

Dynamics of faecal contamination of surface waters

G. MALÁ, V. MATLOVÁ and J. KNIŽEK

Institute of Animal Science, Prague UHřineves, Czech Republic
(e-mail: mala.gabriela@vuzv.cz, matlova.vera@vuzv.cz, knizek.josef@vuzv.cz)

Abstract The aim of this study was to estimate faecal contamination of surface waters at sheep shelter utilization. Two sheep shelters standing near water course were monitored. First shelter (645 m above sea level) is used for winter sheep stabling only. The other shelter (519 m above sea level) is utilized year round. Water samples were taken periodically during the three years for physical, chemical and microbiological analysis. Sheep shelter used yearlong resulted in significant increasing of the number of thermotolerant coliform bacteria (0.11 vs. 4.13 in 1 ml of water sample) and enterococci (1.75 and 1.83 vs. 5.14 in 1 ml of water sample). Statistically significant increase of nitrite concentration was determined in water samples taken in front of farm area (0.03 mg.l⁻¹), behind shelter (0.04 mg.l⁻¹) and behind farm area. While winterizing sheep inside the shelter did not affect significantly surface waters quality.

Key words: *sheep, shelter, water pollution, climatic period*

Introduction

Recently the diffused pollution is considered as the most important problem of water quality. Hydrological parameters and the process of exploitation of the territory are the main factors influencing the nutrient concentration in rivers (Novotný, 2003). The primary reasons of diffused pollution are the excessive or irregular human exploitation of the landscape (Novotný, 2003), diffuse runoff into the surface water from inappropriate land management (Pitter, 2002).

Under standard conditions watershed retention capacity is able to immobilise more than 99 % of potential pollutants inside the soil cover (Salomons and Stingliani, 1995; Salomons and Stol, 1995). These pollutants accumulate in the soil in forms of organic nitrogen and phosphorus, in organic matter of forest and wetland ecosystems. Abundance of pollutants (nitrogen and phosphorus) is released mostly in the form of diffuse water contamination (Novotný, 2003) and have negative influence on the water quality and living environment (Hart et al., 2004).

There is a decrease of the surface layer of the soil profile due to the intensive foot worn of the green-sward by claws and hooves of farm animals, which causes a decrease of soil porosity thereby the decrease of retention capacity and water retention (Gaisler and Hejduk, 2006).

According to Kabelková-Jančárková (2002) water retention capacity is relatively low for nitrates (quick nitrification of ammoniac nitrogen by nitrifying bacteria onto the moving nitrous forms – nitrites and at last nitrates) and in explicit condition relatively high for phosphorus.

Ammoniac nitrogen occurs in waters in the form of ammonium cation (NH₄⁺) and in the non-ionic form as NH₃ (Schejbal and Pitter, 2000). Ammonium is an indicator of water source contamination by the organic matter (Figala and Hanák, 1986). The nitrites are an intermediate product of biochemical decomposition of nitrogen organic matter. Their levels in waters are instable and indicate the fresh organic contamination. Nitrates are final product of aerobic decomposition of organic matter and are the indicators of older faecal contamination of water (Figala and Hanák, 1986). Lord et al. (2002) show, that the concentration of nitrates depends on the way of exploitation of land (meadow, pasture, arable land). The nitrate concentration in pastures depends directly on the way of loaded span (Maticic, 1999). In the time of vegetation rest the nitrates washing is several times higher than during the vegetation season (Trudgill et al., 1991; Kvítek, 2001). Measuring values of nitrate concentration oscillates during the year, maximum level reach during spring, minimum during autumn (Kvítek, 1999). Phosphates proved the contamination and decomposition of organic matter. It comes into the water with the poor filtration capability of the soil (Figala and Hanák, 1986).

The expansion of autotrophic organisms of phytoplankton is caused by high level of concentration of nutrients in water (nitrogen, phosphorus).

The expansion of autotrophic organisms of phytoplankton, biofilm and macrophytes as a result of higher concentration of nutrient in water (Punčochář and Desortová, 2002) has negative influence on the water quality and affects the balance of the whole ecosystem (Rosendorf et al., 2001).

The temperature of the water affects the rate of growth and metabolic activity of nitrificants and concentration of the nitrites in rivers. Phytoplankton activity affects partly pH, due to the facts, that during the high production of biomass the values of pH increase, and partly increases the consumption of oxygen necessary for the oxidation of organic substances contained in the water (chemical oxygen demand), too.

Coliforms, thermotolerant coliforms – *Escherichia coli* and *Enterococci*, which occur in the gastrointestinal tract of man and homeotherms – are hygienic indicators of faecal contamination (Figala and Hanák, 1986). They are eliminated from excrements of grazing animals onto the surface of soil and then transported through the soil water into rivers, lakes and ground waters (McGechan and Vinten, 2004). The presence of classic indicators of contamination as *Escherichia coli*, *Enterococci* and other aerobes can be proved also in surface and ground waters of highland region exploiting pasture (Schaffer and Parriaux, 2002; Vinten et al., 2004). Extensive and intensive pasture of farm animals supports microbiological contamination of water courses that are higher in places, where the animals have access into the watercourses (Vinten et al., 2004; Weaver et al., 2005). The concentration of bacteria (*Escherichia coli*) in water courses is lower in summer than in spring and autumn (Vinten et al., 2004). This is in relation with the incidence and intensity of rainfalls (Deeks et al., 2005).

The aim of this study was to estimate faecal contamination of surface waters at sheep shelter utilization.

Materials and methods

Study areas

Two sheep shelters lying near streams were observed. First shelter (645 m above sea level) is used only for winter stabling of about 15 - 18 sheep with the rearing for approx. 150 days of year. The floor of the shelter is made of coat from compound mould (soil). On the surface of it the remains of non feeding hay are deposit. During winter period this row reaches the height of 0.6 – 0.8 m. Sheep are constantly in pasture without stabling in fencing, which are situated on a slope above the shelter. They are separated from the shelter by 30 m of timber lot and metalled public road. Non grazing vegetation is cropped once a year by cutting

the grass or by mulching. Shelter loading during winterizing was 0.375 great cattle unit per m², the loading of the pasture was during the observation 0.14 great cattle unit per m². In the farm area NV there were observed following supply points: in front of the farm area (NV3), under the shelter (NV6), the stream flowing through the farm area into a lagoon (NV7) and the point outside the farm area.

The observed stream of “Zvonkový potok” flows into the trunk water course “Divoká Orlice” in the river-basin region “Horní a Střední Labe”. Hydrogeological region is Crystallinum of Orlické mountains and geological type siliceous.

The first shelter belongs to the cold climatic zone CH 6 – from very short to up to short summer; from moderately cold, through wet up to drippy long transitional period with cold spring and moderately cold autumn; very long mild cold winter with a long duration of snow cover.

The other shelter (519 m above sea level) is utilized during whole years. This shelter lies in the area of conventional farm (S), where the pastures (14 ha) and cutting meadows (12 ha) slope down into a local brook. The farm is surrounded by the land of co-operative farm intensive exploited for ploughing. Winterize shelter (80m²) is situated in enclosure of owl-run, which serves as night-time sheep-fold. Shelter loading during observation were 0.375 great cattle unit per m², the loading of the pasture (60 sheep) were during observation 1,0 great cattle unit per 1 ha. In the farm area S there were observed following supply points: in front of the farm area (S1), under the slip (S2), under the sheep-fold with shelter (S5) and the point outside the farm area (S4).

The streams supply the “Benešovský potok” in the watershed “Dolní Vltava”. Hydrogeological region is Crystallinum of middle Vltava and geological type siliceous.

The second shelter belongs to the moderately warm climatic zone MT 10 – long summer, warm and dry short transitional period with moderate warm spring and autumn; short, moderately warm and very dry winter with short duration of snow cover.

Water samples were taken periodically during three years for physical, chemical and microbiological analysis.

In observed regions there were determined three climatic periods according to ambient temperatures (table. 1).

Table 1 Climatic period characteristics in the observed shelters

Climatic period	Range of ambient temperature [°C]	Shelter	The average rainfall during observed period [mm]	The range of rainfall during observed period [mm]
Moderate winter (MW)	from -10 to 0	NV	62	42-88
		S	41	16-65
Transitional period (TP)	from 0 to +10	NV	51	15-105
		S	37	9-71
Moderate summer (MS)	from 10 to +20	NV	64	32-93
		S	58	25-94

Water-quality analyses

The samples of water were analysed in the water management laboratory No.4066 conformity assessment by ASLAB (No.333) accredited by National Institute of Public Health for the drinking water analysis (No. S018010115) according to valid procedures.

The physical parameters: values of pH were measured (ČSN ISO 10523).

The chemical parameters: chemical oxygen demands were analysed (COD-Mn) (ČSN EN ISO 8467), NH_4^+ (ČSN ISO 7150-1), NO_3^- (ČSN ISO 7890-3), NO_2^- (ČSN EN 26 777), PO_4^- (ČSN EN ISO 6878).

From the bacterial indicators were analysed: total coliforms (TNV 757837), thermotolerant coliforms bacteria (TNV 757835), enterococci (ČSN EN ISO 7899-2). These parameters were chosen in accordance with their general importance as indicators of water pollution.

Statistical analyses

Obtained data were statistically evaluated by the method GLM using programme Statistica complett.cz (StatSoft, USA).

The water quality characteristics were analysed by using an analytical model with supply points and climatic periods as factors. The relationships between supply points and particular parameters of water quality were tested with General Linear Model in programme Statistica complett.cz (StatSoft, USA). The differences between means were assessed by the use of the POST-HOC Tukey HSD test.

In the tables are presented average values and standard deviations.

The values of water temperature were divided into four categories: I. 0.5 – 4.4 °C; II. 4.5 – 9.0 °C; III. 9.1 - 13.4 °C; IV. above 13.5 °C.

Results

The values of microbiological parameters from separate supply points of surface water during observed period are presented in the table 2.

The amount of coliforms proceeded in the watershed from 1 to 156 per 1 ml of the sample of examined water (shelter S) depending on the supply point, and in the shelter NV from 0 to 103 per 1 ml of the sample of examined water. There was not proved statistically significant difference between the supply points in watershed of individual shelters from the point of amount of coliforms. The incidence of enterococci in the watershed of shelter S achieves much wider range (0-27 ml of the sample of examined water) than in the watershed of shelter NV (0-5 ml of the sample of examined water). There were determined significantly higher numbers ($P < 0.05$) of enterococci in the water leaving the district of the farm (S1) or under the slip (S2, i.e. in front of the shelter and sheep fold). Statistically significantly ($P < 0.05$) higher amounts of thermotolerant coliforms were found in the samples of surface water from under the sheep-fold (S5) than in the samples taken in front of the farm area (S1). The range of total amount of thermotolerant coliforms considerably exceeded

Table 2 The influence of supply point on the microbiological parameters of surface water during the observed period

Supply point	N	Coliform bacteria [count in 1 ml]	Enterococci [count in 1 ml]	Thermotolerant coliform bacteria [count in 1 ml]
sheep shelter for whole-year stabling				
S1	21	38.38 ± 40.87	1.75 ± 2.38 a	0.11 ± 0.32 a
S2	18	45.50 ± 38.04	1.83 ± 1.92 b	2.35 ± 3.66
S4	21	45.29 ± 40.30	5.14 ± 7.81 a,b	3.53 ± 6.23
S5	17	41.63 ± 48.31	2.44 ± 3.61	4.13 ± 8.45 a
sheep shelter for winter stabling				
NV3	23	5.00 ± 4.75	0.05 ± 0.21	0.05 ± 0.21
NV6	21	4.10 ± 5.31	0.05 ± 0.22	0
NV8	22	14.82 ± 17.09	0.10 ± 0.30	0.10 ± 0.31
NV7	20	32.80 ± 31.93	0.74 ± 1.45	0.10 ± 0.31

Statistically significant differences between the supply points: a,b ($P < 0.05$)

Table 3 The influence of watershed of the shelter on microbiological parameters of surface water during observed period

Catchment area of shelter	N	Coliforms bacteria [count in 1 ml]	Enterococci [count in 1 ml]	Thermotolerant coliforms bacteria [count in 1 ml]
S	77	42.66±41.02 A	2.87±4.85 B	2.46±5.55 C
NV	86	14.08±21.31 A	0.22±0.77 B	0.06±0.24 C

Statistically significant differences between the individual shelters: A,B,C ($P < 0.01$)

in the watershed of shelter S (0-26 in the 1 ml of the sample of examined water) in the comparison with the shelter NV (0-1 in the 1 ml of the sample of examined water).

There were not confirmed statistically significant differences in the number of enterococci and thermotolerant coliforms in the watershed of shelter NV.

It stands to reason (table 3), that in the samples of surface water from the watershed of the shelter S there were provided significantly higher ($P < 0.01$) numbers of coliforms, enterococci and thermotolerant coliforms than in the samples of surface water in the watershed of shelter NV.

The range of pH in the samples of surface water from the watershed S were almost consistent and proceeded from 5.86 to 7.69. Wider interval of pH values, from 4.63 to 7.19 was determined in the samples of surface water from the watershed NV, where there were proved significant differences ($P < 0.01$) in pH values. Significantly lowest pH value had a supply point lying under the shelter (NV6) in the comparison with other supply points (NV3, NV8 and NV7).

Chemical oxygen demands (COD) were in the samples of surface water from shelter NV from 0.15 to 4.41 mg.l⁻¹, while in the samples from shelter S oscillate in wider interval from 0.13 to 6.54 mg.l⁻¹. Statistical significant differences in chemical oxygen demand between the individual supply points within the watershed were not proved (table 4).

The concentrations of ammonium ions determined in the samples of surface water from the watershed S (from 0.02 to 0.23 mg.l⁻¹) were up to the samples from the watershed NV (from 0.01 to 0.2 mg.l⁻¹). There were not found statistically significant differences between the individual supply points in the watershed S and NV. Many times higher concentrations of nitrites and nitrates showed the samples from individual supply points from the watershed S (0.01–0.11 mg NO₂⁻.l⁻¹; 21.0–96.0 mg NO₃⁻.l⁻¹) in the comparison with the samples from watershed NV (0.01–0.03 mg NO₂⁻.l⁻¹; 0.3–6.5 mg NO₃⁻.l⁻¹). Samples of surface water from the supply point in front of the farm area (S1) had significantly higher ($P < 0.01$) concentration of nitrates than the samples from the supply point S5 (under the sheep-fold with shelter). The assigned concentrations of nitrites were significantly lower ($P < 0.05$) in the samples of surface water from the supply point S1 (in front of the farm area) than the concentrations of nitrites from the supply points S5 (under the sheep-fold with shelter) and S4 (outside the farm area). The differences in the concentrations of nitrates and nitrites between the supply points in the watershed NV were not statistically significant.

The supply points in the watershed S (0.01–0.21 mg.l⁻¹) achieved the same concentration of phosphates as the supply points in the watershed NV (0.01–0.25 mg.l⁻¹). The concentration differences of phosphates between the individual supply points within the watershed S and NV were not statistically significant.

Table 4 The influence of supply point on selected parameters of chemical examination of surface water during the observed period

Supply point	N	pH	COD [mg.l ⁻¹]	NH ₄ ⁺ [mg.l ⁻¹]	NO ₃ ⁻ [mg.l ⁻¹]	NO ₂ ⁻ [mg.l ⁻¹]	PO ₄ ⁻ [mg.l ⁻¹]
sheep shelter for whpue-year stabling							
S1	21	7.24 ± 0.30	1.93±1.29	0.08±0.04	58.57±13.51 A	0.03±0.01 a,b	0.08±0.05
S2	18	7.28 ± 0.24	2.09±0.99	0.12±0.05	53.83±16.57	0.04±0.02	0.07±0.04
S4	21	7.12 ± 0.50	2.48±1.65	0.09±0.04	50.95±15.08	0.04±0.02 a	0.10±0.06
S5	17	7.14 ± 0.38	2.28±1.07	0.09±0.05	45.00±11.96 A	0.04±0.02 b	0.09±0.05
sheep shelter for winter stabling							
NV3	23	6.61 ± 0.22 A	1.81±1.00	0.08±0.04	2.86±1.31	0.01±0.01	0.06±0.06
NV6	21	5.39 ± 0.31 A,B,C	1.21±0.61	0.09±0.04	4.15±1.54	0.01±0	0.08±0.06
NV8	22	6.67 ± 0.30 B,D	1.61±1.11	0.07±0.02	3.18±1.30	0.01±0	0.05±0.05
NV7	20	6.30 ± 0.37 C,D	2.09±1.44	0.09±0.05	2.74±1.11	0.01±0	0.08±0.08

Statistically significant differences between the supply points: a,b ($P < 0.05$); A,B,C,D ($P < 0.01$)

Table 5 The influence of the shelter watershed on the selected parameters of chemical content of surface water during the observed period

Catchments area of the shelter	N	pH	COD [mg.l ⁻¹]	NH ₄ ⁺ [mg.l ⁻¹]	NO ₃ ⁻ [mg.l ⁻¹]	NO ₂ ⁻ [mg.l ⁻¹]	PO ₄ ⁻ [mg.l ⁻¹]
S	77	7.19±0.37 A	2.19±1.29 B	0.09±0.05	52.49±14.95 C	0.04±0.02 D	0.09±0.05 a
NV	86	6.26±0.59 A	1.68±1.10 B	0.08±0.04	3.23±1.41 C	0.01±0 D	0.07±0.06 a

Statistically significant differences between the individual shelters: a ($P < 0.05$); A,B,C,D ($P < 0.01$)

Table 5 shows, that the surface water from the watershed S has statistically significantly higher values ($P<0.01$) of pH than the surface water from the watershed NV. The water samples from the watershed in the shelter S shown significantly higher ($P<0.01$) consumption of oxygen using for the oxidation of organic substances contained in the water than the water samples from the watershed of the shelter NV.

In the samples of surface water from the watershed of the shelter S there were proved statistically significantly higher contents of nitrites, nitrates and phosphates than in the samples of surface waters in the watershed of the shelter NV.

The development of microbiological parameters in dependence on the climatic period in the observed watershed shows table 6.

The number of coliforms in the samples of surface water were statistically significantly higher during the moderate summer in the comparison with the transitional period in the watershed of both shelters S ($P<0.01$) and NV ($P<0.05$). The highest amount of enterococci ($P<0.01$) were proved in the samples of surface water in the watershed of shelter S during the moderate summer in comparison with the transitional period and moderate winter. During moderate summer there were identified statistically significantly higher concentrations ($P<0.01$) of thermotolerant coliforms in the samples of surface water from the watershed of shelter S in the comparison with transitional period. The differences among the climatic period in the number of enterococci and thermotolerant coliforms in the samples of surface water were not significant.

The value of pH in samples of water from the watershed of the shelters S and NV were significantly higher ($P<0.01$) during the moderate summer in the comparison to the values measured during the transitional period and during the moderate winter.

The lowest values ($P<0.01$) of chemical consumption of oxygen were found in the samples of surface water from the watershed in shelter S during moderate summer in comparison to samples from the transitional period and moderate winter. The samples of surface water from the watershed in shelter NV achieved during moderate summer significantly lower values ($P<0.01$) than during transitional period as well.

The differences in concentrations of ammonium ions in the water samples from watershed S and NV between individual climatic periods were not proved (table 7). The lowest concentrations of nitrates ($P<0.01$) were determined in the samples of water from the watershed of the shelter NV during the moderate summer period in comparison with the transitional period and moderate winter. The concentration of nitrates in the samples of water from the watershed of shelter S during various climatic periods had non-significant differences. There were proved statistically significantly lower concentrations of nitrites in the samples of water from the watershed S during transitional period than the samples from moderate summer. The concentration of nitrites in the samples of water from watershed of shelter NV reached the same value during individual climatic periods.

Table 6 The influence of climatic period on the microbiological parameters of surface waters in the watershed of individual shelters during observed period

Catchments area of shelter	Climatic periods	N	Coliforms bacteria [count in 1 ml]	Enterococci [count in 1 ml]	Thermotolerant coliforms bacteria [count in 1 ml]
S	MS	39	58.10±44.00 A	5.19±5.97 A,B	4.54±7.30 A
	TP	26	18.48±20.97 A	0.38±1.10 A	0.42±1.24 A
	MW	12	42.83±40.61	1.08±1.68 B	0.50±1.08
NV	MS	42	19.95±24.60 a	0.31±0.89	0.08±0.27
	TP	27	8.70±19.61 a	0.08±0.27	0.08±0.28
	MW	17	9.18±10.31	0.24±0.97	0±0

Statistically significant differences between the climatic periods: a ($P<0.05$); A,B ($P<0.01$)

Table 7 The influence of climatic period on the chemical content of surface waters in the watershed of individual shelters during observed period

Catchments area of shelter	Climatic periods	N	pH	COD [mg.l ⁻¹]	NH ₄ ⁺ [mg.l ⁻¹]	NO ₃ ⁻ [mg.l ⁻¹]	NO ₂ ⁻ [mg.l ⁻¹]	PO ₄ ⁻ [mg.l ⁻¹]
S	MS	39	7.39±0.21 A,B	1.67±0.87 A,B	0.09±0.06	52.04±13.26	0.04±0.02 A	0.10±0.06 a
	TP	26	7.02±0.43 A	2.61±1.33 A	0.09±0.04	51.78±12.42	0.03±0.02 A	0.07±0.05 a
	MW	12	6.91±0.28 B	3.04±1.70 B	0.11±0.03	55.73±24.68	0.04±0.03	0.07±0.02
NV	MS	42	6.39±0.51 A,B	1.30±1.04 A	0.08±0.04	2.56±1.17 A,B	0.01±0	0.08±0.07 a
	TP	27	6.16±0.67 A	2.10±1.18 A	0.09±0.03	3.54±1.46 A	0.01±0	0.06±0.05
	MW	17	6.06±0.60 B	1.93±0.82	0.08±0.04	4.38±0.94 B	0.01±0	0.04±0.03 a

Statistically significant differences between the climatic periods: a ($P<0.05$); A,B ($P<0.01$)

The samples of surface water from the watershed of the shelter S significantly differ ($P < 0.05$) by higher concentration of phosphates in the comparison with samples from transitional period. Also the concentration of phosphates in the samples from the watershed of shelter NV reached the statistically significantly higher values during the moderate summer in the comparison with the moderate winter.

Discussion

Our results show that there were significantly higher total amounts of coliform bacteria, enterococci and thermotolerant coliform bacteria in the samples of surface water taken in the watershed with the shelter S in intensively used farming landscape than in the samples taken in extensively used watershed of the shelter NV. Similar results are presented by Maticic (1999) and Vinten et al. (2004). Schaffer et al. (2004) showed that some species of bacteria are frequently present also in surface water in mountain areas which are used for extensive pasture. Total amount of coliform bacteria in the samples taken in watersheds of both shelters S and NV was insignificantly increased. According to an article by the Bilgram Chemikalien, Ltd. (2006) there exist coliform bacteria that ordinarily live and proliferate in external environment, e.g. soil bacteria. This might be the reason why there were found quite high numbers of coliform bacteria in the sample of water from the place NV7 (the stream flowing through the farm area into the lagoon). The stream runs through a wetland area with broadleaved trees and thus works as a biological treatment plant. According to Fiala and Hanák (1986) wetland areas take part in stabilization of ecological balance in landscapes and are of high density of destruent. Wetland areas work as natural vegetative root treatment plants (Sukop, 2006) which come up to insignificantly higher numbers of enterococci and thermotolerant coliform bacteria as well as insignificantly higher chemical oxygen demands.

There was significantly higher total number of enterococci in the samples of water taken outside the farm area (S4) in comparison to the places in front of the farm area (S1) and under the slip (S2). This documents that the surface water was polluted with faecals. Penetrating of enterococci into the surface water of the stream by the shelter S can be connected to free access of sheep to the stream which agrees with results of Tiedemann et al. (1987).

Faecal pollution of the stream of the watershed of the shelter S is also documented by significantly higher total numbers of thermotolerant coliform bacteria in samples of water taken under the sheep-fold with shelter (S5) in comparison to the samples taken in front of the farm area (S1). Contamination of the supply point S5 with thermotolerant coliform bacteria is supposed to have happened during increased runoff as daily use of sheep-fold caused concretion of soil. Gaisler and Hejduk (2006) claim that intensive press-down of upper thickness of soil leads to decreased porosity of soil

and thereby to its decreased retentive capability.

There cannot be excluded possibility of anthropogenic pollution as far as contamination of surface water of the streams of the shelters S and NV are concerned.

Measured values of pH in the samples of water from all supply points from the supply area of the shelter S were in the optimal interval determined by the notice No. 252/2004 Sb. (6.5 to 9.5). On the contrary the samples of water taken under the shelter (NV6; 5.39) and in the stream flowing through the farm area into the lagoon (NV7; 6.30) did not arrive at the lower limit of the mentioned interval. There was statistically significant difference in pH of surface water between the supply area S (7.19) and the supply area NV (6.26). This big difference in pH values was caused by the locality and surrounding vegetation. There are mostly deciduous forests around the shelter S. Leaves of trees in this kind of forests contain sufficient amount of Ca^{2+} , Mg^{2+} and K^{+} ions so that the soil is neutral or alkaline (Sukop, 2006). On the contrary there are conifer forests dominant in the area of the shelter NV. As needles contain acid components and only few cations, the soil is sourer. This is why soil runoffs from conifer forests can acidify water environment (Sukop, 2006). In addition the shelter NV is situated in an area of acid rains which is an important factor of water acidification too according to Sukop (2006).

The samples of water from all supply points of both watershed S (S1, S2, S4, S5) and watershed NV (NV3, NV6, NV8, NV7) fulfilled the limit of chemical oxygen demands in connection with hygienic requirements for drinking water according to the notice No. 252/2004 Sb. (less than 3.0 mg.l^{-1}). The chemical oxygen demands of the samples of water taken in the supply area of the shelter S was significantly higher than the dichromate value of the samples taken in the area of the shelter NV. Higher dichromate value proves organic pollution of water (Figala and Hanák, 1986).

All taken samples of water fulfilled hygienical demands on drinking water according to the notice for maximal concentration of ammonium ions (less than 0.5 mg.l^{-1}). There was proved no significant difference in the concentrations of ammonium between the watersheds of the shelters S and NV.

The concentrations of nitrates in the water samples taken in front of the farm area (S1) and under the slip (S2) as well as at the point outside the farm area (S4) are increased (more than 50 mg.l^{-1}) (notice No. 252/2004 Sb. for drinking water; 50 mg.l^{-1}). According to Figala and Hanák (1986) nitrates indicate older faecal pollution of water. The source of nitrates can also be manured fields as nitrates are not firmly bound in soil (Sukop, 2006) and they are usually drifted out with groundwater (Figala and Hanák, 1986). There were significantly higher concentrations of nitrates in the samples of water taken in front of the farm area (S1) as well as in other supply points of the watershed of the shelter S. They are connected with the location of the farm in an intensively exploited for ploughing. Our results agree with what Lord et al. (2002) claim, that there

is increased over-supply of nitrates in areas exploited for ploughing.

There are many microbial processes among nitrates, nitrites and ammonium which depend on presence or absence of oxygen (Sukop, 2006). Nitrites come into being mostly by biochemical oxidation of ammonia nitrogen (nitrification) or by biochemical reduction of nitrates (Kabelková-Jančárková, 2002; Bilgarm Chemikalien, 2006). Pursuant to appreciation of nitrite concentration in water samples from all supply points in the areas of the shelters S and NV did not get over the limit value (0.5 mg.l^{-1}) allotted by the notice. In the samples of water from the supply area of the shelter S there was significantly higher amount of nitrites than in the samples from the area of the shelter NV. Statistically higher concentrations of nitrites were found in the samples of water taken in the supply point under the sheep-fold with shelter (S5) and at the point outside the farm area (S4) of the watershed of the shelter S in comparison to the supply point in front of the farm (S1). According to Figala and Hanák (1986), this obviously indicates fresh organic pollution. The contamination of the surface water of the stream with nitrites was caused by free access of sheep to the watershed, which agrees with the results of Tiedemann et al. (1987), increased runoff from the sheep-fold as a result of concretion of the upper thickness of soil and decreased ability of absorption of soil (Gaisler and Hejduk, 2006), and absence of vegetation in sheep-folds (Salomons and Stingliani, 1995; Salomons and Stol, 1995).

Although there are no limits for concentration of phosphates in drinking water in the notice there was not found more than 0.10 mg.l^{-1} in any of the samples taken.

As far as our results are concerned we can agree with Weaver et al. (2002) that farm animals take part in polluting water. We think that microbial and chemical quality of surface water can be influenced by regular agricultural methods.

There were found significantly higher concentrations of phosphates in the samples of surface water from the supply area of the shelter S in comparison to the samples from the supply area of the shelter NV. These results confirm that there was high organic pollution with decomposition of organic substances which is in accordance with results of Figala and Hanák (1986). On the contrary Pitter (2002) says that runoff from badly treated fields can be a source of considerable amount of phosphates. This author also says that big amounts of phosphates come to surface water from faeces.

Water in nature always contains certain amount of microorganisms. Their quantity as well as distribution of species widely differs as to environmental conditions. They are necessary to exist in water because many species of microorganisms take part in decomposition of organic substances. When the microorganisms die out, there are biologically active substances unfurled which have stimulant effects on other organisms (Figala and Hanák, 1986).

We proved that there was significantly bigger amount of coliform bacteria (in both supply areas) and thermotolerant coliform bacteria and enterococci (in the supply area with the shelter S) during moderate summer than during transitional period. On the contrary Vinten et al. (2004) and McGechan and Vinten (2004) claim that overall losses of *E.coli* for a period of grazing were generally small during summer but rose to a high level if grazing continued into autumn.

Values of pH measured during moderate summer were statistically significantly higher than values taken during transitional period or moderate winter. This is connected with high production of biomass. Our results agree with the results of Punčochář and Desortová (2002) who additionally proved higher consumption of oxygen necessary for oxidation of organic substances present in water (COD).

Biooxidative processes increase 2 – 3 times at increased temperature. At low temperatures all organic substances is not degraded but deposits on bottoms of rivers where there is often sediment with high portion of organic substances. These substances cause high COD after all degradative processes are finished (Figala and Hanák, 1986). This agrees with our results. COD in the samples from supply areas with shelters S and NV was significantly higher during transitional period than during moderate summer. In the samples from the supply area with the shelter S there was significantly higher value of COD during moderate winter in comparison to moderate summer as well.

Climate explains much of the variability in stream N yield-discharge relations for watersheds. Climate influences the distribution and composition of vegetation and soils, which affect the supply of organic and inorganic N forms to watersheds (Beaulac and Reckhow, 1982; Downing et al., 1999). The weather dominates N loss through the impact of rainfall and temperature on drainage, and N utilisation. The conversion of ammonium to nitrate (nitrification) is temperature dependent and pH (Burton and Turner, 2003).

Optimal temperature for growth of nitrifying bacteria is approximately $25\text{--}30^\circ\text{C}$. Nitrification practically stops at the temperature of 5°C . At higher temperatures the processes are limited by oxidation of nitrites. Temperature influences rate of growth and metabolic activity of nitrifying bacteria and concentration of nitrites in rivers. During winter when oxidation of nitrites was faster than oxidation of ammonium, bacteria oxidizing nitrites consumed nitrites produced by bacteria oxidizing ammonium as well as nitrites that diffused into biofilm from water (Kabelková-Jančárková, 2002). Our results agree with this author except for higher values of nitrites during winter period.

Seasonal fluctuations in stream nitrate loads were not strongly related to the seasonal differences in soil nitrate levels but were more closely related to stream discharge and antecedent climatic conditions. Losses of nitrate from the catchments seemed to be transport limited and independent of variations in soil nitrate supply (Trudgill et al., 1991). Patterns of nitrate leaching are particularly affected by extremes of climate (Reynolds and Edwards, 1995).

We proved significant changes in nitrate concentrations in samples of water from the moderate summer periods in comparison to the samples from transitional periods and moderate winter from the supply area with shelter NV.

The higher phosphate concentrations in the samples of water from the supply area with shelter S were caused by the application of phosphatic fertilizers on arable land.

Conclusion

Sheep shelter used yearlong resulted in significant increasing of the number of thermotolerant coliform bacteria and enterococci. Statistically significant increase of nitrite concentration was determined in water samples taken front of farm area, behind shelter and behind farm area. While winterizing sheep inside of shelter did not affect significantly surface waters quality.

Some ways of farm animal keeping can support contamination of surface water. Good agricultural practice makes it possible to decrease the influence of animal breeding on chemical and microbiological quality of the surface water.

The objective assessment of participation of the agriculture on the nitrogen and phosphorus nutrients in surface water is problematic, due to the hard quantification of sources of water pollution and estimation of the nutrient ratio which remain after the application into the soil.

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