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**Effects of pH on
aquatic
biodegradation
processes**

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Effects of pH on aquatic biodegradation processes

R. F. Krachler¹, R. Krachler¹, A. Stojanovic¹, B. Wielander¹, and A. Herzig²

¹Institute of Inorganic Chemistry, University of Vienna, 1090 Vienna, Austria

²Biological Station Neusiedler See, 7142 Illmitz, Austria

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Correspondence to: R. F. Krachler (rudolf.krachler@univie.ac.at)

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Abstract

To date, little is known about the pH-stimulated mineralization of organic matter in aquatic environments. In this study, we investigated biodegradation processes in alkaline waters. Study site is a large shallow soda lake in Central Europe (Neusiedler See/Ferto). The decomposition rate of plant litter was measured as a function of pH by incubating air-saturated lake-water samples in contact with *Phragmites* litter (leaves) from the littoral vegetation. All samples showed high decomposition rates (up to 32% mass loss within 35 days) and a characteristic two-step degradation mechanism. During the degradation process, the solid plant litter was dissolved forming humic colloids. Subsequently, the humic colloids were mineralized to CO₂ in the water column. The decomposition rate was linearly related to pH. Increasing pH values accelerated significantly the leaching of humic colloids as well as the final degradation process. The observed two-step mechanism controls the wetland/lake/air carbon fluxes, since large quantities of humic colloids are currently produced in the reed belt, exported through wind-driven circulations and incorporated into the open lake foodweb. At present, the lake is rapidly shrinking due to peat deposition in the littoral zone, whereas it has been resistant to silting-up processes for thousands of years. In order to investigate the cause of this abrupt change, the chemical composition of the lake-water was measured during 1995–2007. A thorough analysis of these data revealed that major lake-water discharges through the lake's artificial outlet channel led to a decline in salinity and alkalinity. According to our estimates, the lake's original salinity and alkalinity was 70–90% higher compared to the present conditions, with the consequence of substantially lower pH values in the present lake. The observed pH dependence of reed litter biodegradation rates points to a causal connection between low pH values and accumulation of peat in the lake basin. Our results suggest that the pH stimulated remineralisation of organic matter plays a major role in maintaining the long-term integrity of saline lake/wetland systems.

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1 Introduction

The aerobic mineralization of organic matter generates in all cases organic acids and carbonic acid and hence H^+ ions as a secondary product. Thus decreasing pH values inhibit further mineralization unless the H^+ ions are removed. Our core concept is that, in alkaline aquatic environments, water-soluble carbonates e.g. soda (sodium carbonate) stimulate the bio-mineralization of organic matter by the selective and rapid removal of H^+ ions.

Li et al. (2007) investigated the mineralization of organic carbon in naturally salt-affected sandy soils. They reported that increasing soil pH was associated with increasing CO_2 production and increasing soil microbial biomass. However, little is known about the pH-stimulated mineralization of organic matter in aquatic environments.

Our study site was Lake Neusiedler See (Lake Fertő), a large shallow (maximum depth 2 m) alkaline lake receiving high amounts of organic matter from an extended reed belt of *Phragmites australis*. The long-term stability of the lake depends on the rapid mineralisation of reed debris which otherwise would accumulate forming peat layers in the shallow lake basin. Digital terrain models of the bottom of Lake Neusiedler See by Bacsatyai et al. (1997) show that, at present, the volume of the lake basin is indeed rapidly shrinking due to peat deposition, whereas the lake has been resistant to silting-up processes for thousands of years.

The aim of this study was to verify the cause of the present vulnerability of the lake and to evaluate the significance of the carbonate concentration to the ecological stability of the lake/wetland system. The combination of model experiments under laboratory conditions and “real-world” hydrochemical and hydrological studies has the potential to greatly expand the depth of our understanding of aquatic systems. Our approach included therefore (1) laboratory experiments to investigate the influence of pH on the decomposition rate of reed litter, and (2) a thorough investigation of the long-term changes of the lake’s pH range and chemical composition, including those

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changes which have been caused by human activities.

2 Methods

2.1 Site description

Lake Neusiedler See, the largest water surface in Austria, is located 50 km east of Vienna (Figs. 1 and 2). It is an endorheic, shallow, saline-alkaline lake, extending to 321 km² of which 240 km² are located on Austrian and 81 km² on Hungarian territory. The origin of the lake's salinity is closely related to the marine sediments of the area (Tauber, 1959). There is good evidence that saline waters enter the lake along tectonic fracture zones (Löffler, 1979). About 55% of the lake area is covered by a *Phragmites australis* reed belt. A characteristic feature is the presence of autochthonously precipitated mineral particles, mainly calcite (Blohm, 1974; Preisinger, 1979) which is formed from the water column during aerogenic and biogenic decalcification. Due to the almost perpetual motion of the lake-water, the high pH values of >9.0, and low dissolved Ca²⁺ concentrations, the mineral particles form suspended colloids, which are responsible for the high turbidity of the lake-water ("white water"). Figure 3a shows a REM image of a typical suspended particle in Lake Neusiedler See, a conglomerate of hundreds of small carbonate crystals. In addition, remnants of biomineralisation like diatom shell fragments (Fig. 3b) are important constituents of the lake's suspended matter.

In 1909, an artificial outlet channel has been constructed for drainage purposes to improve the regional agricultural conditions. Since 1965, a weir is operated to control the outflow from the lake. During the 20th century, large volumes of lake-water have been extracted through the artificial outflow and discharged into the River Danube. Simultaneously, a shrinking of the open lake area due to accumulation of organic-rich sediments in the littoral zone has been observed (Weisser, 1973).

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2.2 Chemical analyses and trend determination

In order to estimate the time-dependent changes of the lake's salt stock, we performed a trend analysis on a large base of hydrochemical data. A detailed ion analysis of the lake-water was conducted using flame atomic absorption spectroscopy (F-AAS; Perkin Elmer AA300) and ion chromatography (IC; ICS1000, Dionex Corp., CA, USA). Alkalinity was determined via acid-base titration with 0.1 mol L^{-1} HCl and evaluated by means of Gran derivation (ATI-Orion 940 with ATI-Orion 960-Dispenser). CO_2 partial pressure was measured using a Qubit Systems S157 CO_2 Analyzer. Conductivity (Ecoscan con6, Eutech instruments) and pH (penetration glass electrode VWR, pH100) were measured in situ in the field. Water levels were recorded simultaneously at seven monitoring stations, covering the whole lake area. Wind-driven water circulations and seiches (long standing waves) result in pronounced differences in the water levels at the same moment at different sites. It was therefore necessary to determine average water levels.

The trend determination is based on the following eight parameters: Specific electrical conductivity (EC); alkalinity (A); and the concentrations of Ca^{2+} , Mg^{2+} , Na^+ , K^+ , Cl^- , and SO_4^{2-} . $A = 2[\text{CO}_3^{2-}] + [\text{HCO}_3^-] + [\text{OH}^-]$, concentrations (mol L^{-1}) are indicated by enclosing the species in brackets.

For each of these parameters, about 500 measured values per year were included in the analysis. This large amount of experimental data should allow reliable estimates. A total of 37 sampling stations (Fig. 1) were distributed over the lake in a way covering the central open lake area as well as the littoral zones and the reed belt. Due to the large spatial and temporal variability of the surface water hydrochemical parameters (Dinka et al., 2004), relatively high sampling frequencies in space and time were needed in order to estimate trends with a specified uncertainty. Since precipitation and evaporation are key factors in the water balance of Lake Neusiedler See, the lake's water volume is tightly linked to meteorological conditions and may rapidly increase due to periods of enhanced precipitation, or decrease during hot and dry summer periods,

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respectively. As a consequence, with changing water volume, the concentrations of dissolved species change periodically. In order to include all measurements of a seasonal cycle, the measured ion concentrations have been converted to a reference water level of 115.40 m above the Adriatic Sea. These calculations were performed on the base of a highly reliable digital terrain model of the bottom of Lake Neusiedler See, published by Bacsatyi et al. (1997) (cf. Fig. 4).

2.3 Modelling the carbonate system of Lake Neusiedler See

The pH-value of the lake-water depends on the ionic relationships and concentrations, as well as on the CO₂ concentration in the water column, and is mainly controlled by respiration (CO₂ source), photosynthesis (CO₂ sink) and wind turbulence. The latter tends to establish thermodynamic equilibrium between the lake-water, suspended carbonate minerals and the atmospheric CO₂ partial pressure. As a consequence of these features it is necessary to consider all three phases, aqueous, gas and solid, in order to describe the response of the system to external influences, e.g. dilution. The carbonate system is a weak acid-base system which exists in the lake-water as dissolved carbon dioxide, carbonic acid, bicarbonate and carbonate ions. Aqueous solutions of carbon dioxide contain CO₂ molecules as well as carbonic acid molecules H₂CO₃. However, it has been customary to represent all aqueous carbon dioxide as H₂CO₃^{*}, and we shall follow this practice here. Equations (1) involve the most important chemical species of the lake's carbonate system, the thermodynamic equilibrium constants are approximate values at 25°C (Garrels and Christ, 1965; Hofmann et al., 2008):

$$[\text{H}_2\text{CO}_3^*] = K_H \cdot p(\text{CO}_2) \quad K_H = 3 \times 10^{-2} \text{ M bar}^{-1}$$

$$\frac{[\text{H}^+] \cdot [\text{HCO}_3^-]}{[\text{H}_2\text{CO}_3^*]} = K_1 \quad K_1 = 5 \times 10^{-7}$$

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$$\frac{[\text{H}^+] \cdot [\text{CO}_3^{2-}]}{[\text{HCO}_3^-]} = K_2 \quad K_2 = 5 \times 10^{-11}$$

$$[\text{H}^+] \cdot [\text{OH}^-] = K_W \quad K_W = 10^{-14}$$

$$[\text{Ca}^{2+}] \cdot [\text{CO}_3^{2-}] = K(\text{CaCO}_3) \quad K(\text{CaCO}_3) = 4.8 \times 10^{-9}$$

$$[\text{H}^+] + 2 \cdot [\text{Ca}^{2+}] + 2 \cdot [\text{Mg}^{2+}] + [\text{Na}^+] + [\text{K}^+] =$$

$$5 \quad [\text{OH}^-] + 2 \cdot [\text{SO}_4^{2-}] + [\text{Cl}^-] + [\text{HCO}_3^-] + 2 \cdot [\text{CO}_3^{2-}] \quad (1)$$

Equations (1) can be solved to obtain the unknown equilibrium concentrations. Activity coefficients are assumed to approach unity ($\gamma_i=1$). Input parameters are the experimentally determined molar concentrations $[\text{Mg}^{2+}]$, $[\text{Na}^+]$, $[\text{K}^+]$, $[\text{SO}_4^{2-}]$, $[\text{Cl}^-]$, and the atmospheric CO_2 partial pressure. The lake-water is under-saturated with respect to $\text{Mg}(\text{OH})_2$ and MgCO_3 , respectively. The dissolution products of the latter salts are therefore not included in Eq. (1). For simplicity, the possible precipitation of Mg-calcite is neglected, as well as the possible influence of dissolved constituents such as Ca^{2+} -binding complex ligands (fulvic and humic acids) (Cizkova et al., 1999; Reitner et al., 1999; Balogh et al., 2006). The specific electrical conductivity (EC) indicates the degree of dilution of the dissolved electrolytes. As a first approximation, the ion concentrations of Na^+ , K^+ , Mg^{2+} , SO_4^{2-} and Cl^- can be regarded as proportional to EC. Using the experimental data in Table 1 as input parameters, a model pH curve was calculated (cf. Fig. 5, curve 1).

2.4 Experimentally determined pH values as a function of dilution

20 Figure 5 shows the relationship between the computed dependence of pH on the degree of dilution of the lake-water (curve 1) and experimentally determined EC and

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pH values at 25°C.

Twelve samples (à 5 L) were taken with a clean polyethylene bucket from the surface and transported to the laboratory, where they were concentrated by evaporation at room temperature or diluted with Millipore ultra-clean water, respectively, in order to create a concentration series representative of Lake Neusiedler See's possible states. The (non-filtered) samples were equilibrated with the atmosphere by stirring vigorously. Temperature, EC and pH were measured continuously. Thermodynamic equilibrium was established after 1–2 h. As shown in Fig. 5, the calculated curve (curve 1) is in excellent accordance with the experimental data.

2.5 Litter degradation experiments

A Lake Neusiedler See water sample taken from the open lake surface was divided into 1 L portions, and concentrated by evaporation at room temperature, or diluted with Millipore ultra-clean water, respectively, in order to create a concentration series between pH=8.7 and pH=9.2. In each case, 1.00 g of Lake Neusiedler See reed litter (leaves, dried at 60°C for 12 h) was added to 1 L of the (non-filtered) sample. The samples were filled into open polyethylene bottles, kept in the dark and equilibrated with the atmosphere by stirring vigorously during 5 weeks at 15.0±0.5°C. Water volumes lost by evaporation were currently replaced by Millipore ultra-clean water. Water temperature, EC and pH were measured continuously. After terminating the experiments, the reed litter granules were filtered using a 0.45 µm cellulose nitrate filter, Sartorius, and the mass loss was determined after drying the granules at 60°C using a Sartorius analytical balance. In the filtrate, the dissolved organic carbon (NPOC, Non Purgeable Organic Carbon) content was determined with a TOC analyzer (Shimadzu, TOC-V_{CPH}). Colloids were characterized with a Malvern MasterSizer2000. A series of control experiments done under the same conditions were performed with 1.00 g of Lake Neusiedler See reed litter and 1 L Millipore ultra-clean water.

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3 Results

3.1 Implications of lake-water discharges on pH and chemical composition

The pH of the lake-water depends strongly on the degree of dilution, especially during periods with high water levels and hence lower electrolyte concentrations as can be seen from the slope of the pH versus EC curve (curve 1) in Fig. 5. The lake achieves its typical $\text{pH} > 9$ as soon as the value of EC approaches 2.5 mS cm^{-1} .

In Fig. 6, the annual mean values of the Na^+ concentrations, obtained as described above and converted to a common reference water level of 115.40 m above MSL (Adriatic Sea), are compared with the annual discharge volumes through the Hansag Channel. Obviously, the 1996 lake-water discharge of $150 \times 10^6 \text{ m}^3$ produced a significant dilution of the lake's dissolved salts. We observe an abrupt sodium decrease of -3.5 mmol L^{-1} . After the total stop of discharges in March 2000, a recovery process has started. We find an annual Na^+ regeneration rate of $+0.79 \text{ mmol L}^{-1} \text{ y}^{-1}$ (Table 2). The high standard deviations during 1995 through 1997 are mainly caused by an increase in steepness of the lake's spatial chemical gradients from north to south, due to high flow rates through the drainage channel, which is situated at the southernmost tip of the lake.

Figure 7 shows the annual mean values of concentrations of ionic constituents and alkalinity, converted to water level 115.4 m above MSL (Adriatic Sea), and plotted against time for the years 2000 through 2007. The annual ion concentration changes during this period of hydrologically closed conditions were estimated using linear smoothing functions. Results of the averaging regression are summarized in Table 2. These results indicate an increase of the lake's dissolved salt stock. Only the K^+ ions (which are involved in clay mineral precipitation reactions) show constant concentrations. The trends in Table 2 point to a delayed recovery of the alkalinity concentration with respect to the other parameters (see also Fig. 7). If this current trend is extrapolated into future, the lake's chemical characteristics would substantially change. The anions sulphate and chloride would replace carbonate and bicarbonate,

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and alkalinity would decrease.

The sensibility of the pH value to shifts in the ionic ratios is presented in Fig. 5. (cf. curves 2–3). Curve 2 simulates a state of the lake where the alkalinity concentration has been reduced to one half (i.e. sodium carbonate has been partly replaced by sodium sulphate). In contrast, curve 3 shows the behaviour of a hypothetical Lake Neusiedler See with twofold alkalinity concentration (sodium sulphate has been partly replaced by sodium carbonate).

3.2 Estimating the pristine conditions of the lake

The pristine conditions of the lake were estimated using the analytical data in Table 2, under the assumption of constant salt accumulation rates. We used a scenario where the accumulating salts are periodically removed through large floods which statistically occur in intervals of 50 years (Löffler, 1979). After these floods, salt accumulation is assumed to start from dilute conditions similar to those measured during the year 2000. The reconstructed mean EC value is (converted to water level 115.40 m above MSL (Adriatic Sea)) $EC=4.5\pm 0.5 \text{ mS cm}^{-1}$. That means the original salinity in the lake was 70–90% higher compared to the present conditions, i.e. the lake was less diluted with the consequence of substantially higher pH values in the pristine lake (cf. Fig. 5).

3.3 Effect of pH on reed litter decomposition

Figure 8 shows results of our laboratory experiments. The mass loss of decomposing reed litter (leaves) was fairly rapid and only 68% of the dry mass remained at the end of the experiment during 35 days at pH=9.2. We observed a pronounced effect of pH on the decomposition rate, with lower decomposition rates at lower pH. The decomposition rate was linearly related to pH. During these experiments, the plant debris decomposition was accompanied by a shift in the water colour to brown due to the accumulation of humic substances. The measured DOC values varied between 20 mg L^{-1} and 40 mg L^{-1} . The humic colloids were up to molecular weight

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of 100 kg mol^{-1} or equal to about 20 nm of hydrodynamic diameter.

Control experiments performed with 1.00 g of Lake Neusiedler See reed litter and 1 L Millipore ultra-clean water showed only minor dry mass losses between 0.8% and 1.1%.

4 Discussion

4.1 Losses of salinity and alkalinity

The total lake-water discharge through the drainage channel in the time span 1965–2000 was 10^9 m^3 (Plattner, 2004). The lake basin has thus been five times emptied and, simultaneously, refilled within only 35 years. The observed losses of salinity and alkalinity point to a dominant role of rain- and snowmelt-waters as refill water masses.

In the present investigation, the impact of lake-water discharges on the salt stock of Lake Neusiedler See was quantified, as well as the lake's ability to compensate for anthropogenic salt losses. Our analytical data (Table 2) point to a mean annual salt accumulation in the lake basin of $77.67 \text{ mg L}^{-1} \text{ y}^{-1}$, corresponding to 14 900 tons per year.

The results show that the lake is chronically over-diluted due to water level manipulations, with the consequence of relatively low pH values in the water column.

4.2 Significance of pH to the remineralisation rate

Hietz (1992) studied the aerobic decomposition of reed litter in the reed belt of Lake Neusiedler See using an in situ litter bag technique. Decomposition was fastest at study sites near the open lake (pH values 9.0–9.2) and significantly slower in the reed belt where the waters show markedly lower pH values (8.2–8.4). Our laboratory experiments are in perfect accordance with these field observations.

Recent microbiological studies demonstrate that there is significant evidence

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to support our reconstruction of an originally less diluted soda lake. Bacterial communities associated with decaying plant material of *Phragmites australis* were extensively studied in Lake Neusiedler See by Borsodi et al. (1998, 2003, 2005) and Agoston-Szabo et al. (2006). These authors found microorganisms with remarkable adaptations in terms of salinity- and alkali-tolerance. Results of pH and salt tolerance tests revealed optima at pH=11 and in the presence of 2.5–5% NaCl. These bacteria, which are mainly responsible for the breakdown of reed litter, are obviously adapted to a more saline and alkaline environment.

We found that, in alkaline waters, reed litter is an important source of coloured aquatic humic substances. During our reed litter remineralisation experiments, high concentrations of brown-coloured organic substances (humic colloids) occurred in the water phase. This observation is in accordance with a field study on the decomposition of reed litter in Lake Balaton (a large shallow saline-alkaline lake in Hungary) by Balogh et al. (2006).

The amount of organic carbon which can be removed from the lake by oxidation depends on the O₂ availability. The level of biological oxygen demand of the first step (the production of colloidal-dissolved humic substances) is relatively low, since these degradation products are not yet fully oxidized. Colloidal degradation products accumulate in the oxygen-limited waters of the reed belt. By seiches, the humic colloids are exported from the reed belt (Bouchard, 2007), and distributed over the open lake by wind-driven currents. In contrast to the wind-sheltered reed belt, the open lake is wind-exposed, and is therefore well provided with oxygen. In the open lake, the humic colloids are rapidly incorporated in the food web and oxidized into carbon dioxide. (Amon and Benner, 1996; Reitner et al., 1999; Toth et al., 2007). Thus, wind exposure plays a major role in maintaining the integrity of shallow lake/wetland systems. Wind-driven carbon fluxes may allow the mineralization of 100% of the integrated primary production of the open lake and the reed belt. However, the carbon fluxes are also dependent on two pH-stimulated biodegradation steps: The formation of humic colloids and their subsequent oxidation into carbon dioxide. Our results

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suggest that high dissolved carbonate concentrations are of general importance to the long-term ecological stability of shallow alkaline lakes.

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Table 1. Hydrochemical characteristics of Lake Neusiedler See, extracted from a series of 37 samples taken on 02 May 2005 covering the total lake as given in Fig. 1. Concentrations (\pm SE) of ionic components (meqL^{-1}) as well as specific electric conductivity EC (\pm SE).

Mg^{2+}	K^+	Na^+	SO_4^{2-}	Cl^-	Alkalinity	EC (μScm^{-1})	CO_2 ppm
11.5 ± 0.6	1.10 ± 0.06	16.6 ± 1.1	11.7 ± 0.4	7.70 ± 0.51	12.0 ± 0.6	2650 ± 150	400

SE, standard error of 37 measurements covering the total lake area as given in Fig. 1.

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Table 2. Mean concentrations (\pm SE) of ionic components of Lake Neusiedler See (meqL^{-1}) as well as concentration trends for 2000 through 2007 ($\text{meqL}^{-1} \text{y}^{-1}$). Data converted to 115.40 m above MSL (Adriatic Sea).

year	EC (μScm^{-1})	K ⁺	Na ⁺	Mg ²⁺	Cl ⁻	SO ₄ ²⁻	alkalinity
2000	2082±231	0.92±0.14	13.68±1.64	10.80±1.73	6.12±0.82	8.86±0.94	11.13±1.49
2001	2061±253	0.89±0.11	13.25±1.68	10.87±1.13	5.85±0.67	8.57±0.80	10.79±1.62
2002	2049±237	0.78±0.13	13.76±1.60	10.57±1.23	5.94±0.67	8.58±0.83	10.42±1.45
2003	2113±332	0.82±0.13	14.27±1.54	10.80±1.43	6.37±0.79	9.24±0.73	10.39±1.73
2004	2229±269	0.96±0.09	15.47±1.54	11.20±1.27	6.94±0.86	10.12±0.88	10.60±1.26
2005	2325±280	1.08±0.22	16.25±1.30	11.39±0.97	7.35±0.63	10.98±0.88	10.47±1.12
2006	2721±354	1.16±0.12	18.11±1.87	13.70±1.30	8.23±1.04	11.25±1.01	12.66±1.27
2007	2566±627	1.17±0.15	18.43±2.08	13.95±2.16	8.48±1.04	11.73±1.13	12.56±2.64
trend	90.86 $R^2=0.77$	0.05 $R^2=0.66$	0.79 $R^2=0.91$	0.47 $R^2=0.70$	0.40 $R^2=0.89$	0.49 $R^2=0.90$	0.24 $R^2=0.37$

SE, standard error of 400 to 500 measurements covering the total lake area as given in Fig. 1.

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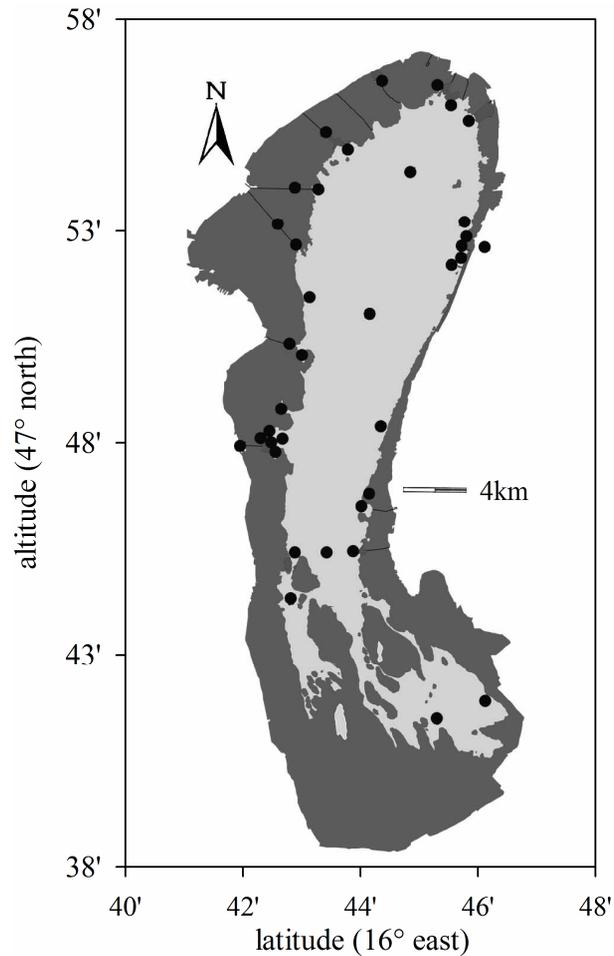


Fig. 1. Map of Lake Neusiedler See, reed belt and open lake. Sampling locations are indicated.

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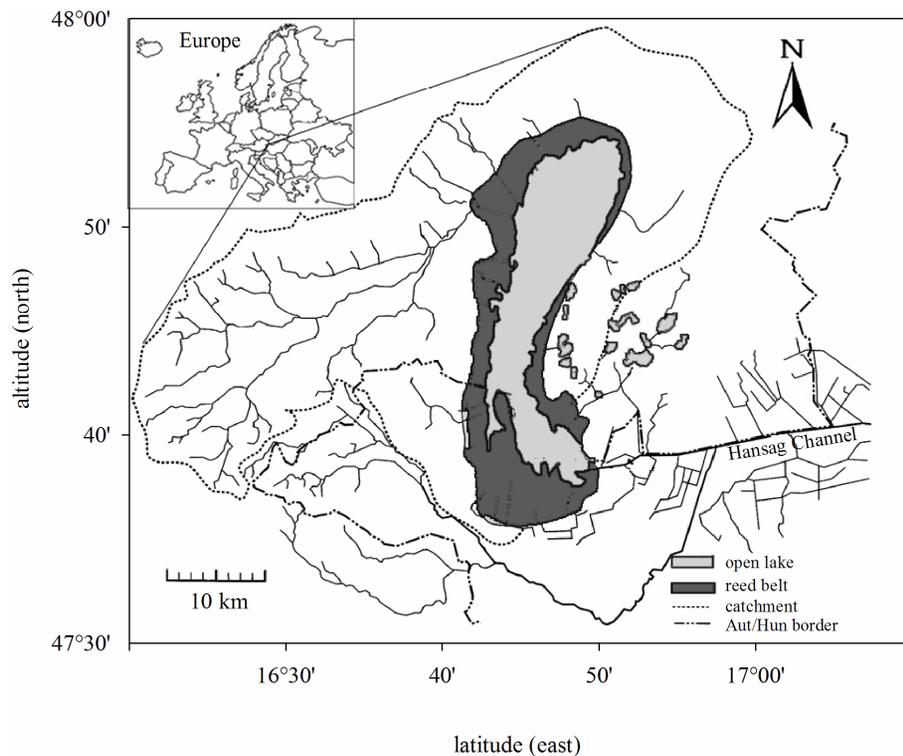


Fig. 2. Hydrogeologic catchment area of Lake Neusiedler See.

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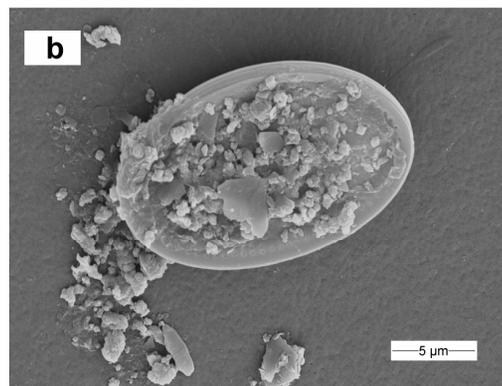
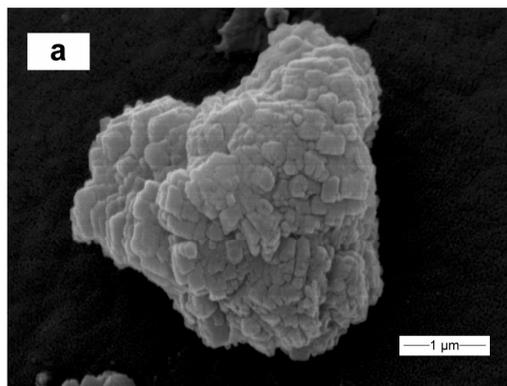


Fig. 3. (a) REM image of a typical suspended mineral particle in Lake Neusiedler See. (b) REM image of a diatom shell fragment together with other suspended particles in Lake Neusiedler See.

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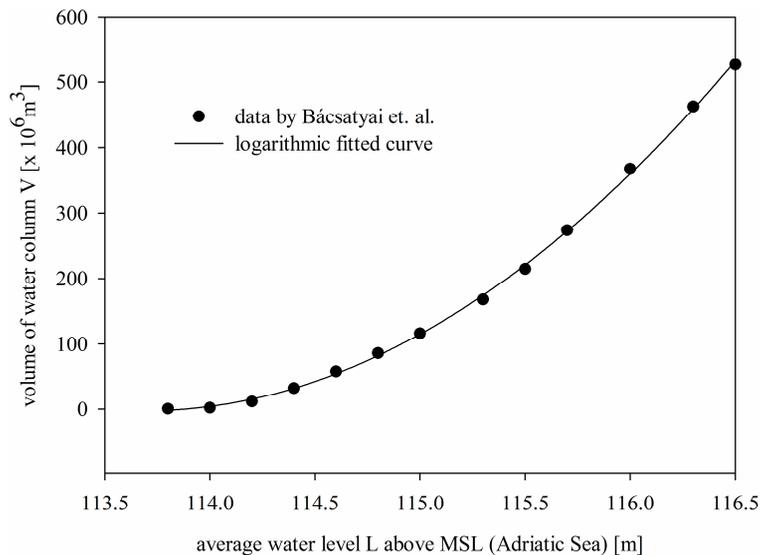


Fig. 4. Water volume plotted against average water levels (data by Bacsatyai et al., 1997).

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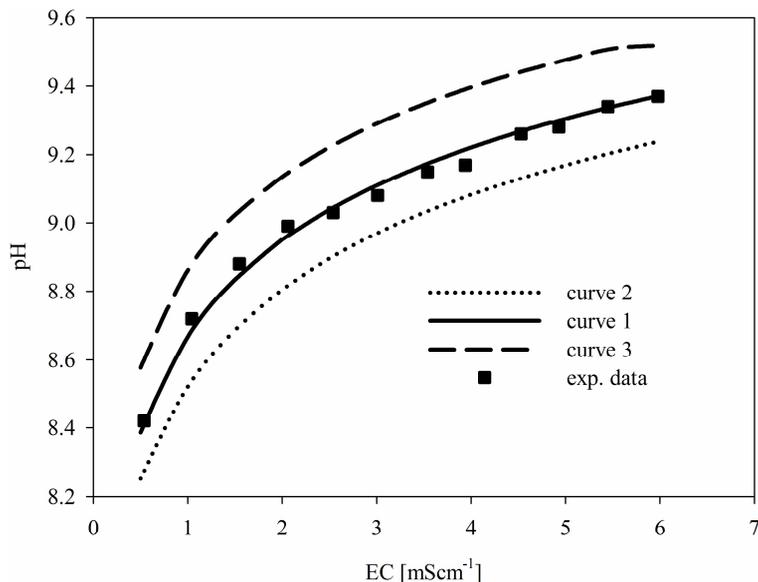


Fig. 5. Equilibrium pH value versus specific electric conductivity (EC) in Lake Neusiedler See water (25°C). Squares indicate experimental points (concentration series). Curve 1: calculated curve, input parameters are the data listed in Table 1. Curve 2 simulates a hypothetical lake-water composition where the alkalinity concentration is reduced to one half (sodium carbonate partly replaced by sodium sulphate). Curve 3 simulates twofold alkalinity concentration (sodium sulphate partly replaced by sodium carbonate).

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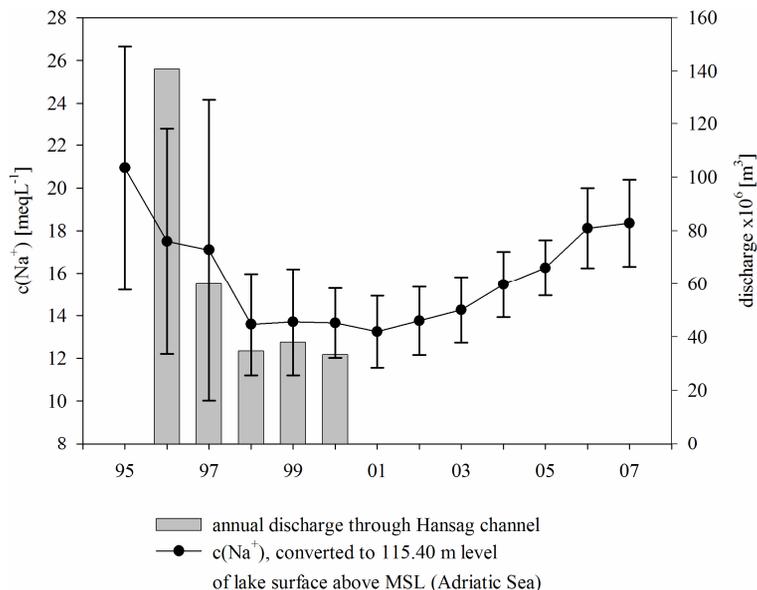


Fig. 6. The columns show the annual water discharges (outflow through the Hansag Channel) in the time span between 1995 and 2007 (Maracek, 1995–2007). The annual mean Na^+ concentrations (full circles) are derived from a number of ca. 500 measured values per year and have been converted to reference water level 115.40 m above MSL (Adriatic Sea). Standard deviations are indicated.

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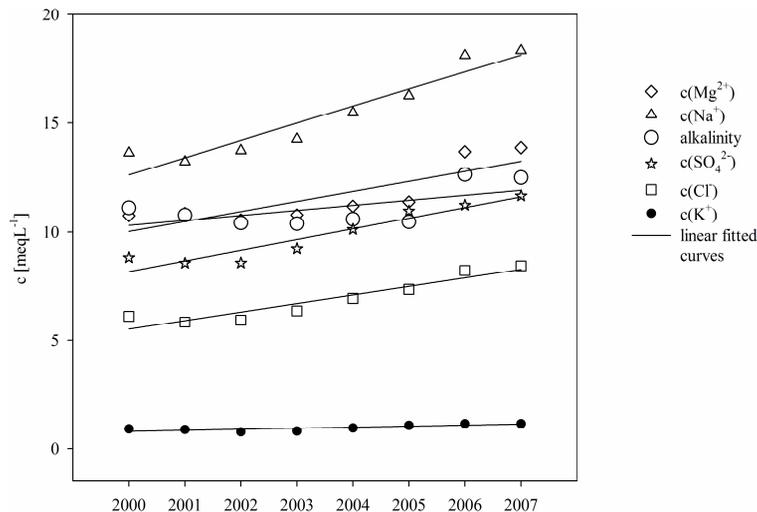


Fig. 7. Mean annual concentrations of major ionic constituents and alkalinity, development during 2000 through 2007. Data converted to reference water level 115.40 m above MSL (Adriatic Sea). Each data point represents approximately 500 measured values.

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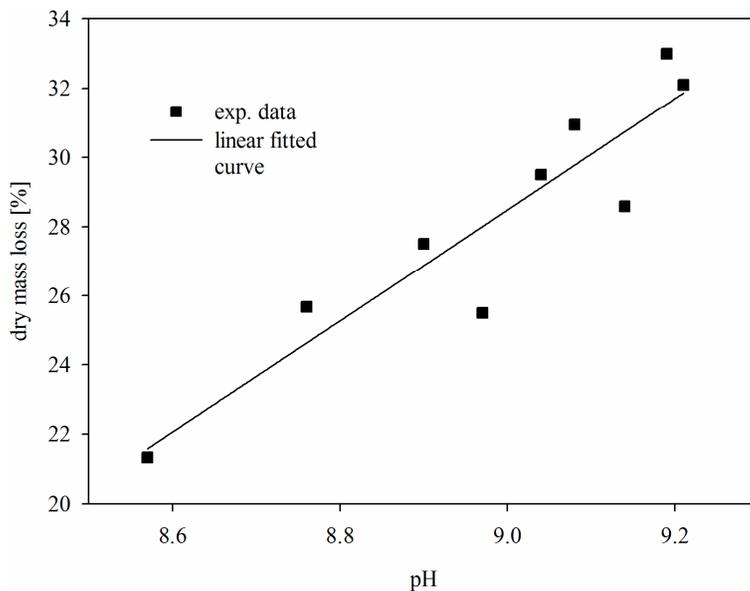


Fig. 8. Decomposition of reed litter granules in unfiltered air saturated Lake Neusiedler See water at 15°C in the dark. Dry mass loss (%) during 35 days as a function of pH.

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