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The influence of agricultural run-off on bacterial populations in a river

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R.M. FERNÁNDEZ-ALVAREZ, S. CARBALLO-CUERVO, M.C. DE LA ROSA-JORGE AND J. RODRÍGUEZ-DE LECEA. 1991. The microbiological quality of the River Riato (Spain) was evaluated. The influence of cattle that roam free in the warm season was marked. The degree of faecal pollution in the river was higher than predicted from the river basin geographical characteristics. The counts of faecal indicators greatly increased when the cattle were allowed to roam free. Counts of enterobacteria and faecal coliforms ranged from 10^3 to 10^6/100 ml. Faecal streptococci counts were smaller (< 10/100 ml). *Escherichia coli* and *Pseudomonas aeruginosa* were isolated from all samples. *Streptococcus bovis* was also isolated but not *Strep. faecalis*.

INTRODUCTION

Tributaries play an important role in the quality of the water of reservoirs because they constitute a way by which pollution reaches lenitic systems (Wetzel 1983). Many authors have attributed the pollution of some reservoirs to polluted rivers (see e.g. Geldreich 1976) and this is especially important when these reservoirs are used for a public water supply, because many Gram-negative enteric pathogens are present in aquatic environments. Moreover numerous works that have been carried out in these environments have demonstrated the existence of drug-resistance factors in these bacteria (e.g. Edberg *et al.* 1986). As reported by Leclerc & Mossel (1989), 50% of enterobacteria in surface waters are antibiotic resistant and this could pose a risk for the consumer when these waters are used for public water supply.

The effect of recreational activities on the bacteriological quality of surface waters is known (Wagenet & Lawrence 1974; Varness *et al.* 1978), as is that of municipal sewage point sources. In the past, the relative contribution from non-point sources was assumed to be small. As remarked by Doran & Linn (1979), the validity of this assumption has recently been questioned. The movement of animal wastes into surface waters is often cited as a major factor contributing to the pollution of available water in many rural areas (Doran & Linn 1979). Remote rural waters are rarely monitored and their microbiological quality is mostly unknown because of the logistical problems associated with access.

The purpose of our study was to evaluate the microbiological quality of water from the River Riato and its influence on the quality of water in the reservoir. Likewise we wanted to check the influence of pasturage of cattle on water pollution.

The microbiological quality of this river has been evaluated within a general study of limnology of a reservoir, El Atazar, located in Madrid (Spain). The Riato is the main tributary of this reservoir and it has a river-basin which is almost totally depopulated. Access to this area is very difficult because it is very rugged. It receives few visitors, campers or tourists. It is therefore far from the more common sources of pollution: recreational activities and municipal sewage.

Another factor which we have studied is the presence of pastureland on the river-basin. Cattle are allowed to roam freely during the warm season, a traditional farming method in this zone called *sesteo*. The livestock takes refuge in the most rugged zones, keeping away from tourists,

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roads, villages, etc. One of the zones they choose is the river-basin of the Riato. The substrata is slate, a very impermeable material that promotes a big surface run-off.

We chose total and faecal coliforms (TC and FC, respectively) as indicators of faecal pollution because coliforms are internationally recognized in assessing microbiological quality of waters (McFeters *et al.* 1986) and the measurement of FC is reported as the most reliable indicator of faecal pollution of water, although it does not identify the precise source of pollution (Doran & Linn 1979). Faecal streptococci (FS) were estimated because the examination of water for both FC and FS has been suggested as a method for determining whether faecal pollution is from human or other animal sources (Doran & Linn 1979). Total enterobacteria (TE) counts were also included. The presence of high numbers of these micro-organisms in water indicates the possible presence of pathogens (Christian & Pipes 1983; Burton *et al.* 1987; Zmirou *et al.* 1987).

MATERIALS AND METHODS

Sampling

The River Riato is 9 km long and is a tributary of El Atazar reservoir (Spain). It is in mountains with elevations up to 1200 m.

Water samples were collected every 30 d from April to September. Samples were transported to the laboratory on ice. All tests were performed within 3 h of sampling. Samples were taken in 1·5 l sterile bottles.

Enumeration of bacterial populations

Plate counts

Samples were shaken and then diluted with sterile buffered water (Anon. 1985). One ml of each decimal dilution was then pipetted on each of four plates of plate count agar. Two plates of each dilution were incubated for 24–48 h at 37°C and the other two for 48–72 h at 22°C (Buck 1979).

Enumeration of faecal bacteria

The multiple tube most-probable number technique was used for total coliforms (TC), faecal coliforms (FC) and total enterobacteria (TE) enumerations. Lactose broth plus 4 ml/l of 0·5% (w/v) bromocresol purple was used for enumeration of TC, after incubation at 37°C for 48 h (Anon. 1976); EC broth was used for enumeration of FC, after incubation at 44°C for 24 h (Anon. 1985); and EE broth for enumeration of enterobacteria, after incubation at 30°C for 48 h (Anon. 1976). From the positive tubes of EC and EE broths, plates of eosin methylene blue (EMB) agar (Anon. 1985) and 1% glucose violet red bile agar (VRBA) (Anon. 1976), respectively, were inoculated to obtain isolated colonies for identification.

Faecal streptococci (FS) concentrations in water samples were determined by membrane filtration (0·45 μm, Millipore membrane filters) using Slanetz and Bartley agar (Rodier 1984). Colonies were confirmed as streptococci by catalase test and then identified (see below).

Identification study

The organisms were identified by the criteria of Krieg & Holt (1984), Sneath *et al.* (1986), Oberhofer (1985) and Bergan (1984).

The following tests were used for the identification of enterobacteria: Gram staining reaction and cell morphology, motility by handing drop, oxidase production, catalase, indole production, methyl red, Voges-Proskauer, citrate on Simmons' agar, hydrogen sulphide on Kligler's agar, urease on Christensen's agar, phenylalanine deaminase, lysine decarboxylase, arginine dihydrolase, ornithine decarboxylase, acid production from D-glucose, gas production from D-glucose, lactose and ONPG.

For the identification of streptococci the following tests were used: Gram staining reaction and cell morphology, oxidase, catalase, oxidation–fermentation (OF) test on Hugh-Leifson medium, gelatine hydrolysis, grow on tellurite broth and starch hydrolysis.

Chemical and physical composition of water

Temperature and dissolved oxygen

Temperature and dissolved oxygen were determined on site with an electronic apparatus (Yellow Spring Instruments).

pH

pH measurements of each water sample were made on site with a Crisson pH-meter.

Ammonia

Ammonia concentrations were determined by the Nessler method (Rodier 1984) in a Hach colorimeter (Hach Instruments, USA).

BOD-5

Biochemical oxygen demand was determined by automatic biometers BIOX-12 (Heto) by the manometric method. Each biometer has an anaerobic barometer in the top, with a scale from 0 to 350 mg of oxygen per litre. We set the barometer to 0 mg/l, and after incubation for 5 d read the results.

Turbidity

Turbidity measurements were made by the nephelometric method with a Hach colorimeter (Hach Instruments, USA).

RESULTS

Bacterial populations

Plate count results are shown in Fig. 1. The plate count increased constantly during our study, from 10^5 cfu/100 ml in the first month to 10^{11} cfu/100 ml in the last two. The differential growth, 22°C/37°C, did not show a significant statistical difference, and was positively correlated ($r = 0{\cdot}999$). The increase in plate count is similar to increases in water temperature.

The lowest enterobacterial count (Fig. 2) was obtained in the first month (MPN = 10^3/100 ml). In May the concentration increased to 10^6/100 ml (when the pasturage or *sesteo* began). From June to August we obtained similar values. In September there was a decrease (concurrent with the *sesteo* ending).

The TC and FC results are shown in Fig. 2. They were similar. Both counts also had a clear increase in May from MPN 10^3/100 ml to 10^5/100 ml, and they decreased in September. The greatest part of TC were FC (59%).

Faecal streptococci concentrations (Fig. 2) showed an increase during the first five months and a slight decrease in September. The counts were smaller than those of the other faecal pollution indicators (10^0–10^1 cfu/100 ml).

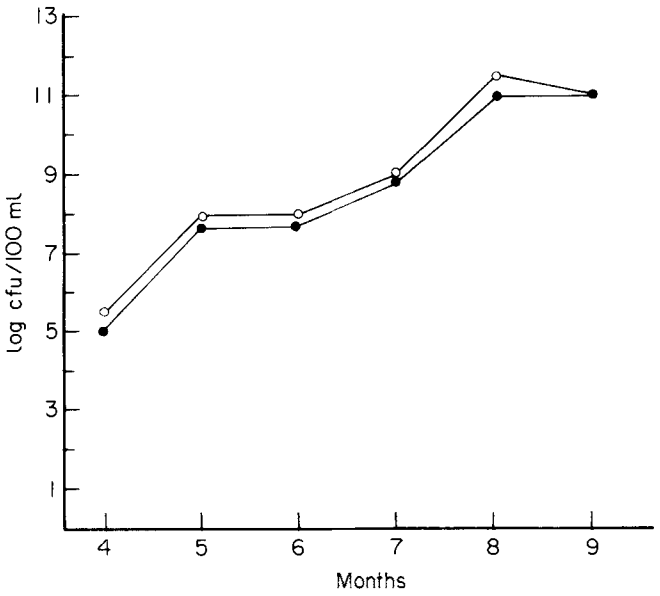

Fig. 1 Variations in aerobic heterotrophic plate count populations that grow at 22°C (○) and 37°C (●) over the period April to September

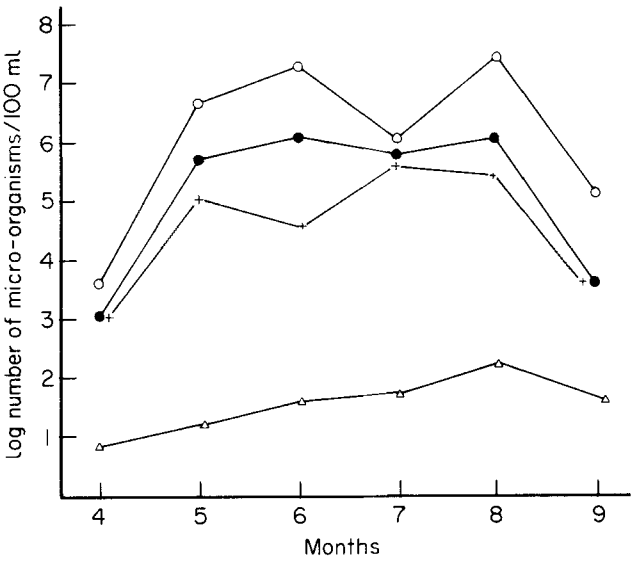

Fig. 2 Concentrations of enterobacteria (○), total coliforms (●), faecal coliforms (+) and faecal streptococci (△) over the period April to September

Physico-chemical parameters

The dissolved oxygen concentration (Table 1) decreased during our study (from 11 mg/l to 7 mg/l) together with a temperature increase. We never detected a 100% saturation. The smallest concentration was 5·6 mg/l in June, with a slight increase in July (7·5 mg/l).

The Riato water was slightly acid in April (pH 6); in May the pH increased to 8·6 and this value remained more or less constant during the other months (Table 1).

Ammonia concentrations are shown in Table 1. In April it was impossible to detect ammonia from the water by our method. In May the ammonia concentration was 0·05 mg/l; in the other months we obtained similar amounts, with a slight increase in September (0·06 mg/l). All concentrations obtained by us were small.

Biological oxygen demand (BOD-5) values are shown in Table 1. As well as ammonia concentrations, it was impossible to detect BOD-5 by our method in the first month of our study. In May there was a dramatic increase to 30 mg/l; the BOD-5 decreased in June and the results obtained in the other months were similar (5–10 mg O/l).

The rainfall figures are shown in Table 1.

Identification of micro-organisms

No pathogenic micro-organisms were detected in River Riato waters during our study. *Escherichia coli* was isolated from all samples. Other enterobacteria isolated included: *Enterobacter cloacae, Ent. intermedium, Citrobacter diversus*

Table 1 Chemical and physical characteristics of water from the river Riato over the period April to September

Variable	Samples					
	April	May	June	July	August	September
Temperature (°C)	7·3	11	18	19·2	21	19
pH	6	8·6	8·3	8·7	8·4	7·9
Turbidity (FTU)	0	16	16	9	30	23
Dissolved oxygen (mg/l)	11	10	5·6	7·5	7·3	7
NH_4^+ (mg/l)	0	0·052	0·065	0·039	0·026	0·065
BOD-5 (mg O_2/l)	0	30	5	0	8·4	8
Rainfall (mm)	58	18	35	5·3	1·3	0·0

and *Edwarsiella* spp. *Streptococcus bovis* was also isolated but *Strep. faecalis* was not found.

In spite of the fact that our study did not include *Pseudomonas* spp. as controls, we isolated these organisms from all samples and they interfered with other counts. These interferences have been reported by different authors (Hsu & Williams 1982; Berlingame *et al.* 1984).

DISCUSSION

It is clear that the degree of faecal pollution in the River Riato is higher than we could expect from the geographical characteristics of its river-basin; according to the Spanish legislation (which agrees with the European Community directive of 16th June 1975) it exceeds the standard conditions of bacteriological quality of surface waters that are going to be used for water supply after a depuration (Anon. 1988). This means that the river-basins have to be protected.

The aerobic heterotrophic microbial populations increased at the same time as the water temperature increased. The differential growth, 22°C/37°C, did not show a significant difference ($r = 0{\cdot}999$). This has been reported by various authors in aquatic ecosystems (Boylen & Brock 1973; Ferroni & Kaminsky 1980).

Enterobacteria, TC, FC and FS counts greatly increased between April and May, just as the *sesteo* began. FC and TC values were higher than those obtained by Doran & Linn (1979) in a similar study, but FS counts were lower. Apparently, human recreational activities are less polluting than livestock, because our results are clearly better than those obtained by Tunnicliff & Bickler (1984) and Varness *et al.* (1978) studying the effects of human recreational activities in rivers.

There was also a simultaneous increase both in BOD-5 and microbial populations (especially the faecal pollution indicators) that confirms the importance of organic matter as an indicator of bacterial changes and suggests a need for detailed analysis of the sources that contribute to the organic matter load (Nuttal 1982).

On the other hand there was a significant decrease in these parameters in September, when the livestock began to leave the river-basin; the greatest decrease was that of coliforms. The middle values we have obtained are superior to those recommended for recreational use of water (200 cfu/100 ml) (Geldreich 1970). The low counts of FS can be explained because we always isolated *Streptococcus bovis* but never *Strep. faecalis*, and it has been reported by many authors (McFeters *et al.* 1974; Feachem *et al.* 1983; Collin *et al.* 1988) that whereas the enterococci (*Strep. faecalis*, *Strep. faecium* and *Strep. durans*) typically survive longer or a little longer than faecal coliforms, *Strep. bovis* dies off considerably faster than faecal coliforms and other species of faecal streptococci.

The results surprised us because they showed that the degree of pollution of the River Riato was considerable. The identification study gave us the solution. Habitual isolation of *Escherichia coli* confirmed the faecal origin of pollution, although this parameter does not identify the source of pollution (Flint 1987).

The identification study of FS gave us the greatest information, as in other studies (Doran & Linn 1979), because it was impossible to detect *Strep. faecalis* (characteristic in human faeces) and in 100% of the samples we detected *Strep. bovis*, characteristic in cattle faeces and rarely isolated from human faeces (Geldreich 1979; Feachem *et al.* 1983). These results confirmed the cattle origin of pollution and discounted the possibility of human origin. The *sesteo* was the real origin of pollution in the River Riato.

The detection of *Pseudomonas aeruginosa* confirms the great degree of pollution of the River Riato because although its origin is not clearly demonstrated it is isolated from surface waters that receive sewage (Geldreich 1978; Feachem *et al.* 1983). This species can grow in distribution systems, mainly in warm countries, when the conditions are appropriate. Its presence in drinking water is not desirable because it is an opportunistic pathogen and can eventually produce enterotoxin (Leclerc & Mossel 1989). Moreover, members of the genus are the most abundant in freshwaters (Quevedo *et al.* 1986). Therefore this species is more and

more important in aquatic studies of public health sciences and we urge other authors to include this genus in the routine analysis of water (La Bonde & Testi 1979).

As usual in rivers, the dissolved oxygen was never oversaturated. Its concentration decreased as water temperature increased because its solubility diminished (Wetzel 1983). The good availability of this gas was undoubtedly responsible for the low ammonia concentrations in spite of the considerable concentration of organic matter which the River Riato had in its waters.

Turbidity was positively correlated with BOD-5 ($r = 0{\cdot}696$) and with FC densities ($r = 0{\cdot}715$), which showed again the relation of rain and run-off with the variations of organic matter levels and with the increase of FC and FS densities (Tunnicliff & Brickler 1984; McDonald *et al.* 1982). The run-off was an important factor in the pollution of these waters because the River Riato basin is very impermeable.

pH was influenced by the increase of pollution and it tended towards basicity as the pollution increased; this effect has been reported by many authors in studies of lakes (Wetzel 1983).

We consider that the results show the importance of non-point sources of pollution, especially livestock and consequently the run-off as its vehicle, as well as the necessity for river basin management to avoid the pollution of public supplies. In other situations good management of pasturage has brought about the reduction of bacterial pollution in freshwaters (Switzer & Evans 1974).

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