Bull. Environ. Contam. Toxicol. (1994) 53:77–84

Environmental Contamination and Toxicology

Estimation of Urobilin as a Fecal Pollution Indicator in the Aquatic Environment

Y. Miyabara, N. Sugaya, J. Suzuki, S. Suzuki

Faculty of Pharmaceutical Sciences, Science University of Tokyo, 12, Ichigaya Funagawara-machi, Shinjuku-ku, Tokyo 162, Japan

Received: 19 March 1993/Accepted: 30 October 1993

In Japan urban rivers are polluted with overflowing raw sewage caused by rainfall (Miyabara et al., a; b), because combined sewers has established in many urban area. These waste waters may also accelerate eutrophication of aquatic environments. Since urban river water may be public water supply source, it is an important public health issue to determine whether urban rivers are polluted with fecal effluent.

Indicators currently used to estimate fecal pollution of water are ammonia nitrogen, the number of total coliform, fecal coliform and coliphage, and coprostanol. However, these indicators suffer from the following disadvantages. Ammonia nitrogen is not a good indicator of water-borne fecal pollution, since other sources may exist (industrial discharges, non point sources, intrinsic generation through denitrification in aerobic sediment). Although the number of total coliform is the conventionally used indicator for fecal pollution, it also originates from sources other than the intestinal tract. In addition, determination of the number of bacteria is time-consuming work (Ashidate, 1988; Tanimoto, 1984). Counts of fecal coliform or coliphage face the same problems as those of total coliform (Ichikawa, 1990; Kusuyama et al., 1987; Borrego et al., 1987). Coprostanol is a sensitive indicator (detection limits of 10 ng/L), but requires a complex methodology (Yde et al., 1982).

In a previous paper (Miyabara et al., 1992), we reported a very sensitive detection method for urobilin by HPLC with fluorescence detection. This method takes advantage of a phosphor formed from urobilin and zinc, based on the Jaffe-Schlesinger reaction (Henry, 1964 a; b). Urobilin is produced in the human spleen and secreted from the duodenum through the gall bladder. Urobilin is excreted together with fecal matter and urine, and therefore specifically indicative of human fecal pollution. We estimated fecal pollution, based on the amount of urobilin present in river water and sediment (Miyabara et al., a; b). However, the fluctuation of the amount of urobilin in aquatic environment was unknown. In this study, we attempted to ascertain whether urobilin is a useful indicator for fecal pollution in aquatic environment. The study objectives were as follows: (1) Measure degradation, over time, of urobilin and other water pollution indicators in river water and sediment, under aerobic and anaerobic condition; and (2) Measure temporal variations in the amounts of urobilin in the aquatic environment (water and sediment) of the Kanda River.

Correspondence to: Y. Miyabara

MATERIALS AND METHODS

Degradation of Urobilin and Other Water Quality Indicators, Under Aerobic and Anaerobic Conditions

To measure degradation, over fine, of urobilin and other water pollution indicators in river water and sediment, under aerobic and anaerobic conditions, river water and sediment samples were collected with a polyethylene bucked and an Ekman–Berge dredge, from the Kanda River at Funagawara bridge on 29th June 1992 and 11th May 1992, respectively.

Portions (12 L) of the river water sample were split in twelve 2 L polyethylene bottles. To maintain aerobic conditions in six bottle, an airspace was provided, and these bottles were shaken 60 rpm by the shaker. To maintain anaerobic conditions in another bottles, the airspace was purged nitrogen gas, and these bottles were shaken at same conditions. Portions (600 g) of the river sediment sample were split in twelve polyethylene bottles, and the sediment (50 g) suspended in distilled water (1000 mL). To maintain aerobic and anaerobic conditions, these bottles treated as mentioned above. All bottles with river water and suspended sediment were incubated at room temperature (20 °C) for a 16 days period. Concentration of urobilin and other water pollution indicators in aerobic and anaerobic river water and sediment were measured after 1, 2, 4, 8 and 16 days incubation.

To elute urobilin from sediment, 12.1 g of Tris–HCl added to the suspended sediment and shook in a polyethylene bottle at 80 rpm for 1 hr at room temperature. The supernatant was extracted with chloroform at pH 1.0 and evaporated in chloroform. The residue was then dissolved in methanol and filtered through a 0.45 um membrane filter, whereupon the urobilin was measured by HPLC. River water was extracted with chloroform at pH 1.0, and the content of urobilin was measured in a similar manner.

Numbers of total coliform and fecal coliform were calculated by the most probable number (MPN) method as follows. Both river water and sediment samples were diluted with sterilized water from 1/10 to $1/10^5$, with 9 mL of five lactose broths (Eiken Chemical Co., Ltd., Tokyo, Japan) subsequently added to each 1mL of sample. After 48 hr incubation at 37°C, a portion of positive lactose broth, identified by yellow color and gas production, was added to 10 ml of brilliant green lactose bile (BGLB) broth (Eiken Chemical Co., Ltd., Tokyo, Japan). After an additional 48 hrs incubation at 37°C, the number of total coliform was calculated from the positive number of BGLB broths that had turned yellow and produced gas. At the same time, positive lactose broth was added to 10 ml of *Escherichia coli* (EC) broth (Eiken Chemical Co., Ltd., Tokyo, Japan). After 24 hr incubation at 44.5°C, the number of fecal coliform was calculated from the positive number of EC broths that had become murky and produced gas.

Dissolved oxygen (DO) in water and ammonia nitrogen concentration in river water and eluted from sediment were measured by the Standard Methods of Analysis for Hygienic Chemists (Pharmaceutical Society of Japan, 1990).

The amount of coprostanol in water and sediment was measured by high–performance liquid chromatography with fluorescence labeling (Goto et al., 1983).

Temporal Variations in Urobilin Concentrations in Kanda River Water and Sediment

To measure temporal variations in urobilin concentrations, river water and sediment samples were collected with a polyethylene bucked and an Ekman–Berge

dredge, during the April 1990 to January 1993 period from the Kanda River at Funagawara bridge in Japan (lat. 35°42' N, long. 139°45' E). Samples were brought back to the laboratory and refrigerated at 4°C.

A mixture of 100 g of sediment and 1000 mL of Tris–HCl buffer was shaken in a polyethylene bottle at 80 rpm for 1 hr at room temperature. The supernatant was extracted with chloroform at pH 1.0 and evaporated in chloroform. The residue was then dissolved in methanol and filtered through a 0.45 um membrane filter, whereupon the urobilin was measured by HPLC. River water was extracted with chloroform at pH 1.0, and the content of urobilin was measured in a similar manner.

The HPLC conditions for measurement of urobilin were as follows. A Shiseido CAPCELL PAK C18 (250 X 4.6 mm I.D., 5 um) column packed with ODS–Hypersil was used. The flow–rate, injection volume, excitation and emission wavelengths for fluorescence detection, and column temperature were 1.0 mL/min, 10 uL, 485 nm and 513 nm, and 20°C, respectively. The eluent consisted of 0.1% zinc acetate, 75 mM boric acid, 15 mM sodium chloride, and 75% methanol, and was adjusted to pH 6.0 with hydrochloric acid (Miyabara et al., 1992). Urobilin used as the standard was purchased from Porphyrin Products Inc. (Logan, Utah, USA).

RESULTS AND DISCUSSION

Degradation of Urobilin and Other Water Quality Indicators, Under Aerobic and Anaerobic Conditions

Fluctuations in the quantities of urobilin and other water pollution indicators in Kanda River water under aerobic and anaerobic conditions are shown in Figure 1. Except for the degradation of coprostanol under anaerobic conditions, same water sample was used. Dissolved oxygen (DO) concentration indicate that aerobic and anaerobic conditions were properly maintained through the incubation period. After 2 days, the amount of urobilin in river water decreased more than 90% under both aerobic and anaerobic conditions. Urobilin degradation rate under aerobic and anaerobic conditions appear similar. Interesting to that total coliform and fecal coliform degradation rates are similar to those for urobilin. It appears that urobilin, total coliform, and fecal coliform are all good indicators of recent (less than approximately 2–4 days) fecal pollution in river water, if lab. results are indicative of actual degradation in the field. As with urobilin, aerobic and anaerobic degradation of both total and fecal coliform are very similar. Other 2 indicators (coprostanol and ammonia nitrogen) degrade more slowly than urobilin, total and fecal coliform. Degradation of ammonia occurs more slowly under anaerobic conditions. Ammonia nitrogen had the longest half–life among the fecal pollution indicators for both aerobic and anaerobic conditions.

Fluctuations in the quantities of urobilin and other water pollution indicators in Kanda River sediment are shown in Figure 2. Under anaerobic conditions, the initial ammonia nitrogen concentration (4.7 mg/L) increased to 20.6 mg/L (four–fold) after 8 days, and to 30.1 mg/L (six–fold) after 16 days. Under aerobic conditions, the initial ammonia nitrogen concentration increased to 39.4 mg/L (eight–fold) after 8 days, but subsequently decreased to 10 mg/L (two–fold), after 16 days. After 4 days, the aerobic coprostanol concentration (905 ug/kg) was approximately 120% of the initial concentration (775 ug/kg), and the anaero–

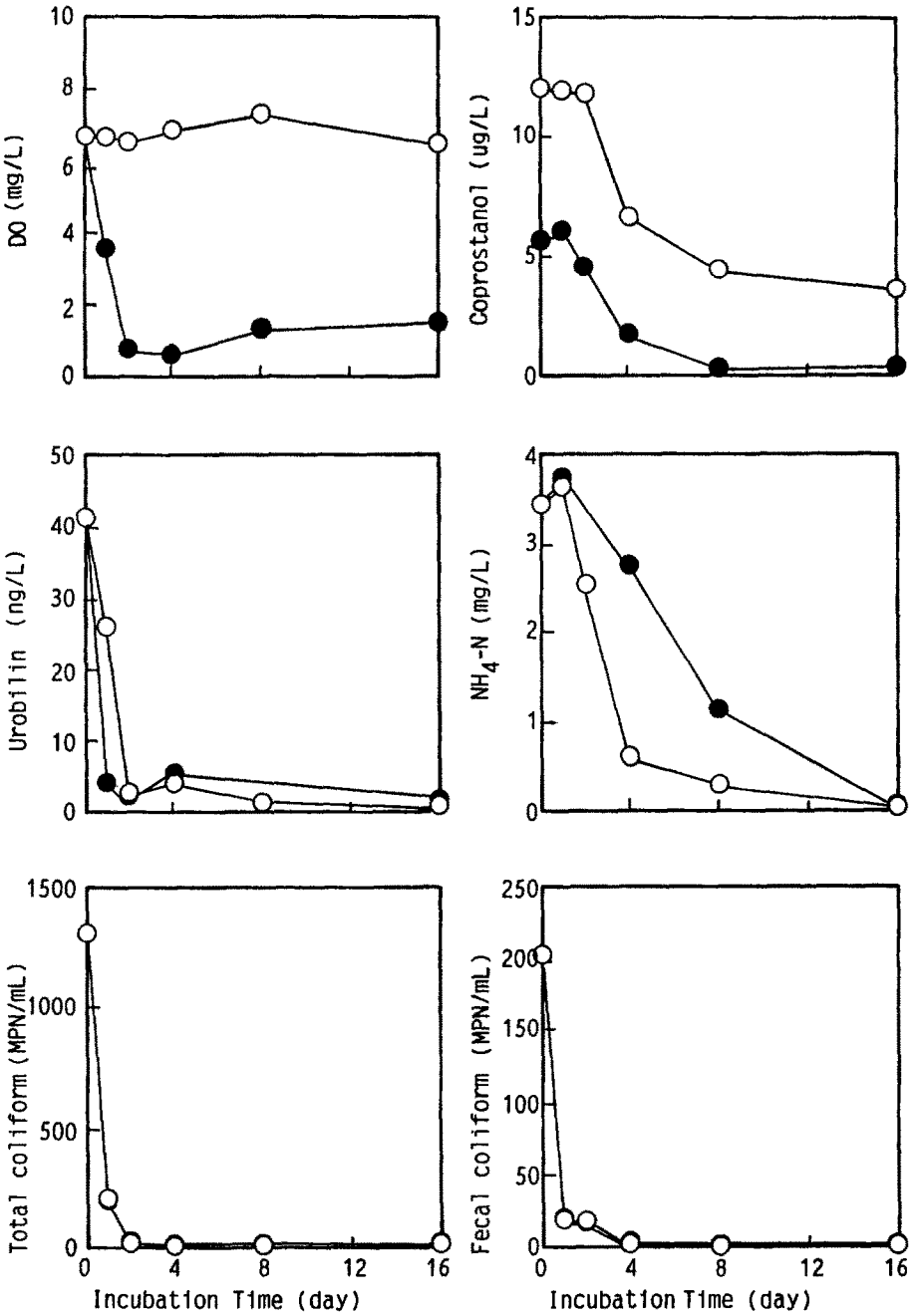

Figure 1. Degradation of water pollution indicators in river water from the Kanda River at Funagawara Bridge. Aerobic condition (○), anaerobic condition (●).

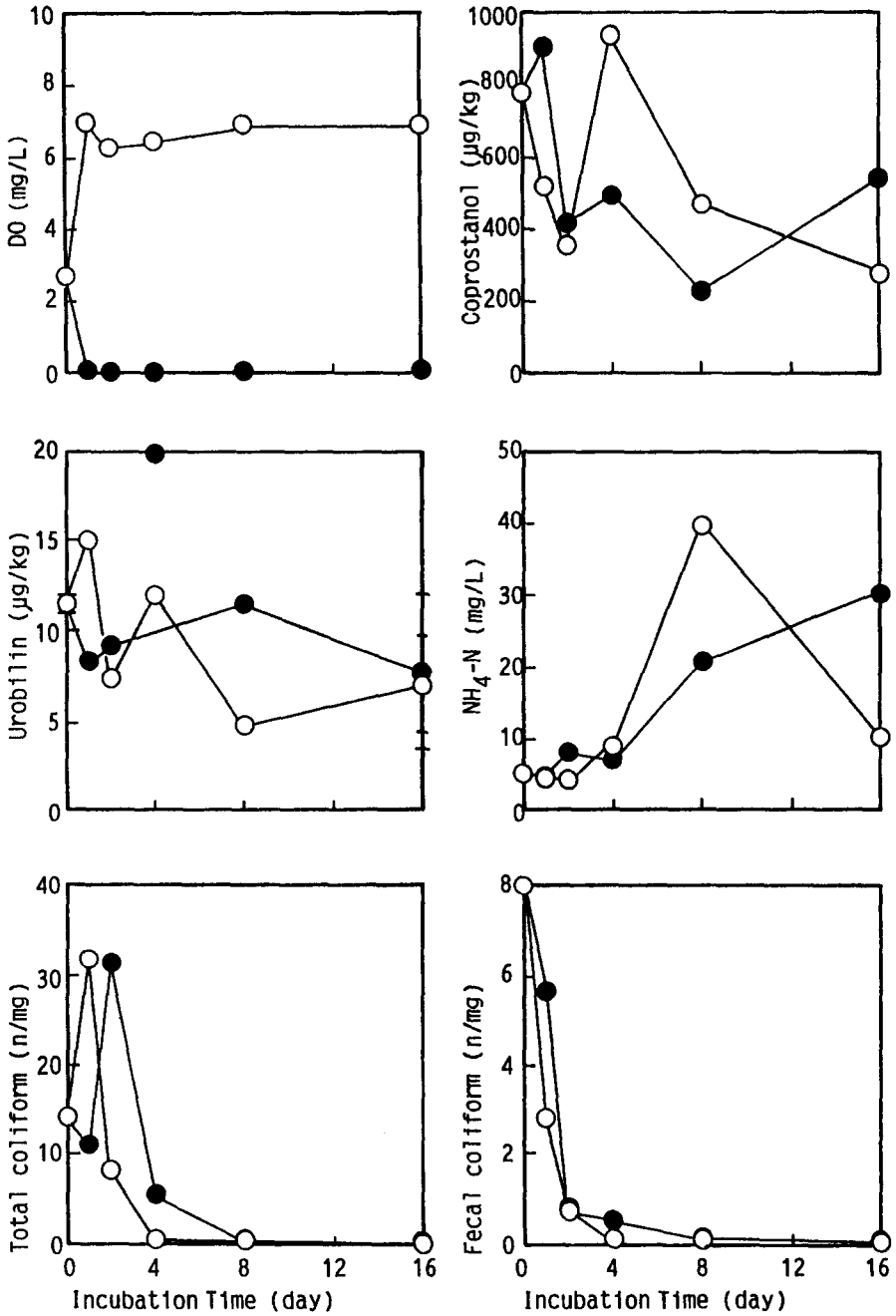

Figure 2. Degradation of water pollution indicators in sediment from the Kanda River at Funagawara Bridge. Aerobic condition (○), anaerobic condition (●).

bic concentration (516 ug/kg) was approximately 65% of the initial concentration. Coprostanol, like urobilin, also appears to be fairly stable in sediment, whereas total and fecal coliform degrade fairly rapidly. The vale of urobilin measurement in sediment is that, because of its relative stability in sediment, discharges of fecal pollution to the river can still be detected after two weeks (16 days), and beyond, whereas degradation of urobilin, total coliform in river water, and total and fecal coliform in sediment occurs rapidly (<4 days), making these indicators (especially bacteria) much less useful for detecting recent pollution.

On the other hand, the amounts of urobilin in sediment were almost constant under both aerobic and anaerobic conditions after 16 days, and urobilin had the longest half-life, under anaerobic conditions, of all the indicators except ammonia nitrogen. This indicates that urobilin in river sediment is relatively stable, and that it accurately reflects fecal pollution of the river at the sampling location.

Temporal variations in Urobilin Concentrations in Kanda River Water and Sediment

Table 1 shows the variation of in urobilin concentrations in the aquatic environment (water and sediment) of the Kanda River in 9th November 1992. Coefficients of variation for urobilin in water and sediment were both about 30%, indicating that the amount of urobilin in the Kanda River is homogeneously distributed between river water and sediment.

Table 1. Variation in the amount of urobilin in the Kanda river in 9th Nov. 1992.

Sample No.	Urobilin	
	River water ng/L	Sediment ug/kg
1	9.53	2.70
2	17.3	4.70
3	12.1	3.39
4	10.8	2.19
5	9.67	2.20
Average	11.9	3.04
S.D.	3.19	1.05
C.V. (%)	26.9	34.7

S.D.: Standard deviation,
C.V.: Coefficient of variation.

Table 2 shows the fluctuation of urobilin concentrations in Kanda River water and sediment from 1990 to 1993. Urobilin concentrations in river water ranged from 4.6 to 16,400 ng/L while sediment concentration ranged from 1.27 to 16.3 ug/kg. Coefficients of variation for urobilin in water and sediment were 250 and 79%, respectively. These results indicated that urobilin concentrations in sediment are relatively constant in that period, thereby better-reflecting the degree of fecal pollution.

Table 2. Fluctuation in the amount of urobilin in the Kanda river from 1990 to 1993.

Sampling date	River water ng/L	Sampling date	Sediment ug/kg
14 Apr. '90	33.9		
16 Apr. '90	1880		
20 Apr. '90	43.8		
8 May '90	128		
22 May '90	67.4		
4 June '90	67.6		
5 June '90	134.1		
21 Jan. '91	68.0	4 Dec. '91	16.3
20 Apr. '92	6650	20 Apr. '92	10.0
28 Apr. '92	57.5	11 May '92	11.4
24 June '92	16400	6 July '92	1.27
29 June '92	40.7	9 Nov. '92	3.04
22 Sep. '92	4.6	6 Jan. '93	3.12
9 Nov. '92	11.9		
Average	1830		7.52
S.D.	4570		5.94
C.V. (%)	250		79.0

S.D.: Standard deviation,
C.V.: Coefficient of variation.

Fecal pollution indicators in water and sediment have different requirements. If river water is a public water supply source, the indicators must reflect current pollution. On the other hand, the degree of pollution is offer based on the concentration of indicator present in sediment. Therefore, the amounts of urobilin in water and in sediment must reflect current pollution, and be stable, respectively.

The behavior of urobilin in river water and sediment satisfies the above prerequisites. Furthermore, the analytical method for measuring the amount of urobilin in sediment is simple, whereas all the many methods for measuring coprostanol and bacteria which have been published over the past decade are time-consuming and tedious. It is suggested that urobilin is a useful indicator for fecal pollution.

REFERENCE

Ashidate N (1988) Evaluation of total coliform for water quality standards. *J Water Waste* **30:** 229-38

Borrego J J, Morinigo M A, Vicente A D, Cornax R and Romero P (1987) Coliphage as an indicator of fecal pollution. *Wat Res* **21:** 1473-80

Goto J, Saito M, Chikai T, Goto N and Nambara T (1983) CLXXXVII. Determination of serum bile acids by high-performance liquid chromatography with fluorescence labeling. *J Chromatogr* **276:** 289-300

Henry R J (1964 a) Determination of urobilin in urine by the zinc fluorescence reaction. Clinical Chemistry, Harper-Row, New York p 618-9

Henry R J, Fernandez A A and Berkman S (1964 b) Studies on the determination of bile pigments VI. urobilinogen in urine as urobilinogen-aldehyde. *Clin*

Chem (Winston–Salem, North Carolia) **10:** 440–6
Ichikawa H (1990) Microbiological indicators for evaluating treatability on water purification process. *Jap J Water Pollut Res* **13:** 477–83
Kusuyama K, Yamamoto K, Itoh N and Ohtani H (1987) Enterococcal species isolated from river water using m–enterococcus agar. *J Water Waste* **29:** 131–5
Miyabara Y, Sakata Y, Suzuki J and Suzuki S (in press a) Estimation of fecal pollution based on the amounts of urobilin in urban rivers. *Environ Pollut*
Miyabara Y, Miyata K, Suzuki J and Suzuki S (in press b) Evaluation of fecal pollution of river sediment by detection of urobilin. *Environ Pollut*
Miyabara Y, Tabata M, Suzuki J. and Suzuki S (1992) Separation and sensitive determination of i–urobilin and l–stercobilin by high–performance liquid chromatography with fluorimetric detection. *J Chromatogr* **574:** 261–5
Pharmaceutical Society of Japan (1990) Standard Method of Analysis for Hygienic Chemists. With Commentary. Kanehara Shuppan Co, Ltd, Tokyo
Tanimoto K (1984) Studies on the fecal *E.* coliform in the environmental waters I. On the selectivity of fecal *E.* coliform in the river water by BGLB/44.5°C method. *Nihon Koueishi* **31:** 199–206
Yde M, De Wulf E, De Maeyer–Cleempoel S and Quaghebeur D (1982) Coprostanol and bacterial indicators of fecal pollution in the Scheld Estuary. *Bull Environ Contam Toxicol* **28:** 129–34