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Heavy contamination of a subsurface aquifer and a stream by livestock wastewater in a stock farming area, Wonju, Korea

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"Capsule": *Deep aquifers were heavily contaminated with infiltrated livestock wastewater.*

Abstract

A survey of groundwater and stream water quality was undertaken in a stock farming area where livestock wastewater infiltrates into sandy unsaturated zones and saturated bedrock aquifers containing fractures. To determine the degree of contamination and track the effect of livestock wastewater on groundwater and stream water quality, the population of indicator bacteria (total coliforms, fecal coliforms, fecal streptococci, *Staphylococcus* spp., and sulfite-reducing clostridia) together with relevant physicochemical parameters were monitored along the wastewater flow-pathways over a 19-month period. The stream water was severely contaminated with livestock wastewater. Nearly all physicochemical and bacteriological parameters in the stream water were much greater than those in the groundwater. Nitrate-N concentrations ranged from 10.0 to 20.0 mg l^{-1} in boreholes located downstream (site C) from the livestock waste disposal site, while those in the background borehole (W2) were below 1.0 mg l^{-1}. Densities of indicator bacteria in boreholes at site C were two or three orders of magnitude higher than those in W2 borehole. In boreholes located downstream from the livestock waste disposal site, the concentration of ammonium-N, nitrate-N, and pollution indicator bacteria increased as groundwater level rose due to infiltration of rainwater. In W2 borehole, however, physicochemical parameters and the number of pollution indicator bacteria had no correlation with the groundwater level. Collectively, these results suggest that the deep aquifers were heavily contaminated with infiltrated livestock wastewater, which consequently must be adequately treated to minimize groundwater pollution.

Keywords: Livestock wastewater; Groundwater; Indicator bacteria; Fecal contamination; Groundwater recharge

1. Introduction

Approximately 95% of water used in Korea is currently taken from surface water sources, but the quality of this surface water has been deteriorating for several decades and utilization of groundwater has greatly increased. The rural population, in particular, depend mostly on groundwater from private wells for domestic water and drinking. Consequently, attention has recently been directed to the potential health problems caused by exposure to bacterial and chemical contaminants in the groundwater. Many stock farms are located in rural areas and livestock wastewaters are not treated adequately. In addition, urea fertilizers and livestock manure are often spread onto the cropped fields. Therefore, intensive stock farming represents a potential source of contamination of groundwater in these areas. Although studies on groundwater contamination in and around wastewater and sludge disposal sites have been undertaken in the context of environmental pollution and human health (Walsh and LaFleur, 1995; Chan et al., 1997; Lee and Yoon, 1998), only a few papers (Withers et al., 1998; Goody et al., 1998; Nikolaidis et al., 1998) have been dedicated to the study of impact of livestock waste on groundwater quality. Consequently, the impact of livestock wastewater caused by stock farming on groundwater quality in rural areas is not well understood.

Livestock wastewater contains high concentrations of inorganic and organic nitrogen (N) compounds, other nutrients, and pathogenic or indicator bacteria (Bitton and Gerba, 1984; Gustafsson, 1997). The primary inorganic compound of concern to public health in groundwater contaminated with livestock wastewater is nitrate-N, which may cause methemoglobinemia, the

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so-called blue-baby syndrome, in infants (Canter, 1997). In addition, livestock wastewater also contains high concentrations of ammonium-N. In aerobic conditions, nitrate concentrations increase due to nitrification of ammonium by nitrifying bacteria and in anaerobic conditions, nitrate is denitrified to gaseous N compounds (Martikainen et al., 1993). Therefore, measurement of different types of inorganic N is important in evaluating the effect of livestock wastewater on groundwater quality.

When large volumes of wastewater are applied to the surface soil, bacteria pass through the vadose zone and act as a potential reservoir for contamination of groundwater. Heavy rainfall facilitates the transport of pathogenic bacteria, and such pathogens introduced into groundwater can survive in a culturable state or a viable but nonculturable state (Cho and Kim, 1999). As a result, severe health problems may arise after drinking or coming into contact with contaminated groundwater containing pathogenic bacteria. Most bacteriological data for studying groundwater ecosystems have concerned conventional heterotrophic bacteria, total microscopic count, and functional groups such as nitrifying, denitrifying, and sulfate-reducing bacteria (Balkwill et al., 1989; Francis et al., 1989; Fredrickson et al., 1993; Sarbou et al., 1994). However, few studies have been dedicated to specifically investigating the effect of livestock wastewater on pollution indicator bacteria in groundwater. In the pollution study, usually two to three indicator bacteria (such as total coliforms, fecal coliforms, and fecal streptococci) have been measured for evaluating water quality. Indicator bacteria for fecal pollution are important for the determination of the hygienic status of ecosystems. The combined use of different kinds of pollution indicator bacteria, therefore, provides more precise information on fecal pollution in a given environment. In the present paper, we investigated the variation of five pollution indicator bacteria in the groundwater and stream water.

A ca. 2-year sampling program of boreholes located in a livestock-farming area in Wonju, Korea, was carried out to measure the degree of contamination of groundwater contaminated by livestock wastewater and determine the key parameters for evaluation of groundwater pollution.

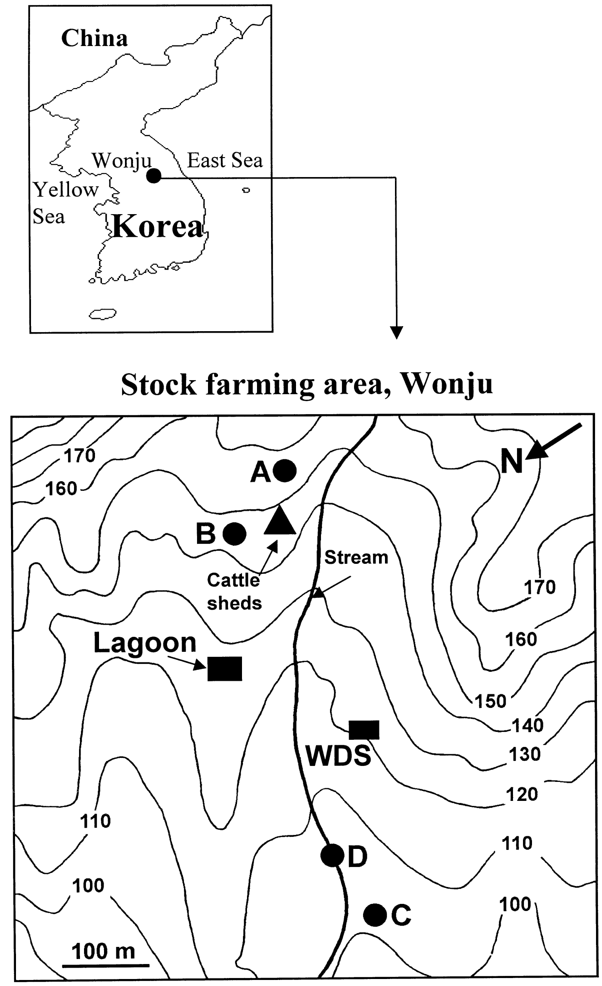

Fig. 1. Locations of sampling sites and boreholes drilled in a livestock farming area in Wonju, Korea, for long-term monitoring of groundwater quality. Numbers on the contours show altitude (m) above sea level. Site A contains W2 borehole, site B contains W3 borehole, and site C contains four boreholes (WI, B6, B7, B8). Site D is a stream contaminated with livestock wastewater. The locations of cattle sheds (▲), a livestock wastewater storage lagoon (Lagoon) and a solid animal waste dump site (WDS) are also shown.

2. Materials and methods

2.1. Research area

The research site is situated about 20 km south of Moonmak (37° 14′ N, 127° 46′ E), Wonju, Kangwon Province, Korea, in a stock-farming and agricultural area where medium or coarse-grained biotite granites of Jurassic origin predominate (Fig. 1). Cattle sheds housing ca. 1500 herds of pigs, 100 herds of cows, and 200 herds of goats are located in this area and since 1992, livestock waste and wastewater have been recycled to the surrounding land surface from livestock waste disposal sites. Livestock wastewater disposal sites consist of a wastewater storage lagoon and a solid animal waste dump site (Fig. 1). The livestock wastewater storage lagoon was designed as a biological digester for raw animal slurry stabilization in 1992, which continually receives ca. 25 m^3 of wastewater per day by an aboveground pipeline from the cattle sheds throughout the year. The morphology of the lagoon is a hexahedron type and its capacity is approximately 1250 m^3 with a depth of 5 m. The surface livestock wastewater has been removed from the lagoon bimonthly and ca. 25 m^3 of them has been spread to the surrounding land surface.

Solid animal wastes have been deposited to a surface soil for utilizing them as manure by mixing with rice hulls and spread to the cultivated land surface about once a month. The total amount of animal wastes in a solid animal waste dump site was ca. 230 m^3 in 1996 and has increased until now. Leachate from this dump site has contaminated a surface soil and groundwater. Consequently livestock wastewater has been introduced directly or indirectly into the saturated zone. Groundwater flows northwestward from livestock waste disposal sites (Fig. 1). Within the study area, 10 boreholes were core-drilled with a core of 152 or 76 mm diameter in January 1995 for research purposes, including hydraulic tests and texture analyses (Jeong and Kim, 1996). A selection of these boreholes were monitored over a 19-month period.

The locations of four sites which were sampled during the present study are given in Fig. 1. Site A, located upstream from the livestock waste disposal sites, consists of a single borehole (W2) and was regarded as an uncontaminated groundwater control. The borehole depth of W2 was 57 m and the depth of the aquifer was 7 m below the land surface. Site B, located adjacent to the cattle sheds, was a 120 m deep domestic well (W3 borehole) from which groundwater has been extracted for drinking. W3 borehole can also be regarded as an uncontaminated control as W2 borehole. Site C is an area located downstream from the livestock waste disposal sites and contaminated with livestock wastewater; here, four boreholes (W1, B6, B7, B8) were monitored. Site C is surrounded by cultivated land, but site C itself has never been cultivated and is in permanent pasture. The borehole depths of B6, B7, and B8 were all 35 m, while that of W1 was 57 m. Site D is a small stream adjacent to site C. This stream is regularly highly contaminated with livestock wastewater. The water quality of this stream could be inferred from the brownish color and a foul smell of water. In the summer season, the stream was in a relatively large volume of water, whereas in a very small volume in the winter, early spring, and late fall. Samples (S1) representative of surface water quality in the study area were collected at a small stream (site D) with a bucket sampler.

2.2. Sampling and physicochemical analyses

During the period from May 1995 to November 1996, the effects of stock farming on groundwater quality were traced by sampling at monthly or bimonthly intervals at different points along the wastewater flow-pathways at each site. Prior to groundwater sampling, groundwater level was checked using a water level sensor, after which each borehole was flushed by pumping until at least three well volumes of water had been evacuated, and the pH and conductivity were stable. Thereafter, groundwater samples were collected from within the saturated zone at each borehole with a suction-lift pump. However, in W3 borehole, water level could not be measured because the well was directly connected to the tap by a pipe and there was no possibility to insert a water level sensor. All samples were taken carefully in sterile polypropylene bottles avoiding any contamination, stored in ice during transit to the laboratory and analyzed immediately. Water temperature, pH, and electric conductance (EC) were measured in situ using portable electrode-carrying devices (Checkmate 90, Corning, NY, USA). After filtration of groundwater samples through a membrane filter (0.45 μm, 47 mm, Nuclepore, MA, USA), alkalinity, hardness, phosphate-P, ammonium-N, nitrite-N, and nitrate-N were measured according to standard methods (APHA, 1995). The concentrations of total organic carbon (TOC) were measured by combustion methods using a TOC analyzer (Shimadzu-5000A, Shimadzu, Kyoto, Japan). Dissolved organic carbon (DOC) was also measured by the combustion method after filtrating through GF/F glass microfiber filter (Whatman, Maidstone, UK).

2.3. Total bacterial number, heterotrophic plate counts, and bacterial secondary production

The numbers of heterotrophic bacteria were determined by the plate count technique on a R2A medium (Reasoner and Geldreich, 1985). Samples were serially diluted and spread on the R2A agar plate in triplicate.

Plates were incubated at 20°C, and colonies were enumerated after 14 days. Total bacterial numbers were measured using the acridine orange direct count method (Hobbie et al., 1977). Bacterial secondary production was measured by the ^{3}H-thymidine incorporation method (Fuhrman and Azam, 1982). The production rates of bacterial numbers per mole of thymidine incorporated were calculated using a conversion factor of 1.8×10^{18} cells (moles of thymidine)$^{-1}$ measured in W1 borehole.

2.4. Pollution indicator bacteria

Pollution indicator bacteria were enumerated in triplicate according to generally accepted basic laboratory procedures using membrane filtration techniques (APHA, 1995). Total coliforms, fecal coliforms, fecal streptococci, and *Staphylococcus* spp. were, respectively, enumerated using m-Endo LES Agar (Difco laboratories, MI, USA), m-FC Agar (Difco laboratories, MI, USA), m-Enterococcus Agar (Difco laboratories, MI, USA), and Baird-Parker medium (Merck, Darmstadt, Germany) supplemented with egg-yolk tellurite. All plates were incubated and typical colonies enumerated. Sulfite-reducing clostridia were enumerated by membrane filtration using egg yolk-free tryptose–sulfite–cycloserine (TSC) agar (Merck, Darmstadt, Germany).

The plates were anaerobically incubated in Anaerocult (Merck, Darmstadt, Germany) for 48 h at 37°C. All black colonies were counted as sulfite-reducing clostridia (Sartory et al., 1993).

2.5. Statistical analysis

Relationships among physicochemical and bacteriological parameters were obtained by calculating Spearman rank correlation coefficients. Spearman rank correlation coefficients were calculated for each individual data set of each borehole ($n=16$), and for all data sets of four boreholes at site C ($n=64$).

3. Results

3.1. Physical and chemical analyses

The mean values and standard deviations of physicochemical parameters measured during the 19 months of study are given in Table 1. Groundwater temperature and pH did not vary significantly at any site, although values in stream water at site D did fluctuate more. Other physicochemical parameters showed a high degree of variation during the sampling period. pH was below 6.5 in samples from all boreholes at site C but was over 7.0 at W2 borehole. Physicochemical values were considerably greater in stream water adjacent to site C than those in groundwater sampled from the boreholes. In boreholes W2 and W3 located upstream from the livestock waste disposal sites, the concentrations of TOC, DOC, and inorganic constituents in the groundwater were much lower than in the boreholes located at site C. For example, TOC ranged from 2.71 to 6.54 mg l^{-1} (mean, 4.28; SD, 1.27) at boreholes W2 and W3, while those at site C boreholes ranged from 3.81 to 33.68 mg l^{-1} (mean, 13.61; SD, 7.92). The concentrations of ammonium-N in both stream water and groundwater samples were significantly lower than those of nitrate-N. Concentrations of ammonium-N were generally less than 300 μg l^{-1} in each borehole, whereas those of nitrate-N ranged from ca. 10.0 to 20.0 mg l^{-1} in boreholes at site C, below 5.0 mg l^{-1} in borehole W3, and below 1.0 mg l^{-1} in borehole W2. Similar differences in EC, nitrite-N and phosphate-P were recorded (Table 1). Concentrations of phosphate-P were generally low except in the stream water samples. These differences suggest that the boreholes at site C were heavily contaminated with livestock wastewater.

The depth of groundwater table varied dramatically during the sampling period depending on rainfall pattern. After rainfall, groundwater levels increased significantly and in a similar pattern across all boreholes (Fig. 2). Nearly all chemical constituents increased in concentrations as groundwater recharged from the infiltration of rainwater containing livestock contaminants; a typical example of data from the B6 borehole representing the co-variation of inorganic nitrogen with groundwater level is shown in Fig. 3. Groundwater level correlated significantly with ammonium-N ($r^2=0.85$, $n=16$, $p<0.001$) and nitrate-N ($r^2=0.64$, $n=16$, $p<0.001$) concentrations in the B6 borehole. Relationships between measured parameters ($n=64$ in each parameter) in boreholes located at site C were obtained by calculating Spearman correlation coefficients (Table 2). Groundwater levels correlated significantly with alkalinity ($r^2=0.66$, $p<0.01$), TOC ($r^2=0.42$, $p<0.01$), ammonium-N ($r^2=0.64$, $p<0.001$), and nitrate-N ($r^2=0.52$, $p<0.01$) concentrations, and other microbiological parameters. However, in the W2 borehole (the background borehole), groundwater levels did not correlate with physicochemical parameters except for water temperature (Table 3). Similar patterns

Table 1
Physicochemical parameters in groundwater and stream water samples in a livestock farming area from May 1995 to November 1996

Physicochemical parameters	Site A	Site B	Site C				Site D
	W2	W3	W1	B6	B7	B8	S1
Groundwater level (cm)	301.3 ± 28.2[a]	ND[b]	312.5 ± 70.0	379.3 ± 66.1	80.3 ± 30.2	228.5 ± 57.3	ND
Temperature (°C)	13.2 ± 1.7	14.1 ± 2.7	13.5 ± 1.3	13.5 ± 1.5	13.4 ± 1.3	13.6 ± 1.3	15.4 ± 7.8
pH	7.8 ± 0.3	6.8 ± 0.5	6.0 ± 0.5	6.0 ± 0.4	6.4 ± 0.3	6.1 ± 0.4	8.3 ± 1.0
Electric conductance (μS cm^{-1})	165.1 ± 21.5	154.3 ± 19.4	374.5 ± 113.4	457.4 ± 328.3	411.3 ± 335.4	335 ± 244.1	982 ± 679.9
Alkalinity (meq l^{-1})	92.8 ± 59.7	78.8 ± 18.1	54.1 ± 20.3	56.7 ± 20.5	122.9 ± 48.6	81.0 ± 9.2	203.3 ± 120.3
Hardness (meq l^{-1})	90.7 ± 36.7	123.9 ± 17.7	161.6 ± 96.2	226.8 ± 141.1	215.1 ± 134.1	126.7 ± 35.9	278.6 ± 252.3
Total organic carbon (mg l^{-1})	4.7 ± 1.3	3.7 ± 0.8	12.1 ± 6.5	9.0 ± 4.5	14.0 ± 6.3	12.4 ± 6.3	138.8 ± 41.5
Dissolved organic carbon (mg l^{-1})	3.9 ± 1.2	3.0 ± 0.8	8.7 ± 4.2	6.3 ± 2.8	9.7 ± 3.3	7.1 ± 4.0	97.3 ± 32.8
Phosphate-P (μg l^{-1})	9.4 ± 7.7	7.0 ± 5.6	35.7 ± 22.1	38.7 ± 29.1	113.4 ± 61.3	17.8 ± 15.5	849.7 ± 838.8
Ammonium-N (μg l^{-1})	3.9 ± 5.5	1.4 ± 1.3	3.1 ± 5.2	3.4 ± 6.5	42.0 ± 49.4	17.3 ± 22.5	131.4 ± 131.1
Nitrite-N (μg l^{-1})	0.3 ± 0.6	2.6 ± 5.7	17.0 ± 26.0	25.9 ± 35.7	58.9 ± 143.7	25.0 ± 79.0	414.3 ± 902.7
Nitrate-N (mg l^{-1})	0.2 ± 0.1	3.5 ± 3.1	13.9 ± 5.7	19.7 ± 7.1	15.9 ± 8.6	14.2 ± 8.1	35.1 ± 19.4

[a] Mean ± SD.
[b] Not determined.

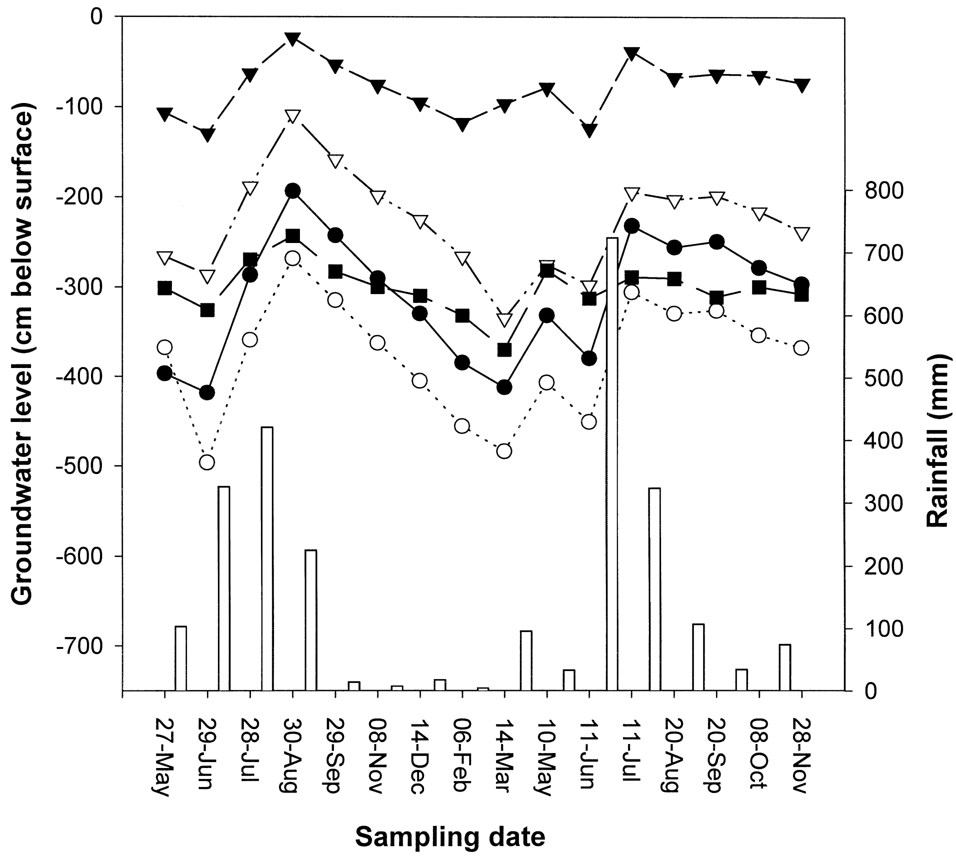

Fig. 2. Variation in groundwater level in each borehole and a rainfall pattern from March 1995 to November 1996. Symbols: W2 boreholes (■), W1 borehole (●), B6 borehole (○), B7 borehole (▼), B8 borehole (▽), and rainfall (vertical bars).

to the W2 borehole were expected in the W3 borehole, however, we could not verify it because had no data for groundwater levels in the W3 borehole. Above results indicate that fluctuation in groundwater level by the infiltration of rain water containing livestock wastewater is a key factor in evaluating the degree of contamination of groundwater by livestock wastewater in this area.

3.2. Bacteriological analyses

Table 4 shows the mean values and standard deviations of bacteriological parameters. Heterotrophic plate counts, total bacterial numbers, and bacterial secondary production indicated that substantial numbers of bacteria were present and active in groundwater samples. Direct counts were two or three orders of magnitude higher than the heterotrophic plate counts on R2A agar. The number of heterotrophic bacteria, total bacterial numbers, and bacterial secondary production in samples from W3 borehole at site B ranged from 1.67×10^{1} to 4.71×10^{3} CFU ml^{-1}, from 4.63×10^{3} to 6.52×10^{5} cells ml^{-1}, and from 2.20×10^{5} to 2.03×10^{6} cells l^{-1} h^{-1}, respectively. These values were considerably lower than those from boreholes at site C (Table 4). In samples from the W2 borehole located at site A, however, the bacterial numbers and activity were higher than those from the W3 borehole. Also, the effect of livestock wastewater could not be clearly demonstrated from the differences in bacterial number and activity between the W2 borehole and the four boreholes at site C. However, groundwater contamination by the infiltration of livestock wastewater was clearly evident from the significant differences in the numbers of pollution indicator bacteria between boreholes at sites A and C. The abundance of total coliforms, fecal coliforms, fecal streptococci, sulfite-reducing clostridia, and *Staphylococcus* spp. in the boreholes at site C were two to three orders of magnitude higher than those in the W2 borehole. On the basis of the much greater number of indicator bacteria detected, stream water samples were heavily contaminated with livestock wastewater.

All bacteriological parameters in the contaminated boreholes located at site C varied according to the groundwater level. As groundwater level rose by recharge from surface water, the number of heterotrophic and indicator bacteria also increased. Representative data from the W1 borehole illustrating the common pattern are shown in Fig. 4. As with the chemical determinants, the depth of the water table showed significant correlation with heterotrophic plate counts ($r^2=0.49$, $p<0.05$), total coliforms ($r^2=0.59$, $p<0.01$), fecal streptococci ($r^2=0.69$, $p<0.001$), sulfite-reducing

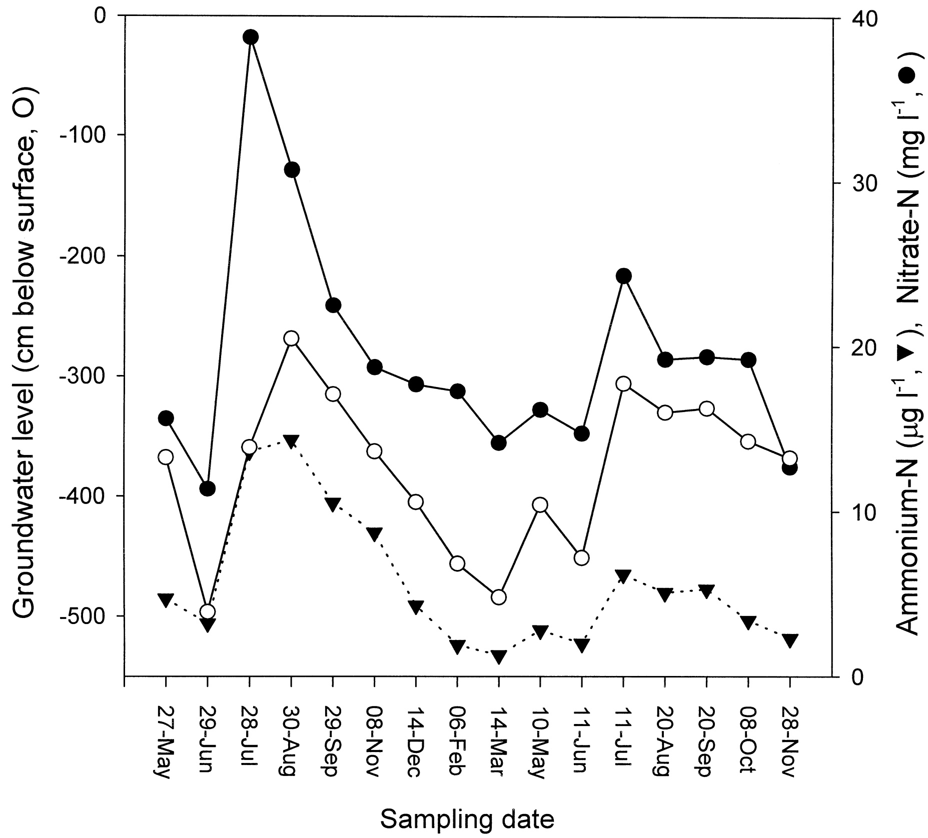

Fig. 3. Co-variation patterns between groundwater level (○), ammonium-N (▼), and nitrate-N (●) measurements in B6 borehole from March 1995 to November 1996. Note the differences in scales on the *y*-axes and the units of concentration of inorganic nitrogen.

clostridia ($r^2=0.48$, $p<0.05$), and *Staphylococcus* spp. ($r^2=0.56$, $p<0.01$). In addition, bacteriological parameters correlated significantly with ammonium-N and nitrate-N at samples from boreholes located at site C (Table 2). However, there was no correlation between groundwater level and inorganic constituents or bacteriological parameters in W2 borehole (Table 3).

4. Discussion

The infiltration of livestock wastewater clearly adversely affected groundwater quality at sampling sites downstream of the livestock waste disposal sites; the degree of contamination largely depending on rainfall patterns. The infiltration of rainwater containing livestock contaminants caused an elevation of groundwater level and a severe contamination of aquifer with nitrate and indicator bacteria. The difference in groundwater level was about 225 ± 3 cm in three boreholes at site C, except for B8 borehole in which the difference was 106.5 cm. In Korea, rainfall tends to be concentrated in the summer season causing a large rise in the groundwater table between June and August in all boreholes. This tendency leads to much variation in physicochemical and biological contamination of the aquatic environment (Cho et al., 1997). Although known to affect surface water quality, our data indicate that significant contamination of subsurface water quality can also occur as a result of this rainfall pattern.

Precipitation in the form of rainfall to the surface soil infiltrates into the vadose zone or becomes overland flow (Freeze and Cherry, 1979). In the study area, overland rainwater after infiltration into the vadose zone flows toward the stream. Consequently, the concentrations of physicochemical parameters and indicator bacteria in the stream water became much greater than in the groundwater. The abundance of heterotrophic bacteria and bacterial secondary production rates in the stream water samples were one to two orders of magnitude higher than those in the boreholes at site C and various indicator bacteria were two to four orders of magnitude higher. This is due to a storm surface run-off component in the sloping land causing direct contamination. Generally, sands have a higher hydraulic conductivity than silty or clay-rich soils and thus provide high percolation rates (Wilhelm et al., 1996). The soils of the study area are very free draining with typically 92–97% of sand and an irregular sandy layer at a depth of 4 m that can be considered as 'sandy aquifer'. The deep rocks within the saturated zone consist of biotite granite and biotite gneiss containing

Table 2
Spearman rank correlation coefficients among physicochemical and bacteriological parameters in boreholes (W1, B6, B7, B8) at site C ($n = 64$)

	GWL[a]	TM	pH	ALK	HARD	TOC	DOC	PO4	NH4	NO2	NO3	HPC	TBN	BSP	TC	FC	FS	STA	CLO
GWL	1.00	–[b]	0.45[c]	0.66	0.24	0.42	0.31	–	0.64	–	0.52	0.46	0.38	0.32	0.55	0.52	0.72	0.67	0.68
TM		1.00	–	–	–	–	–	–	–	–	–	0.31	–	–	0.23	0.24	–	–	0.21
pH			1.00	0.57	0.22	–	–	–	0.34	–	–	0.34	–	–	0.34	0.29	–	–	–
ALK				1.00	0.43	–	–	–	0.56	–	–	0.53	–	–	–	–	0.32	0.26	0.40
HARD					1.00	–	–	–	0.43	0.33	–	0.32	–	–	–	–	–	–	–
TOC						1.00	0.88	–	0.51	–	0.46	0.44	0.31	0.22	–	0.34	0.41	0.45	0.39
DOC							1.00	–	0.63	–	0.50	0.56	0.25	0.49	–	0.45	0.53	0.59	0.52
PO4								1.00	–	0.38	0.21	–	–	–	0.35	0.23	0.38	0.27	–
NH4									1.00	–	0.28	0.45	–	0.45	0.57	0.54	0.74	0.79	0.49
NO2										1.00	–	–	–	–	–	–	–	–	0.23
NO3											1.00	0.34	–	0.23	0.43	0.39	0.48	0.51	0.45
HPC												1.00	–	0.32	0.37	0.51	0.65	0.62	0.61
TBN													1.00	0.73	–	0.23	0.34	0.49	0.38
BSP														1.00	–	0.37	0.42	0.54	0.32
TC															1.00	0.84	0.45	0.51	0.40
FC																1.00	0.52	0.58	0.51
FS																	1.00	0.92	0.88
STA																		1.00	0.91
CLO																			1.00

[a] GWL, goundwater level; TM, temperature; ALK, alkalinity; HARD, hardness; TOC, total organic carbon; DOC, dissolved organic carbon; PO4, phosphate-P; NH4, ammonium-N; NO2, nitrite-N; NO3, nitrate-N; HPC, heterotrophic plate counts; TBN, total bacterial numbers; BSP, bacterial secondary production; TC, total coliforms; FC, fecal coliforms; FS, fecal streptococci; STA, *Staphylococcus* spp.; CLO, sulfite-reducing clostridia.
[b] $p > 0.05$ or $r^2 < 0.20$.
[c] $p < 0.05$.

Table 3
Spearman correlation coefficients among physicochemical and bacteriological parameters in W2 borehole at site A ($n = 16$)

	GWL[a]	TM	PH	ALK	HARD	TOC	DOC	PO4	NH4	NO2	NO3	HPC	TBN	BSP	TC	FC	FS	STA	CLO
GWL	1.00	0.65[c]	–[b]	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
TM		1.00	–	–	–	–	–	–	–	–	–	0.35	–	0.32	–	–	–	–	–
pH			1.00	0.55	0.32	–	–	–	0.23	0.44	–	–	–	–	–	–	–	–	–
ALK				1.00	0.43	–	–	–	–	–	–	0.53	–	–	–	–	0.32	0.26	0.40
HARD					1.00	–	–	–	–	–	–	0.32	–	–	–	–	–	–	–
TOC						1.00	0.67	–	–	–	–	0.54	0.32	0.28	0.31	–	–	–	–
DOC							1.00	–	–	–	–	0.61	0.27	0.25	–	–	–	–	–
PO4								1.00	–	–	–	–	–	–	0.54	–	–	–	–
NH4									1.00	–	–	–	–	–	0.32	–	–	–	–
NO2										1.00	–	–	–	–	0.64	–	–	–	–
NO3											1.00	–	–	–	–	–	–	–	–
HPC												1.00	0.52	0.41	–	–	–	–	–
TBN													1.00	0.67	–	–	–	–	–
BSP														1.00	–	–	–	–	–
TC															1.00	–	–	–	–
FC																1.00	0.53	0.37	0.57
FS																	1.00	0.75	0.63
STA																		1.00	0.51
CLO																			1.00

[a] GWL, goundwater level; TM, temperature; ALK, alkalinity; HARD, hardness; TOC, total organic carbon; DOC, dissolved organic carbon; PO4, phosphate-P; NH4, ammonium-N; NO2, nitrite-N; NO3, nitrate-N; HPC, heterotrophic plate counts; TBN, total bacterial numbers; BSP, bacterial secondary production; TC, total coliforms; FC, fecal coliforms; FS, fecal streptococci; STA, *Staphylococcus* spp.; CLO, sulfite-reducing clostridia.
[b] $p > 0.05$ or $r^2 < 0.20$.
[c] $p < 0.05$.

fractures. The hydraulic conductivity of the aquifers is consequently high, ranging from 3.5×10^{-4} to 3.5×10^{-3} cm s^{-1}. Recharge to the water table therefore occurred due to infiltration of rainwater and lateral movement of groundwater northwest from the livestock waste disposal site. These characteristics of the study area rendered sand and bedrock aquifers heavily contaminated with livestock wastewater and manure.

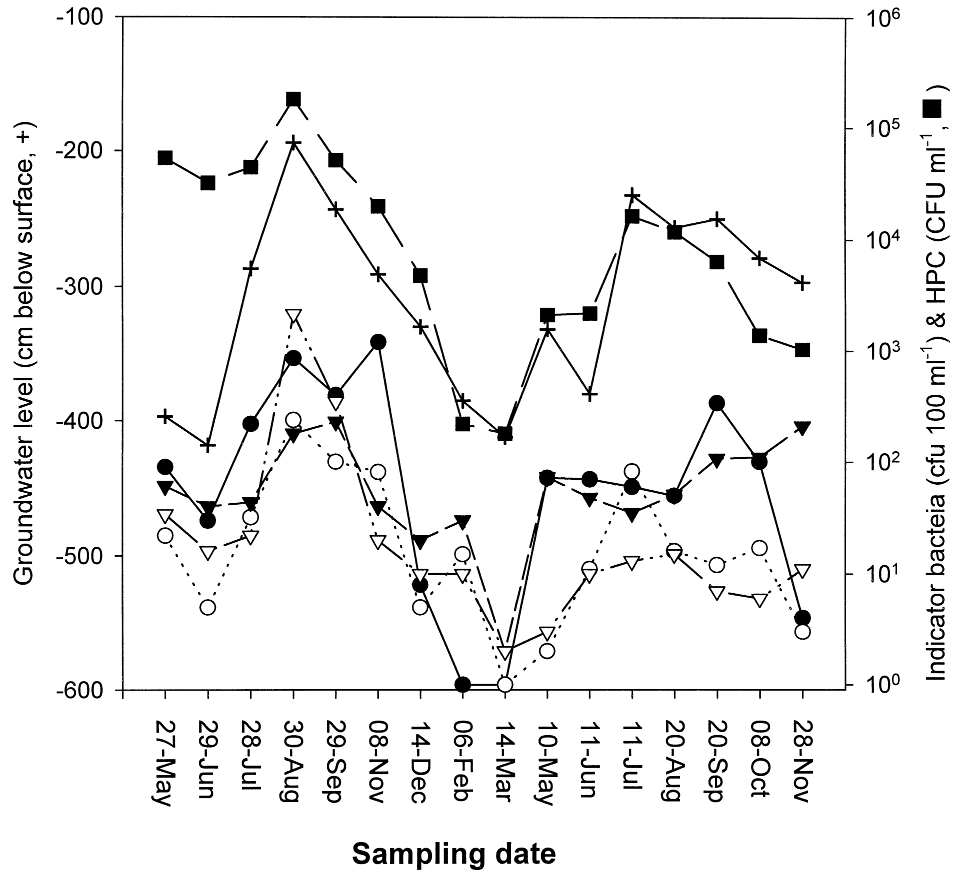

Fig. 4. Co-variation patterns between groundwater level and bacteriological parameters in W1 borehole from March 1995 to November 1996. Symbols: groundwater level (+), heterotrophic plate counts (HPC, ■), total coliforms (●), fecal streptococci (○), *Staphylococcus* spp. (▼), sulfite-reducing clostridia (▽). Note the differences in scales on the *y*-axes and the units of bacterial densities.

Table 4
Bacteriological parameters in goundwater and stream water samples in a livestock farming area from May 1995 to November l996

Bacteriological parameters	Site A	Site B	Site C				Site D
	W2	W3	W1	B6	B7	B8	S1
Heterotrophic plate counts ($\times 10^4$ CFU ml^{-1})	2.95 ± 4.15[a]	0.07 ± 0.13	2.63 ± 4.51	2.82 ± 4.49	18.21 ± 40.13	25.35 ± 28.03	118.1 ± 166.2
Total bacterial numbers ($\times 10^6$ cells ml^{-1})	8.32 ± 9.22	0.45 ± 0.61	9.89 ± 10.33	7.65 ± 9.11	98.39 ± 101.34	108.33 ± 232.12	ND[b]
Bacterial secondary production ($\times 10^6$ cells l^{-1} h^{-1})	8.21 ± 22.63	0.95 ± 0.49	9.64 ± 11.48	8.94 ± 12.77	13.75 ± 14.34	10.53 ± 12.74	113.0 ± 120.1
Total coliforms ($\times 10^3$ CFU l^{-1})	0.06 ± 0.20	0.17 ± 0.33	2.19 ± 3.43	5.96 ± 17.8	16.85 ± 32.87	26.3 ± 60.6	1628 ± 2246
Fecal coliforms ($\times 10^3$ CFU l^{-1})	0.04 ± 0.12	0.14 ± 0.26	0.74 ± 0.61	1.34 ± 1.22	9.59 ± 8.14	12.51 ± 15.33	1022 ± 1693
Fecal streptococci ($\times 10^3$ CFU l^{-1})	0.09 ± 0.13	0.18 ± 0.56	0.40 ± 0.61	6.32 ± 7.80	7.43 ± 25.6	7.3 ± 12.8	404.1 ± 876.8
Staphylococcus spp. ($\times 10^3$ CFU l^{-1})	0.24 ± 0.55	0.45 ± 1.42	0.81 ± 0.72	5.41 ± 17.91	24.41 ± 74.81	1.18 ± 1.92	1705 ± 3734
Sulfite-reducing clostridia ($\times 10^3$ CFU l^{-1})	0.39 ± 0.75	0.10 ± 0.25	1.68 ± 5.37	1.12 ± 3.84	94.8 ± 34.73	1.28 ± 3.14	272.3 ± 820.1

[a] Mean ± SD.
[b] Not determined.

The concentrations of nitrate-N in the contaminated boreholes at site C exceeded the acceptable limit of 10 mg l^{-1} imposed by the Ministry of Environment in Korea for drinking water, while ammonium-N concentrations did not exceed the maximum acceptable limit of 0.5 mg l^{-1}. However, although ammonium-N concentrations were much lower than those of nitrate-N in the contaminated aquifer there was a significant positive correlation between ammonium-N and the numbers of contamination indicator bacteria (Fig. 3, Table 2). Therefore, ammonium-N can be used as a good indicator in evaluating acute contamination of groundwater. The absence of high ammonium-N concentrations in the contaminated boreholes is probably due to oxidation of ammonium-N to nitrate-N in the livestock waste disposal sites or aerobic subsurface by nitrifying bacteria. It has been reported that ammonium-N is oxidized to nitrate-N in septic tanks, wastewater stabilization ponds (Harman et al., 1996; Lai and Lam, 1997), and a cow slurry storage lagoon (Goody et al., 1998; Withers et al., 1998). In the study area, nitrification process is also suggested by the relatively higher nitrite-N concentrations than ammonium-N, the intermediate product in the nitrifying process in the study area. The lower pH values in contaminated boreholes than uncontaminated boreholes are also further evidence of nitrification. Recently, we detected the presence of ammonia-oxidizing bacteria of the β subdivision of the *Proteobacteria* in the contaminated boreholes and raw livestock wastewater by amplifying the ammonia monooxygenase gene (*amo A*) and the 16S rDNA gene (unpublished data). Nitrification process in the study area can be clearly verified by measuring nitrification activities in the near future. High nitrate-N concentrations of contaminated boreholes are thought to be a result of nitrification of ammonium-N or direct introduction of nitrate-N from the livestock wastewater disposal site and recycled animal manure to the land surface. Because chemical N fertilizers have not be applied in the cultivated land but livestock manure has been applied, nearly all nitrate-N in the boreholes may be originated from the livestock wastewater and manure. The concentrations of nitrate-N and ammonium-N were co-varied with sampling time; however, nitrate-N concentrations decreased during July 1995 as groundwater level and ammonium-N concentrations increased (Fig. 3). This pattern is probably due to denitrification as a change to anoxic conditions following the introduction of livestock wastewater containing high concentrations of carbon substrates. These results suggest that nitrification and denitrification of N compounds introduced by livestock wastewater control nitrogen cycling of the study area.

Although pathogens and indicator bacteria were introduced by the animal manure applied to agricultural soil adjacent to the study site, the contamination can be considered as point-source pollution because a considerable amount of livestock wastewater contaminating the boreholes actually originated from the livestock waste disposal site. As such, the high concentrations of pathogenic bacteria can be attributed to livestock wastewater and manure. It has been reported that indicator and pathogenic bacteria are efficiently retained in soils and are detected at only low levels in groundwater under field conditions (Liu, 1982; Alhajjar et al., 1988). However, a large number of indicator bacteria were detected in these sites. Such unusually high densities of indicator bacteria indicate contamination of groundwater with bacteria of fecal origin. Heavy rainfall promotes the movement of bacteria and other inorganic contaminants through soil (Zyman and Sorber, 1988; Nikolaidis et al., 1998). Therefore, a large number of the indicator bacteria which survived in the vadose zone were rapidly introduced into the saturated zone by heavy rains, thus contaminating the groundwater. The results showing that bacterial densities, activities, and the number of indicator and pathogenic bacteria in contaminated groundwater increased with elevation of groundwater level clearly supported this point.

Total coliforms and fecal coliforms have been used as indicators of pathogens (APHA, 1995). However, it has been reported that estimation of total coliforms and fecal coliforms are not sufficient to confirm fecal contamination (Fuzioka and Shizumura, 1985; Hazen, 1988). For this reason, pollution indicators chosen in this study included total coliforms, fecal coliforms, fecal streptococci, *Staphylococcus* spp., and sulfite-reducing clostridia. Fecal streptococci are known to be more resistant than coliforms in the natural environment, and may be an indicator of fecal pollution (Rice et al., 1993). Sulfite-reducing clostridia may be a better indicator of fecal pollution due to the resistant nature of its spore, allowing lengthened survival period (Bisson and Cabelli, 1979; Fuzioka and Shizumura, 1985). Also, *Staphylococcus* spp., which are commonly found in normal inhabitants of human or animal skin, do not occur naturally in groundwater habitat and thus may be an indicator of contamination of the subsurface aquifer (Capuano et al., 1995). By detecting a large number of pollution indicator bacteria in the groundwater, heavy fecal contamination of this subsurface aquifer was indicated. In addition, fecal streptococci, *Staphylococcus* spp., and sulfite-reducing clostridia showed significant correlation with the elevation of groundwater level, total coliforms, and fecal coliforms. These indicator bacteria, therefore, could be utilized as bacterial indicators for livestock wastewater pollution of groundwater.

This study showed that shallow and deep subsurface aquifers in a livestock farming and agricultural area were heavily contaminated with nitrate and pollution indicator bacteria from livestock wastewater. Correlation analyses confirmed that infiltration of rainwater after summer rainfall was a major factor accelerating groundwater pollution from the livestock waste disposal sites. It can be suggested that should the groundwater be qualified as drinking water, livestock wastewater must be fully treated and livestock production must be controlled accordingly.

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