Storage induced changes in coliform, heterotrophic groups of bacteria and nutrient levels of human urine for its safe use in biological production

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Human urine is a potential source of various nutrients, minerals and trace elements. Its use as a fertilizer is growing popular among farmers. Here, we examined the pattern of changes in the counts of coliform, heterotrophic bacteria as well as physico-chemical characteristics of human urine during different days of storage under closed conditions at ambient temperature. We observed that after 253 days of storage under closed condition, the coliform counts were reduced significantly and remained within the safe limit to be used as fertilizer. With increase in storage period, the concentration of phosphate showed decline coupled with rise in pH, alkalinity and electrical conductance. Our study revealed that human urine can be used as safe fertilizer after 8 months of storage under closed conditions at ambient temperature ranging 25-35°C.

Keywords: Excreta, Mineral fertilizer, Recycling, Sewage, Sustainability

Human urine is an aqueous solution containing major and minor constituents such as sodium chloride, urea, calcium, potassium, magnesium, sulphate and phosphate and some growth promoting agents such as amino acids, glucose, vitamins, dissolved micro pollutants, hormone as well as some pharmaceutical drugs. It is estimated that each person, on an average, contributes to fertilizer resources amounting to 4.6 kg of phosphorus and 11 kg of potassium per year through excreted urine. In other words, each person excretes almost 6 kg of pure plant nutrients via urine. This is almost equivalent to the amount of nutrients contained in 15 kg of compound NPK fertilizer.

Use of night soil (human feces) and urine has been an age old practice in China, Vietnam and other south Asian countries. In view of the promotion of organic farming worldwide, use of human urine and feces has been greatly emphasized in recent years, particularly human urine as liquid fertilizer in Europe and in some Scandinavian countries and also in Africa. The amount of human urine generated daily in a highly populated country like India can be an important resource if it is managed properly as liquid fertilizer for economic driven activities in rural areas.

The added advantage of urine as fertilizer is the absence or low levels of hazardous chemical compounds or heavy metals in human urine compared to commercial chemical fertilizers. In fact, agriculture bulk fertilizer may have cadmium concentration up to 36 g per kg phosphate fertilizer which are several magnitudes higher than those of typical human urine.

Contrary to the key benefits of reuse of human urine in various agricultural production through recycling of nutrients in urine, presence of certain pathogens such as Leptospira interrogans, Salmonella typhi, Salmonella paratyphi, Schistosoma haematobium and Escherichia coli in the urine of diseased persons has raised some questions. The possibility of infection by S. typhi and S. paratyphi through urine is less as urine oral transmission is probably unusual compared to faecal oral transmission. Moreover, the risk of transmission of Salmonella sp. is low, even with short storage time, due to the rapid inactivation of fecal bacteria. Human urine is generally free of pathogens when excreted by a healthy person. However, post excretion cross contamination has to be challenged through proper treatment and management.

Storage of human urine is prudential for inactivation of some pathogens. In this process, the microbial population in the urine is killed without the need for any preservative. On the other hand,
microbial activity is responsible for the observed changes in the stored human urine and vice versa. The Gram negative bacteria such as Aeromonas hydrophila, Escherichia coli, Pseudomonas aeruginosa, Salmonella senftenberg, Salmonella typhimurium have been reported to be killed within a few days of storage. Likewise, Rhesus rotavirus and Salmonella typhimurium phage 28 B as well as the oocyte of the pathogenic protozoa Cryptosporidium parvum fail to survive few weeks of storage. Further, bacteriophages have some beneficial effects in reducing the number of pathogens from contaminated wastes. On the other hand, some bacteria such as Clostridium and sulphate reducing bacteria appear in the stored urine despite their absence in fresh urine. Presence of Clostridium has been reported to have some beneficial effects such as slowing down the colonization of root by fungal mycelium. Human urine undergoes microbiological and associated chemical changes, several microbial-dependent or independent processes such as urea hydrolysis, precipitation, ammonia volatilization, etc. during the storage period. In fresh urine, about 95% of nitrogen is in the form of urea and 5% as total ammonia (unionized and ionized). Urea hydrolysis results in the formation of bicarbonate and ammonia within few days of storage. Stored urine then becomes highly alkaline with pH values changing from 6 to about 9. A small fraction of aqueous ammonia ionizes into ammonium and hydroxyl ion until the concentration of free ammonia increases up to 2000 mg-N L\(^{-1}\). With increase in the values of pH and ammonia-N, the concentration of phosphate decreased throughout the storage period. About 80% of the nutrients contained in human urine can be concentrated in 25% of the original volume by freezing which would facilitate transport and storage. Acidification at the beginning of storage with 60 meq/L sulphuric or acetic acid per litre of undiluted urine resulted in the inhibition of decomposition of urea in the stored urine during 100 days of storage.

Though storage has been advocated for inactivation of bacteria for using human urine as safe fertilizer in agriculture or aquaculture, little studies have been made so far to examine the pattern of changes of some bacterial population during the different periods of storage under tropical conditions. This is important in view of tremendous nutrient potentials of human urine and its growing demand for agriculture or aquaculture in view of the promotion of organic farming worldwide. With a view to identifying specific (optimal) storage period of human urine as safe fertilizer, in this present study we examined the responses of coliform and heterotrophic groups of bacteria along with associated changes in physico-chemical characteristics of urine that occurred during different periods of storage.

Materials and Methods

The urine was collected from non-hydro urinals used by non-medicated and healthy post-graduate male students (21-25 years) in the department of Zoology, University of Kalyani. Fresh human urine was collected in triplicate in high quality plastic jars closing its mouth tight with a super fit plastic lid and kept for 2-22 months in the laboratory at room temperature (20-35°C). Samples of urine collected in triplicate on the day of experiment were used as samples of fresh urine.

All the required chemicals, glassware were of high quality grade and obtained from Merck India (Mumbai, India) and Hi Media (Mumbai, India). Routine procedures were followed for sterilization of glassware, serial dilution, media preparation, inoculation of the samples and incubation, etc. according to the methods described in APHA. Viable counts of heterotrophic bacteria were obtained by culturing the colony forming units (cfu) on standard nutrient agar media (Hi Media) having composition as follows: peptic digest of animal tissue 5 g, beef extract 1.5 g, yeast extract 1.5 g, sodium chloride 5 g and agar 15 g; pH 7.2.

The counts of coliform groups of bacteria were enumerated by growing them on Eosin Methylene Blue (EMB) agar media consisting of peptic digest of animal tissue 10 g, di-potassium phosphate 2 g, lactose 5 g, sucrose 5 g, eosin Y 0.4 g, methylene blue 0.065 g, agar 13.5 g and distilled water 1 l; pH 7.2±0.2 at 25°C. The prepared medium was sterilized in the autoclave at 15 lb/in\(^{2}\) pressure for 15 min.

For enumeration of total coliform groups of bacteria, about 15 mL of EMB agar was added to 0.1 mL urine sample in sterilized Petri dishes. The colonies of coliform/E. coli grown on EMB agar medium were identified with the characteristics of dark centre and distinctive metallic green sheen colonies. Four replicate plates were used for each group of bacteria. Incubation of Petri dishes was done at 37°C for 48 h. After incubation period, bacterial colonies were visualized and enumerated manually. In
case of coliform, some characteristic colonies of coliform bacteria were considered for enumeration. The arithmetic means from four plates were obtained and used in the study.

Different physico-chemical parameters such as pH, carbonate, bicarbonate, total hardness, ammonia-N, nitrate-N and phosphate were analyzed following the methods described in the APHA.

One way analysis of variance was performed to analyze the data obtained. If the effect was found significant, the ANOVA was followed by a least significant difference (LSD) test. All statistical tests were performed at 5% probability level using statistical package SPSS-10.0.

Results

Heterotrophic bacterial population

The counts of heterotrophic bacteria ranged from $140.66 \times 10^6$ cfu ml$^{-1}$ to $0.00266 \times 10^6$ cfu ml$^{-1}$ in the samples of urine stored for different months of the study (Fig. 1). The counts were maximum in case of fresh urine, but tended to decline steadily as the storage period progressed till day 75 (ANOVA; $P < 0.05$). However, no difference (ANOVA; $P > 0.05$) was found in the counts of bacteria between fresh and stored urine beyond 75 days of storage.

Total coliform bacterial population

The mean count of total coliform of bacteria was maximum ($174.66 \times 10^4$ cfu ml$^{-1}$) in case of fresh urine and lowest ($0.033 \times 10^4$ cfu ml$^{-1}$) in old urine stored for 638 days (Fig. 1). There was steady decline (98%) in the counts of bacteria from the day 0 to day 75 of storage (ANOVA: $P < 0.05$). The counts then remained fairly low ($0.233-0.033 \times 10^4$ cfu ml$^{-1}$) which did not differ (ANOVA; $P > 0.05$) between the fresh and stored urine.

Gradual changes in physico-chemical parameters of urine were well pronounced with increase in storage period (Table 1), and the changes were comparable to that of data compiled by Deutsche Gesellschaft für Technische Zusammenarbeit (GTZ). The concentrations of phosphate in the stored urine (Table 1) had been declined by 56% from its initial value in fresh urine (Table 1). This was due to the fact that phosphorus, calcium, magnesium, ammonium, and potassium are precipitated during the storage and that precipitation was triggered by the progressive rise in alkaline condition during the storage process.

Several factors that influence the storage process are time, temperature and pH. From the inactivation curves for Rhesus rotavirus, Campylobacter jejuni, Campilobacter parvum, it can be concluded that urine stored at 20°C for at least 6 months may be considered as safe to use as a fertilizer for any crop. Initial counts of total coliform and E. coli have been reduced and remained less than detection limit after 4 h of storage. Though it is encouraging, practically the time needed for certain reduction is hardly to

![Fig. 1—Changes in the counts of heterotrophic bacteria and total coliform bacteria in relation to pH and storage period (days). [The number within parenthesis indicates the days of storage]](image)

<table>
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<th>Parameters</th>
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<td>5.0</td>
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<td>200</td>
<td>220</td>
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<td>160</td>
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<tr>
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<td>1.46</td>
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<td>0.598</td>
<td>0.588</td>
<td>0.484</td>
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<td>PO$_4$-P (mg l$^{-1}$)</td>
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predict as the conditions may vary depending upon the ambient temperature, moisture, design of storage container, competition between the naturally occurring microorganisms and the pathogen, etc.\textsuperscript{21}

One of the possible risks of using fresh human urine for fish culture or agriculture is the presence of ammonia that occurs either in ionized (NH\textsubscript{4}\textsuperscript{+} or unionized (NH\textsubscript{3}) form depending upon pH. In general, unionized form of ammonia is more toxic to fish and occurs in greater proportions at high pH and higher temperature\textsuperscript{30} Ammonia is highly volatile and it escapes from the air face layer of the aqueous solution, but the loss of ammonia is marginal (1-10\%) in closed storage tanks\textsuperscript{31}. It is estimated that open storage led to nitrogen losses of 90\%, whereas the closed storage containers retained 93\% of total nitrogen. Under storage, the N:P ratio of 8:1 in fresh urine has been changed to 14:1 in the supernatant and to 1:12 in the precipitate. Though sulphate was not measured in the present investigation, sulphate that occurred in the stored urine was possibly the most favorable electron acceptor as element oxygen, nitrite and nitrate were missing and concentration of iron was very low\textsuperscript{32}.

It appears from the results of the study that sharp reduction in the counts of coliform groups of bacteria or death of pathogenic bacteria was due to highly alkaline condition that developed during storage period. As the counts of pathogenic groups of bacteria gradually declined until day 75 of storage and then remained extremely low (0.233 \times 10^4 \text{ cfu ml}^{-1}), it becomes clear that storage for approximately 75 days might be considered suitable, or 250 days for conservative measure, for inactivation of pathogenic groups of bacteria in human urine to be used as safe fertilizer in agriculture or aquaculture. This is in alignment with the recommendations of World Health Organization (WHO)\textsuperscript{33} as the counts of coliform groups of bacteria in the stored urine remained far less than the recommended counts of coliform groups of bacteria for wastewater fed aquaculture.

Conclusion

With the results of the present study, it may be concluded that human urine can be used as low risk fertilizer after its storage for 253 days under prescribed conditions.

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