

## Microbiological irrigation water quality of the Marchfeld Canal system

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### Abstract

The Marchfeld basin with a size of approximately 1000 km<sup>2</sup> represents the Austrian “granary”. To prevent shortage of water as a result of increased ground water removal for irrigation, industrial purposes and drinking water supply, a canal being 18 km in length was constructed from the Danube to the center of the Marchfeld. From there, water is further distributed via two creeks (Rußbach and Stempfelbach).

This study was intended to evaluate whether the surface water of the Marchfeld canal system can be classified into hygienic-microbiological categories as proposed by DIN (Deutsche Industrienorm) standards for irrigation water. For this purpose, water sampled monthly from three different sampling sites from 1996 to 1999 was examined for *E. coli* and enterococci. In addition, water samples were examined for salmonella twice a year from 1996 to 1998 and for cryptosporidia six times during the year 1999.

Though the water showed varying degrees of fecal load, the results of the examinations revealed that only one of the three sampling sites showed constant quality levels according to the DIN classification system over prolonged periods of time. However, exceeding of the limit values was occasionally observed indicating the need for regular bacteriological examinations. The high variation of the results from the other sampling sites hardly permits a definite classification in one of the quality classes.

**Keywords:** Surface water – microbiological quality – irrigation – risk assessment

### Introduction

The Marchfeld basin is a flat region in the East of Austria where the urban periphery of Vienna and the agriculturally dominated area of Lower Austria meet. The Marchfeld is about 1000 km<sup>2</sup> in size representing about 2 percent of the Austrian territory. The climate is moderate. Ideal temperature during summer ensures optimal growth of many plants and vegetables. The limiting factor is the low rainfall, especially in the

growing season. On average, rainfall reaches 500 to 600 mm per year. The geohydrological situation in the Marchfeld is characterized by a steadily decreasing ground water level due to removal of water for irrigation, local need of drinking water and for industrial purposes. To prevent a shortage of water in the Marchfeld, a 18 km canal with a width of about 15 m was built in the years 1987–1992 to bring additional water from the Danube river into the Marchfeld (Figure 1). From the center of the Marchfeld, two creeks (the

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Rußbach and the Stempfelbach via the Obersiebenbrunner canal) ensure linear water distribution throughout the region (Figure 1).

In this water management project, distribution of water from the Danube via decentral water enrichment plants (Menschel et al., 1991) plays a key role in the stabilization of the ground water level. In addition, future withdrawal of surface water for agricultural irrigation purposes for an area of about 10.000 ha will reduce the increasing demand of ground water.

The use of surface water for irrigation is preferred for agricultural purposes because adequate water temperature plays a key role for the prospering of many plants. As regards hygienic aspects, microbiological quality of surface water is difficult to assess because of the lack of stable water purification conditions as in the case of ground water due to the migration through the soil. Physico-chemically, the water of the Marchfeld canal system was shown to be well suited for irrigation purposes and exceeds the quality of ground water available so far (Schöller et al., 1997).

The aim of this study was to examine whether microbiological quality differs during prolonged obser-

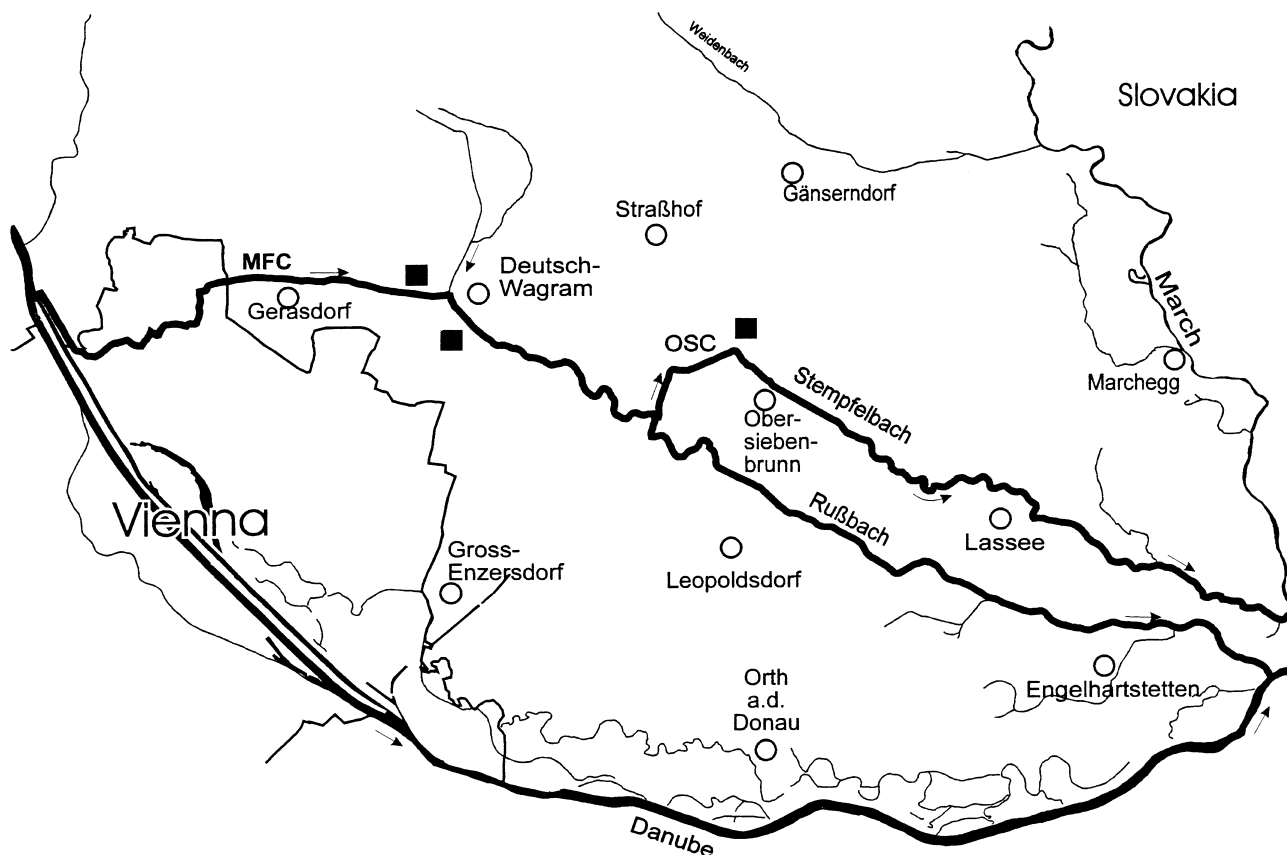
vation periods and whether surface water can be classified into the categories for irrigation water established by DIN standards (DIN 19650, 1999).

## Materials and methods

In the period between 1996 and 1999 examinations of surface water for *Escherichia coli* and enterococci were performed at three different sampling sites in the Marchfeld canal system (see Figure 1). In addition, examinations for salmonella were performed twice a year from 1996 to 1998, and 6 examinations for cryptosporidia were done in the Marchfeld canal and the Rußbach creek in the year 1999. Apart from the microbiological evaluations, physico-chemical parameters such as temperature, conductivity (ISO 7888;1285), and turbidity (ISO 7027;1884) were also evaluated.

## The Marchfeld canal system

The Marchfeld canal system consists of three different sectors: 1. the Marchfeld canal: contains only surface water from the Danube; 2. water of the Rußbach creek



**Fig. 1.** The Marchfeld Canal System. ■ indicates the site of sampling, ○ indicates a village/town, MSC: Marchfeld canal system; OSC: Obersiebenbrunner canal.

(known to have a poor water quality from previous investigations) after junction with the Marchfeld canal: contains water of the Marchfeld canal and the Rußbach creek at a mixing ratio of about 50:1; 3. the Obersiebenbrunner canal: the same water after a flow of approximately 8–12 hours.

## Bacteriological investigations

*E. coli* and enterococci: The membrane filtration method was used. Hundred, 10 and 1 ml of the sample were filtered through a membrane filter with a pore size of 0.45 µm (Millipore, France). The membrane filters were incubated both on EMX agar (Biotest, Germany) for detection of *E. coli* and kanamycin-esculin-azide agar (Merck, Germany) for detection of enterococci. Suspected colonies were further differentiated by standard biochemical means.

Salmonella: 1000 ml sample were filtered through a cellulose-nitrate filter with a pore size of 0.45 µm and the filter was incubated in a buffered non-selective peptone broth at 37°C for 24 h. Then culture on a selective solid culture medium was performed (SMID agar plates; BioMerieux, France) and 10 ml of the peptone broth were added to 90 ml of a selective enrichment broth (Rappaport-Vassiliadis, Merck, Germany). The selective broth was incubated for further 4 days and cultures on SMID agar plates were performed daily. The identification of suspected colonies were performed by biochemical and serological methods.

Cryptosporidia: Water samples ranging from 30–45 liters were filtered on site under moderate vacuum through a polycarbonate membrane with a pore size of 2.0 µm (Millipore, France). The filtrate was discarded and the membrane was transferred to the laboratory. Sample purification and enrichment of Cryptosporidia was performed as described elsewhere (Hansen et al., 1991). The final suspension of 110 µl was processed as follows:

1. One part (5 µl) was suspended on glass slides, dried, and stained in a modified Ziehl-Neelsen staining.

2. One part (5 µl) was suspended on glass slides, dried, fixed in methanol (10 min), and stained with a conjugated monoclonal antibody according to the manufacturer's instructions (Meriflour Cryptosporidium/Giardia; Meridian Diagnostics Inc, USA). If one or more shining spheric objects with 3–4 µm diameter were detected, the sample was considered positive.

3. Hundred µl of the sample material were frozen at –196°C and thawed three times. Then DNA was extracted according to the tissue protocol of the QIAmp DNA Mini Kit (Qiagen GmbH, Germany).

Five µl of the DNA suspension were amplified in a 25 µl single tube PCR according to Morgan et al. (Morgan et al., 1998) with some modifications. The template is a 390 bp long part of the acetyl-CoA synthetase gene of *Cryptosporidium* spp. Primers were produced by VBC-Genomics (Austria). Ready-To-Go PCR Beads (Amersham Pharmacia Biotech, Germany) were used as reaction mix. An inhibition check was performed with the included controls.

Amplification detection was done by flat-bed electrophoresis on PAA gels and silver staining (Amersham Pharmacia Biotech, Germany). If one band between 387 and 395 bp was visible, the sample was considered positive.

To evaluate the recovery of the method,  $5 \times 10^5$  *Cryptosporidia* oocysts were added in the laboratory to 10 liters of each creek water sample and processed as described above.

## Evaluation

The DIN standard 19650 “Irrigation – hygienic quality of irrigation water” was taken as the basis for evaluation. According to the respective fecal load, this standard defines quality classes of water permitted for overhead irrigation of agricultural cultivation (Table 1). DIN standard 19650 does not specify the number of samples required for classifying water, especially surface water, in any given quality class. Moreover, this standard also does not describe any specific approach

**Table 1.** Extract from DIN standard 19650. Hygienic-microbiological classification and use of irrigation water.

Suitability class	Use	Admissible count <i>E. coli</i> /100 ml	Admissible count Enterococci/ 100 ml
I (Drinking water)	all greenhouse and outdoor crop without restriction	undetectable	undetectable
II	outdoor and greenhouse crop for raw consumption	< 200	< 100
III	vegetables up to 2 weeks prior to harvest outdoor crop for raw consumption until time of fructification	< 2000	< 400
IV	sugar beets, starch potatoes, oil-producing plants until industrial processing, grain until lactescence (not for raw consumption)	waste water having passed at least one purification step	

to be used, if the levels identified are exceeding the respective quality limits for some time.

**Statistical Analysis:** The coefficient of correlation between counts of *E. coli* and enterococci and temperature, conductivity and turbidity was calculated by the Pearson correlation test. Statistical significance was set at  $P = 0.01$  and testing was 2-sided.

## Results

At the sampling sites 1 and 2, 48 (once every month) and 42 measurements, respectively, were performed. At sampling site 3 altogether 64 samples were taken and investigated.

Of the 48 samples taken at sampling site 1, 39 and 43 samples (81.2% and 89.6%) were within the limit values for *E. coli* (< 200/100 ml; Table 2) and enterococci (< 100/100 ml; Table 3), thus indicating water quality

category II. In 8 cases (16.7%) the values were above the limit for *E. coli* with only 5 of these 8 samples showing enterococci levels exceeding 100 cfu/100 ml. In these 8 samples *E. coli* concentrations reached levels between 200 and 400/100 ml, and enterococci levels measured between 100 and 200/100 ml. Only one sample (2.1%) revealed > 2000 *E. coli*, while the respective enterococci level was below 400 cfu/100 ml.

At sampling site 2, 17 samples (40.4%) showed *E. coli* levels exceeding 200/100 ml and 11 samples (26.2%) 2000 cfu/100 ml. Enterococci levels of more than 100 were obtained in 16 (38.1%) and of more than 400 cfu/100 ml in 11 of the samples (26.2%) (Table 2). During the 3-year study, no salmonella could be detected in any sample of these two sampling sites.

At sampling site 3, 37 (57.8%) of *E. coli* levels were below 200 cfu and 53 (82.8%) of the enterococci levels below 100 cfu. Twenty samples showed *E. coli* levels above 200 cfu and 6 samples (9.4%) enterococci

**Table 2.** Counts of *E. coli* at the 3 sampling sites. Minimum, maximum, mean, median and according to DIN standards

Sampling site (number of samples)	<i>E. coli</i>		mean	median	Bacterial counts according to DIN standards ( ) indicates the percentage of samples falling into this class		
	range				< 200	> 200–2000	> 2000
	min.	max.					
MFC (n = 48)	10	2800	259	150	39 (81.2)	8 (16.7)	1 (2.1)
RB (n = 42)	20	20000	1762	850	14 (33.3)	17 (40.4)	11 (26.2)
OSC (n = 64)	3	17000	667	250	37 (57.8)	20 (31.3)	7 (10.9)

MFC: Marchfeld canal; RB: Rußbach creek; OSC: Obersiebenbrunner canal.

**Table 3.** Counts of Enterococci at the 3 sampling sites. Minimum, maximum, mean, median and according to DIN standards.

Sampling site (number of samples)	Enterococci		mean	median	Bacterial counts according to DIN standards ( ) indicates the percentage of samples falling into this class		
	range				< 100	> 100–400	> 400
	min.	max.					
MFC (n = 48)	0	300	67	50	43 (89.6)	5 (10.4)	0
RB (n = 42)	10	20000	1209	250	15 (35.7)	16 (38.1)	11 (26.2)
OSC (n = 64)	5	2500	199	50	53 (82.8)	6 (9.4)	5 (7.8)

MFC: Marchfeld canal; RB: Rußbach creek; OSC: Obersiebenbrunner canal.

levels above 100 cfu. At the time of sampling, 7 samples (10.9 %) exceeded 2000 cfu/100 ml of *E. coli* and 5 samples (7.8 %) 400 cfu/100 ml enterococci.

No cryptosporidia or giardia cysts could be detected in any of the 6 samples obtained from the sampling sites at the Marchfeld canal and the Rußbach creek in the year 1999. However, tests showed that the recovery rate was indirectly proportional to turbidity and only ranged between 8.2 and 17.3 %.

Water temperature varied within a range of 1.3 °C and 22.5 °C, conductivity ranged between 340–620 µS and turbidity between 2–54 FNU. For turbidity, values (means ± standard deviation) of  $12.6 \pm 7.2$ ,  $31.1 \pm 19.3$  and  $12.3 \pm 7.5$  FNU were observed in the Marchfeld canal, Rußbach creek and Obersiebenbrunner canal, respectively.

A rather moderate correlation could only be observed between the counts of *E. coli* or enterococci and turbidity with coefficients of correlation of 0.418 and 0.499, respectively ( $P < 0.01$ ).

## Discussion

Only the Marchfeld canal itself showed microbiological stability, with more than 80 % of the samples classifying for class II.

No salmonella and no cryptosporidia or giardia cysts were found. Salmonella were not suspected, because there are no typical bird breeding places along the canal. Cryptosporidia, which could not be detected either, seem to be rare in public water supply systems in Lower Austria, as could be shown recently (Klenner et al., 2000). One possible reason could be, that – in obvious contrast to the Western part of Austria – cattle are held in cowsheds throughout the year. In the Marchfeld, also called the Austrian “granary”, there is very little cattle-breeding. On the other hand, our method was limited by a low recovery rate due to the high water turbidity and therefore lower oocysts counts would have remained undetected.

However, in occasional instances even the surface water of the Marchfeld canal showed values exceeding these thresholds.

Hygienically, the question arises, whether using the Marchfeld canal water for irrigation may increase the risk of spreading infection. In our opinion, this is of primary importance for all vegetables intended for raw consumption.

The 1989 WHO “Health guidelines for the use of waste water in agriculture and aquaculture” (WHO, 1989) recommend a mean value of 1000 fecal coliforms/100 ml for waste water used for irrigating vegetables intended for raw consumption. This threshold is five times higher for vegetables for raw consumption

than that stipulated by the DIN standard. Thus, occasional exceeding of the thresholds used in this study does not seem to affect human health.

Downstream sampling point 2 microbiological parameters got worse. In this segment, only 33.3 % of the samples fulfilled the criteria for quality class II. This deterioration in quality is understandable, considering that sewage plants drain into the Rußbach creek.

In this part of the Marchfeld canal system, the water was classified as class III, i. e. unsuitable for irrigation of vegetables intended for raw consumption; and even this class can not be achieved regularly.

In the Obersiebenbrunner canal, a better water quality could be observed. One explanation for this improvement in quality could be the settling of particles during their passage of the Rußbach creek and the Obersiebenbrunner canal. This hypothesis was also supported by the considerable better values seen for turbidity in the Obersiebenbrunner canal.

Of all physical parameters, only turbidity could be correlated positively to bacteriological indicators.

Since the Obersiebenbrunner canal sampling site is only a few kilometers downstream of the Rußbach sampling site, and so far no definite explanation for the improvement of microbiological water quality has been found, this raises the question of appropriate classification of water quality. If the water is classified as class II – as achieved for both bacteriological parameters in nearly 60 % of all samplings – this quality has to be guaranteed for the time of overhead irrigation. On the other hand, classification as quality class III would mean limited use of the surface water, thus requiring increased use of well water and consequently increased withdrawal of ground water.

Hence, reliable reduction of microbiological counts is the only possibility to ensure sustained use of surface water. If such a reduction of counts is directly related to the number of particles, as suggested by us, adequate sedimentation must be ensured in this section of water flow.

In conclusion, the results of our study show that surface water of the Marchfeld canal system can not definitely be categorised into different classes. This is only possible in the Marchfeld canal, but even there regular controls of fecal bacteria as indicator for the fecal load are required.

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