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Bacteriological indicators of faecal contamination: result of a loading experiment with untreated urban wastewater

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A. RAMOS-CORMENZANA, A. CASTILLO, C. INCERTI AND L.F. GOMEZ-PALMA. 1994. Some observations were made on the behaviour of total coliforms, faecal coliforms, enterococci, numbers of aerobic bacteria, salmonellas and sulphur-reducing clostridia as bacterial indicators of faecal contamination of groundwater. A controlled irrigation experiment was carried out with untreated residual water in the alluvial aquifer of the Vega of Granada (Spain). The results obtained confirm the value of these parameters as useful indicators of very recent faecal contamination; and changes were detected as the level of the freatic layer increased and the chemical composition of the groundwater changed. These groups of micro-organisms persisted for about 200 h, with the exception of the aerobes which survived for much longer. Salmonellas were present at levels too low to calculate the extent of faecal contamination and sulphur-reducing clostridia were not detected. The results obtained show that irrigation with untreated wastewater offers a lower risk of microbiological contamination of groundwater compared with the direct addition of waters decanted and/or previously filtered.

INTRODUCTION

Many studies have been carried out on pollution of groundwater by urban wastewater in which total coliforms, faecal coliforms, enterococci, enteroviruses and bacteriophages (Schaub and Sorber 1977; Moore *et al.* 1981) have been detected.

These parameters have frequently been used as indicators of faecal pollution in groundwater and have been particularly valuable as trace indicators in hydrological studies (Keswick *et al.* 1982). Nevertheless, the arrival of the micro-organisms at the saturated aquifer is highly dependent on the self-purifying action of the physical medium especially in detritic aquifers (Hagedorn *et al.* 1981; Trevors *et al.* 1990; Yates and Yates 1990).

There is evidence that untreated urban wastewater has been used in the city of Granada (300 000 inhabitants) since the 14th century when the Moors irrigated its extensive fertile plain (200 km^2). At the time this provoked numerous outbreaks of faecal-water epidemics, mainly as a result of the consumption of horticultural crops but also because of the contaminated drinking water supply. In the 1990s, however, a large proportion of these waste waters are purified.

The aquifer from the Granada valley, a depression in the Beticas mountain range, is composed of alluvial layers of gravel, sand and clays. The aquifer water resources are of 200 hm^3/year and reserves of 2000 hm^3 (Castillo 1986).

The objective of this study was to increase the knowledge about self-purifying, mainly microbiological processes which are performed by natural processes in the fertile plains of Granada. This paper focused mainly on the behaviour shown by the classic bacteriological indicators of faecal contamination in the groundwater studied.

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MATERIAL AND METHODS

Description of the experiment

The experiment consisted of loading plots 1000 m^2 (50 × 20 m) in the 'cortijo Trevijano', in the 'Vega de Granada' with untreated urban wastewater from the city of Granada. The wastewaters used had mean values of BOD, COD and S/solids of 320, 630 and 240 mg l^{-1} respectively. Some bacteriological content values are shown in Table 1.

Table 1 Bacteriological content of wastewater used*

Hour	Coliforms (10^5 cfu ml^{-1})	Enterococci (10^3 cfu ml^{-1})	Aerobes (10^6 cfu ml^{-1})
3 : 00	0·79	1·56	2·14
9 : 00	1·23	8·29	2·74
15 : 00	1·49	3·94	2·97
21 : 00	2·45	3·69	4·75

* Average weekly values obtained from daily analyses determined.

The inflow applied was 60 l s^{-1} which created a layer of water 40 cm deep. The water was fed in over a period of 128 h although the inflow was zero after 38 h; the investigation continued for 550 h. Twenty-three metres in front of the plot, in the direction of groundwater flow, a well was set up with an unsaturated depth of 21 m. This was used for the analytical and piezometric control of the groundwater. In this sector a layer of surface clay 80 cm deep followed by layers of gravel and sand. The transmissivity in this section is approximately 900 m^2 d^{-1} (Castillo 1986).

Periodic analytical tests were carried out on water entering and leaving the plot and on water in the well. Also the piezometric surface and flows were measured in order to quantify the inflow. Similarly, edaphological analyses were undertaken before and after soil flooding.

Detection of micro-organisms

Total coliforms (TC) and faecal coliforms (FC) were determined by the membrane filtration method (Anon. 1989), 100 ml volumes of the appropriate dilutions of each samples were filtered through Millipore[R] filters with 0·45 μm pore diameter. These were then placed on Petri dishes on Endo-MF basal medium (ADSA-Micro) and incubated for 24 h, for faecal coliforms at 44°C and total coliforms at 37°C. Coliforms were identifiable as red-pink colonies (lactose-positive), with or without production of metallic green pigment.

Enterococci (E) were determined by filtering sample dilutions through the same filters and with KF-Streptococcus agar (Difco) as basal medium and incubating at 37°C for 24 h. Enterococci were identifiable as salmon red colonies.

To determine aerobic bacteria (A), tryptic soy agar (Difco) was inoculated with serial dilutions, which were then incubated at 37°C for 24 h.

For the sulphur-reducing clostridia the samples were heated to 80°C and 20 ml were filtered. The filter was placed on ferric sulphite agar (ADSA-Micro) and incubated at 37°C in anaerobic conditions for 48 h. The sulphur-reducing colonies were black in colour.

For the detection of salmonellas the samples were pre-concentrated. Filters were placed in selenite broth (Difco) for enrichment and incubated at 39°C for 18–20 h. Hektoen medium (ADSA-Micro) was then inoculated, and identification of the strains was confirmed by oxidase, Kligler, urease, ornithine, phenylalanine, motility and Gram stain tests (Cowan and Steel 1974).

Physical and chemical analyses

Redox potential, pH, temperature and dissolved oxygen determinations were carried out '*in situ*' by direct methods by Hanna instruments (Limena, Italy). Nitrates, ammonia, and phosphate determinations were also carried out '*in situ*' by preliminary tests of a semiquantitive nature. The appropriate corresponding tests were undertaken and measurements were carried out with a 'Lasa Aqua' field spectrophotometer made by Lange.

In the laboratory the following parameters were determined (Rodier 1981): chlorides by titration with silver nitrate, sulphates by visible spectrophotometry (VS) with barium ions, bicarbonates by titration with hydrochloric acid, calcium and magnesium by atomic absorption spectrophotometry (AAS); sodium and potassium by flame spectophotometry; nitrates by ultraviolet spectrophotometry (UVS) with brucina; nitrites by UVS with Zambelli's reagent; ammonia by VS with Nessler's reagent; and phosphates by VS with molybdenum blue.

RESULTS AND DISCUSSION

The initial analysis of the groundwater controls gave salinity values of 1 g l^{-1} and were of calcium sulphate type. The chemical values obtained showed a mild level of pollution of agricultural origin, with minimum levels of pollution by organic matter and toxins. The microbiological examination showed a mild level of organic pollution, with the presence of TC, FC and A, and the absence of E, clostridia and salmonellas.

Once the loading began the infiltration rate was 800 mm in the first 24 h. This rate gradually but noticeably diminished from 25 to 35 h; and after 35 h the inflow was practically nil until the end of the experiment. The volume loaded was therefore calculated to be approximately 1000 m^3.

The load took 40 h to appear as an increase in the piezometric surface of the control well (Fig. 1).

The groundwater did not show indications of external contamination at any time, made evident by the lack of colour change, odours and turbidity. Chemical parameters analysed remained relatively constant (including salinity) with the exception of nitrates, sulphates and nitrites (Fig. 2).

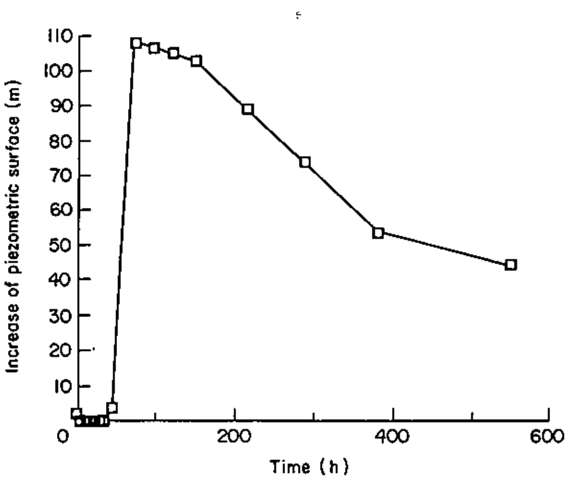

Fig. 1 Relative increase of piezometric surface for 600 h period of the study

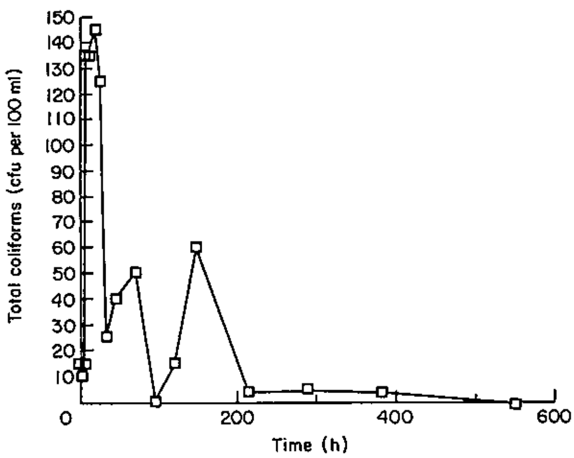

Fig. 3 Results of total coliform analyses for 600 h period of the study

The change in nitrate concentration was similar to that shown by the piezometric surface whereas the nitrite concentration seemed to follow the concentrations of the TC, FC and E (Figs 3, 4 and 5). The concentration changes of the TC, FC and E were all very similar, while that shown by the A was significantly different from these observed previously (Fig. 6).

The condition induced was most accurately reflected by the coliform levels (TC and FC); 9 h after loading began the first significant increase was detected. The E behaved in a very similar manner, and the first increase was detected somewhat later, 21 h after loading began. The aerobes A first significantly increased after 33 h, which also coincided with nitrite concentrations. In two samples salmonellas were isolated after 140 h. Sulphur-reducing clostridia were not detected in any of the samples.

About 200 h after initial loading TC, FC, E and nitrite concentrations diminished to reach minimum levels after displaying strong oscillations and they remained like that until the end of the experiment. Meanwhile the A gradually

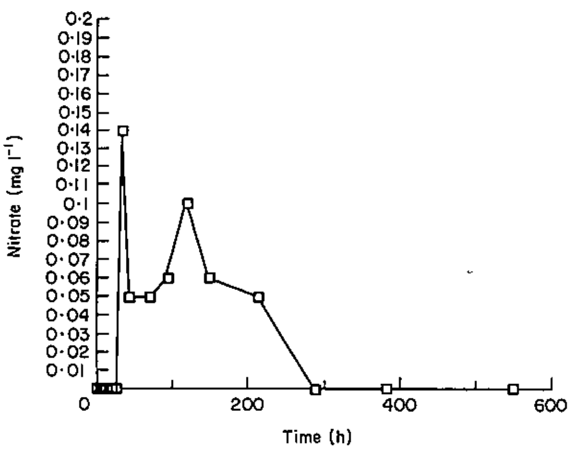

Fig. 2 Results of nitrate analyses for 600 h period of the study

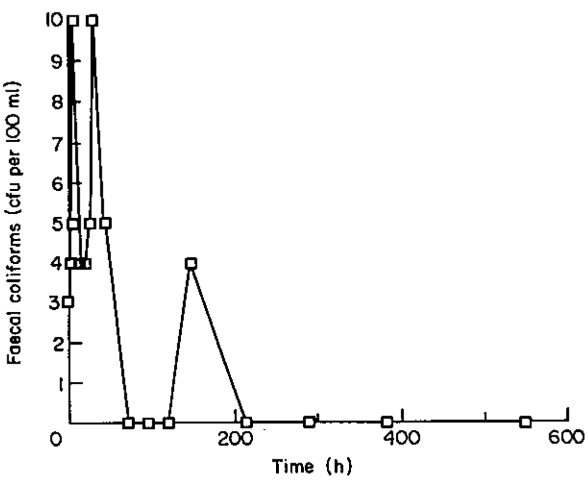

Fig. 4 Results of faecal coliform analyses for 600 h period of the study

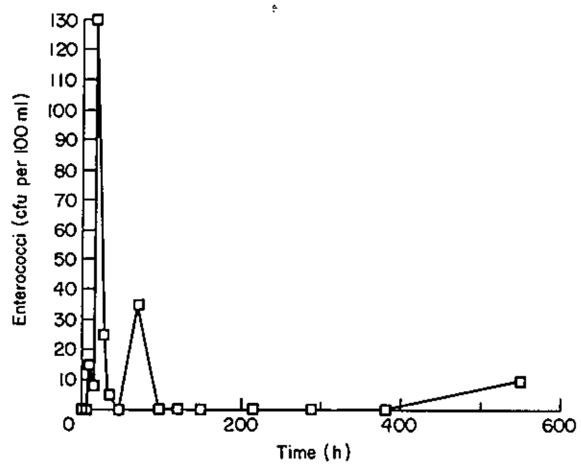

Fig. 5 Survival of enterococci for 600 h period of the study

increased until 382 h, continuing at high concentrations until 550 h after the beginning of the experiment.

These results verify the high value of TC, FC and E as indicators of recent faecal pollution or faecal pollution actually occurring at that moment in groundwaters (Brown and Broghton 1981; El-Zanfaly *et al.* 1989; Ogan 1989). The average persistence of the TC, FC and E in the absence of infiltration of new inputs was approximately 200 h.

In the wastewater used, levels of TC were found to be 100 times E levels (Table 1), while analysis of the groundwaters showed similar concentrations for both. These results indicate that the E have a greater capacity for invasion and survival (McFeters *et al.* 1974; Schaub and Sorber 1977), even enabling the identification of different species and serotypes (Khalaf and Muhammad 1989).

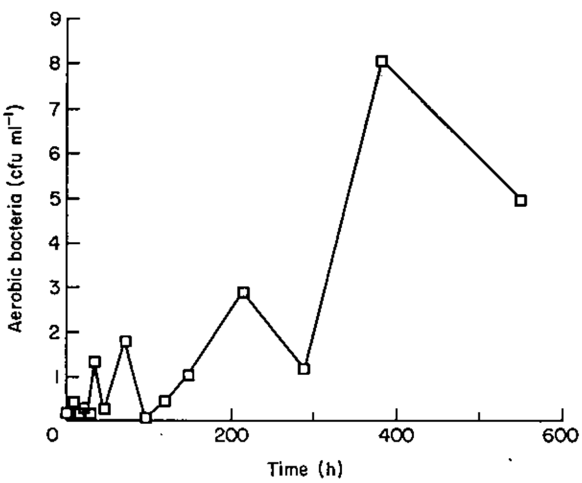

Fig. 6 Changes in number of aerobes during period of the study

The A grew more slowly, and showed a longer period of survival in the medium studied. Maximum increases in concentration were observed once minimum initial concentrations were established for the other biological parameters determined. Thus with low concentrations or absence of TC, FC and E, and a high number of aerobic bacteria relatively recent pollution may be suspected.

Although clostridia and salmonellas are not usually detected in groundwaters (Moore *et al.* 1981), the soil is a suitable habitat for the growth of the latter (Chandler and Craven 1981). In fact, the continuous flow of wastewater caused an increase in the number of these bacteria in the bottom of the water layer, which were detected in some of the groundwater samples long after maximum values were obtained for TC and FC. The somewhat random identification of salmonella colonies, whose presence was detected in only two of the 18 samples examined, showed the limited diagnostic value of these bacteria compared with the FC and E groups.

Nitrites, whose correlation with organic wastes is well documented, were the best chemical indicator of the induced faecal contamination. However, they were not sensitive to low levels of bacteria at the beginning of the infection period and disappeared when bacteria were still present.

As expected, the change in the piezometric surface showed the long physical duration of the induced wastewater input, detected analytically only after 200 h by the high A values. Incidentally, an increase in nitrate and sulphate levels was observed which correlated with the increase in piezometric level caused by a leaching effect on the fertilizers present in the unsaturated border.

The relatively cyclical oscillations of the concentrations of bacteria, as opposed to the steady change in the piezometric surface level could be due to differences in the composition of the wastewater inflow with time (Table 1). These oscillating values support the need to undertake a number of consecutive examinations in order to guarantee the absence of health risks by faecal contamination of groundwater.

The input values obtained, along with the physical, chemical, microbiological and edaphological analyses undertaken, show that there is a low risk of bacteriological contamination of groundwaters by irrigation with untreated urban wastewater compared with that caused by the input of water which has only undergone primary treatment. The soil filtration rate gradually decreases as it blocks up with organic matter, which in turn decreases the concentration of the bacteria in the filtered water thus naturally limiting the intensity and extent of bacterial infection in the groundwater. In addition, the deposited organic mud provides a valuable nutritional input to the irrigated soil and at the same time improves its physical and chemical qualities. Providing that it does not contain any foreign or undesirable elements (Reynolds *et al.* 1980) it should not in any way damage the soil.

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