Use of human urine in phytoplankton production as a tool for ecological sanitation

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ABSTRACT

Measurements of primary productivity of phytoplankton and enumeration of the counts of coliform and heterotrophic bacteria (HB) were made in the water of 12 experimental tanks used for 3 treatments and control in triplicate as follows: (a) fresh human urine (0.02%), (b) stored human urine (0.02%), (c) mixed urine of fresh and stored human urine (0.02%) and (d) control without input of urine. The gross primary productivity of phytoplankton was highest in the stored urine treated tanks (508 mg C m^{-2} h^{-1}) followed by fresh urine (353 mg C m^{-2} h^{-1}), mixed urine (303 mg C m^{-2} h^{-1}) and control (215 mg C m^{-2} h^{-1}). Similar was the response of net primary production of phytoplankton. The mean count of HB observed in stored urine fed tanks was significantly higher (59–184%) than the remaining urine fed treatments. The mean count of *Escherichia coli* did not differ from urine treated tanks to control implying the good quality of water. The concentration of dissolved oxygen of water (7.6 to 12.8 mg L^{-1}) in these tanks remained satisfactory for aquaculture. The mean concentration of ammonium-N observed in fresh urine treated tanks was more than 10 times higher than the remaining treatments employed. In contrast, the level of phosphate and electrical conductivity in the stored urine treated tanks were significantly higher than the remaining treatments. It is proposed that stored urine with a significantly reduced load of *E. coli* might be an effective low cost liquid fertilizer for algal biomass production.

Key words | *E. coli*, fertilizer value, phytoplankton, primary productivity, stored and fresh human urine

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INTRODUCTION

Human urine is an aqueous solution containing sodium chloride and urea as major constituents and calcium, potassium, magnesium, sulfate and phosphate as minor constituents (Altman & Dittmer 1974). Each person, on an average, contributes via urine per year about 2.5–4.3 kg nitrogen, 0.7–1.0 kg phosphorus and 0.9–1.0 kg potassium (Kirchmann & Petterson 1995). In addition, some growth-promoting substances are also present in human urine in the form of amino acids, glucose and vitamins (Shigang 1989; Vinneråsa *et al.* 2003). Although human urine comprises less than 1% of the total domestic wastewater by volume, it contributes significantly in the form of major nutrients (94% of N, P and K in direct toilet water and 80% of N, 50% of P and 60% of K in wastewater) in domestic waste and, therefore, may cause a source of pollution. In Israel, fish production has been reported to the tune of 3.8 tons ha^{-1} $year^{-1}$ by utilizing the kitchen and laundry wastes. Recycling of domestic sewage produced 4 tons ha^{-1} $year^{-1}$ of fish in Java. In India, fish yield in sewage fed ponds was still higher yielding about 7 ton ha^{-1} $year^{-1}$ of fish with carp (Jhingran 1995).

Eco-sanitation and organic farming are interlinked with each other as the former is crucial in closing the loop between the nutrients and crops in agriculture or in aquaculture. As a result, organic farming utilizing a variety of organic wastes including human wastes has become a boon in many Asian countries with a huge burden of waste disposal problems.

As the composition of human urine reflects the average requirement of essential nutrients for plant growth (Feachem *et al.* 1983; Heinonen-Tanski & van Wijk-Sijbesma 2005), its use as a fertilizer in agricultural production has been highly cost effective. Human urine, night soil and domestic sewage has been effectively recycled for biological production in China, many countries in South East Asia the

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Middle East and Far East Asia, and many parts of Europe (Jana 1998; Jana *et al*. 2000).

The rationale for using human urine in agriculture was based on the fact that the hazardous chemical compounds or heavy metals are generally absent or extremely low in human urine (Wolgast 1993; Kirchmann & Petterson 1995; Hellström & Kärrman 1996; Jönsson *et al*. 1997; Hellström *et al*. 1999), but they occur in high concentrations in chemical phosphate fertilizer despite the fact that the cadmium content in phosphate fertilizer may vary depending upon the source of rock material used in phosphate fertilizer. In general, agricultural bulk fertilizers may have cadmium concentrations of up to 36 mg/kg phosphate fertilizer which is several magnitudes higher than that of typical urine (Rogowski *et al*. 1999; Mc Bride & Spiers 2001).

Despite the immense nutrient potentials of human urine for agriculture or aquaculture, some pathogens and parasites such as *Leptospira interrogans, Salmonella typhi, Salmonella paratyphi, Schistosoma haematobium* have, however, been detected in excreted urine on some occasions. As a result, some guidelines have been developed in order to ensure that human urine and faeces are used as safe and low cost fertilizers.

In fresh urine, the greater part of the nitrogen appears as urea [$CO(NH_2)_2$] (Hellström *et al*. 1999) which, on storage, is hydrolyzed microbiologically into ammonia causing pH augmentation from ≈ 6 to ≈ 9 accompanied by ammonium (NH_4^+) and bicarbonate ions (Alexander 1977; Hellström *et al*. 1999; Maurer *et al*. 2006). This rise in pH triggers the precipitation of calcium and magnesium in the form of carbonate and phosphate compounds (Udert *et al*. 2003a, b) and this is clearly visible with reduced level of Ca and Mg in stored urine (Maurer *et al*. 2006). According to Gethke *et al*. (2006), throughout the storage period of human urine, the pH value and concentration of ammonia-N increased, the concentration of phosphate decreased and these variants can be sped up by addition of urease. It is suggested that storage of human urine in combination with elevated pH (~9.0) and ammonia has been a useful strategy to inactivate enteric microorganisms such as *Salmonella* (Heinonen-Tanski *et al*. 1998) and some other viruses (Turner *et al*. 1999) and, hence, may be used in fish culture.

It is reported that if the urine is stored at 20 °C for at least 6 months, the urine may be considered safe for using as fertilizer for any crop (Höglund *et al*. 2002). Based on the results of study (Vinnerås *et al*. 2011), a storage time of five weeks at a temperature below 20 °C or of two weeks at a temperature above 20 °C is sufficient to prevent transmission of mycobacteria when recycling human urine. Moreover, pH of stored urine below 4 seems to further reduce the number of pathogens (Hellström *et al*. 1999). Studies of Hellström & Kärrman (1996) and Haneaus *et al*. (1996) have shown that increased storage temperature accelerated urea decomposition and ammonia evolution. Urea hydrolysis in stored human urine at optimal pH of around 7.0 continues till the rise of the concentration of free ammonia-N up to 2,000 mg L^{-1}: an inhibitory effect on urea hydrolysis, was observed above pH 9 (Hotta & Funamizu 2008). Kabdasli *et al*. (2006) reported that at pH from 2 to 7.5, about 25% of urea of stored urine could be hydrolyzed within 30 days.

Because of possible ammonia toxicity in fresh urine, stored urine was thought to be better for aquaculture activities including algal production due to the fact that initial microbial degradation has already been progressed, resulting in considerable ammonia detoxification, pathogen inactivation, and nutrient stability for its use as safe fertilizer (Maurer *et al*. 2006; Feng *et al*. 2008).

Despite the immense nutrient potentials of human urine, ease of availability and almost no costs being involved for procurement of human urine from urine diverted dehydration eco-toilets, no systematic studies have so far been made for evaluation of the fertilizer value of human urine in aquatic systems for the primary production of phytoplankton, which plays an important role as the natural diet of fishes in pond ecosystems. The nutrient potentials of human urine should not be overlooked and underestimated. This situation is important because of the fact that fish farming is a very common method of livelihood for millions of poor fishers in the tropical developing countries where the production cost of fish using conventional fertilizers and chemicals has been quite high. On the contrary, use of human urine as fertilizer would reduce the production cost if standardized as a protocol. The basic mechanism by which human urine acted on fish growth was mediated through induction of phytoplankton diversity and abundance used for food for fishes. The purpose of the present study was to compare the responses of primary productivity of phytoplankton in experimental tanks fed with stored human urine and that of fresh human urine or mixed human urine.

MATERIALS AND METHODS

The study used 12 experimental tanks (3 m × 1.5 m × 1 m). Twelve tanks (5,000 L) were provided with dry soil and filled with ground water (pH 7.2–7.4) a week prior to experiment. Tanks were grouped into four treatments in triplicate

as follows: (a) fresh urine, (b) stored urine of 11 months old, (c) fresh and stored urine mixed (1:1) and (d) a set without any input of fertilizer as control.

Fresh human urine was collected, without flushing water, from urinals used by non-medicated and healthy male students (22–25 years) in the Department of Zoology, University of Kalyani, West Bengal, India. Urine was collected in a 10 L high quality PVC container which was kept at room temperature (20–30 °C) for 11 months by closing its mouth tight with a super fit plastic lid. Urine collected and used on the day of experiment was considered as fresh urine. The composition of both fresh and stored urine (Table 1) was determined following the standard methods (APHA 1995).

A pilot study was performed to determine the dose of human urine by monitoring the general performance of biological productivity including the survival of fish in experimental tanks. Accordingly, the dose of 0.02% of human urine was selected in the present study. Fresh or stored human urine was procured and the required dose of urine (1 L) was applied every week in experimental tanks. The trial was conducted for 16 weeks.

Samples of water were collected every or two weeks at a fixed hour of the day (9:00 am) by collecting the samples of water from three places of each tank and then pooled into one for each tank before final analysis.

Primary productivity of the phytoplankton was determined using the light and dark bottle method described by Vollenweider (1974). Samples of plankton (5 L) were collected every week from each tank using a plankton net made of standard bolting silk cloth (60 μm) and the samples were concentrated to a suitable volume for qualitative and quantitative determinations following the methods described in APHA (1995). Water quality parameters were examined following the standard methods described by APHA (1995).

Standard procedure (APHA 1995) was used to enumerate heterotrophic bacteria (HB) of water samples grown on nutrient agar media having the following standard composition: peptone –10 g, beef extract – 1.5 g, NaCl – 2.0 g, agar – 20 g, pH – 7.2. The arithmetic means from four plates were then obtained after incubation of the Petri dish at 37 °C for 48 h.

The counts of colony forming units (cfu) of *Escherichia coli* were obtained from the culture tanks on EMB (Eosine Methylene Blue) agar media consisting of agar – 13.5 g, pancreatic digest of casein – 10.0 g, lactose – 5.0 g, sucrose – 5.0 g, K_2HPO_4 – 2.0 g, Eosin Y – 0.4 g, Methylene blue – 0.065 g, distilled water – 1,000 mL.

From samples of water collected from the urine treated tanks, 0.1 mL of water were added to 10 mL of EMB agar media in a sterilized Petri dish and that was spread uniformly by a sterilized glass spreader. After incubation for 48 h at 37 °C, blue-black coloured bacterial colonies were visualized on the agar plates and were enumerated. The arithmetic means from four plates were then obtained after incubation of the Petri dish at 37 °C for 48 h.

A one way ANOVA (Gomez & Gomez 1984) was used for analysis of data. If the main effect was found significant, the ANOVA was followed by a least significant difference (LSD) test. Accepted statistical risk was 5% ($p < 0.05$).

Table 1 | Basic composition of fresh and stored human urine used in the study ($N = 5$)

Parameters	Fresh urine	Stored urine (11 months old)
pH	6.95	9.38
CO_3 alkalinity (mg L^{-1})	0	510 ± 13.33
HCO_3 alkalinity (mg L^{-1})	410 ± 6.67	6,449 ± 16.86
Total hardness (mg L^{-1})	280 ± 6.67	105.8 ± 1.67
Ca^{2+} (mg L^{-1})	32 ± 1.33	24 ± 1.33
NH_4-N (mg L^{-1})	125.99 ± 0.00	365.34 ± 0.01
NO_2-N (mg L^{-1})	12.20 ± 0.13	9.8 ± 0.11
NO_3-N (mg L^{-1})	82.70 ± 0.05	13.50 ± 0.21
PO_4-P (mg L^{-1})	205.05 ± 0.01	0.442 ± 0.02
Electrical conductivity (mS cm^{-1})	160 ± 0.001	220 ± 0.001

RESULTS

Primary productivity

Qualitatively, important phytoplankters were represented by *Chlorella* spp., *Scenedesmus* spp., *Selenastrum* spp., *Closterium* spp., etc. These phytoplankta are favoured food items for zooplankton as well as for fish.

Application of stored urine in the tanks resulted in significant rise in the values of gross primary productivity of phytoplankton (508 mg C m^{-2} h^{-1}) followed by fresh urine (353 mg C m^{-2} h^{-1}), mixed treatment (303 mg C m^{-2} h^{-1}) and control (215 mg C m^{-2} h^{-1}). Differences in the mean primary productivity of phytoplankton were significant ($p < 0.05$) between the stored urine and the mixed urine fed treated tanks. However, there was no difference ($p > 0.05$) between the fresh and mixed urine fed treatments.

The responses of net primary productivity of phytoplankton remained the same as that of gross primary productivity of phytoplankton (Figure 1), being maximal in the stored urine fed tanks (414 ± 36.4 mg C m^{-2} h^{-1}) followed by fresh urine fed tanks (301 ± 53.87 mg C m^{-2} h^{-1}), mixed urine fed tanks (243 ± 36.02 mg C m^{-2} h^{-1}) and control (170 ± 30.22 mg C m^{-2} h^{-1}).

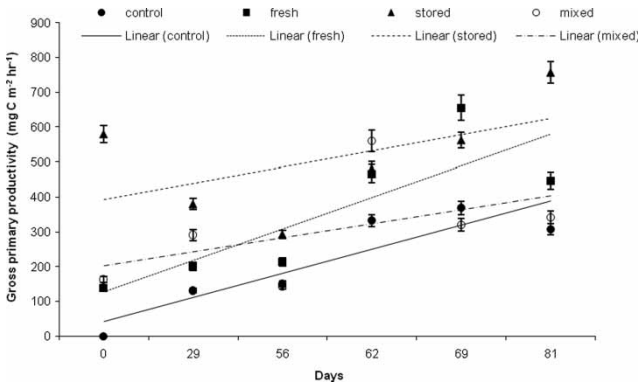

Figure 1 | Temporal course of variations in gross primary productivity in different treatments employed. Bar (T) represents the standard error of mean (±SE) of three samples ($n = 3$) (• - control, ■ - fresh urine, ▲- stored urine and ○- mixed urine). Trend lines p of the data are also indicated.

Heterotrophic bacterial population

The count of HB ranged from 13.53 ± 1.873 to $38.38 \pm 7.951 \times 10^3$ cfu ml^{-1} in the different treatments employed. The mean counts of HB enumerated in the stored urine fed tanks were 74, 59 and 184% higher ($p < 0.05$) than mixed, fresh and control tanks, respectively (Table 2). This implied that degradation activity was maximum level in the former compared with the later treatments.

E. coli population

The counts of *E. coli* in different treatments ranged from 244 ± 28 to 274 ± 54 cfu ml^{-1} (Table 2). Differences in the mean count of *E. coli* among the different treatments were not significant ($p > 0.05$). Considering the loads of *E. coli* in the water media as criteria, it appears that human urine might be used as a safe fertilizer for aquaculture activities for algal production.

Nutrient parameters

The concentration of ammonium-N increased by three-fold ($p < 0.05$) in the culture tank fed with fresh urine compared to stored urine fed tanks (Table 2). However, the mean

Table 2 | Water quality after 16 weeks weekly application of 0.02% of fresh, stored and mixed urine. Different superscripts (a, b, c and d) among treatments denote significant ($p < 0.05$) difference between treatments

Parameters	Control	Fresh urine	Stored urine	Mixed urine
pH	$8.65^b \pm 0.038$	$8.89^a \pm 0.052$	$8.94^a \pm 0.063$	$8.54^a \pm 0.054$
Total alkalinity (mg L^{-1})	$81.94^c \pm 6.13$	$132.66^b \pm 7.65$	$157^a \pm 9.01$	$128.44^b \pm 12.55$
Dissolved oxygen (mg L^{-1})	$10.08^a \pm 0.26$	$8.52^b \pm 0.56$	$8.87^b \pm 0.44$	$9.90^b \pm 0.37$
NH_4-N (mg L^{-1})	$0.024^c \pm 0.001$	$0.244^a \pm 0.045$	$0.08^b \pm 0.014$	$0.075^b \pm 0.012$
NO_2-N (mg L^{-1})	$0.03^a \pm 0.005$	$0.062^a \pm 0.013$	$0.048^a \pm 0.009$	$0.041^a \pm 0.009$
NO_3-N (mg L^{-1})	$0.133^b \pm 0.005$	$0.214^b \pm 0.013$	$0.303^a \pm 0.005$	$0.163^b \pm 0.018$
PO_4-P (mg L^{-1})	$0.09^c \pm 0.007$	$0.17^c \pm 0.026$	$0.439^a \pm 0.083$	$0.281^b \pm 0.048$
N_i/PO_4-P	0.703 ± 0.04	2.022 ± 0.116	0.504 ± 0.029	0.581 ± 0.033
Electrical conductivity (μS cm^{-1})	$120.3^c \pm 8.6$	$203.8^b \pm 7.08$	$226.62^a \pm 8.4$	$200.27^b \pm 3.67$
Gross primary productivity (GPP) (mg C m^{-2} h^{-1})	$215^d \pm 38.09$	$353^b \pm 58.66$	$508^a \pm 39.87$	$303^c \pm 43.17$
Net primary productivity (NPP) (mg C m^{-2} h^{-1})	$170^d \pm 30.22$	$301^b \pm 53.87$	$414^a \pm 36.39$	$243^c \pm 36.02$
Community respiration (CR) (mg C m^{-2} h^{-1})	$44^d \pm 9.0$	$61^b \pm 8.29$	$65^a \pm 6.22$	$53^c \pm 6.02$
Heterotrophic bacteria (cfu $\times 10^3$ mL^{-1})	$13.53^c \pm 1.87$	$24.15^b \pm 5.59$	$38.38^a \pm 7.95$	$22^b \pm 3.97$
E. coli (cfu mL^{-1})	$244^a \pm 28$	$274^a \pm 54$	$250^a \pm 37$	$253^a \pm 37$

concentration did not differ ($p > 0.05$) between the stored and mixed treatments.

The mean concentration of nitrite-N did not differ ($p > 0.05$) from one treatment to another. The values ranged from 0.030 mg L^{-1} (control) to 0.0629 mg L^{-1} (fresh urine treated tanks) (Table 2).

The mean concentration of nitrate-N varied by a factor of 2.3 (0.133–0.303 mg L^{-1}) between the treatments and control. The mean concentration observed in the stored urine-fed tanks was significantly higher ($p < 0.05$) than the remaining treatments employed.

The mean concentration of phosphate observed in the stored urine (0.439 mg L^{-1}) was significantly higher ($p < 0.05$) than the mixed (0.281 mg L^{-1}) or fresh urine treated tanks (0.170 mg L^{-1}) (Table 2).

The ratio between total –N (NH^4-N + NO^2-N + NO^3-N) to PO^4-P was maximum in fresh urine treated tanks (2.022) and minimum (0.504) in stored urine treated tanks. The values in the remaining two treatments were 0.703 for control and 0.581 for mixed urine fed treatment. This clearly indicates the dominance of ammoniacal-N over the phosphate in fresh urine fed treatment and vice versa in stored urine fed treatment.

Water quality

Application of urine (fresh or stored) resulted in significant rise of water pH compared to control (Table 2). The mean concentration of total alkalinity ranged from 81.94 mg L^{-1} (control) to 157.00 mg L^{-1} (stored urine treatment) (Table 2). Mean differences of total alkalinity were significant ($p < 0.05$) among all treatments except between fresh urine and mixed treatment ($p > 0.05$).

The concentration of dissolved oxygen of water ranged from 7.6 to 12.8 mg L^{-1} in all the treatments during the period of study. The mean concentration of dissolved oxygen of water remained highest in the control (10.08 mg L^{-1}) and lowest (8.5 mg L^{-1}) in the fresh urine treated tanks ($p < 0.05$).

The values of electrical conductivity were consistently high ($p < 0.05$) in the stored urine treated tanks and low in the control (Table 2). Lack of difference ($p > 0.05$) was demonstrated between the mixed treatment and fresh urine fed treatments.

DISCUSSION

The results of the study revealed that the primary productivity of phytoplankton developed in these treatments was in clear response to the nutrient status of the tank, especially the phosphate and nitrogen added to the tanks via allochthonous input of human urine. As anticipated, changes in the productivity of phytoplankton were highly dependent upon the phosphate concentration of water (Figure 2) in the different treatments employed. Further, the primary productivity of phytoplankton was a function of increased level of PO^4-P to nitrogen or vice versa. This implied that the phosphate level was perhaps more important than nitrogen in determining the primary productivity of phytoplankton. Evidently, the increased level of phosphate in the stored urine was responsible for the highest values of primary productivity of phytoplankton in that treatment. This shows that stored human urine with reduced load of *E. coli* may be used for the production of algal diversity and abundance. Several experimental studies have demonstrated that the composition of the phytoplankton community was strongly influenced by the N/P content of the aquatic environments (Bulgakov & Levich 1999). The high N/P weight ratios (20–50) favour the development of Chlorococcales, while lower values (5–10) lead to a community dominated by Cyanophyta. In the present study, N_i/PO_4 ratio in the urine fed treated water ranged from 0.504 to 2.022 during the period of the experiment.

It is likely that stored urine, upon transfer to experimental tanks, was fast mineralized and liberated the nutrients responsible for boosting the autotrophic production. The detritus particle of the stored urine also favoured the growth of HB which might have served as the source of food for zooplankton (Golder *et al.* 2007). Distinctly higher counts of HB in the waters of the stored urine-fed system than in fresh or mixed treatments, clearly reflected that microbial degradation in the former was in a more active phase than in the latter. As the stored urine required a

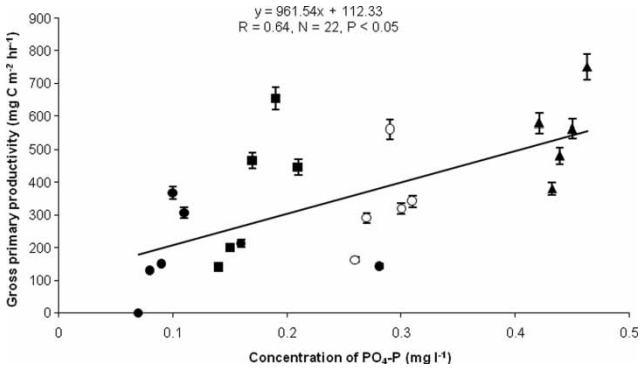

Figure 2 | Relationship between the gross primary productivity of phytoplankton and concentration of PO_4-P in different treatments employed (• - control, ■ - fresh urine, ▲- stored urine and ○ - mixed urine treatment).

considerably shorter period for microbial degradation and maintained reduced load of *E. coli*, improved nutrient load and water quality, it can be safely used for algal productivity serving as food for fishes. The counts of *E. coli* remained within the recommended limits for safe use of wastewater, excreta and grey water (WHO 2006).

CONCLUSION

It may be reasonable to conclude from the results of the present study that urine, especially the stored urine, may be used to induce the growth of primary production of phytoplankton, which forms the basis of the grazing food chain of fishes. Another advantage of using human urine was that it favoured the dominance of HB which contributed to the detritus food chain of fishes.

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