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BioMed Central
1980

Acta Veterinaria Scandinavica. 1980. 21: 43-54

http://hdl.handle.net/1975/737

Acta vet. scand. 1980, *21*, 43—54.

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THE EFFECT OF CADMIUM ON INDICATOR BACTERIA IN SEWAGE

By

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KORKEALA, H. and J. HIRN: *The effect of cadmium on indicator bacteria in sewage.* Acta vet. scand. 1980, *21*, 43—54. — The effect of cadmium (Cd) on the number of different faecal indicator bacteria in sewage, and on species composition of different indicator bacteria, was studied. Different amounts of Cd were added to aliquots of a sewage sample, and after 0, 4½ and 24 h of Cd exposure at 20°C coliforms, faecal coliforms, faecal streptococci and Clostridium perfringens were enumerated by the membrane filter method.

The Cd-induced reduction in the number of coliforms and faecal coliforms during exposure was found to be greater than the decrease in the number of faecal streptococci. In the case of C. perfringens the Cd concentrations used produced no observable effect on the cell number. The addition of Cd changed the faecal coliforms and faecal streptococci density relationship. Escherichia coli seems to be more resistant to Cd than other coliforms and Streptococcus faecalis var. liquefaciens and Streptococcus durans more resistant to Cd than other faecal streptococci. No influence of Cd on gas production by faecal coliforms was observed.

Faecal streptococci and C. perfringens seem to be better indicator bacteria than coliforms and faecal coliforms in evaluating the hygienic quality of Cd polluted sewage.

coliforms; faecal coliforms; faecal streptococci; Clostridum perfringens.

The isolation of faecal indicator bacteria is commonly used to signify the potential presence of intestinal pathogens. When in water these bacteria are in an environment with possible stress factors and not favourable to maintaining the viability of most bacteria. Studies have been performed to compare the survival of faecal indicator bacteria and pathogens in different kinds of waters. The absence or numerical reduction of faecal indicator bacteria in water do not necessarily indicate the absence or similar reduction of pathogens, because of the dif-

ferent sensitivity of bacteria to varying environmental stress (*Gyllenberg et al.* 1960, *Gordon* 1972, *Cohen & Shuval* 1973, *McFeters et al.* 1974).

Toxic metals are known to have an effect on the viability of bacteria (*Winslow & Hotchkiss* 1922, *Mitra et al.* 1975). For example the sensitivity of bacteria to cadmium (Cd) varies between species (*Babich & Stotzky* 1977, *Korkeala & Pekkanen* 1978), and even within the same strain (*Novick & Roth* 1968).

The purpose of this investigation was to study the effect of Cd as a stress factor on different indicator bacteria in water and also on the species composition of different faecal indicator bacteria in water.

MATERIAL AND METHODS

The untreated sewage sample used in this study was taken at the Saarioinen-Sahalahti treatment plant, which is located about 200 km north of Helsinki. The sample represents the sewage of the rural Sahalahti community (pop. 500), which has no special industrial activity. The sample was taken in a sterilized bottle at 4 p.m. and stored at 4°C until the experiments were started on the following morning.

Effect of different Cd concentrations on the number of coliforms, faecal coliforms, faecal streptococci and Clostridium perfringens

One ml of the sewage sample was transferred to 99 ml of a modified nutrient broth developed for the preservation of bacteria (*Hirn & Pekkanen* 1977). The nutrient broth contained 0, 0.3, 3 and 30 mg of added Cd/litre broth. Ten parallel samples from each Cd concentration were taken. The samples were kept at room temperature (20°C). The numbers of the different faecal indicator bacteria were determined immediately, after 4.5 h and after 24 h. Each 1 ml sample taken from the nutrient broth was diluted with 99 ml of sterile physiological NaCl solution (pH 7.2) before membrane filtration.

The membrane filter (MF) method (*American Public Health Association* 1975) was used throughout the study for the determination of coliforms, faecal coliforms, faecal streptococci and Clostridium perfringens. Millipore HC filters (Millipore Corporation, Mass., USA) (porosity 0.70 μm) were used for faecal coliforms and Gelman GN-6 filters (Gelman Instrument Company, Mich., USA) (porosity 0.45 μm) for the other groups. The growth media used were LES Endo agar (Orion Diagnostica, Espoo, Finland) for coliforms, mFC agar for faecal coliforms and KF-Streptococcus agar for faecal streptococci. The last two media were from Difco Laboratories, Detroit, Mich., USA. Coliforms were incubated at 35°C for 24 h, faecal coliforms at

44.5°C for 24 h and faecal streptococci at 35°C for 48 h. For the enumeration of C. perfringens the tryptose-sulfite-cycloserine-egg yolk (TSCEY) agar membrane filter method was used (*Hirn & Raevuori* 1978). C. perfringens was incubated at 35°C for 24 h in GasPak jars equipped with GasPak disposable hydrogen+carbon dioxide generator envelopes (BBL, Cockeysville, Md., USA).

Identification of coliforms, faecal coliforms and faecal streptococci

The incubated LES Endo, mFC and KF-Streptococcus agar plates were obtained after membrane filtration of the sewage sample in modified nutrient broth (1:100) containing 30 mg of Cd/l. The filtration was performed after 0 and 24 h of exposure of the sewage sample to Cd at 20°C. From both sets of the incubated plates 120 colonies of coliforms, faecal coliforms and faecal streptococci were randomly isolated for further studies. The isolated strains from LES Endo agar and from mFC agar were stored at 4°C on nutrient agar (Difco), and the strains from KF-Streptococcus agar were similarly stored on brain heart infusion agar (Difco) until identification. The 240 coliform strains and 240 faecal coliform strains thus obtained were identified with the API 20E (Analytab Products Inc., La Balme les Grottes, France). For the identification of the 240 isolates of faecal streptococci, biochemical and physiological tests as described by *Facklam* (1972) were used. The final identification was carried out according to the outlines of *Facklam, Geldreich* (1976) and *Clausen et al.* (1977).

Gas production by faecal coliforms

Gas production by faecal coliforms from lactose was studied in a broth containing 3.0 g of beef extract (Oxoid, London, England), 5.0 g of peptone (Difco) and 5.0 g of lactose (BDH Chemicals, Poole, England) per 1000 ml of distilled water (pH 7.0). The tubes were incubated at 44.5°C for 48 h.

Cd analysis

Cd was determined by flameless atomic absorption spectrophotometry. The apparatus used was the Perkin-Elmer 303 atomic absorption spectrophotometer (Norwalk, Conn., USA), equipped with a graphite furnace and graphite cell power supply HGA 72 (Überlingen, German Federal Republic).

Statistical method

In all statistical calculations Student's t-test was used.

RESULTS

The effect of different Cd concentrations on the number of coliforms, faecal coliforms, faecal streptococci and Clostridium perfringens in sewage after 4½ and 24 h of incubation at 20°C

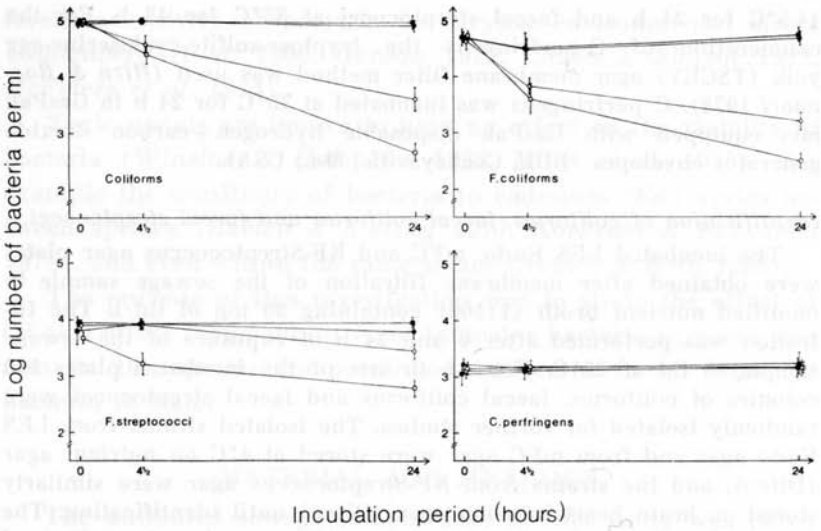

Figure 1. The effect of cadmium (Cd) on the number of coliforms, faecal coliforms, faecal streptococci and Clostridium perfringens of sewage diluted (1:100) with modified nutrient broth. The enumeration

When the results presented in Table 3 are examined, the coliform distribution pattern is similar to that found in other studies (*Vlassoff* 1977). Table 3 further shows that Escherichia coli seems to be the most resistant of coliforms to Cd exposure, since the decrease in numbers occurs mostly within the other coliform species. The decrease in the share of Klebsiella pneumoniae is particularly noticeable.

The proportion of the relatively Cd resistant E. coli among faecal coliforms was so great that no selection during incubation between faecal coliform species could be seen (Table 3). The share of K. pneumoniae among faecal coliforms is quite similar to that found by *Bagley & Seidler* (1977). According to the present results it is obvious that the Klebsiella population, which can grow at 35°C but not at 44.5°C, is more sensitive to Cd than the E. coli population.

Why is the decrease in density greater in faecal coliforms than in coliforms after 4½ h of Cd exposure (Fig. 1 and Table 1), although E. coli seems to be most resistant to Cd of these species (Table 3)? Evidently a part of the E. coli population was able to tolerate Cd better during 4½ h of exposure at 20°C than the others of the same species. This could be due to a better ability of the cells to repair the Cd-induced cellular damage and form colonies at 35° than at 44.5°C. After 24 h of Cd exposure the Cd-sensitive E. coli cells may have been damaged so seriously that they were unable to form colonies either at 35°C or at 44.5°C. Therefore the mean decrease in cell number after 24 h of exposure to Cd did not differ significantly between coliforms and faecal coliforms as it did after 4½ h of exposure.

The faecal streptococci were found to be more resistant to Cd than the coliforms and faecal coliforms (Fig. 1 and Table 1). This is somewhat accentuated in the present study by the absence of the relatively stress-sensitive Streptococcus bovis and Streptococcus equinus (*McFeters et al.*) from the sewage sample (Table 3). The results in Table 3 further show that in particular Streptococcus equinus (*McFeters et al.*) from the sewage sample durans seem to tolerate Cd exposure better than Streptococcus faecium. The good persistence of S. faecalis var. liquefaciens in the sample during exposure is in agreement with the results obtained by *Geldreich & Kenner*.

Gas production from lactose by faecal coliforms at 44.5°C, which is of diagnostic significance, could be influenced by Cd.

The results in Table 4 show that this is not the case regarding E. coli. The rest of the faecal coliforms did not produce gas even at 0 h.

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SAMMANFATTNING

Inverkan av kadmium på avloppsvattens indikatorbakterier.

Inverkan av kadmium (Cd) på antalet i avloppsvatten förekommande olika fekala indikatorbakterier samt på artsammansättningen av olika indikatorbakterier undersöktes. Olika Cd mängder tillsattes till delar av ett avloppsvattenprov och förekomsten av coliforma bakterier, fekala coliforma bakterier, fekala streptokocker och Clostridium perfringens undersöktes med membranfiltertekniken, sedan bakterierna hade blivit utsatta för Cd i 0, 4½ och 24 timmar vid 20°C.

Efter inkubering med Cd var minskningen av coliforma och fekala coliforma bakterier större än förminskningen av fekala streptokocker. De använda Cd koncentrationerna hade ingen betydande inverkan på antalet av C. perfringens. Tillsatsen av Cd förändrade relationerna i antalet mellan fekala coliforma bakterier och fekala streptokocker. Escherichia coli var tydligen mera resistent mot Cd än andra coliforma bakterier och Streptococcus faecalis var. liquefaciens och Streptococcus durans mera resistent mot Cd än andra fekala streptokocker. Ingen inverkan av Cd observerades på gasbildingen av fekala coliforma bakterier.

Fekala streptokocker och C. perfringens verkar vara bättre indikatorbakterier än coliforma och fekala coliforma bakterier, när den hygieniska kvaliteten av Cd förorenade avloppsvatten undersökes.

(Received September 11, 1979).

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