mix, the process of oxidation of ammonium ion is more intensive than in the summer, which may be related to losses – through leaching - of the nitrogen introduced.

Figure 22e presents the annual average concentrations of nitrate ion. Analysis of variance showed a significant difference in the concentration of $N\text{-}NO_3^-$ between 1998 and the other two years of the experiment. The concentration exceeds the value of concentration of NO_3^- ions introduced with wastewater. A similar tendency was also observed in the case of field 2 (Fig. 10e), which suggests that the strongest effect on the concentration of nitrates(V) could be that of the weather, conducive to the accumulation of $N\text{-}NO_3^-$ in the soil profile.

11.4. Comparative analysis of nitrogen transformations with respect to the plant grown

In various environments, the availability of soil nitrogen in the form of NH_4^+ or NO_3^- differs greatly with relation to the environmental conditions that affect the transformation of NH_4^+ to NO_3^- in the biological process called nitrification. For example, in the waterlogged soils of the tundra almost all nitrogen occurs in the form of NH_4^+ (*Barsdate and Aleksander,1975*), while in deserts and in forests on the NO_3^- form is of significance (*Virginia and Jarrell, 1983; Nadelhoffer et al. 1984*).

Numerous species show a preference for NO_3^-, although species occurring in areas where nitrification is slow or inhibited they often display increased growth with availability of NH_4^+ ions (*Haynes and Goh 1978; Adams and Attwill 1982; Falkengren-Grerup 1995*).

One of the adopted objectives of the study was to determine the contribution of plants in the process of wastewater purification after the 2nd stage of treatment. The subject of this work is the determination of the degree of depletion of the ammonium and nitrate(V) forms of nitrogen due to their transformations in the soil profile with relation to the depth in the profile, dosage of wastewater applied, and the plants grown. The problem applies specifically to the excess of nitrate(V) ion introduced to the soil environment with the wastewater.

11.4.1. Transformations of the ammonium form

1997 year

Figure 23 presents the balance of $N\text{-}NH_4^+$ transformations in the period from the 3rd hour to the 7th day after irrigation with wastewater, expressed in the form of reduction or increase in the amount of the analysed ion, in absolute values, for

the three plant species grown – willow, rape and grass mix – in the first year of the experiment.

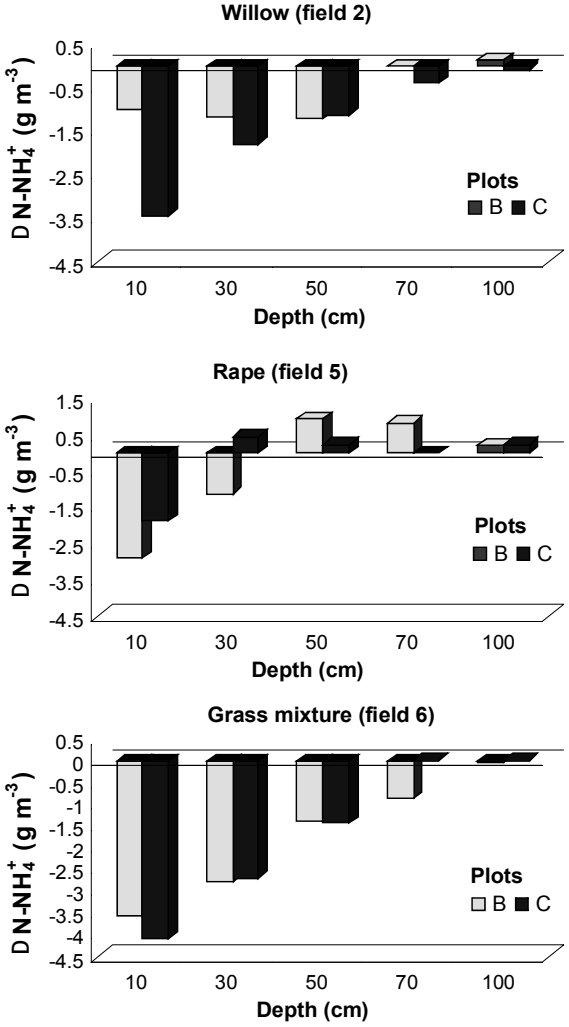


Fig. 23. Changes in $N\text{-}NH_4^+$ concentrations (g m^{-3}) in soil solution expressed like a decrease or increase after 7 days after flooding under willow (field 2), rape (field 5) and grass mixture (field 6) as dependent on the wastewater dose (single B and double C)

Analysis of the results presented indicates a similarity of the character of transformations in the soil under willow and grasses, though in differing ranges of concentrations. The biggest reductions observed in the amount of ammonium ion were under the grass mix, at the depth of 10 cm. In that case the dosage of waste-

water was of little significance only at the depth of 10 cm manifested in a slightly more pronounced drop in the concentration of ammonium ion. The effectiveness of the reduction decreases with depth, which is undoubtedly related with the lower biological activity in the lower parts of the soil profile.

Of much greater importance in the process of ammonium ion sorption, when compared to rape and the grass mix, was the dosage of wastewater applied in the case of willow, where a much greater reduction in the amount of $N\text{-}NH_4^+$ was observed in the case of the double dose, especially at the depth of 10 cm. The tendency decreased with depth. It seems that the structure of the root system could play a significant role in that process. The much more developed root system of the grasses efficiently utilized both the wastewater doses. A totally different course of changes in ammonium ion concentration was observed in the soil under rape, where at single dose of wastewater the reduction in the amount of the ion was higher. In the case of rape cultivation, increase in the concentration of ammonium nitrogen was observed down from the depth of 50 cm, and in plot (C) already from 30 cm. The reasons for this phenomenon may be numerous, including the mineralization of organic mattrer in amounts exceeding biological sorption, as well as, in the case of deeper soil horizons, favourable conditions for the occurrence of dissimilative reduction of nitrates(V) and the production of the NH_4^+ ion as the end product.

11.4.2. Transformations of nitrate(V) form

1997 year

Figure 24 presents the balance of nitrogen transformations between the 3rd hour and the 7th day from irrigation, expressed in the form of reduction or increase in the amount of nitrate(V) ion, in absolute values, for the fields with willow, rape, and the grass mix irrigated with single or double doses of wastewater. Analysis of the results presented indicates that, in most cases included in the analysis, the reduction in the amount of nitrate(V) ion decreases with depth in the soil profile, proportionally do the concentration of nitrates(V) in the soil profile (Fig. 7). The strongest depletion of nitrates(V) in the whole profile was observed in the case of the field under the grass mix, somewhat weaker under rape – except at the depth of 10 cm, and the weakest in the case of willow. And considering the dosage of wastewater and its efficiency in wastewater purification, two trends can be identified. Better utilization of nitrates(V) introduced with the single dose of wastewater was observed in the fields under willow and grass mix, but only down to the depth of 50 cm, while in the case of rape a stronger reduction in the amount of $N\text{-}NO_3^-$ was observed with the double irrigation dose, whose utilization effi-

ciency, as a rule, increases below the depth of 50 cm irrespective of the plants grown.

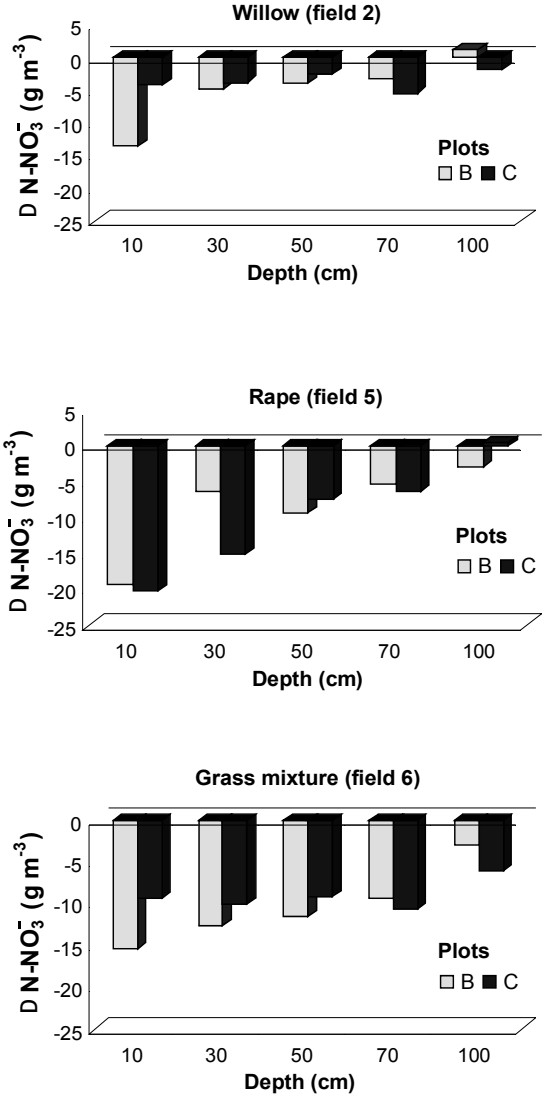


Fig. 24. Changes in $N\text{-}NO_3^-$ concentrations (g m^{-3}) in soil solution expressed like a decrease or increase after 7 days after flooding under willow (field 2), rape (field 5) and grass mixture (field 6) as dependent on the wastewater dose (single B and double C)

11.5. Redox potential of the experimental object

The placement of electrodes in the soil profile permitted the observation of changes in the redox processes in the soil, expressed as Eh values, under the conditions of natural environment of plant and microbial habitat. The values were a resultant of all the environmental factors under the conditions of soil flooding with wastewater.

11.5.1. Dynamics of redox potential changes in the object prior to the irrigation

Changes in the values of redox potential (Eh) reflect changes in oxygen content. A decrease in oxygen concentration in the soil profile entails a drop of the Eh value and the development of such microorganisms that can live under conditions of oxygen deficit. Every biochemical process, including transformations of oxidized forms of nitrogen, takes place at specific values of the redox potential.

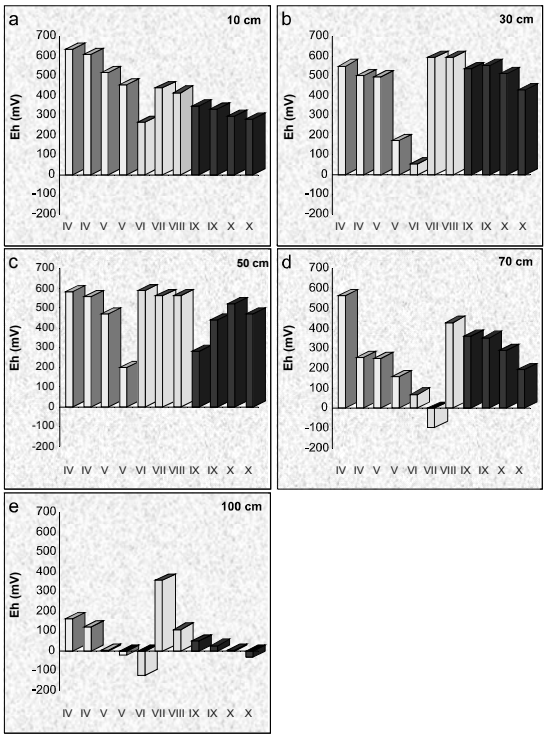


Fig. 25. Initial redox potential value in various profile depths (10, 30, 50, 70 and 100 cm) from spring to autumn 1996 period

Figure 25 a, b, c, d, e presents changes of redox potential values during the period under analysis, i.e. from spring (April) till autumn (October) within the whole soil profile (10-100 cm). The results obtained clearly indicate a drop in redox potential values from spring till summer at all depths. The reason for this phenomenon is likely related to the higher soil moisture during the spring season, accompanied by temperature increase and rapid utilization of the accumulated organic matter. Intensified biochemical processes of aerobes and facultative anaerobes entail rapid depletion of oxygen in the soil, which is reflected in the drop of redox potential values.

Lowered content of oxygen, the main acceptor of electrons in the process of aerobic respiration, activates microorganisms of the dissimilative path in which nitrates act as acceptors of electrons. Therefore, the drop in redox potential values is accompanied by a reduction in the content of nitrates in the soil solution (Section 10.2.2). This finds a reflection in the narrowing ratio of NO_3^- / NH_4^+ (Section 10.2.3).

The largest drop in Eh values within the period from spring to summer was observed at the depth of 70 cm (Fig. 25d), where the drop was initially 660 mV, which supports our supposition that it was at that depth that the most intensive denitrification of nitrates took place. Improvement of aeration conditions at that depth between July and August entailed a violent growth of the Eh values, by as much as 526 mV. Another drop in the redox potential values, observed from summer till autumn, had a much gentler course and amounted to 263 mV, which could be related to the exhaustion of available organic matter.

The least decrease in redox potential values in the spring-summer period was observed at the depth of 100 cm (287 mV), i.e. at the ground water table, which is related to the very low initial value of Eh (+164 mV) and the lowest biological activity at that depth.

The highest initial value of redox potential was found at depths down to 10 cm, i.e. the best oxidized zone in the soil (+635 mV). Within that layer and at 50 cm the lowest drop in Eh values during the spring months was observed, not exceeding 390 mV. A much lower value of redox potential was found at the depth of 30 cm (Δ Eh $\approx$ 500 mV), which may result from poorer aeration and greater intensity of nitrogen mineralization processes as evidenced by the highest value of the NO_3^-/NH_4^+ ratio (Fig. 3) observed at that depth.

Decrease in soil water content in the summer season, and therefore easier oxygen diffusion in the soil, cause a considerable increase in the value of Eh. The highest increase of redox potential in that period was observed at the depth of 30 cm (by 537 mV), and the lowest at the depth of 10 cm (by 176 mV). Another drop in the value of redox potential can be observed in the months from the summer till October, but it was much more moderated. This could be related to slower oxygen

depletion by heterotrophic bacteria that utilize less readily available organic matter. In the upper parts of the soil profile (down to 50 cm), the decrease did not exceed 170 mV, while at the depth of 70 and at 100 cm the decrease in Eh values was about 300 and 400 mV, respectively. During that period there was, probably, intensive growth of autotrophic bacteria that include nitrifiers especially sensitive to oxygen deficit. This hypothesis is supported by the increase in the NO_3^-/NH_4^+ ratio, especially at the depth of 30 cm (Section 10.2.3), which indicates intensive nitrification of ammonium ion.

Analysing the average values of Eh from the whole period of investigation, we can observe notable aeration deficit in the deepest part of the soil profile, i.e. at 100 cm. Values of Eh below 200 mV may suggest domination of anaerobic reactions over aerobic ones that periodically took place at depths from 30 to 100 cm, but definitely for longest periods at the depth of 100 cm. The lowest average value of Eh was observed at the depth of 100 cm (Fig. 26).

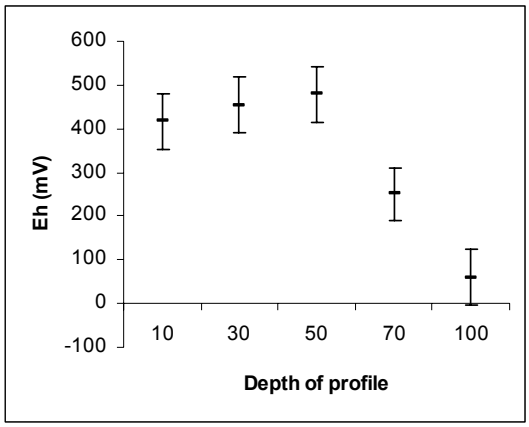


Fig. 26. Average redox potential value from spring to autumn 1996 period as a function of soil depth. Bars indicate 95% coincidence intervals

The value of redox potential at the depths of 70 and 100 cm was significantly lower than the Eh values at depths from 10 to 50 cm. That zone in the soil profile can be assumed to be an area of clear domination of anaerobic biochemical processes.

11.5.2. Dynamics of redox potential changes during the 1st year of the experiment on the example of fields under willow (Field 2), rape (Field 5) and grass mix (Field 6)

Introduction of wastewater to the soil in amounts causing its total flooding causes radical changes in the water-air relations in the soil. As shown by research

by numerous authors (*Gliński and Stępniewski, 1985; McKenney et al., 2001*), under the conditions of soil flooding with water exhaustion of oxygen occurs within several hours, resulting in a variety of changes in the soil. In particular, the redox potential of the soil decreases, with the drop of Eh values below +300 mV being caused by reduction of the soil and successive activation of redox couples other than the O_2/H_2O system, e.g. NO_3^-/NO_2^- (*Gliński and Stępniewski, 1985*).

As a result of oxygen content in the soil decrease below 1% (v/v), anaerobic microorganisms begin to dominate over aerobes. *Gliński and Stępniewski* (1985) conclude that soil flooding with water causes a gradual drop of redox potential until a certain fairly stable level is reached. The rate of the decrease and the value of Eh depend on the intensity of the process of reduction, determined by temperature and by the content of easily decomposable organic substrate and of oxidized inorganic compounds functioning as acceptors of electrons, including nitrates(V). The dynamics of redox potential changes was described on the example of three selected fields (2, 5 and 6) in the first year of the experiment.

Figure 27 presents the change of redox potential after 3 hours, and two and seven days from the application of the single and double irrigation doses in fields under willow, rape, and grass mix. Redox potential in the control soil profiles was stabilized down to the depth of 50 cm and varied within the range from about +400 mV to +500 mV, i.e. in the range of aerobic oxidation of carbon (*Tate III 1995*). At depths below 50 cm the value of Eh rapidly drops by 300 to 360 mV, reaching negative values close to the process of methanogenesis. Such a drastic drop of the redox potential is related with the appearance of ground water table at the depth of 50-60 cm. That is why the values of Eh at the depths of 70 and 100 cm were relatively low and varied from +160 to –170 mV.

Irrigation of the fields with the single and double doses of wastewater resulted in a change to the redox potential, with observable influence of the plants and of the ground water table on the values of Eh, which is especially evident in field 2 (under willow) at depths down to 30 cm. Analysis of changes in the aeration status in plots A, B, C down to the depth of 30 cm on the 3rd hour showed a slightly higher value of Eh in the combinations with irrigation than in the control plot (A), which may be related with the lower ground water table (70 cm) in plots B and C and with better aeration of the upper part of the soil profile as compared to the control object. In the lower parts of the soil profile of field 2, at the depths of 50 and 70 cm, the value of Eh decreased in plots B and C with relation to the control. In the other cases analysed (fields 5 and 6), redox potential measured down to the depth of 50 cm was lower in plots B and C with relation to the control, which may also be related to the different levels of ground water table in the objects studied and to the process of wastewater application on the soil.

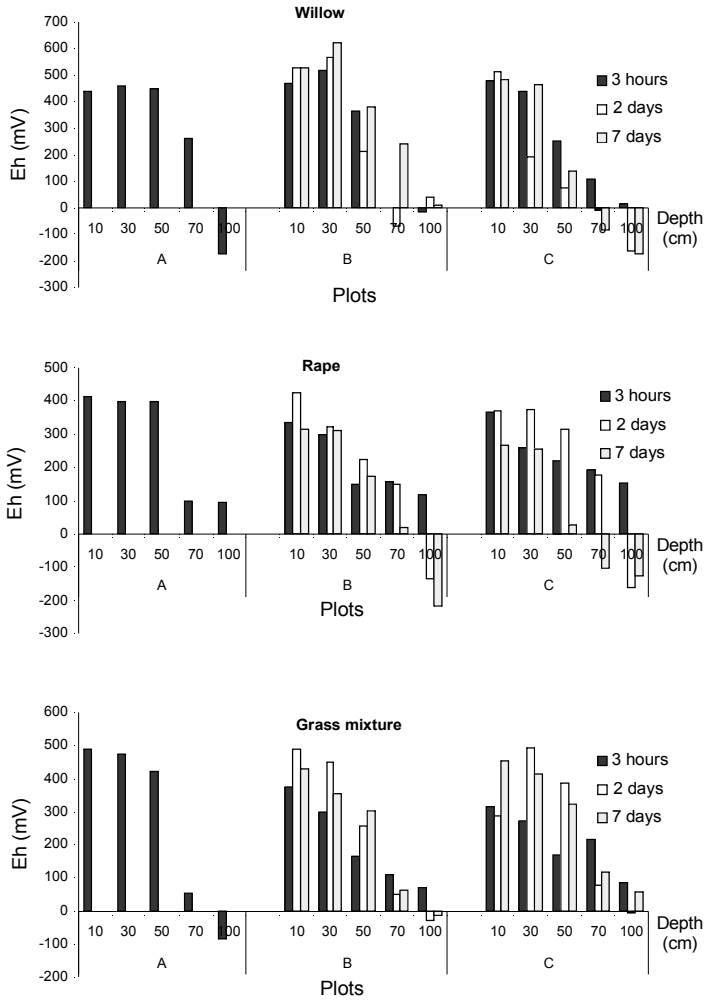


Fig. 27. Redox potential value in various profile depths (10, 30, 50, 70 and 100 cm) as dependent on the wastewater dose (A - control, B – single dose, C – double dose), time after flooding (3 h, 2 and 3 days) under willow (field 2), rape (field 5) and grass mixture (field 6)

Generally it can be stated that the value of redox potential, at the 3rd hour from irrigation, decreased with depth in all the objects studied. The decrease depended more on the ground water table than on the wastewater applied.

Wastewater introduced into the soil migrate down the soil profile at various rates depending on the profile structure and filtration coefficient, causing changes in the water-air relations i.e. the ratio of pores filled with water to pores filled with air. The

soil under study is characterized by varied air and water permeability, both between and within the fields depending on the depth. This feature of the soil is related to the dynamics of redox potential decrease and of reoxidation of the soil profile, observed with the migration of wastewaters and with improvement of the aeration conditions in the soil. Analysis of the oxygenation status of the field under willow at single irrigation dose down to 30 cm showed a slow increase of the value of Eh from the 3rd hour to the 7th day from the irrigation, which may indicate improvement of the air-water relations and the beginnings of the process of reoxidation, observed already from the second day after irrigation application. The process could also be affected by the water permeability of the soil whose index of filtration distinctly increases at the depth of 20-40 cm (*Final Report Ref. PBZ-31-03*). But the process of reoxidation of the lower horizons of the soil (from 50 to 70 cm) took place with a certain delay, on the seventh day of wastewater effect, which was likely related with the process of wastewater migration down the soil profile. The process did not take place at the depth of 100 cm, i.e. below the ground water table. In plot (C) there was a notable effect of the double dose of wastewater on the redox status of the soil. There was a slowing-down of the process of reoxidation of the soil profile, and the effect was observable already from the depth of 30 cm. At the depths of 30-50 cm a pronounced decrease was observed in the value of Eh, by 243 and 182 mV, respectively, on the second day of the experiment, with an increase appearing only on day seven. The slowing down of the process of soil reoxidation in plot C must have had also been affected by the notably lower filtration coefficient of about 45 $cm \cdot d^{-1}$ as compared to plot (B) where its value was approximately 140 $cm \cdot d^{-1}$ (*Final Report Ref. PBZ-31-03*). At the depths of 70 and 100 cm the very low redox potential value at the moment of irrigation with wastewater decreased drastically after 2 and 7 days, to the values of –83 and –173 mV.

Somewhat different dynamics of redox potential changes was observed in the fields under rape and grass mix. In both of those fields, in the initial stage of wastewater application, down to the depth of 50 cm a much lower Eh value was observed in the plots irrigated with wastewater as compared to the control, in extreme case even by 250 mV. In the lower soil horizon such a phenomenon was not observed. Perhaps that was due to lower level of ground water table, or its period lowering. On the second day after flooding the soil with wastewater, for both the single and double dosage in most of the analysed soil horizons down to the depth of 50 cm the phenomenon was observed of fairly rapid increase in the redox potential value reversing to a decrease by the seventh day from flooding. The rapid decrease after flooding could be related to high microbial activity that is always accompanied by fast depletion of oxygen in the environment and a drop of Eh before the microorganisms can adapt to anaerobic conditions and the system involving the NO_3^-/NO_2^- pair of electrons (next in line after O_2) can activate. This

phenomenon occurs most frequently when the soil gets flooded following thorough drying. The engineering works performed on the experimental object in 1996 caused considerable shifting and mixing of the soil material, which could result in the phenomenon described above. Thus, in the case of fields under rape and grass mix, in the profile down to the depth of 50 cm (except for field 6B at 50 cm) no distinctly observable phenomenon of reoxidation, so important for proper development of the root systems of plants grown.

Figure 28 presents average values of redox potential in the profile with relation to the plant grown, irrigation dosage applied, and time elapsed from irrigation. The results obtained indicate that average Eh values in the whole soil profile in the control plots of all the plants grown were similar, and therefore, in this case, the plant itself had no significant effect on the value of Eh. The effect of plants on the redox potential of the soil under study became apparent only in combination with wastewater application and time from flooding.

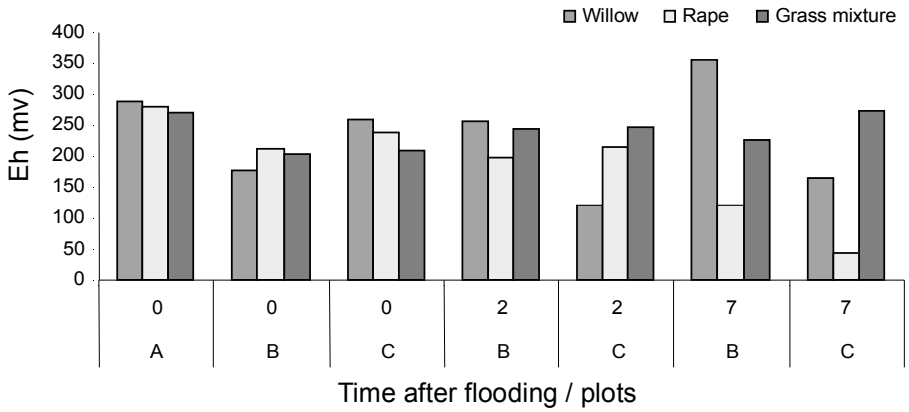


Fig. 28. Average redox potential value as a function of soil depth and the wastewater dose (A - control, B – single dose, C – double dose), time after flooding (3 h, 2 and 3 days) under willow (field 2), rape (field 5) and grass mixture (field 6)

The results obtained showed that the soil under rape had the lowest capacity for reoxidation, while the highest was noted in the field under willow.

The character of nitrogen transformations, and especially of its ammonium form, is strictly related to the status of soil oxygenation. In Section 10.3. we discussed the transformations of nitrates(V) with relation to the depth in the soil profile, to the plant grown, and to the wastewater dosage applied. Analysis of the dynamics of those processes pointed generally towards a decreasing tendency of their concentration. Characterization of soil oxygenation status through the determination

of redox potential values provides the possibility of identification of the biochemical path that may be entered by nitrates(V). Down to the depth of 50 cm, the control plots under all the plants grown were characterized by relatively high redox potential, close to +400 mV or much above that value. Eh values above +400 mV are characteristic of aerated soils, where oxidation reactions predominate. It is to be supposed, therefore, that under those conditions nitrates entered the path of assimilative reduction nitrogen was reduced by plants or microorganisms to the ammonium form before being built into cells. The process involves participation of enzymes from the reductase group, called nitrate reductases. Redox potential decrease below 400 mV has been conventionally adopted as the limit of nitrate reduction on the path of dissimilative reduction where nitrates are the respiratory substrate under conditions of deficient oxygen content in soil environment. The process is called denitrification when the end products are N_2O or N_2 emitted to the environment. The process involves also activity of nitrate reductases, from the dissimilative path, that are inactive in the presence of oxygen.

It is to be supposed, therefore, that below +400 mV the decrease in $-NO_3^-$ concentration was caused by the process of denitrification. Literature reports indicate that the beginning of the process of denitrification in various soils occurs at different values of Eh, sometimes below +200 mV (*Van Cleemput and Patrick, 1974; Kralova et al., 1992; Włodarczyk, 2000*).

Under favourable conditions (min. low Eh value, available organic matter), nitrates(V) entered the path of dissimilative reduction, where the final product was the ammonium ion, periodically increasing its concentration in the soil profile, especially in its lower horizons.

11.5.3. Concentration of native nitrogen compounds versus redox potential in the soil prior to the experiment

The collected redox potential data were used as a basis for statistical analysis of the relationship between the concentration of native nitrates(V) and the ammonium form in the soil prior to the flooding of the soil with wastewater and the values of Eh in the period from spring till autumn (Fig. 29 and 30). The dependence of concentration of nitrates(V) on the redox potential is described by a positive logarithmic function, which means that the value of Eh increases in a curvilinear manner with the concentration of $N\text{-}NO_3^-$. This relation is best described for the depth of 10 cm, where the coefficient of determination (R^2) is 0.9 with correlation coefficient significant at the level of 0.001. The relation is accompanied by high concentration of nitrates(V) – above 100 gm^{-3} (Fig. 29).

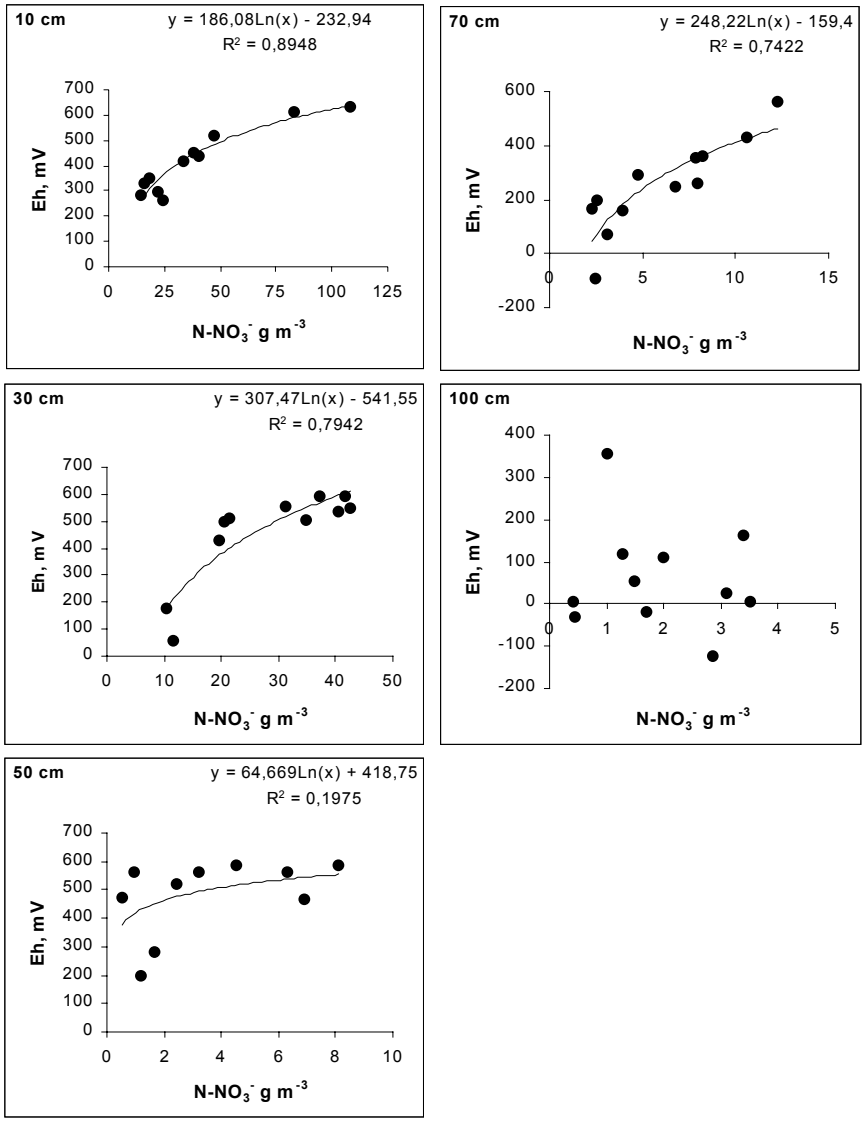


Fig. 29. Average redox potential value versus $N\text{-}NO_3^-$ concentrations ($g \cdot m^{-3}$) in various profile depths (10, 30, 50, 70 and 100 cm) from spring to autumn 1996 period under consideration

Good oxygenation conditions, evidenced by the high redox potential above +600 mV, and high biological activity of the surface horizon of the soil profile were conducive to the process of nitrification and accumulation of nitrates. The highest concentrations of $N\text{-}NO_3^-$ were observed at Eh values above +600 mV and a drop in the con-

centration of $N\text{-}NO_3^-$ by half at Eh values below +600 mV. This appears to be the boundary value between nitrification optimum and conditions that cause the process of nitrification to slow down. High level of significance ($P<0,001$) with determination coefficient $R^2=0,79$ is held at the depth of 30 cm, where maximum concentration of $N\text{-}NO_3^-$ dropped by a factor of 2.5 in comparison to the depth of 10 cm, accompanied by a decrease in the value of Eh below +100 mV. Deteriorating with depth aeration conditions and weaker nitrification resulted in a slow but steady decrease of redox potential. The concentration of nitrates(V) at the depth of 50 cm had no significant effect on redox potential, though the character of the curve remained similar to those for the depths discussed earlier. At the depth of 70 cm decrease of $N\text{-}NO_3^-$ concentration below 15 $g \cdot m^{-3}$ significantly affected the value of redox potential, and the determination coefficient R^2 (0,74) was only slightly lower than at the depth of 30 cm. As follows from the curve of regression, in the case of the soil under analysis a drop of $N\text{-}NO_3^-$ concentration to about 5 $g \cdot m^{-3}$ meant reaching a critical concentration that caused a drop of the value of Eh below +200 mV. This observation is supported by the clear drop of redox potential below the value of +200 mV at the depth of 100 cm, where the concentration of $N\text{-}NO_3^-$ did not exceed 4 $g \cdot m^{-3}$.

Figure 30 presents the concentration of ammonium ion in the function of redox potential in the whole soil profile in the period from spring till autumn. The results presented show that significant relations between the soil oxygenation status and the content of ammonium ion occur at depths of 30–70 cm. The relation is described by a negative logarithmic function, which means that the content of ammonium ion decreases in a curvilinear manner with increasing values of Eh.

Intensive process of nitrification at low values of redox potential, in which ammonium ion is the substrate and NO_3^- the product, causes a lowering of the relation. The highest determination coefficient was noted for the depth of 50 cm ($R^2=0,86$), and the lowest at the depth of 30 cm ($R^2 = 0,42$).

The data obtained shows that accumulation of $N\text{-}NH_4^+$ above 3 gm^{-3} takes place at Eh value of about +200 mV, i.e. under conditions that are conducive to the growth of microorganisms active in the path of dissimilative reduction of nitrates(V). The lower content of ammonium ion and low Eh at the depth of 100 cm may result from the effect of dilution and permanent oxygen deficit in that part of the soil profile.

Figure 31 presents the relation of the index of nitrogen mineralization, expressed in the ratio of nitrate(V) ion to ammonium ion, to the value of redox potential. The relation is described by a positive logarithmic function down to the depth of 70 cm. The highest determination coefficient $R^2 = 0,87$ was noted for the depth of 70 cm. The broad range of values of the NO_3^-/NH_4^+ ratio, indicating intensive process of oxidation of ammonium ion, correlated with higher values of Eh.

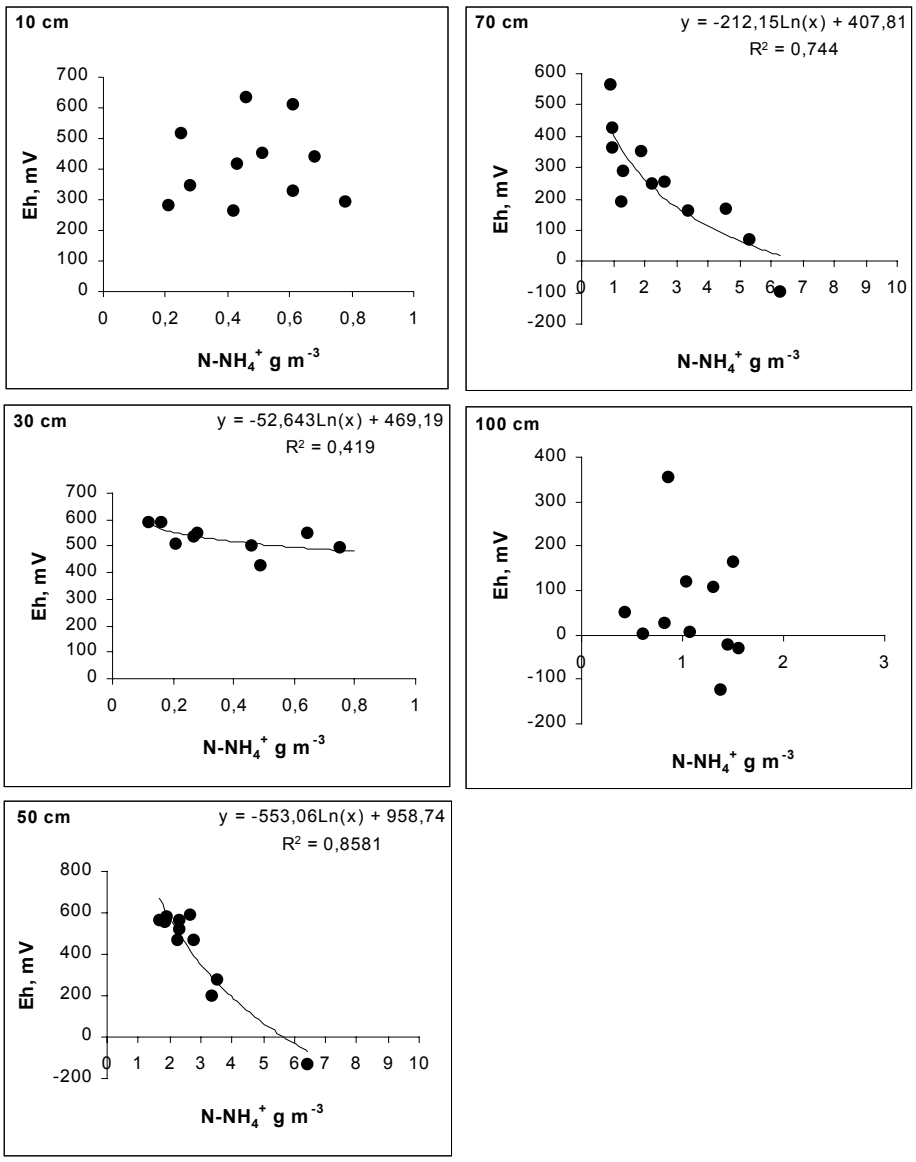


Fig. 30. Average redox potential value versus $N\text{-}NH_4^+$concentrations (g m^{-3}) in various profile depths (10, 30, 50, 70 and 100 cm) from spring to autumn 1996 period under consideration

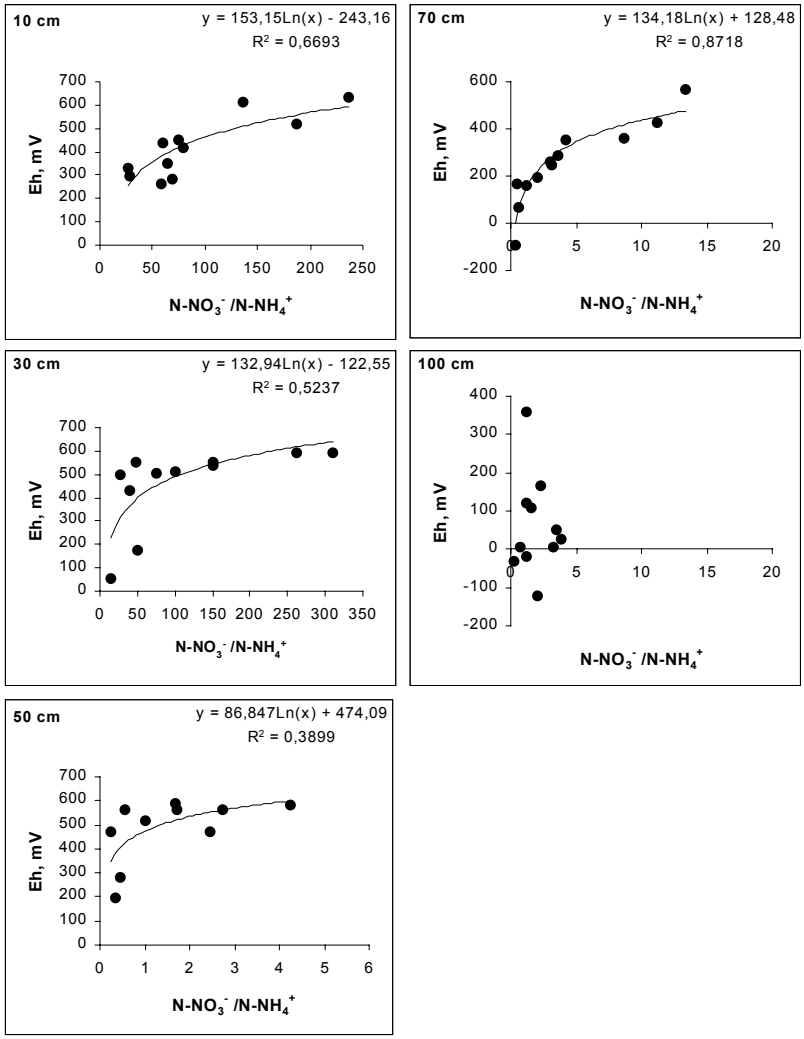


Fig. 31. Average redox potential value versus ratios of $N\text{-}NO_3^-$ to $N\text{-}NH_4^{+-}$ concentrations (g m^{-3}) in various profile depths (10, 30, 50, 70 and 100 cm) from spring to autumn 1996 period under consideration

11.5.4. Concentration of nitrates(V) versus redox potential in the soil irrigated with single and double dose of wastewater after 2nd stage of treatment

Soil flooding may cause a gradual decrease of redox potential until a certain relatively stable level is reached. The rate of the decrease and the minimum value of Eh depend on the intensity of processes of reduction, determined by temperature and the

amount of easily available organic substrate, and on the amount of bioreducible inorganic compounds which are acceptors of electrons (nitrates, three- and four-valent manganese compounds, iron oxides). The presence of such compounds maintains redox potential on a certain constant level which e.g. in the case of nitrates is from +100 to +200 mV (*Bailey, Beauchamp, 1971; Gliński, Stępniewski, 1985*).

In 1997, in parallel to analysis of concentration of nitrate(V) ions, data concerning the redox potential were collected and an attempt was made at determining the correlation between the concentration of nitrates(V) introduced with the single and double doses of wastewater and the value of the redox potential. The relations obtained are presented on the example of average values of $N\text{-}NO_3^-$ concentration and values of Eh measured in the fields in the first year of the irrigations.

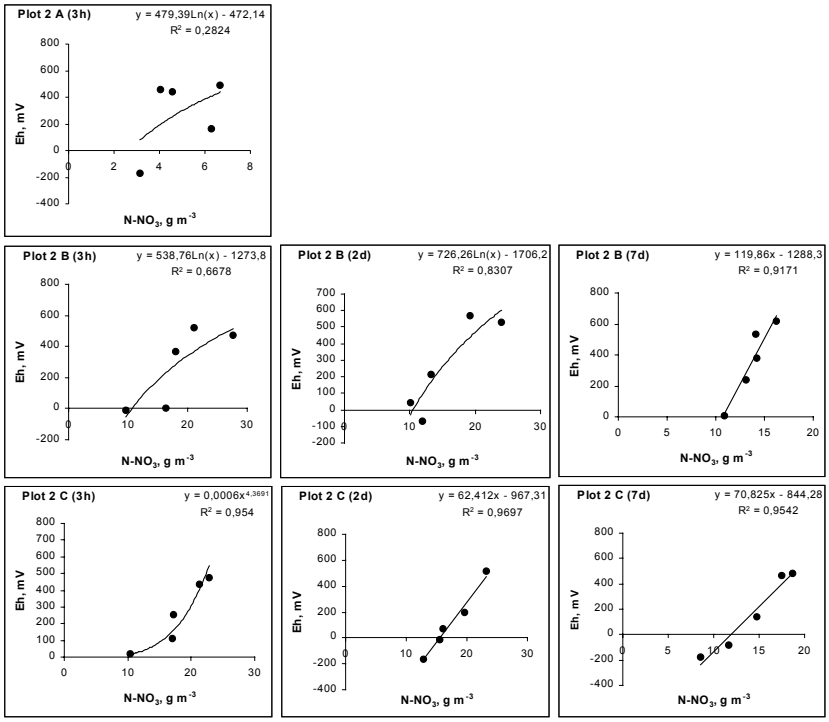


Fig. 32. Redox potential value after 3h, 2 and 7 days after flooding versus $N\text{-}NO_3^-$ concentration and wastewater dose (A - control, B – single dose, C – double dose) under willow (field 2)

Analysis of regression of the relationship under study, performed for the control plots of fields 2, 5 and 6, in two cases (willow and rape) showed a lack of dependence between the content of native nitrates(V) and the value of Eh, and a slight correlation ($P<0,05$) in the case of the field under grass mix (Fig. 32, 33, 34).

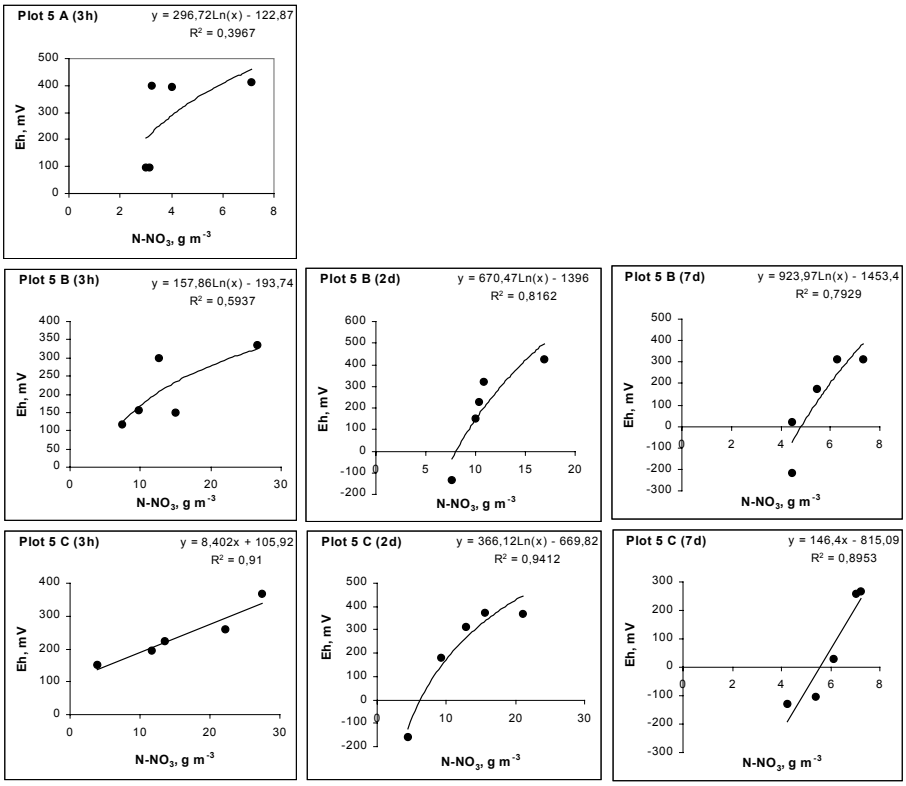


Fig. 33. Redox potential value after 3h, 2 and 7 days after flooding versus $N\text{-}NO_3^-$ concentration and wastewater dose (A - control, B – single dose, C – double dose) under rape (field 5)

An earlier analysis of the concentration of nitrates(V) in the fields after the engineering works performed (that strongly affected, among other things, the filtration properties of the soil and modified its physical status) showed a considerable decrease in the concentration of $N\text{-}NO_3^-$ in comparison to the soil before the works were performed. It seems that one of the reasons for the lack of correlation between nitrates(V) and the redox potential was the low concentration of the nitrates. Probably, under the conditions of the control plots, the value of Eh was more strongly affected by the soil moisture and by oxygen concentration in the soil profile, which is supported by the decreasing values of redox potential with depth in the profile.

The introduction of wastewater drastically altered the air-water relations, and therefore also oxygen concentration, which resulted in a change of redox potential. Under anaerobic conditions, the dynamics of changes in the value of Eh depends, among other things, on bioreducible oxidized inorganic compounds, pri-

marily nitrates(V), which are electron acceptors alternative to oxygen. Therefore, it appears to be of interest to trace the relation between the content of nitrates(V), brought in with wastewater, and the value of Eh.

The relationship is described by four mathematical functions: linear (y= a + bx), logarithmic (y= alnx + b), power (y = ax^b), and exponential (y = e^{a+bx}). In all the cases analysed the functions are positive, which means higher concentration of $N\text{-}NO_3^-$ is accompanied by a higher value of Eh. The highest correlation was observed in the fields under willow and rape (Fig. 32, 33), where the determination coefficient R^2 in many cases reached values above 0.1 ($P < 0{,}001$), and the lowest under the grass mix (Fig. 34).

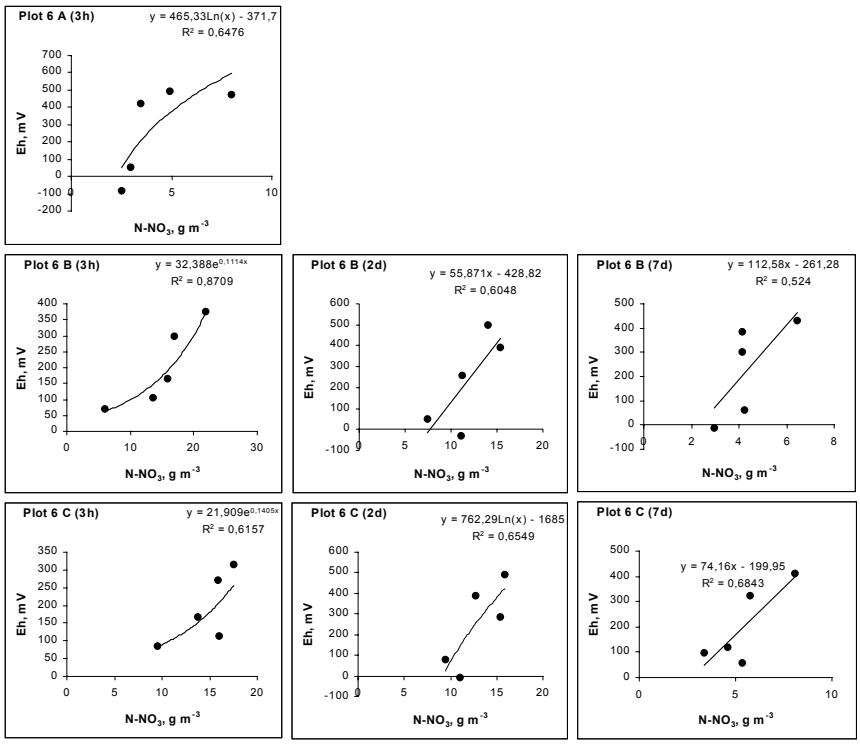


Fig. 34. Redox potential value after 3h, 2 and 7 days after flooding versus $N\text{-}NO_3^-$ concentration and wastewater dose (A - control, B – single dose, C – double dose) under grass mixture (field 6)

A decidedly higher coefficient of determination was observed in fields irrigated with the double dose of wastewater, in the amount of 1200 mm. Analysis of the diagrams presented shows that up to two days from wastewater application in all the cases analysed the value of Eh held at the level of +200 mV within the range of $N\text{-}NO_3^-$ concentrations of 10–20 $g \cdot m^{-3}$. With high soil moisture, that concentration

could protect the soil against the process of reduction. In the case of soil flooding with wastewater in amounts from 400 to 1800 mm, there occurred a restriction of oxygen influx, first of all in the surface horizons. In that situation the presence of nitrates affected the value of Eh proportionally to their concentration.

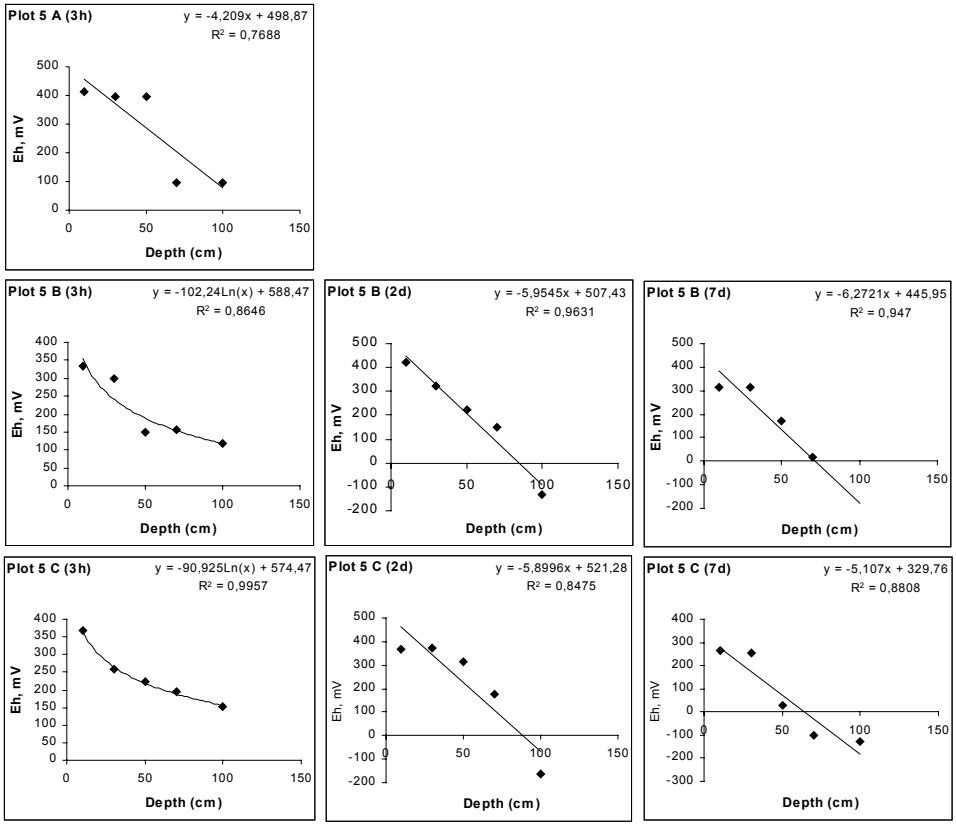


Fig. 35. Redox potential value after 3h, 2 and 7 days after flooding versus depth of profile and wastewater dose (A - control, B – single dose, C – double dose) under willow (field 2)

Włodarczyk (2000) found that with a decrease in the content of nitrates within the range from about 100 to approximately 10 mg $N\text{-}NO_3^-\cdot kg^{-1}$, redox potential value decreased from 250 to 190 mV. For mineral soils, the limit value which is accompanied by a clear decrease in Eh value is the level of approximately 100 mg $N\text{-}NO_3^-\cdot kg^{-1}$. The highest diurnal reduction of nitrates occurred at Eh values within the narrow range between 200 and 210 mV.

In the data presented in Section 10.5, the effect of depth on the value of Eh could be observed. Statistical analysis of Eh values in the function of depth showed their close correlation.

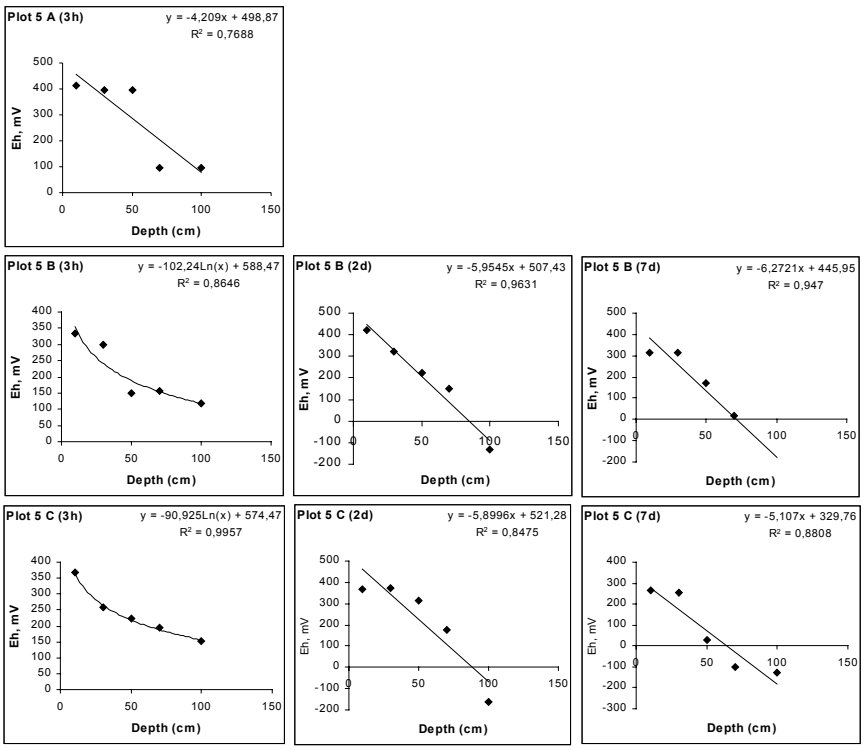


Fig. 36. Redox potential value after 3h, 2 and 7 days after flooding versus depth of profile and wastewater dose (A - control, B – single dose, C – double dose) under rape (field 5)

Figures 35, 36, 37 present the dependence of redox potential on depth for willow, rape, and grass mixture, respectively. The dependence is described by three mathematical function: linear ($y = a + bx$), the most common, logarithmic ($y = a\ln x + b$), and exponential ($y = e^{a + bx}$). In all four cases under analysis the functions are negative, which means that redox potential decreases with depth. In eleven cases out of 21 analysed, the coefficient of determination R^2 is close to 0.9 or even higher, which, with five replications, gives significance at the level of 0.001. The remaining relations are significant at the level of 0.01, except for field 6 C on the second day from irrigation ($P > 0,05$).

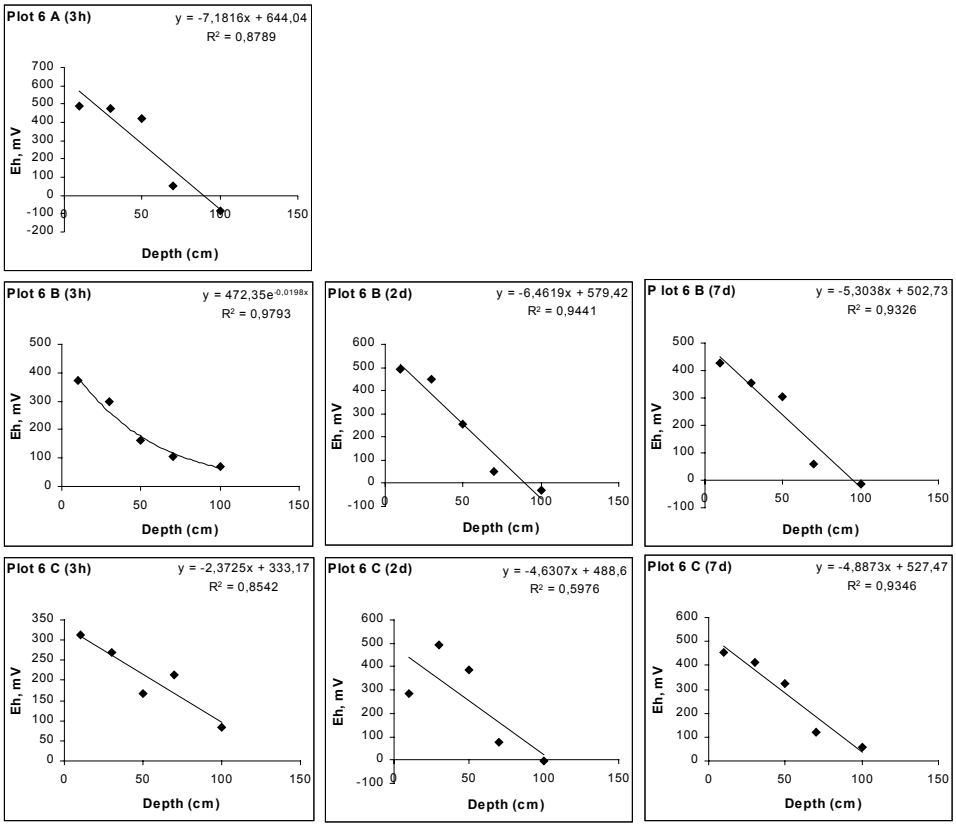


Fig. 37. Redox potential value after 3h, 2 and 7 days after flooding versus depth of profile and wastewater dose (A - control, B – single dose, C – double dose) under grass mixture (field 6)

11.5.5. Statistical analysis of redox potential values in the fields under willow and grass mix based on data from 1997-2000

The analysis of variance of changes in redox potential with relation to such factors as type of plant, wastewater dosage, depth in the soil profile, flooding duration, season of the year, and time measured in years of experiment, performed with the use of large datasets (frequently exceeding n = 500), provided the basis for the determination of redox characteristics of an organic soil subjected to periodic flooding and drying for a period of 4 years.

Figures 38 a, b, c and 39 a, b, c present the analysis of variance of Eh values in the function of the factors mentioned above, based on data from the period of 1997-2000.

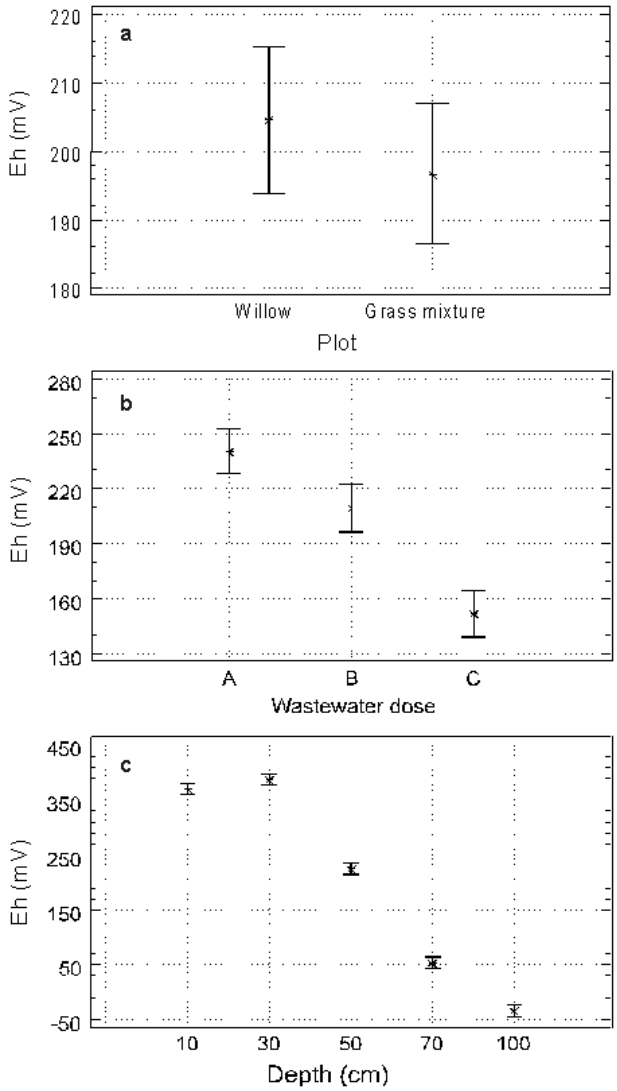


Fig. 38. Average redox potential value as a function of kind of plant (a) the wastewater dose (b) and soil depth (c). Bars indicate 95% coincidence intervals

Figure 38a presents the relation of redox potential to the plant grown. Statistical analysis did not reveal any significant effect of the type of plant on the redox potential, though somewhat lower Eh values were observed in the field under the grass mix. This phenomenon may be connected with higher microbial activity of the rhizosphere of grasses, faster transformation of organic matter that is the

source of electrons transmitted in redox processes (*Gliński and Stępniewski, 1985; Brzezińska, 1998; Januszek, 1999*).

Figure 38b illustrates the effect of wastewater dosage on the value of Eh. Analysis of variance showed a significant negative effect of wastewater dosage on the redox potential, expressed as a decrease of Eh values. This phenomenon was particularly disturbing in the case of the double dose of wastewater, when the redox potential dropped below +200 mV, i.e. to the level marking the beginning of conditions for dissimilative reduction of nitrates(V) with the emission of N_2O. The data conform earlier conclusions that the application of the double dose of wastewater intensifies the process of soil reduction, negatively affecting its biological activity and crop yields.

Figure 38c presents the value of Eh in the function of depth. The data obtained indicate that in the soil profile, at depths from 10 cm to 100 cm, biochemical processes can take place, performed by microorganisms with varied oxygen requirements – from aerobes, through facultative anaerobes, to anaerobes. The best oxidized part of the soil profile, where the value of Eh held above +350mV, was the soil layer down to 30 cm. From the depth of 50 cm, redox potential decreased significantly, creating good conditions for the growth of denitrifiers, and at the depth of 100 cm reached negative values, conducive to methanogenesis and the production of sulphides.

Figure 39a presents changes in the redox potential in the function of time since flooding. The data illustrate the process of soil reduction and reoxidation initially caused by flooding and then by the drainage of wastewaters from the soil profile and reoxidation of the soil. The process is extremely important from the viewpoint of plant growth and crop yielding, as prolonged oxygen stress has a negative effect on the uptake of macro components and on plant crop yields. *Stępniewski and Przywara* (1992) found that the uptake of N, P, K, Ca, Mg and Na by winter rye drops under the conditions of reduced oxygen content in the soil.

The lowest decrease of redox potential was observed after the first day since the moment of soil flooding with wastewater, when high soil moisture held within the whole profile. As the wastewater migrated down the profile and the upper soil horizons dried, the value of Eh increased to reach, on the second day, a value lower from the initial level by only 10 mV. On successive days of soil reoxidation, the value of redox potential increased, with certain drops on days 3 and 6. On day 7, the redox potential reached the value of about +260 mV, i.e. higher by 20 mV than that at 3 hours from the moment of flooding. Considering that that value includes the average for the whole profile, one can assume that the soil oxygenation conditions have been considerably improved, especially in the surface horizon.

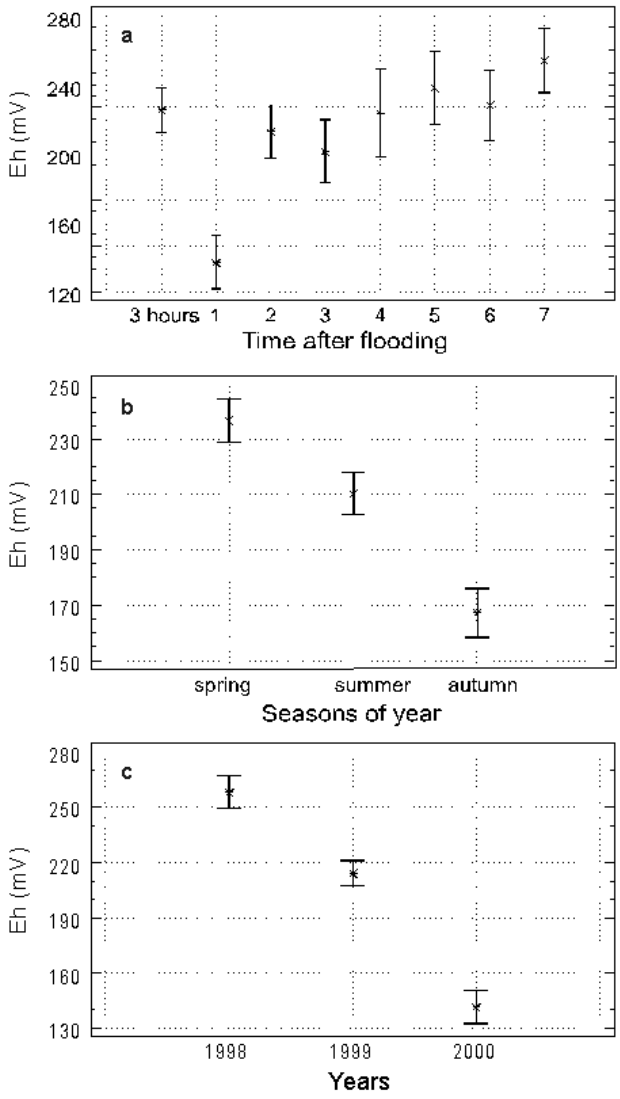


Fig. 39. Average redox potential value as a function of time after flooding (a) season of year (b) and year of experiment (c). Bars indicate 95% coincidence intervals

Figure 39b illustrates the distribution of Eh values with relation to the season of the year, that is primarily to climatic factors. In the period under analysis, a significant effect of the season of the year was observed on the intensification of

the redox processes. The highest value of Eh was observed in spring (~+240 mV), and the lowest in autumn (below +170 mV). The high value of redox potential in spring corresponds to high concentration of $N\text{-}NO_3^-$ (Fig. 40 a) which, as it is known, through their biological reduction counteract the reduction of the values of Eh. Perhaps a significant factor in this case is, among other things, the effect of temperature on the course of microbiological processes.

Figure 39c presents the values of Eh in the particular years of the experiment. Analysis of variance revealed significant differences in Eh values between the particular years. The year 2000 has been additionally included to identify the trends in the processes of oxygenation and reduction taking place in the experimental object. The results presented indicate that from the year 1998 a distinct decrease is observed in the redox potential, which could indicate that the soil was losing its buffering properties, probably due to the alternating cycles of flooding and drying and to a decrease in its microbial activity. Lower values of Eh were also accompanied by a lower concentration of $N\text{-}NO_3^-$

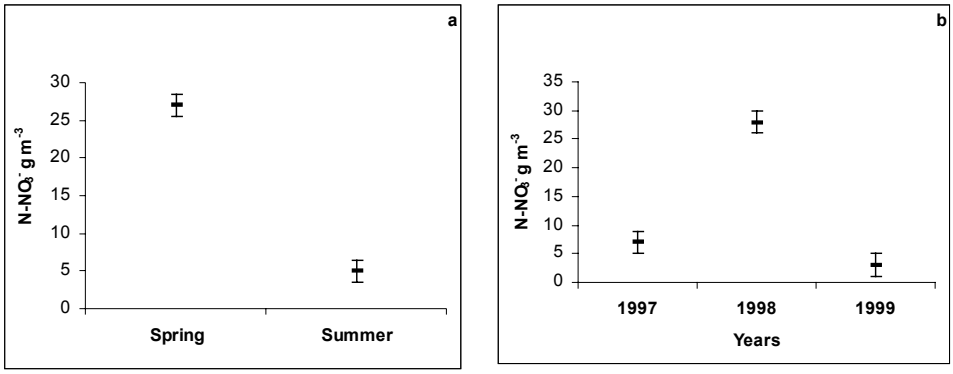


Fig. 40. Average $N\text{-}NO_3^-$ (g m^{-3}) concentration in soil solution as dependent on season of the year (a), and years of experiment (b). Bars indicate 95% coincidence intervals

(Fig. 40b), which did not protect the soil from the drop in the redox potential. This hypothesis is also supported by the decrease in the concentration of nitrates(V) in the drainage waters (Fig. 41), which may suggest intensification of the process of denitrification of $N\text{-}NO_3^-$ in the object studied.

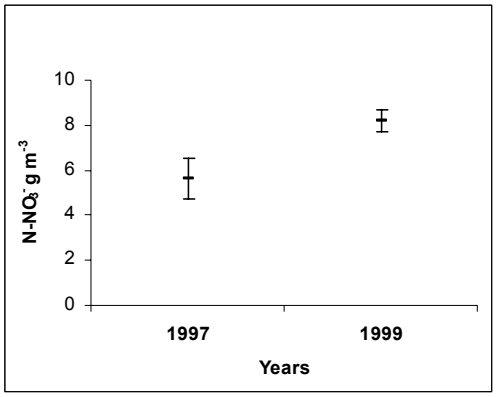


Fig. 41. Average $N\text{-}NO_3^-$ (g m^{-3}) concentration in drainage wastewater as dependent on years of experiment. Bars indicate 95% coincidence intervals

12. CONCLUSIONS

The study conducted in the period of 1997-1999 in the „Hajdów" experimental object with organic soil and various plants cover on changes in the redox potential and on the content of various forms of nitrogen permitted the formulation of the following conclusions:

1. Decrease in the concentration of $N\text{-}NH_4^+$ in the soil profile indicates that, with respect to their suitability in the process of wastewater purification, the plants grown in the experiment can be arranged in the following decreasing order: grass mix, willow, and rape; in the case of concentration of the $N\text{-}NO_3^-$ ion, the corresponding series would be as follows: grass mix, rape, and willow.
2. Considerably higher concentration of $N\text{-}NO_3^-$ and lower concentration of $N\text{-}NH_4^+$ in spring were demonstrated, which may suggest intensification of the
3. process of nitrification. For this reason, wastewater doses should be reduced in spring to prevent the process of nitrates(V) migration to the ground waters.
4. In a great majority of cases discussed, the concentration of $N\text{-}NO_3^-$ in drainage waters did not exceed 15 g $N \cdot m^{-3}$, which meets the requirements imposed by the European Union.
5. Significant relationships have been shown between the redox potential and nitrogen transformations taking place in a soil irrigated with wastewater after 2nd stage of treatment.
6. The best aeration conditions were found down to the depth of 30 cm, where Eh values held above +350 mV, with the possibility of nitrifiers development; below the depth of 50 cm redox potential decreased to a value permitting the development of denitrifiers; at the depth of 100 cm redox potential reached

negative values, characteristic for methanogenesis and for the formation of sulphides.

7. A significant negative effect of wastewater dosage on redox potential was observed, especially in the case of the double dose, causing the reduction of redox potential value below the level of +200 mV, corresponding to dissimilative reduction of nitrates(V) to the forms of N_2O and N_2.
8. From the second year of irrigations, a distinct lowering of the redox potential was observed, which could indicate that the soil was loosing its buffering properties, probably due to the alternating cycles of flooding and drying and to the decrease in microbial activity. Lowering of the concentration of nitrates(V) in the drainage waters may suggest intensification of the process of denitrification of $N\text{-}NO_3^-$ in the object studied.
9. Wastewater application in the single dose may provide a valuable source of nitrogen in the cultivation of plants for industrial purposes.

13. REFERENCES

1. **Abou-Seada, M.N.I., Ottow, J.G.C. 1985.** Effect of increasing oxygen concentration on total denitrification and nitrous oxide release from soil by different bacteria. Biol. Fertil. Soils 1:31-38.
2. **Achtnich, C., F. Bak, R. Conrad. 1995.** Competition for electron donors among nitrate reducers, ferric iron reducers, sulphate reducers, and methanogenesis in anoxic paddy soil. Biology and Fertility of Soils 19: 65-72.
3. **Adams M.A., Attiwill P.M. 1982.** Nitrate reductase activity and growth response of forest species to ammonium and nitrate sources of nitrogen. Plant and Soil., 66: 373-381.
4. **Ambus P., Lowrance R. 1991.** Comparison of denitrification in two riparian soil. Soil Sci. Soc. Am. J., 55, 994-997.
5. **Albers B.P., Beese F., Hartman A. 1995.** Flow-micro-calorimetry measurements of aerobic and anaerobic soil microbial activity. Biol. Fertil. Soils 19, 203-208.
6. **Aleem, M.I.H. 1985.** Biochemistry of chemolitotrophic nitrogen cycle. In Proc. Int. Symp. Nitrogen and the Environment, Malik, K.A., Mujtaba Naqvi, S.H., and Aleem, M.I.H., Eds. Nuclear Institute for Agriculture and Biology, Faisalabad, Pakistan, 29.
7. **Al-Hadhrami, M.N., H.M. Lappin-Scott, P.J. Fisher. 1997.** Studies on the biodegradation of three groups of pure n-alkanes in the presence of molasses and mineral fertilizer by *Pseudomonas aeruginosa*. Mar. Pollut. Bull. 34:969–974.
8. **Amundson, R.G. Davidson, E.A. 1990.** Carbon dioxide and nitrogenous gases in the soil atmosphere. J.Geochem.Explor.38:13-41.
9. **Anderson, J.C. Levine, J.S. 1987.** Simultaneous field measurements of biogenic emission of nitric oxide and nitrous oxide. Journal of Geophysical Research 92:965-976.

10. **Anonymous. 1992.** Keys to soil taxonomy. Agency for Int. Development/ USDA Soil Conservation Service Soil Management Support Services Tech. Monogr. no. 19. 5th ed. Pocahontas Press, Blacksburg, VA.
11. **Arah, J.R.M. Smith, K.A. 1990b.** Factors influencing the fraction of the gaseous products of soil denitrification evolved to the atmosphere as nitrous oxide. In Bouwman, A.F. ed Soil and the greenhouse effect. Proceedings of the International Conference Soils and the Greenhouse Effect. International Soil Reference and Information Centre ISRIC. John Wiley and Sons, New York pp. 475-480.
12. **Aulakh, M.S., Rennie, D.A. Paul, E.A. 1984a.** Acetylene and N-serve effects upon N_2O emissions from NH_4^+ and NO_3^- treated soils under aerobic and anaerobic conditions. Soil Biol. Biochem. 16; 351-356.
13. **Aulakh, M.S., Rennie, D.A. Paul, E.A. 1984b.** Gaseous nitrogen losses from soils under zero-till as compared with conventional-till management systems. J. Environ. Qual. 13; 130-136.
14. **Bailey L.D., Beauchamp E.G. 1971.** Nitrate reduction, and redox potentials measured with permanently and temporarily placed platinum electrodes in saturated soils. Can. J. Soil Sci., 51, 51.
15. **Bailey, L.D. 1976** Effects of temperature and root denitrification in a soil. Canadian Journal of Soil Science 56:79-87.
16. **Baker A.J. M., Mcgrath S. P., Sidoli C. M. D., Reeves R. D. 1995.** The potential for heavy metal decontamination. Mining Environ. Mgt 3, 12-14.
17. **Banerji, M.R., D.L. Burton, S. Depoe. 1997.** Impact of sewage sludge application on soil biological characteristics. Agric. Ecosyst. Environ. 66:241–249.
18. **Baran S., Turski R. 1999.** Wybrane zagadnienia z utylizacji i unieszkodliwiania odpadów, Wydawnictwo Akademii Rolniczej Lublin, 194-246.
19. **Barsdate R.J., Aleksander V. 1975.** The nitrogen balance of Arctic tundra: Pathways, rates, and environmental implications. Journal of Environmental Quality 4: 111-117.
20. **Bartlett R.J. 1986.** Soil redox behaviour. pp. 179-207. In D.L. Sparks ed., *Soil Physical Chemistry*. CRC Press, Boca Raton, Florida.
21. **Belser L.W. 1979.** Population ecology of nitrifying bacteria. Annu. Rev. Microbiol., 33, 309.
22. **Benckiser G., Haider K. Sauerbeck D. 1986.** Field measurements of gaseous nitrogen losses from an Alfisol planted with sugar-beets. Z. Pflanzenernähr. Bodenk. 149; 249-261.
23. **Benckiser G., Simarmata T. 1994.** Environmental impact of fertilizing soils by using sewage and animal wastes. Fert. Res. 37.
24. **Berner R.A. 1984.** Sedimentary pyrite formation: An update. Geochimica et Cosmochimica Acta 48: 605-615.
25. **Betlach M.R. Tiedje J.M. 1981.** Kinetic explanation for accumulation of nitrite, nitric oxide, and nitrous oxide during bacterial denitrification.. Appl. Environ. Microbiol. 42; 1074-1084.
26. **Białkowicz F. 1995.** Oczyszczanie i utylizacja ścieków miejskich na plantacjach wierzby krzewiastej połączone z produkcją drewna opałowego. Zeszyt Problemowy PZITS Nr 672, Technika Sanitarna Wsi, Wrocław.

27. **Blackmer A.M. Bremner J.M. 1978a.** Inhibitory effect of nitrate on reduction of N_2O to N_2 by soil microorganisms. Soil Biology and Bichemistry 10; 187-191
28. **Blackmer A.M., Robbins S.G. Bremner J.M. 1982.** Diurnal variability in rate of emission of nitrous oxide from soils. Soil Science Society of America Journal 46:937-942
29. **Bloomfield C. 1952.** The distribution of iron and aluminium oxides in gley soils. J. Soil Sci. 3:167–171.
30. **Błażejewski R. 1993.** Złoża trzcinowe – proste i tanie oczyszczanie ścieków. Gospodarka ściekami i odpadami w gminach, Poznań
31. **Bohn H.L. 1971.** Redox potentials. Soil Sci., 112, 1, 39-45.
32. **Bohn H. L., B.L. McNeal G.A. O'Connor. 1985.** *Soil Chemistry*. 2nd ed. Wiley, New York,
33. **Bonin P., Gilewicz M. Bertrand J. 1989.** Effects of oxygen on each step of denitrification on *Pseudomonas nautica*. Can. J. Microbiol. 35; 1061-1064
34. **Bouwman A.F. 1990b.** Exchange of greenhouse gases between terrestrial ecosystem and the atmosphere 4.5. nitrous oxide In: Soil and the greenhouse effect. Proc. Of Int. Conf. Soils and the Greenhouse Effect. International Soil Reference and Information Centre ISRIC. Ed A.F. Bouwman.
35. **Boyajian G.E., Sumner R.B. 1997.** Phytoremediation: a cost-effective cleanup solution. Chem. Waste Litigation Rep. 34, 967-974,
36. **Brandyk T. 1978.** Oczyszczanie i wykorzystanie rolnicze ścieków i osadów cukrowni. Rozprawa habilitacyjna. IMUZ Falenty.
37. **Breitenbeck G.A., Blackmer A.M. Bremner J.M. 1980.** Effects of different nitrogen fertilizer on emission of nitrous oxide from soil. Geophysical Research Letters. 7; 85-88.
38. **Breitenbeck G.A., Bremner J.M. 1986.** Effects of various nitrogen fertilizer on emission of nitrous oxide from soils. Biology and Fertility of Soils 2;195-199.
39. **Bremner J.M. Blackmer A.M. 1978.** Nitrous oxide: emission from soils during nitrification of fertilizer nitrogen. Science 199:295-296.
40. **Braun C. Zumft W.G. 1991.** Marker exchange of the structural genes nitric oxide reductase blocks the denitrification pathway of *Pseudomonas Stutzeri* at nitric oxide. J.Biol.Chem. 266:22785-22788.
41. **Bricker O.P. 1982.** Redox potential: Its measurement and importance in water systems. pp. 55-83. In R.A. Minear and L.H. Kieth eds., *Water Analysis*. Vol. 1. Academic Press, New York,
42. **Brzezińska M., Stępniewska Z., Stępniewski W. 1998.** Soil oxygen status and dehydrogenase activity. Soil Biol. Biochem. Vol. 30. No 13 pp. 1783-1790.
43. **Buol S.W., Hole F.D., McCracken R.J. 1989.** Soil genesis and classification, 3rd ed. Iowa State Univ. Press, Ames.
44. **Buresh R.J., Austin E.R. 1988.** Direct measurement of dinitrogen and nitrous oxide flux in flooded rice fields. Soil Sci. Soc. Am. J. 52;681-687
45. **Burford J.R., Dowdell R.J. Grees R. 1981.** Emission of nitrous oxide to the atmosphere from direct-drilled and ploughed clay-soil. Journal Science Food Agriculture 32:219-223

46. **Burton D.L., Beauchamp E.G. 1985.** Denitrification rate relationship with soil parameters in the field. Commun. Soil Sci. Plant Anal. 16; 539-549
47. **Caccavo F., Blakemore R.P., Lovley D.R. 1992.** A hydrogen-oxidizing, Fe III-reducing microorganism from the Great Bay Estuary, New Hampshire. Applied and Environmental Microbiology 58: 3211-3216.
48. **Callebaut F., Gabriels D., Winjauw W., De Boodt M. 1982.** Redox potential, oxygen diffusion rate, and soil gas composition in relation to water table level in two soils. Soil Science 134: 149-15.
49. **Carter J.P., Hsiao Y.H., Spiro S., Richardson D.J. 1995.** Soil and sediment bacteria capable of aerobic nitrate respiration. Applied and Environmental Microbiology 61: 2852-2858.
50. **Casella S., Leporini C., Nuti M.P. 1984.** Nitrous oxide production by nitrogen-fixing fast growing *Rhizobia*, Microbiol. Ecol., 10, 107.
51. **Cates R.L., Keeney D.R. 1987b.** Nitrous oxide production throughout the year from fertilized manured maize fields. Journal of Environmental Quality 16:443-447
52. **Cate R.B. 1964.** New data on the chemistry of submerged soils: Possible relationship to bauxite genesis. Econ. Geol. 59:161–162.
53. **Colbourn P., Harper I.W. 1987.** Denitrification in drained and undrained arable clay soil. J. Soil Sci., 38, 531-540,
54. **Cole J.A. 1988.** Assimilatory and dissimilatory reduction of nitrate to ammonia. In J.A. Cole and S.J. Ferguson eds. The nitrogen and sulphur cycles. Cambridge University Press, Cambridge. pp. 306-308.
55. **Conrad R. Seiler W., Bunse G. 1983.** Factors influencing the loss of fertilizer nitrogen in the atmosphere as N^2O. Journal of Geophysical Research 88:6709-6718
56. **Couto W., Sanzonowicz C., de Barcellos. O.A. 1985.** Factors affecting oxidation-reduction processes in an Oxisol with a seasonal water table. Soil Science Society of America Journal 49: 1245-1248.
57. **Cunningham S. D, Berti W. R., Huang J. W. 1995.** Phytoremediation of contaminated soils. Trends Biotechnol. 13, 393-397.
58. **Cunningham S. D., Anderson T. A., Schwab A. P., Hsu F. C. 1996.** Phytoremediation of soils contaminated with organic pollutants. Adv. Agron. 56, 55-114,
59. **Daniel, R.M. Grey, J. 1976.** Nitrate reductase from anaerobically grown *Rhizobium japonicum*. J. Gen. Microbiology, 96, 247.
60. **Davidson E.A., Swank W.T., Perry T.O. 1986.** Distinguishing between nitrification and denitrification as a sources of gaseous nitrogen production in soil. Applied Environmental Microbiology 52:1280-1286.
61. **Davidson E.A. 1992.** Sources of nitric oxide and nitrous oxide following wetting of dry soil. Soil Science Society of America Journal 56:95-102.
62. **Davidson E.A., Matson P.A., Vitousek P.M., Riley, R., Dunkin, K., Garcia-Mčndez, G., Maass, J.M. 1993.** Processes regulating soil emissions of NO and N_2O in a seasonally dry tropical forest. Ecology 74; 130-139
63. **Delwiche C.C., Bryan, B.A. 1976.** Denitrification. Annu. Rev. Microbiol., 30, 241.

64. **Denmead O.T., Freney J.R., Simpson J.R. 1979.** Nitous oxide emission from a grass sward. Soil Science Society of America Journal 43:726-728.
65. **Dévai I., Felföldy L., Wittner I., Plósz S. 1988.** Detection of phosphine: New aspects of the phosphorus cycle in the hydrosphere. Nature 333: 343-345.
66. **Dix M. E., Klopfernstein N. B., Zhang J.-W., Workman S. W., Kim M.-S. 1997.** Potential use of populus for phytoremediation of environmental pollution in Riparian zones. (In:) Micropropagation, genetic engineering, and molecular biology of Populus. Klopfernstein N. B., Chun Y. W., Kim M.-S., Ahuja M.R. red. USDA Forest Service Gen. Tech. Rep. RM-GTR-297, 206-211.
67. **Dorland S., Beauchamp E..G. 1991.** Denitrification and ammonification at low soil temperatures. Can. J. Soil Sci. 71, 293-303.
68. **Dowdell R.J., Smith K.A. 1974.** Field studies of the soil atmosphere II. occurrence of nitrous oxide. Journal of Soil Science 25:231-238
69. **Dunbabin J. S., Bowmer K.H. 1992.** Potential use of constructed wetlands for treatment of industrial wastewaters containing metals. Sci. Total Environ. 111, 151-168.
70. **Duxbury J.M., Mc Connaughey P.K. 1986**. Effect of fertilizer source on denitrification and nitrous oxide emissions in a maize field. Soil Sci. Soc. Am. J. 50:644-648.
71. **Ensley B. D., Raskin J., Salt D. E.** 1997. Phytoremediation applications for removing heavy metal contamination from soil and water. (In:) I. Sayler et al. eds. Plenum Press, New York, str. 59-64.
72. **Erich M.S., Bekerie A. 1984.** Activities of denitrifying enzymes in freshly sampled soils. Soil Sci. 138: 25-32.
73. **Eswaran H., Van Den Berg E., Reich P., Kimble J. 1995.** Global soil carbon resources. pp. 27-43. In R. Lal, J. Kimble, E. Levine, and B.A. Stewart eds., *Soils and Global Change.*CRC Lewis Publishers, Boca Raton, Florida.
74. **Evans, C.V, Franzmeier D.P. 1988.** Colour index values to represent wetness and aeration in some Indiana soils. Geoderma 41: 353-368.
75. **Falkengren-Grerup U.** 1995.Interspecies differences in the preference of ammonium and nitrate in vascular plants. Oecologia 102: 305-311.
76. **Fashchevsky B.V., Fashchevskaya G. 2003.** Ecological Hydrology: New Scientific Direction for Water Resource Management, http://www.wrrc.dpri.kyoto-u.ac.jp/~aphw/APHW2004/proceedings/OHS/56-OHS-M162/56-OHS-M162.pdf
77. **Fashchevsky B.V., 1996.** Fundamentals of ecological hydrology. Ecoinvest.
78. **Faulkner S.P., Patrick W.H., Gambrell R.P. 1989.** Field techniques for measuring wetland soil parameters. Soil Science Society of America Journal 53: 883-890.
79. **Fava F., Gioia D.D. 1998.** Effects of Triton X-100 and Quillaya Saponin on the ex situ bioremediation of a chronically polychlorobiphenyl-contaminated soil. Appl. Microbiol. Biotechnol. 50: 623–630.
80. **Fenchel T., Blackburn T.H. 1997.** Bacteria and Mineral Cycling. Academic Press, London. chapter 5.

81. **Fillery I.R.P. 1983.** Biological denitrification. In Gaseous loss of nitrogen from plant soil systems. Developments in Plant and Soil Sciences, vol.9, ed. Frey, J.R. and Simpson, J.R. The Hague. Martinus Nijhoff/Dr W. Junk. pp.33-64.
82. **Firestone M.K. Tiedje J.M. 1979.** Temporal changes in nitrous oxide and dinitrogen from denitrification following onset of anaerobis. Applied Environmental Microbiology 38:673-679
83. **Firestone M.K., Firestone R.B. Tiedje J.M. 1980.** Nitrous oxide from soil denitrification: Factors controlling its biological production. Science 208: 749-751
84. **Firestone M.K.** 1982 Biological denitrification. In Stevenson, F.J. Nitrogen in agricultural soils. Number 22 in the series agronomy. American Society of Agronomy, Inc., Crop Science Society of America, Inc., Soil Science Society of America, Inc. Publisher Madison, Wisconsin USA; 289-326
85. **Firestone M.K., Davidson E.A. 1989.** Microbiological basis of NO and N_2O production and consumption in soil. In Andrea, M.O. and Schimel, D.S. eds Exchange of the trace gases between terrestrial ecosystems and the atmosphere. pp. 7-22. Report for the Dahlem Workshop on Exchange of Gases between terrestrial Ecosystems and the Atmosphere, Berlin 1989. J. Wiley and Sons.
86. **Focht D.D. 1974.** The effect of temperature, pH and aeration on production of nitrous oxide and gaseous nitrogen - a zero order kinetic model. Soil Sci. 118; 173-179.
87. **Focht D.D., Verstreate D. V. 1977.** Biochemical ecology of nitrification and denitrification. Adv. Microbiol. Ecol., 1.
88. **Folorunso O.A. Rolston D.E. 1985.** Spatial and spectral relationships between field-measured denitrification gas fluxes and soil properties. Soil Science Society of America Journal 49;1087-1093.
89. **Ford H.W. 1971**. Interrelationships between sulphides oxygen and iron in ground water. Soil Crop Sci. Soc. Fla. Proc. 31:7–9.
90. **Ford H.W. 1974.** Biochemical and physical factors contributing to resistance in drain outflow in a modified spodosol. Soil Crop Sci. Soc. Fla. Proc. 34:10–12.
91. **Ford H.W. 1975.** Blockage of drip irrigation filters and emitters by iron sulphur bacterial products. Hort Science 10:62–64.
92. **Freney J.R.; Denmead O.T. Simpson J.R. 1979.** Nitrous oxide emission from soils at low moisture contents. Soil Biology and Biochemistry 11:167-173.
93. **Freney J.R., Simpson J.R., Denmead O.T., Muirhead W.A., Leuning, R. 1985.** Transformation and transfer of nitrogen after irrigation a cracking clay soil with a urea solution. Aust. J. Agric. Res. 36; 685-694
94. **Gale P.M., Gilmour J. T. 1988.** Net mineralization of carbon and nitrogen under aerobic and anaerobic conditions. Soil Society of America Journal 52: 1006-1010.
95. **Galsworthy A.M., Burford, J.R. 1978.** A system for measuring the rates of evolution of nitrous oxide and nitrogen from incubated soil during denitrification. J.Soil Sci. 29; 537-550.
96. **Gassmann G., Glindemann D. 1993.** Phosphane PH_3 in the biosfere. Angewandte Chemie, International Edition in English 32: 761-763

97. **Gauthier D.K, Clark-Walke, G.D., Garrara, W.T., Lascelles,J. 1970.** Nitrate reductase and soluble cytochrome c in *Spirillum itersonii*. J. Bacteriol. 102, 797.
98. **Gliński J., Stępniewski W. 1984.** Procesy biologiczne i chemiczne w glebie uzależnione od stanu natlenienia. Problemy Agrofizyki, 44.
99. **Gliński J., Stępniewski W. 1985.** Soil Aeration and its Role for Plants. CRC Press Inc.
100. **Gliński J., Stępniewski W., Stępniewska Z., Ostrowski J., Włodarczyk T., Brzezińska M. 2000a.** Agroekologiczne aspekty warunków tlenowych gleb ornych. Monografia. Acta Agrophysica No. 32, in Polish.
101. **Gliński J., Stępniewski W., Stępniewska Z., Włodarczyk T., Brzezińska M. 2000b.** Characteristic of aeration properties of selected soil profiles from Central Europe. Int. Agrophysics, vol.14, No 1, 17-31.
102. **Golterman H.L. 1991.** Influence of FeS on denitrification in shallow waters. Verh. Int. Ver. Theor. Angew. Limnol. 24:3025-3028.
103. **Goodroad, L.L., Keeney, D.R. 1984a.** Nitrous oxide emission from soils during thawing Canadaian Journal of Soil Science 64:187-194
104. **Goodroad L.L., Keeney, D.R. 1984c.** Nitrous oxide production in aerobic soils under varying pH, temperature and water content. Soil Biology and Biochemistry 16:39-43
105. **Granli T., Bøckman O. 1994.** Nitrous oxide from agriculture. Norw. J. Agricul. Sci. Suppl., 12.
106. **Groffman P.M., Tiedje, J.M. 1988.** Denitrification hysteresis during wetting and drying cycles in soil. Soil Sci. Soc. Am. J. 52; 1626-1629
107. **Groffman P.M., Tiedje J.M. 1991**. Relationships between denitrification, CO_2 production and air-filled porosity in soils of different texture and drainage. Soil Biol. Biochem. 23; 299-302
108. **Grundman G.L., Rolston D.E. 1987.** A water function approximation to degree of anaerobiosis associated with denitrification. Soil Sci. 144; 437-441.
109. **Hansen S., Mćhlum J.E., Bakken L.R. 1993.** N_2O and CH_4 fluxes in soil influenced by fertilization and tractor traffic. Soil Biol. Biochem. 25; 621-630
110. **Hao W.M., Scharffe D., Crutzen P.J., Sanhueza, E. 1988.** Production of nitrous oxide, methane, and carbon dioxide from soils in tropical savannah during the dry season. J. Atmos. Chem. 7; 93-105.
111. **Hasselblad S., Hallin S. 1998.** Intermittent addition of external carbon to enhance denitrification in activated sludge. Water Sci. Technol. 37:227–233.
112. **Haynes R.J., Goh K.M. 1978.** Ammonium and nitrate nutrition of plants. Biological Reviews 53: 465-510.
113. **Haynes R.J., Naidu R. 1998.** Influence of lime, fertilizer and manure applications on soil organic matter content and soil physical conditions: A review. Nutr. Cycling Agroecosyst. 51:123–137.
114. **Heinemeyer O., Haider K. Mosier A. 1988.** Phytotron studies to compare nitrogen losses from corn-planted soil by the 15-N balance or direct dinitrogen and nitrous oxide measurements. Biol. Fertil. Soils 6; 73-77

115. **Hillel D. 1998.** Environmental Soil Physics. Academic Press, San Diego-London.
116. **Horn R., Stępniewski W., Włodarczyk T., Walenzik G., Eckhardt F.E.W. 1994.** Denitrification rate and microbial distribution within homogenous model soil aggregates. Int. Agrophysics 8: 65-74.
117. **Howeler R.H., Bouldin D.R. 1971.** The diffusion and consumption of oxygen in submerged soils. Soil Science Society of America Proceedings 35: 202-208.
118. **Hutchinson G.L., Brams E.A. 1992.** Nitric oxide versus nitrous oxide emissions from an ammonium ion-amended Bermuda grass pasture. J. Geophys. Res. 97; 9889-9896
119. **Januszek K. 1978.** Potencjał oksydoredukcyjny wybranych gleb leśnych Polski Południowej w świetle badań polowych i laboratoryjnych. Praca doktorska, AR Kraków.
120. **Januszek K. 1999.** Aktywność enzymatyczna wybranych gleb leśnych Polski południowej w świetle badań polowych i laboratoryjnych. Rozprawa habilitacyjna. Zeszyty Naukowe AR w Krakowie, zeszyt 250, Kraków.
121. **Jeffery J.W.O. 1960.** Iron and the Eh of waterlogged soils with particular reference to paddy. J. Soil Sci. 11:140–148.(
122. **Jørgensen S.E. 1994.** Fundamentals of Ecological Modelling, 2nd Edn. Elsevier Amsterdam, London, New York, Tokyo, 117-118.
123. **Ingraham J.L. 1981.** Microbiology and genetics of denitrifiers. In Denitrification, Nitrification and Atmospheric Nitrous Oxide, Delwiche, C.C., Ed., John Wiley and Sons, New York, 67.
124. **Kadlec R.H. 1987.** Nutrient dynamics in wetlands, in aquatic plant for water treatment and resource recover. K.R. Reddy & W.H. Smith, Magnolia Publishing, Inc., Orlando, Florida.
125. **Kalisz L. 1993.** Wykorzystanie roślin korzeniowych do oczyszczania ścieków. Gospodarka ściekami i odpadami w gminach, Poznań.
126. **Kaplan W.A., Wofsey, S.C. 1985.** The biogeochemistry of nitrous oxide: A review. Adv. Agric. Microbiol. 3:181-206.
127. **Keeney D.R., Fillery I.R., Marx, G.P. 1979.** Effect of temperature on gaseous N products of denitrification in soil. Soil Science Society of America Journal 43:1124-1128.
128. **Kerner M. 1993.** Coupling of microbial fermentation and respiration processes in an inertial mudflat of the Elbe Estuary. Limnology and Oceanography 38: 314-330.
129. **Killman K. 1986.** Heterotrophic nitrification. In J.I. Prosser ed.. Nitrification. IRL Press, Oxford. pp.117-126.
130. **Kirschbaum M.U.F. 1995.** The temperature dependence of soil organic matter decomposition, and the effect of global warming on soil organic C storage. Soil Biol. Biochem. 27, 753-760.
131. **Klemedtsson L. Svensson B.H., Rosswall T. 1988.** Relationship between soil moisture content and nitrous oxide production during nitrification and denitrification. Biology and Fertility of Soils 6:106-111
132. **Klinkhammer G.P. 1980.** Early diagensisin sediments from the eastern equatorial Pacific. II. Pore water metal results. Earth and Planetary Science Letters 49: 81-101.
133. Knowles R. 1982. Denitrification. Microbial Rev. 46:43–70.

134. **Kondzielski I., Buczkowski R., Szymański T. 1996.** Biologiczne metody remediacji wód i gleb zanieczyszczonych metalami ciężkimi. Ekologia i Technika 5/6, 23-27.
135. **Korom S.F. 1992.** Natural denitrification in the saturated zone: A review. Water Resour. Res. 28:1657–1668.
136. **Kowalik P.J., Lewis S. 1995.** Złoża trzcinowe i wiklinowe jako czyszczalnie odcieków z wysypisk. Konferencja naukowa nt. „Oczyszczalnie hydrobotaniczne", Gdańsk.
137. **Kowalik P.J., Obarska-Pempkowiak H. 1997.** Oczyszczalnie hydrofitowe w Polsce. W: Wawrentowicz D. Ed., Oczyszczanie ścieków – nowe trendy, modernizacja istniejących oczyszczalni i gospodarka osadowa, Białystok.
138. **Kralova M., Masscheleyn P.H., Lindau C.W., Patrick W.H. jr. 1992.** Production of dinitrogen and nitrous oxide in soil suspensions as affected by redox potential. Water, Air, Soil Pollut., 61, 37-45.
139. **Kreitinger J.P., Klein T.M., Novick N.J., Alexander M. 1985.** Nitrification and characteristics of nitrifying microorganisms in an acid forest soil. Soil Sci. Soc. Am. J., 49, 1407-1410.
140. **Letey J., Hadas A., Valoras N., Focht D.D. 1980a.** Effect of preincubation treatments on the ratio of N_2O/N_2 evolution. Journal Environmental Quality 9:232-235.
141. **Letey J., Hadas A., Valoras N., Focht D.D. 1980b.** Effect of air-filled porosity, nitrate concentration, and time on the ratio of N_2O/N_2 evolution during denitrification. Journal Environmental Quality 9:227-231.
142. **Li C., Frolking S., Frolking T.A. 1992a.** A model nitrous oxide evolution from soil driven by rainfall events; 1. Model structure and sensitivity. J. Geophys. Res. 97; 9759-9776.
143. **Li C., Frolking S., Frolking T.A. 1992b.** A model nitrous oxide evolution from soil driven by rainfall events; 2. Model applications J. Geophys. Res. 97; 9777-9783
144. **Lidster W.A., Ford H.W. 1981.** Rehabilitation of ochre iron clogged agricultural drains. p. Q.36–R.27, 451–463. In 11th Congr. Int. Commission on Irrigation and Drainage, New Delhi, India. ICID, New Delhi.
145. **Linn D.M., Doran J.W. 1984.** Effect of water-filled pore space on carbon dioxide and nitrous oxide production in tilled and no tilled soils. Soil Sci. Soc. Am. J. 48; 1267-1272.
146. **Lovley D.R., Klug M.J. 1986.** Model for the distribution of sulphate reduction and methanogenesis in freshwater sediments. Geochimica et Cosmochimica Acta 50: 11-18.
147. **Lovley D.R., Phillips E.J.P. 1987.** Competitive mechanisms for inhibition of sulfate reduction and methane production in the zone of ferric iron reduction in sediments. Applied and Environmental Microbiology 53: 2636-2641.
148. **Lovley D.R., Phillips E.J.P. 1988a.** Novel model of microbial energy metabolism: Organic carbon oxidation coupled to dissimilatory reduction of iron or manganese. Applied and Environmental Microbiology 54: 1472-1480,
149. **Lovley D.R., Phillips E.J.P. 1988b.** Manganese inhibition of microbial iron reduction in anaerobic sediments. Geomicrobiology Journal 6: 145-155.

150. **Lovley D.R., Phillips E.J.P. 1989.** Requirement for a microbial consortium to completely oxidize glucose in Fe III-reducing sediments. Applied and Environmental Microbiology 55: 3234-3236.
151. **Lovley D.R. 1995.** Microbial reduction of iron, manganese, and other metals. Advances in Agronomy 54: 175-231.
152. **Malhi S.S., McGill W.B., Nyborg, M. 1990.** Nitrate losses in soils: effect of temperature, moisture and substrate concentration. Soil Biol. Biochem. 22; 733-737.
153. **Malicki M., Walczak R. 1983.** A gauge of the redox potential and oxygen diffusion rate in the soil with an automatic regulation of cathode potential. ZPPNR. 220, II 447-451.
154. **Mancino C.F., Torello W.A. and Wehner D.J. 1988.** Denitrification losses from Kentucky bluegrass sod. Agron.J. 80; 148-153.
155. **Masscheleyn P.H. DeLaune R.D., Patrick W.H. 1993.** Methane and nitrous oxide emissions from laboratory measurements of rice soil suspension - effect of soil oxidation-reduction status. Chemosphere 26; 251-260.
156. **Matthews E., I. Fung. 1987.** Methane emission from natural wetlands: Global distribution, area, and environmental characteristics of sources. Global Biogeochemical Cycles 1, 61-86.
157. **Megonigal J.P., Patrick W.H., Faulkner S.P. 1993.** Wetland identification in seasonally flooded forest soils: Soil morphology and redox dynamics. Soil Science Society of America Journal 57: 140-149.
158. **McKenney D.J., Shuttleworth K.F., Findlay W.J. 1980.** Nitrous oxide evolution rates from fertilized soil: effects of applied nitrogen. Canadian Journal of Soil Science 60:429-438.
159. **McKenney D.J., C.F. Drury and S.W. Wang. 2001.** Effect of oxygen on denitrification inhibition, repression, and depression in soil columns. Soil Sci. Soc. Am. J. 65:126-132.
160. **Mosier A.R., Hutchinson B.R. 1981.** Nitrous oxide emission from cropped field. Journal of Environmental Quality 10;169-173
161. **Mosier A.R., Stillwell M., Parton W.J., Woodmansee R.G. 1981.** Nitrous oxide emissions from a native short grass prairie. Soil Science Society of America Journal 45; 617-619.
162. **Mosier A.R., Parton W.J., Hutchinson G.L. 1983.** Modelling nitrous oxide evolution from cropped and native soils. In Hallberg, R. ed Environmental biogeochemistry. Ecol. Bull. Stockholm 35:229-241.
163. **Mosier A.R., Guenzi W.D., Schweizer E.E. 1986.** Soil losses of dinitrogen and nitrous oxide from irrigated crops in north-eastern Colorado. Soil Sci. Soc. Am. J. 50; 344-348.
164. **Mosier A.R. 1989.** Chamber and isotope techniques. In Andrea, M.O. and Schimel, D.S. eds.. Exchange of trace gases between terrestrial ecosystems and the atmosphere pp. 175-187, J. Wiley and Sons.
165. **Mulvaney R.L., Kurtz L.T. 1984.** Evolution of dinitrogen and nitrous oxide from nitrogen-15 fertilized soil cores subjected to wetting and drying cycles. Soil Science Society of America Journal 48:596-602.
166. **Murakami T.N., Kumazowa K. 1987.** The effects of soil conditions and nitrogen form on nitrous oxide evolution by denitrification. Soil Sci. Plant Nutr. 33; 35-42.

167. **Myrold D.D., Tiedje J.M. 1985.** Establishment of denitrification capacity in soil: Effect of carbon, nitrate and moisture content. Soil Biol. Biochem. 17:819-822.
168. **Myrold D.D. 1988.** Denitrification in ryegrass and winter wheat cropping system of western Oregon. Soil Sci. Soc. Am. J. 52; 412-416.
169. **Nadelhoffer K.J., Aber J.D., Melillo J.M. 1984.** Seasonal patterns of ammonium and nitrate uptake in ine temperature forest ecosystems. Plant and Soil 80, 321-335.
170. **Nealson K.H., Myers C.R. 1992.** Microbial reduction of manganese and iron: New approaches to carbon cycling. Applied and Environmental Microbiology 58: 439-443.
171. **Nicholas D.J.D. 1985.** Recycling of N_2 and H_2 in a denitrifying photosynthetic bacterium. In Proc. Int. Symp. Nitrogen and the Environment, Malik, K.A., Mujtaba Naqvi, S.H., and Aleem, M.I.H., Eds. Nuclear Institute for Agriculture and Biology, Faisalabad, Pakistan, 93.
172. **Nõmmik H. 1956.** Investigations on denitrification in soil. Acta Agricultura Scandinavia 6: 195-228
173. **Nugroho S.G., Kuwatsuka S. 1992.** Concurrent observation of several processes of nitrogen metabolism in soil amended with organic materials. 2. Effect of farmyard manure on ammonification, nitrification, denitrification and N_2-fixation at different levels of soil moisture. Soil Sci. Plant Nutr. 38; 593-600
174. **Obarska-Pempkowiak H. 1991.** Seasonal variations in the efficiency of nutrient removal from domestic effluent in a quasi-natural field of reed Phragmites communis. W: Etnier C. & Guaterstarm B. Eds., Ecological Engineering for Wastewater Treatment, Bakskogen, Szwecja, 239-247.
175. **O'Hara G.W., Daniel R.M. 1985.** Rhizobial denitrification: a review. Soil Biology and Biochemistry 17:1-9.
176. **Opracowanie WIOŚ 1997** pod kierunkiem Żelaznego L., Bańkowskiej-Królikowskiej J.: Pięciolecie działalności WIOŚ, Biblioteka Monitoringu Środowiska Lublin
177. **Ottow J.C.G. 1971.** Iron reduction and gley formation by nitrogen-fixing *Clostridia*. Oecologia 6: 164-175.
178. **Ottow J.G.C., Glathe, H. 1973.** Pedochemie und Pedomikrobiologie hydromorpher Böden: Merkmale Voraussetzungen und Ursachen der Eisenreduktion. Chem.. ERde 32:1-44.
179. **Parkin T.B., Tiedje J.M. 1984.** Application of soil core method to investigate the effect of oxygen concentration on denitrification. Soil Biol. Biochem. 16; 331-334.
180. **Parkin T.B. 1987.** Soil microsites as a source of denitrification variability. Soil Science Society of American Journal 51:1194-1199.
181. **Parsons L.L., Murray, R.E., Scott S. 1991.** Soil denitrification dynamics: Spatial and temporal variations of enzyme activity, populations and nitrogen gas loss. Soil Sci. Soc. Am. J. 55; 90-95.
182. **Parton W.J., Mosier A.R. Schimel D.S. 1988.** Rates and pathways of nitrous oxide production in a short grass steppe. Biogeochemistry 6; 45-58.
183. **Patrick W.H., Tusneem M.E. 1972.** Nitrogen loss from flooded soil. Ecology 53: 735-737.
184. **Patrick W.H., Jugsujinda A. 1992.** Sequential reduction and oxidation of inorganic nitrogen, manganese, and iron in flooded soil. Soil Science Society of America Journal 56: 1071-1073.

185. **Patten D.K., Bremner J.M., Schimel, D.S. 1980.** Effects of drying and air-dry storage of soils on their capacity for denitrification of nitrite. Soil Science Society of America Journal 44:67-70.
186. **Paul E.A., Clark F.E.:** Soil Microbiology and Biochemistry. eds E.A Paul, F.E. Clark. Academic Press, Toronto, 1996.
187. **Payne W.J. 1973.** Reduction of nitrous oxides by micro- organisms. Bacteriological Revives 37: 409-452.
188. **Payne W.J. 1981.** Denitrification. John Wiley, New York. 214 pp.
189. **Perttu K.L., Kowalik P.J. 1997.** Salix vegetation filters for purification of waters and soils. Biomass and Bioenergy 12, 9-19.
190. **Ponnamperuma F.N. 1972.** The chemistry of submerged soils. Advances in Agronomy 24: 29-96.
191. **Peters V., Conrad R. 1996.** Sequential reduction processes and initiation of CH_4 production upon flooding of oxic upland soils. Soil Biology and Biochemistry 28: 371-382.
192. **Post W.M., Emanuel W.R., Zinke P.J., Strangenberger A.G. 1982.** Soil carbon pools and world life zones. Nature 298, 156-159.
193. **Postgate J.R., Kent H.M., Robson R.L. 1988.** Nitrogen fixation by *Desulfovibrio*. pp.457-471. In J.A. Cole and S.J. Ferguson eds., *The Nitrogen and Sulphur Cycles*. Cambridge University press, Cambridge.
194. **Punshon T., Dickinson N M. 1997.** Acclimation of Salix to metal stress. New Phytol. 137, 303-314.
195. **Raich J.W., Rastetter E.B., Melillo J.M., Kicklighter D.W. Steudler P.A., Peterson B.J., Grace A.L., More B., Vörösmarty B.J. 1991.** Potential net primary productivity in South America: Application of a global model. Ecological Applications 1, 399-429.
196. **Raport końcowy PBZ 31-03. 1998.** Sprawozdanie merytoryczne z realizacji projektu badawczego zamawianego. Opracowanie zintegrowanego systemu oczyszczania ścieków miejskich połączonego z nawadnianiem upraw przemysłowych. Red.: Filipek T., Lublin.
197. **Reed S.C., Brown D. 1992.** Constructed wetland design – The first generation, Research Journal of the WEF, vol. 64, no. 6, 776-781.
198. **Reddy K.R., Patrick W.H. 1975.** Effect of alternate aerobic and anaerobic conditions on redox potential, organic matter decomposition and nitrogen loss in a flooded soil. Soil Biology and Biochemistry 7: 87-94.
199. **Reddy K.R., Patrick W.H. 1976.** Effect of frequent changes in aerobic and anaerobic conditions on redox potential and nitrogen loss in a flooded soil. Soil Biology and Biochemistry 8: 491-495.
200. **Report WIOŚ. 1997.** Report of Regional Institute for Environment Protection,
201. **Richardson C.J., Davies J.A. 1987.** Natural and artificial wetland systems: Ecological opportunities and limitations, in aquatic plants for water treatment and resource recovery. K.R. Reddy & W.H. Smith, Magnolia Publishing, Inc., Orlando, Florida
202. **Robertson L.A., Kuenen J.G. 1984b.** Aerobic denitrification: a controversy revived. Arch. Microbiol. 139; 351-354.

203. **Robertson, L.A., Van Kleeff, B.H.A. and Kuenen, J.G.** 1986 A microcomputer-based method for semi-continuous monitoring of biological activities. J. Microbiol. Methods 5; 237-242.
204. **Robertson G.P., Vitousek P.M., Matson P.A., Tiedje J.M. 1987.** Denitrification in a clear cut Loblolly pine plantation in the south-eastern US. Plant Soil, 97, 119-129.
205. **Robertson L.A., Kuenen J.G. 1990.** Physiological an ecological aspects of aerobic denitrification, a link with heterotrophic nitrification?. Revsbech, N.P. and Sorensen, J. eds Denitrification in soil and sediment. Plenum Press, New York. pp. 91-104.
206. **Robertson L.A., Kuenen J.G. 1991.** Physiology of nitrifying and denitrifying bacteria.In Rogers, J.E. and Whitman, W.B. eds Microbial production and consumption of greenhouse gases: methane, nitrogen oxides, and halomethanes. American Society for Microbiology, Washington D.C. 189-235.
207. **Rolston D.E., Hoffman D.L., Toy, D.W. 1978.** Field measurement of denitrification: I. Flux of N_2 and N_2O. Soil Science Society of America Journal 42, 863-869.
208. **Rolston D.E., Sharpley D.W., Toy, D.W. 1982.** Field measurement of denitrification: 3. Rates during irrigation cycles. Soil Science Society of America Journal 46, 289-296.
209. **Rowell D.L. 1988.** Flooded and poorly drained soils. W: W. Russells „Soil Condition and Plant Growth", A. Wild Wyd., 11th Edition, Longman Scientific & Technikal.
210. **Ruiz-Herrera, J., DeMoss, J.A. 1969.** Nitrate reductase complex of *Escherichia coli* K-12; participation of specific formate dehydrogenase and cytochrome b1 components in nitrate reduction. J. Bacteriol., 99, 720.
211. **Ruiz-Herrera, J., Showe, M.K., DeMoss, J.A. 1969.** Nitrate reductase complex of *Escherichia coli* K-12; isolation and characterization of mutants unable to reduce nitrate. J. Bacteriol., 97, 1291.
212. **Ryden J.C., Lund L.J. 1980.** Nitrous oxide evolution from irrigated land. J. Environ. Qual. 9; 387-393.
213. **Ryden J.C. 1983.** Denitrification loss from grassland in the field receiving different rates of nitrogen as ammonium nitrate. J.Soil Sci., 34, 355-365.
214. **Sahrawat K.L., Keeney D.R. 1986.** Nitrous oxide emission from soils In Stewart, B.A. ed.1986 Advances in soil science volume 4 Springer-Verlag, New York pp. 103-148York; 127-149.
215. **Salt D. E., Blaylock M., Kumar N. P. B. A., Dushenkov V., Ensley B. D., Chet I., Raskin I. 1995.** Phytoremediation: a novel strategy for the removal of toxic metals from the environment using plants. Bio/Technology 13, 468-474.
216. **Samson M.I., Buresh R.J., De Datta S.K. 1990.** Evolution and soil entrapment of nitrogen gases formed by denitrification in flooded soil. Soil Sci. Plant Nutr., 36, 299-307.
217. **Scott Smith M., Zimmmerman K. 1981.** Nitrous oxide production by nondenitrifying soil nitrate reducers. Soil Sci. Soc. Am. J. 45:865-871.
218. **Schlesinger W.H., Marks P.L. 1977.** Mineral cycling and the niche of Spanish moss. *Tillandsia usneoides* L. American Journal of Botany 64: 1254-1262.
219. **Schlesinger W.H. 1997.** Biogeochemistry and Analysis of Global Change. Academic Press.

220. **Schmidt J., Seiler W., Conrad, R. 1988.** Emission of nitrous oxide from temperature forest soils into the atmosphere. Journal of Atmospheric Chemistry. 6, 95-115.
221. **Schuster M., Conrad R. 1992.** Metabolism of nitric oxide and nitrous oxide during nitrification and denitrification in soil at different incubation conditions. FEMS Microbiol. Ecol. 101; 133-143.
222. **Smith M.S., Tiedje J.M. 1979.** The effect of roots on soil denitrification. Soil Science Society of America Journal 43:951-955.
223. **Smith K.A. 1980.** A model of extent of anaerobic zones in aggregated soils, and its potential application to estimates of denitrification. J. Soil Sci. 31; 263-277
224. **Smith K.A., Arah, J.R.M. 1990.** Losses of nitrogen by denitrification and emission of nitrogen oxides from soils. Proceedings 299 The Fertiliser Society pp. 1-34.
225. **Slemr F., Conrad R., Seiler W. 1984.** Nitrous oxide emission from fertilized and unfertilised soils in a subtropical region Andalusia, Spain. Journal of Atmospheric Chemistry 1:159-169.
226. **Sławiński C., Sokołowska Z., Walczak R. 2000. Effects** of secondary transformation of peaty-moorsh soils on their physical properties. Acta Agrophysica, 26, 85-94.
227. **Sherlock R.R., Goh, K.M. 1983.** Initial emission of nitrous oxide from sheep urine applied to pasture soil. Soil Biology and Biochemistry 15: 615-617.
228. **Shoun H., Kim D-H., Uchiyama H., Sugiyama J. 1992.** Denitrification by fungi. FEMS Microbiol. Lett. 94: 277-282.
229. **Smirnov P.M. Kidin V.V., Pedishyus A. 1979.** Loss of nitrogen by denitrification. Biol. Bull. Acad. Sci. USSR 6; 450-459.
230. **Smith C.J., Chalk, P.M. 1980.** Gases nitrogen evolution during nitrification of ammonia fertilizer and nitrite transformations in soil. Soil Science Society of America Journal 44:277-282.
231. **Smith C.J. Wright M.F., Patrick W.H. jr. 1983.** The effect of soil redox potential and pH on the redaction and production of nitrous oxide. Journal Environmental Quality 12:186-188.
232. **Skiba U., Hargreaves K.H., Fowler D., Smith K.A. 1992.** Fluxes of nitric and nitous oxide from agricultural soils in a cool temperate climate. Atmos. Environ. 26A; 2477-2488.
233. **Sort X., Alcaniz J.M. 1999a.** Effect of sewage sludge amendment on soil aggregation. Land Degrad. Develop. 10:3–12.
234. **Sort X., Alcaniz J.M. 1999b.** Modification of soil porosity after application of sewage sludge. Soil Tillage Res. 49:337–345.
235. **Straub K.L., Beng M., Schink B., Widdel F. 1996.** Anaerobic, nitrate-dependent microbial oxidation of ferrous iron. Applied and Environmental Microbiology 62: 1458-1460.
236. **Stępniewska Z. 1988.** Własności oksydoredukcyjne gleb mineralnych Polski. Problemy Agrofizyki, 56, 1-104.
237. **Stępniewski W., Przywara G. 1992.** The influence of oxygen availability on yield and nutrient uptake N, P, K, Ca, Mg, Na by winter rye *Secale cereale*. Plant and Soil 143, 267-274.
238. **Stępniewski W., Stępniewska Z., Przywara G., Brzezińska M., Włodarczyk T., Varallyay G. 2000.** Relation between aeration status and physical parameters of selected Hungarian soils. Int. Agrophysics, 14, No 4, 439-447.

239. **Stępniewska Z., Stępniewski W., Gliński J., Ostrowski J. 1997.** Atlas właściwości oksydo-redukcyjnych gleb mineralnych Polski. Morpol, Lublin.
240. **Stępniewska Z., Brzezińska M., Gliński J., Stępniewski W., Włodarczyk T., Čurlik J., Houškova B. 2000**. Aeration status of some Slovakian soils. Int. Agrophysics, 14, 327-339.
241. **Stumm W., Morgan J.J. 1981.** Aquatic Chemistry 2nd ed. Wilej, New York.
242. **Stolzy L.H. Focht D.D., Flühler H. 1981.** Indicators of soil aeration status. Flora 171: 236-265.
243. **Stouthamer, A.H. 1976.** Adv. Microb. Physiol. 14, 315.
244. **Stouthamer A.H. 1988.** Dissimilatory reduction of oxidized nitrogen compounds. in A.J.B. Zehnder ed. Biology of anaerobic microorganisms. John Wiley & Sons Ltd.,New York, NY. pp.245-303.
245. **Sweerts J., Bär-Gilissen M.J., Cornelese A.A., Cappenberg T.E. 1991.** Oxygen-consuming processes at the profundal and littoral sediment-water interface of a small meso-eutrophic lake Lake Vechten, The Netherlands. Limnolopgy and Oceanography 36: 1124-1133.
246. **Tamm C.O. 1991.** Nitrogen in Terrestrial Ecosystems. Ecological Studies, Vol.81. Eds. W.D.Billigs, F. Golley, O.L. Lange, J.S. Olson, H. Remmert. Springer-Verlag Berlin, Heidelberg, New York, London, Paris.
247. **Tate III R.L. 1995.** Process control in soil. In: Soil Microbiology Eds Robert L., Tate III. John Wiley & Sons inc. New York, Chichester, Brisbane, Toronto, Singapore.
248. **Terry R.E., Tate, R.L. 1980.** The effect of nitrate on nitrous oxide reduction in organic soils and sediments. Soil Science Society of America Journal 44, 744-746.
249. **Terry R.E., Tate R.L., Duxbury J.M. 1981.** The effect of flooding on nitrous oxide emission from an organic soil. Soil Sci. 132; 228-232
250. **Tiedje J.M. 1981.** Use of nitrogen-13 and nitrogen-15 in studies on the dissimilatory fate of nitrate. In: Genetic engineering of symbiotic nitrogen fixation and conservation of fixed nitrogen Eds J.M Lyons et al.. Plenum Press, New York, 481-497.
251. **Tiedje J.M. 1988.** Ecology of denitrification and dissimilatory nitrate reduction to ammonium. In Zehner, J.B. ed Biology of anaerobic microorganisms. Wiley, New York pp.179-244.
252. **Umarov M.M. 1990.** Biotic sources of nitrous oxide in the context of the global budget of nitrous oxide. in A.F. Bouwman ed. Soils and the greenhouse effect. John Wiley & Sons Ltd., Chichester. pp. 263-268.
253. **Van Cleemput O., Patrick W.H. 1974.** Nitrate and nitrite reduction in flooded, gamma-irradiated soil under controlled pH and redox potential conditions. Soil Biol. Biochem., 6, 85-88.
254. **Van de Geijn S.C., Van Neen J.A. 1993.** Implications of increased carbon dioxide levels for levels for carbon input and turnover in soils. Vegetatio 104/104, 283-292
255. **Verstraete W. 1981.** Nitrification. In: Clark F.E., Rosswall T. eds Terrestrial nitrogen cycles. Ecol. Bull. Stockh., 33, 303-314.
256. **Verstraete W., Philips S. 1998.** Nitrification–denitrification processes and technologies in new context. Environ. Pollut. 102: 717–726.

257. **Vinther F.P. 1984.** Total denitrification and the ratio between N_2O and N_2 during the growth of spring barley. Plant Soil 76; 227-232.
258. **Virginia R.A Jarrell W.M. 1983.** Soil properties in a mesquite-dominated Sonoran desert ecosystem. Soil Science Society of America Journal 47:138-144.
259. **Webster C.P., Dowdell R.J. 1982.** Nitrous oxide emission from permanent grass swards. J. Sci. Food Agric. 33; 227-230.
260. **Weier K.L., J.W. Gillam. 1986.** Effect of acidity on denitrification and nitrous oxide evolution from Atlantic coastal plain soils. Soil Science Society of America Journal 50: 1202-1206.
261. **Weier K.L., Doran J.W. Power J.F., Walters D.T. 1993.** Denitrification and the dinitrogen/nitrous oxide ratio as affected by soil water, available carbon and nitrite. Soil Sci. Soc. Am. J. 57; 66-72.
262. **Winkler J.P., Cherry R.S., Schlesinger W.H. 1996.** The Q_{10} relationship of microbial respiration in a temperate forest soil. Soil Biol. Biochem. 8, 1067-1072.
263. **Włodarczyk T. 2000.** Emisja i absorpcja N2O na tle emisji CO_2 w glebach brunatnych w zróżnicowanych warunkach oksydoredukcyjnych. Acta Agrophysica, 28.
264. **Włodarczyk T., Stępniewska Z., Brzezińska M. 2001.** Wpływ temperatury na emisję N_2O i CO_2 z gleb brunatnych i czarnoziemnych wytworzonych z lessu. Acta Agrophysica 57, 169-176.
265. **Witkowska-Walczak B., Bieganowski A., Rovdan E. 2002.** Water-air properties in peat, sand and their mixtures. Int. Agrophysics, 16, 4,313-318.
266. **Wojciechowski K. 1995. Ocena** jakości biologicznego oczyszczania ścieków na oczyszczalniach korzeniowych, Praca naukowo-dydaktyczna W. S. Inż., Zielona Góra.
267. **Yu Tian Ren. 1985.** Physical chemistry of paddy soils. Science Press, Beijing, Springer-Verlag, Berlin.
268. **Zhou X., Mackenzie A.F., Madramootoo C.A., Kaluli J.W., Smith D.L. 1997.** Management practices to conserve soil nitrate in maize production systems. J. Environ. Qual. 26:1369–1374.
269. **Zumft W.G., Kroneck P.M.H. 1990.** Metabolism of nitrous oxide. In: Denitrification in soil and sediment Eds N.P. Revsbech and J. Sorensen. Plenum Press, New York, 37-55.