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Phosphorus and Bacterial Growth in Drinking Water

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The availability of organic carbon is considered the key factor to regulate microbial regrowth in drinking water networks. However, boreal regions (northern Europe, Russia, and North America) contain a large amount of organic carbon in forests and peatlands. Therefore, natural waters (lakes, rivers, and groundwater) in the northern hemisphere generally have a high content of organic carbon. We found that microbial growth in drinking water in Finland is highly regulated not only by organic carbon but also by the availability of phosphorus. Microbial growth increased up to a phosphate concentration of 10 μg of PO_4-P liter^{-1}. Inorganic elements other than phosphorus did not affect microbial growth in drinking water. This observation offers novel possibilities to restrict microbial growth in water distribution systems by developing technologies to remove phosphorus efficiently from drinking water.

The hygienic quality of drinking water is reduced if pathogenic microbes penetrate water treatment or if the conditions in the water distribution network allow a high level of microbial growth. Most of the microbes in drinking water are heterotrophic, i.e., they need organic compounds for their carbon and energy sources. Microbial occurrence and growth in drinking water are controlled by different disinfection agents such as chlorine, hypochlorite, and ozone. However, the oxidizing agents have been found to split organic compounds of high molecular weight to simple organic acids, thus increasing the possibilities for growth of heterotrophic microbes in the drinking water network (4, 24). This microbially unstable fraction of organic carbon, i.e., assimilable organic carbon (AOC), has been suggested to be the main nutrient for microbial regrowth in distribution networks (15, 25). Boreal regions (northern Europe, Russia, and North America) contain large amounts of organic carbon in forests and peatlands (8, 11). Therefore, natural waters (lakes, rivers, even groundwater) in the northern hemisphere generally have a high content of organic carbon (2). Water technologies are under development to reduce not only total organic carbon but also the remaining labile organic compounds in drinking water.

Little attention has been paid to the effects of inorganic nutrients on microbial growth in drinking water. We have found previously that inorganic nutrients can have importance for microbial growth in drinking water (17). Here we complete that preliminary study and show the importance of phosphorus on the growth of heterotrophic microbes in different Finnish drinking water samples produced from surface water or groundwater.

MATERIALS AND METHODS

Water samples. The waterworks studied were the largest ones in Finland. Three surface waterworks (A, B, and C), two artificially recharged groundwater works (D and E), and one groundwater works (F) were included in the study (Table 1). Surface waters were treated by a polyaluminum flocculation purification process, and the drinking water was disinfected. None of the artificially recharged groundwater works or the groundwater works used disinfectants. At waterworks A ozonation was applied, and waterworks B had biologically activated carbon filtration (Table 1). At all waterworks pH was adjusted with lime. The effect of phosphorus on microbial growth was studied in water samples taken from fresh drinking water leaving the waterworks and in water samples from the main pipelines of the distribution networks at a distance of 10 km from the waterworks.

Effect of nutrient addition. The chemical and microbiological analyses were performed four times during 1995. The effect of nutrient additions on heterotrophic microbial growth was studied twice by in vitro laboratory tests (17) from 1995 to 1996. Microbial growth in water was measured by incubating water samples at 15°C in the dark in acid-washed and heat-treated (8 h at 250°C) glass flasks. Free chlorine in water samples was removed at the beginning of incubation with 50 μl of 0.02 M sodium thiosulfate (10 nM sodium thiosulfate, final concentration) (Merck). Bacterial growth was monitored by counting bacteria every second day by a spread plate technique (see below). Microbial growth was tested once at every waterworks. Tests included duplicate samples of untreated water, water treated with phosphorus (50 μg of PO_4-P liter^{-1}) (Na_2HPO_4; Merck), and water amended with a mixture of inorganic nutrients [$(NH_4)_2SO_4$, KH_2PO_4, $MgSO_4 \cdot 7\ H_2O$, $CaCl_2 \cdot 2H_2O$, and NaCl] (Merck). The concentrations of nutrients in samples were 970 μg of NH_4-N liter^{-1}, 50 μg of PO_4-P liter^{-1}, 60 μg of K liter^{-1}, 10 μg of Mg liter^{-1}, 30 μg of Ca liter^{-1}, 80 μg of Na liter^{-1}, and 120 μg of Cl liter^{-1}.

The effect of phosphorus concentration on heterotrophic microbial growth was studied by adding different amounts of phosphorus (Na_2HPO_4; Merck). The final concentrations of added phosphorus were 0, 1, 2, 5, and 10 μg of PO_4-P liter^{-1}. The effect of phosphorus concentration on microbial growth was studied by testing fresh drinking water from all surface waterworks (A, B, and C), one artificially recharged groundwater works (D), and one groundwater works (F). The effect of phosphorus concentration was also studied by testing water samples from distribution networks of all surface waterworks and one groundwater works (F). Microbial growth was monitored by using R2A plate counting as described below.

Microbial growth. Heterotrophic bacterial growth in water samples was measured by plate counting (R2A agar; Difco) (9, 21). Microbial growth was monitored in every test for 2 weeks. Agar plates were incubated for 1 week at 20 ± 3°C before the colonies were counted.

Analyses of organic carbon. Total organic carbon (TOC) content in the water samples was analyzed by a high-temperature combustion technique with a Shimadzu 5000 TOC analyzer (Kyoto, Japan). The content of AOC was measured by a modification of the standard method (9). The maximum growth of *Pseudomonas fluorescens* P17 (ATCC 49642) and *Spirillum* sp. strain NOX (ATCC 49643) in water samples was used to correspond to the amount of AOC (in micrograms of AOC-C per liter). The modification included an amendation with inorganic nutrients (see above) to ensure that only the AOC content restricted microbial growth.

Phosphate and nitrate. The analyses of phosphate and nitrate concentrations were done in the laboratories of each waterworks. The concentration of phosphate was analyzed by a colorimetric ascorbic acid method (5), and nitrate concentration was analyzed by a photometric salicylate method (6). These Finnish standard methods are based on the "Standard Methods for the Examination of Water and Wastewater" (9).

RESULTS

Contents of TOC and AOC. The average content of TOC in fresh drinking water produced from surface water was higher than that in drinking water produced from groundwater (Table

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TABLE 1. Main characteristics of the waterworks

Waterworks	Production volume (m^3/day)	Chemical and biological purification method	Disinfection agent
Surface waterworks			
A	95,000	Polyaluminum flocculation	Ozone and monochloramine
B	34,500	Polyaluminum flocculation, BAC[a]	Chlorine dioxide
C	3,500	Polyaluminum flocculation	Chlorine
Artificially recharged groundwater works			
D	10,000	Slow sand filtration	No disinfection
E	10,000	Slow sand filtration	No disinfection
Groundwater works			
F	4,000		No disinfection

[a] BAC, biologically activated carbon filtration.

2). TOC concentration was also higher in fresh drinking water produced from artificially recharged groundwater (D and E) than in the drinking water produced from groundwater. The AOC content was higher in fresh drinking water produced from surface water than in drinking water produced from artificially recharged groundwater or from groundwater (Table 2). The AOC content was highest in drinking water from waterworks A, at which ozonation was applied (Table 2).

Phosphate and nitrate. Phosphate concentrations were below the detection limit (<2 μg of P liter^{-1}) in all samples of fresh drinking water leaving the waterworks (Table 2). The concentrations of nitrate in fresh drinking water processed from surface water ranged from 0.2 to 7 mg of NO_3-N liter^{-1}, and those in all other drinking water ranged from 0.2 to 2.0 mg of NO_3-N liter^{-1} (Table 2).

Effect of phosphorus addition on microbial growth. The laboratory tests showed that microbes grew in every water sample. Though the AOC content in drinking water produced from surface water was generally higher than that in drinking water produced from groundwater, the microbial growth was higher in the water leaving groundwater works (Table 2; Fig. 1).

The addition of phosphorus (50 μg of PO_4-P liter^{-1}) increased microbial growth in fresh drinking water produced from surface water or groundwater (Fig. 1). This was also true for the water in the distribution network. Other inorganic nutrients (N, K, Mg, and Ca) did not significantly affect the microbial growth (Fig. 1).

Even the addition of 1 μg of phosphate phosphorus increased the microbial growth (Fig. 2). The microbial growth was increased by phosphate addition up to a concentration of 10 μg of PO_4-P liter^{-1} (Fig. 3).

DISCUSSION

Surface water in boreal regions generally contains large amounts of organic matter (2). Finnish surface waters are rich in humic substances (22). Therefore, drinking water processed from surface water and even groundwater has a high content of organic carbon (26). The availability of AOC for microbial growth was high in the drinking water studied (Table 2). Although it has been suggested that the content of AOC should affect microbial growth (15, 25), we noticed in this study that the content of AOC correlated poorly with microbial growth in drinking water. Therefore, some factors other than the availability of carbon could affect microbial growth in drinking water. The addition of phosphorus to our drinking water samples greatly increased the growth of heterotrophic bacteria. This was true not only for the drinking water produced from surface water but also for that produced from groundwater (Fig. 1).

The concentration of phosphorus needed to stimulate growth was exceptionally low. Even the addition of 1 μg of PO_4-P liter^{-1} increased microbial growth significantly both in fresh drinking water (Fig. 3A) and in water from distribution networks (Fig. 3B). The addition of other nutrients such as nitrogen had negligible effects on the microbial growth (Fig. 1).

Phosphorus has a major ecological role in nature, because it is an essential element for microbes and because it is commonly the least abundant element compared to carbon (27). In

TABLE 2. TOC, AOC, nitrate, and phosphate in fresh drinking water

Waterworks	TOC (mg liter^{-1})[a]	AOC (μg of AOC-C liter^{-1})[a]	NO_3-N (mg liter^{-1})[a]	PO_4-P (μg liter^{-1})[b]
Surface waterworks				
A	3.1 ± 0.1	470 ± 70	0.25 ± 0.03	<2
B	2.6 ± 0.2	315 ± 135	0.5 ± 0.5	<2
C	3.7 ± 0.3	425 ± 150	5.7 ± 1.2	<2
Artificially recharged groundwater works				
D	1.9 ± 0.3	145 ± 60	2.3 ± 0.1	<2
E	1.8 ± 0.2	160 ± 80	0.3 ± 0.05	<2
Groundwater works				
F	0.7 ± 0.1	155 ± 40	1.3 ± 0.4	<2

[a] Data are means ± standard deviations for four measurements.
[b] Data are means of four measurements.

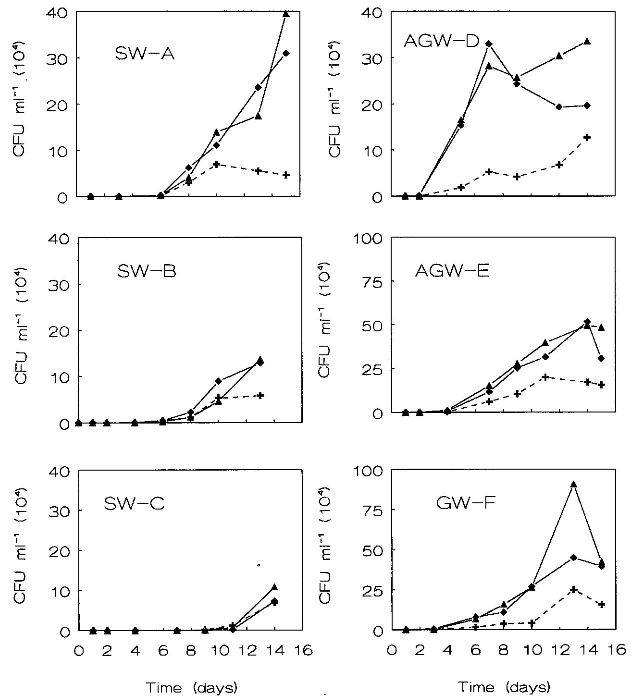

FIG. 1. Growth of heterotrophic bacteria in fresh drinking water from three surface waterworks (SW-A to -C), two artificially recharged groundwater works (AGW-D and -E), and one groundwater works (GW-F). Tests included those on untreated samples (+---+), on samples with added phosphorus (50 μg of P liter^{-1}) (▲——▲), and on samples amended with a mixture of inorganic nutrients [$(NH_4)_2SO_4$, KH_2PO_4, $MgSO_4 \cdot 7\,H_2O$, $CaCl_2 \cdot 2H_2O$, NaCl] (◆—◆). The figure is adapted in part from data presented earlier in *Nature* (17) and is used with the permission of the publisher.

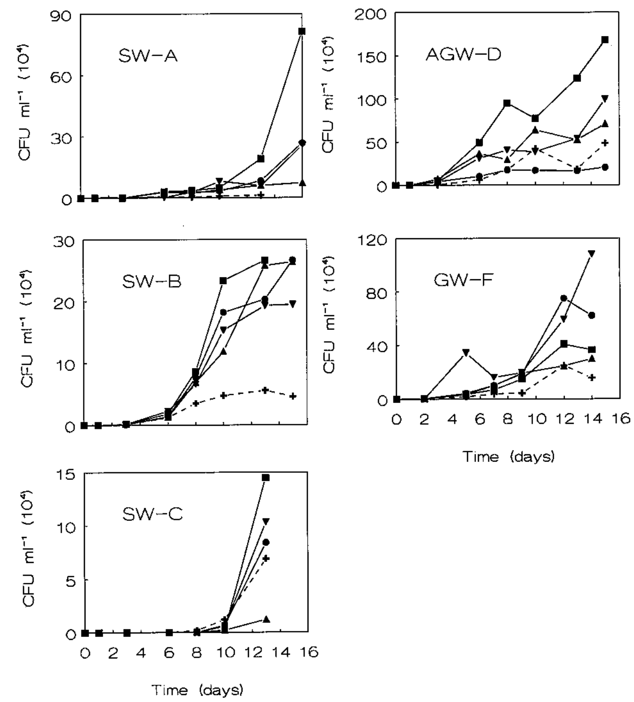

FIG. 2. Growth of heterotrophic bacteria in different fresh drinking water samples with different phosphorus additions. Tests included those on samples from three surface waterworks (SW-A to -C), one artificially recharged groundwater works (AGW-D), and one groundwater works (GW-F). Symbols: ✚, no phosphorus addition; ▲, 1 μg of P liter^{-1} added; ●, 2 μg of P liter^{-1} added; ▼, 5 μg of P liter^{-1} added; and ■, 10 μg of P liter^{-1} added.

natural surface water ecosystems the lack of either nitrogen or phosphorus can be the principal factor limiting microbial productivity (1, 3, 10). Our results show the importance of phosphorus on microbial growth in processed drinking water in Finland. This might be true for drinking water in general in the boreal regions. Possibly, low phosphorus levels could explain earlier observations of a poor correlation between AOC and microbial growth in distribution networks (7, 13). However, in many regions in Europe and the United States, natural water has a higher content of mineral nutrients relative to AOC than that which exists in boreal regions. There, AOC, not phosphorus or nitrogen, can be the most important factor to limit microbial growth in drinking water (12, 19).

The total phosphorus concentration in unpolluted surface water is generally from 10 to 50 μg of P liter^{-1} (27). In Finland the total phosphorus content in surface water is on average 15 μg of P liter^{-1} (14). Most of the total phosphorus is associated with particulate matter, and only a minor part exists dissolved, i.e., directly usable for microbes (27). Finnish waterworks have paid little attention to phosphorus content in drinking water because drinking water produced from surface water that receives coagulation/flocculation purification has a low phosphorus content compared to the guideline value of <100 μg of PO_4-P liter^{-1} (18). Often, the phosphorus concentration in drinking water is below the detection limit (<2 μg of PO_4-P liter^{-1}) of the standard methods for phosphorus measurement (9). At least some of the current water treatment techniques remove most of the phosphorus from surface water. However, different waterworks treat raw water with different techniques. Every water plant should therefore test for the importance of phosphorus to microbial growth in finished water. Only minor changes in phosphorus concentration might greatly affect the microbial growth potential in the distribution networks. The purity of chemicals used in water treatment can vary, thus affecting the concentration of residual phosphorus in finished drinking water. For example, liming agents used in pH adjustment of water may contain phosphorus.

Should the waterworks in boreal regions pay more attention to phosphorus in water treatment by developing purification techniques to ensure a very low concentration of phosphorus (e.g., below 1 μg liter^{-1})? Billions of dollars are spent annually by waterworks for drinking water purification in the United States alone (23). One of the important goals in drinking water production is to reduce the content of organic carbon, especially its microbially labile fraction, AOC, to an acceptable level. For example, according to Van der Kooij (25) the content of AOC-C should be below 10 μg liter^{-1} to ensure that drinking water is biologically stable. This task is often difficult, especially if ozonation is applied in the water purification process (20). The results of the present study suggest that in addition to AOC the content of phosphorus greatly affects microbial growth in drinking water. The microbial growth in the drinking water distribution network is the sum of several factors such as concentration of different nutrients and disinfection agents. Also, the corrosion of pipelines affects microbial growth. High doses of phosphate to counteract the corrosion of pipelines have successfully been used to reduce the occurrence of total coliforms in water distribution networks (16). However, the present results suggest that the use of phosphate against corrosion can cause problems for the microbiological quality of drinking water in boreal regions. We suggest that the waterworks, at least in boreal regions, keep the

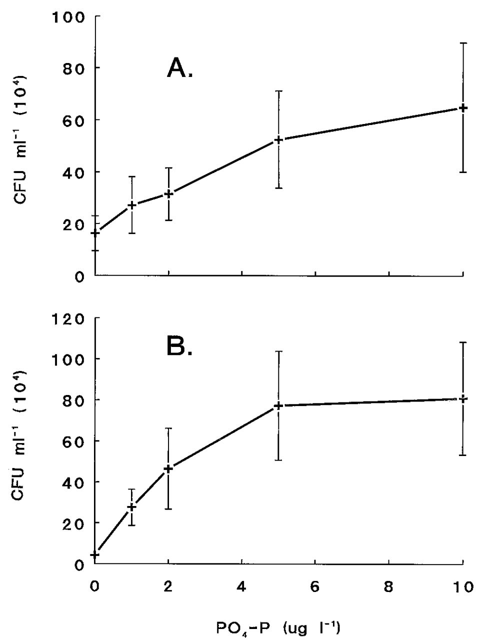

FIG. 3. Maximum growth of heterotrophic bacteria (viable counts) after addition of phosphorus to the processed drinking water (fresh water) leaving the waterworks (five different surface waterworks and groundwater works) (A) and to the water samples from distribution networks (four different surface waterworks and groundwater works) (B). Standard errors are shown by bars.

phosphorus level as low as possible to increase the biological stability of drinking water.

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