Introduction

Saline lake ecosystems

Saline lakes are common landscape features on every continent. The volume of athalassic (inland) saline waters (104 000 km$^3$) is almost as great as the volume of the world’s freshwater lakes (125 000 km$^3$). Many saline lakes are small and shallow and may be ephemeral. Williams (1972) pointed out the uniqueness of saline lake ecosystems. They are very discrete in that they have no outlets and usually only temporary inflows. Their most important feature is the salt accumulation within the lake by evaporation. Sodium carbonate/sulphate dominated alkaline waters are mainly found in East Africa and North America, but also in Central Europe (Pannonian Plain in Hungary and eastern Austria) (Hammer, 1986). Mineralogical, limnological and biological aspects of saline-alkaline lake ecosystems have been studied since the beginning of the 20th century however, a surprising paucity of information on minor chemical constituents exists. In particular, with the exception of a few papers on humic substances in saline-alkaline lakes in the semiarid regions of the Canadian prairies, there is almost no mention of colloidal-dissolved coloured humic substances, which nevertheless can occur in very high concentrations >100 mg L$^{-1}$ in saline-alkaline lakes (“black waters”).

The origin of colloidal humic substances in saline-sodic shallow lakes

Formation of humic substances as by-products of anaerobic degradation processes

The activity of sulphate-reducing microbes forms near-surface horizons of sulphate reduction in water saturated sediments of sulphate-rich shallow saline lakes (Fig. 1).

Figure 1: The plot shows the total reduced inorganic sulfur concentration (TRIS) (mg kg$^{-1}$ of wet sediment) versus sediment depth (cm) in a typical Seewinkel soda pool (Oberer Stinkersee, eastern Austria) at two different sampling sites. The peak in 1 cm depth points to a near-surface microbial sulphate reduction horizon (Mantler, 2008).
Organic debris that escapes aerobic mineralization settles and accumulates on top of the lake floor, where it fuels microbial sulphate reduction and other anaerobic degradation processes in the anoxic surficial sediment layer. Typically, much of the organic debris originates from the littoral vegetation. This plant litter (contributed e.g. by reed or halophytes) contains high molecular weight (HMW) lignin. The biodegradation of HMW lignin is widely believed to be prevented under anaerobic conditions. However, in the Seewinkel salt pans, rapid degradation of reed leaves and stems in anaerobic sediments in the presence of elevated concentrations of Na$_2$SO$_4$ have been observed (Mantler, 2008). A recent publication by Ko et al. (2009) corroborates this observation. The most important aspects of lignin biodegradation are depolymerisation and solubilisation, which result from oxidative reactions following $\beta$-ether linkage cleavage by extracellular enzymes. Accumulation of a water-soluble lignin polymer (APPL, acid-precipitable polymeric lignin) is observed during the process of lignin degradation. As the concentration of APPL reaches a certain level, degradation of APPL into monomeric aromatic compounds occurs (e.g. vanillic acid, caffeic acid, hydrocinnamic acid, gallic acid). These compounds are known as chemical constituents of humic and fulvic acids.

Formation of humic substances under aerobic conditions in the riparian zone

During aerobic reed litter degradation by alkaliphilic microorganisms at the water/plant material interface under conditions of high salinity and high pH>8.5, lignins and other biopolymers are solubilised rather than fully mineralized. The products, APPL and other humic matter precursors are further degraded into aquatic humic substances (HS) and exported from the extended reed belts into the open waters by wind-driven currents. E.g. in Lake Neusiedler See, trails of brown-coloured humic-rich water extend far out into the main body of the lake (Dokulil, 1979).

In saline-alkaline soils, the high pH and high concentrations of monovalent cations decrease the formation of solid organo-clay complexes. As a consequence, the humic compounds are not stabilized against leaching by chemical bonding to soil minerals. This behaviour is in sharp contrast to the behaviour of non-alkaline, non-saline soils. The solubilisation of soil humic material leads to the accumulation of humic colloids in the water phase (Figs. 2 and 3). Waters passing through the riparian soils of saline-alkaline lakes transport huge amounts of humic colloids into the lake basin.

Figure 2: Coloured humic substances in a small pool on top of the dried-up lake floor of a soda lake. The white-coloured crystals is mainly washing soda (natron), Na$_2$CO$_3$$\cdot$10 H$_2$O

Figure 3: Coloured humic substances in a drainage channel near the shallow soda lake “Lange Lacke” located in the Seewinkel region in eastern Austria.
Pometto and Crawford (1986) showed that actinomycetes play a key role in lignin and lignocellulose degradation and the production of water soluble lignin– and/or cellulose-derived humic substances in alkaline aquatic environments. Lignin, which is covalently linked to cellulosic polysaccarides in lignocellulosic biomass, is resistant to degradation by most microorganisms and is degraded by a few fungi and filamentous bacteria. In contrast to ligninolytic fungi which usually thrive in more acidic environments and seem to be not highly competitive in alkaline soils, filamentous actinomycetes (e.g. *Streptomyces viridosporus*) show optimal lignocellulose degradation rates in the pH range of 8.4 to 8.8. *S. viridosporus* degrades lignin under aerobic conditions, releasing APPL as the initial intermediate of lignin catabolism. Lignin and cellulose solubilisation rates are greatest when the pH is>8.5. Further degradation of the solubilised products leads to the formation of various humic substances which can be regarded as lignin biodegradation intermediates which are slowly degraded to CO₂ by other microorganisms.

**Adverse effects of humic substances on health-related bacteria in saline-alkaline lakes**

There is consensus in the literature that, in freshwater lakes, humic matter is generally a poor substrate for bacterioplankton and that the turnover of humic substances is low compared to the nonhumic DOC (Wetzel 1992). In contrast, in a typical shallow saline-alkaline lake (Lake Neusiedler See) humic DOC, derived from the riparian reed vegetation, is the main fuel for bacterial growth in the open lake as has been reported by Reitner et al. (1999). Steinberg et al (2006) emphasized that humic DOC may interact directly with organisms. Several adverse effects of humic substances on algae and bacteria in freshwater ecosystems have been described, including light-induced effects. Experiments with NOM isolates from Scandinavian surface waters yielded significant growth retarding effects upon irradiation on *E. coli* at environmental relevant concentrations (Paul et al., 2006): the stronger the humification the slower the growth. The authors attributed this effect to the production of reactive oxygen species of which H₂O₂ is the longest-lived one and may account for a longer-lasting bactericidal effect. Humic substances were shown to suppress cyanobacteria more than eukaryotic algae in circum-neutral and slightly acidic freshwater ecosystems (Steinberg et al., 2008). In contrast to these systems, in saline-alkaline lakes, cyanobacteria play a dominant role even at high DOC concentrations (Kusel-Fetzmann, 1979).

Recent studies suggested that humic substances in the water column as well as in the sediment interstitial water may have the potential to inhibit the growth of pathogenic bacteria and fecal indicator bacteria in shallow saline-alkaline lakes. Kirschner et al., (2008) could show that in Lake Neusiedler See increasing concentrations of humic substances reduce the growth rate and growth yield of endemic *Vibrio cholerae* strains, belonging to non cholera causing serogroups (nonO1/nonO139; Figure 4).
Figure 4: Negative correlation between the concentration of humic high molecular weight substances (HMW) and growth rate (expressed in relative units to make results from several experiments comparable) of *Vibrio cholera* in lake water microcosms (Kirschner et al., 2008)

Shallow soda lakes are among the world's most productive environments (Eiler et al., 2003). They provide plentiful supply of food for various species of organisms and fulfil an important function as resting places for migratory birds. They are, however, hot spots for migratory-bird associated microbial import and are therefore prone to wildlife health issues. The high population density of birds leads to faecal contamination and can be a contributing factor to epizootics. E.g. the soda pools in the National Park Neusiedler See/Seewinkel in eastern Austria have been heavily afflicted with avian botulism since the 1980s. Their soda concentrations as well as humic/fulvic acids concentrations have declined since 1980 due to anthropogenic impacts which may be causally linked to the outbreaks of avian botulism. Data on the increase of pathogens have been published by Zechmeister et al. (2006). High groundwater tables combined with arid or semi-arid climate conditions result in upward movement of saline groundwater by capillary action. Solochak (white alkali) and solonetz (black alkali) soils contain accumulations of saline and sodic salts. Precipitation waters passing through these soils transport their salts (along with their HS) into the lake basin. Drainage measures for intensification of agriculture and an increase in sprinkling irrigation has led to severe lowering of the groundwater levels in the catchment areas of the shallow soda lakes in the Seewinkel. As a consequence of the lowering of the groundwater table, upward movement of salts by capillary action has more or less ceased and the soils have lost much of their saline and sodic salts during the past decades. Large quantities of HS can be mobilized in saline wetland soils at pH values >8.5. Decreasing soda concentrations lead to lower pH values and HS become sorbed onto soil minerals due to pH reduction.

Whether or not the colloidal humic substances in shallow saline-alkaline lakes have the potential to inhibit in-situ the growth of pathogenic bacteria is a question of major interest, since in many of these lakes the anthropogenic water management measures have caused severe disturbances on the chemical composition of the water and the riparian soils, with the consequence of declining humic DOC
concentrations. These shifts may lead to increased risk to wildlife. Wildlife (e.g. fish or waterfowl) diseases are regarded as severe threats to biodiversity and may also pose risks to human health.

**Abbreviations:**
- APPL = acid-precipitable polymeric lignin
- DOC = dissolved organic carbon
- HMW = high molecular weight
- HS = humic substances
- NOM = natural organic material

**References**


