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A Simple Resource-oriented Sanitation of Cattle Urine as a Value-added Liquid Fertilizer

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***Abstract*: Evaporation of malodourous ammonia formed due to urease-decomposition of urea of cattle urine causes loss of nitrogen and creates difficulty in handling the urine for application as a liquid fertilizer. Here we show that phosphoric acid (PA), along with neutralization of urine, can inhibit urease-decomposition of cattle urine while converting urine into pH-neutral, odourless, nitrogen-intact, and phosphorous-enriched liquid fertilizer with an additional advantage of utilization of the urine-water in water scarce regions or seasons. Pre-dosing of cattle urine with the optimized doses of PA has been shown to restrict the pH of the liquid fertilizer within 8.0 for at least seven weeks retaining undecomposed urea in cow, buffalo, and pig urine as 4.25gL^{-1}, 5.74gL^{-1} and 4.78gL^{-1}, respectively. A small precipitate of struvite, magnesium ammonium phosphate hexahydrate, obtained during PA-treatment of the cattle urines may be used as a solid N-P fertilizer. The addition of optimum doses of PA increases the P-content of the urine of lactating cattle from 25mgL^{-1}, 46mgL^{-1} and 48mgL^{-1} to 1.47gL^{-1}, 1.87gL^{-1}, and 1.33gL^{-1}, respectively. Application of PA is a potential green way to resource-oriented sanitation of cattle urine converting the urine into a convenient value-added liquid fertilizer at almost no cost due to its atom efficiency.**

Keywords: Cattle urine, Liquid fertilizer, Resource-oriented sanitation, Urease inhibition, Urine malodour

I. INTRODUCTION

Cattle urine, which is usually a waste and flows over the floor and drain, is a potential liquid fertilizer rich in nitrogen, potassium, and other micronutrients in addition to its valuable water in water-scarce regions. It consists of approximately 95% water, 2.5% urea and 2.5% other minerals, hormones, enzymes, etc., while the amount of urea mainly depends on the diet and hydration level of the animals (Sigurdarson et al., 2018; Jadhav et al., 2016). Under practical cattle-shed settings, the urine usually becomes alkaline due to formation of ammonia from urea decomposition by ubiquitous urease. This increases the pH of the urine above 9.2 which can harm to tender plants, especially at the time of seed germination and growth (Ray et al., 2018; Mobley & Hausinger 1989; Krishnamoorthy et al., 2020). Loss of nitrogen in the form of evaporation of ammonia is also associated with environmental pollution and unpleasant odour causing difficulty in handling and storage of the urine as a fertilizer (Senecal & Vinnerås 2017; Ciurli et al., 1999; Tabatabai & Bremner 1969; Hutchinson & Viets 1969; Whitehead & Raistrick 2019; ApSimon et al., 1939; Randall et al., 2016). The malodour of cattle urine is mainly due to ammonia, though there are other odorous compounds present in urine, which vary depending on the gender, age, diet, physiological and hormonal status, and use of drugs (Li et al., 2017; Edman & Brooks 1983; Guernion et al., 2001). Direct application of urine to the crop field is also reported to lower the nitrogen fixing capacity of the soil (Di et al., 2002).Thus, it is worthwhile to tap this potential organic liquid fertilizer by controlling the pH and stopping the malodour through inhibition of the enzyme (Márquez et al., 2017).

Urease enzyme is present in various species of bacteria, algae, fungi, invertebrates, and plants (Bekheet & Syrett 1977; Cook 1976; Frankenberger & Tabatabai 1982; Hanlon1975). It is a metalloenzyme with bi-nickel active sites which selectively binds with urea to decompose it into ammonia and carbon dioxide (Mobley & Hausinger 1989; Dixon et al., 1926). The time of urea hydrolysis mainly depends on the amount of urease present in the urine and the surrounding environment (Ray et al., 2018).There are several reports on methods of prevention of urea hydrolysis using urease inhibitors such as acids; *viz.*, 30mM

sulfuric acid and 60mM citric acid; base, *viz.*, calcium hydroxide; electrochemical treatment and use of peroxydisulfate (Randall et al., 2016; Kot et al., 2001; Hellström et al., 1999; Lv et al., 2020). Hellström et al., 1999 reported results of one-time dosage of 60 meq sulphuric or acetic acid per litre undiluted urine at the beginning of the storage period which could inhibit the decomposition of urea for more than 100 days of storage in cans. Randall et al., 2016, used $Ca(OH)_2$ to increase the pH of urine to above 12.5 to stop urea hydrolysis. Senecal et al., 2017 reported a technique to increase the N concentration (from 0.6% to >6%) through urine dehydration to produce a dry fertilizer of monetary value and avoid the need for liquid disposal from the toilet. Safitri et al., 2019 reported a study of fermented cow urine as a potential liquid fertilizer using effective microorganism and local microorganisms that derived from organic waste for the fermentation. To determine the quality of fermented cow urine they analyzed the concentrations of N, P, K, and ammonia (NH_3).

Liu et al., 2024 have recently developed a urine stabilization product consisting of camphor, kathone, and citric acid in specific ratios to stop the urea hydrolysis process in waterless urinal. Kafarski and Talma, 2018 in a recent review on various urease inhibitors including compounds bearing fragment of urea in their structure, quinolones, flavonoids, heterocyclic compounds, compounds which bind covalently with urease, coordination complexes, and organophosphorous compounds, opined that urease inhibition is still an active field of medicinal chemistry.

Application of N-n-butyl thiophosphorictriamide (NBPT) and N-(n-butyl) phosphorictriamide has been reported to inhibit urease activity (Kot et al., 2001; Hellström et al., 1999; Zaman et al., 2013). Zaman et al., 2013 provides a method to minimize both ammonia (NH_3) and nitrous oxide (NO_2) losses from cow urine patches by using N-(n-butyl) thiophosphorictriamide (nBTPT) as urease inhibitor and dicyandiamide (DCD) as nitrification inhibitor. It was reported that double inhibitor (DI) (nBTPT plus DCD) is effective in minimizing NH_3 and NO_2 losses from applied urine. NBPT, after binding to the S. pasteurii urease enzyme, undergoes hydrolysis to produce monoamidothiophosphoric acid, which is effectively bound to the two Ni ions in the active site and thus inhibits the enzyme (Kafarski & Talma 2018; Mazzeiet al., 2017; Benini et al., 1999). Phosphate and phosphoric acid (PA) have been long known as urease inhibitors (Howell & Sumner 1934; Todd & Hausinger 1989). Benini et al., 2001 suggested that coordination binding of phosphate ($H_2PO_4^-$) with Ni ions in the active sites inhibits urease. Nevertheless, low-cost stabilization of urea and control of pH of urine is remaining as challenges for application of cattle urine as a liquid fertilizer (Comadran-Casas et al., 2022).

Since phosphorous-containing compounds, including phosphate and PA, are known to inhibit urease decomposition, it was thought worthwhile to explore the possibility of using PA to neutralize alkaline cattle urine and, at the same time, to inhibit further decomposition of urea for the purpose sanitation of cattle urine converting it into a convenient-to-handle and value-added liquid fertilizer. PA was chosen also due to its low cost. A single-step addition of PA may have a three-fold value-addition to cattle urine as a liquid fertilizer through. Firstly, through control of pH of urine as suitable for fertilizer, secondly, through reduction of malodour and loss of nitrogen from urine by reducing production and evaporation of ammonia by inhibition of decomposition of urine by urease, and finally, through an increment in the P-content of cattle urine. The study was conducted with urine collected from cow, buffalo, and pig sheds under different practical conditions.

II. MATERIALS AND METHODS

85%w/v orthophosphoric acid was purchased from Rankem, India and used as such (Nath & Dutta 2010; Gogoi et al., 2015). All other chemicals were of analytical grade and used without any further purification. Cow urine, buffalo urine and pig urine were collected from at Bharalipariya Kanyaka Multipurpose farm (BKMF), Tezpur, 10km away from Tezpur University. Its well-maintained cowshed had around 50 mostly lactating cows. The piggery had 15 pigs and twenty piglets. The buffalo shed had 35 buffalos. Fresh cow urine and buffalo urine were collected in clean plastic containers directly while urinating at the respective cattle-sheds. Drained cow urine was collected overnight, before flushing of the cowshed for cleaning in the morning, in an 80L plastic container containing pre-added PA dose for minimizing loss of ammonia. Fresh cow urine was used for characterization of urine while drained cow urine was used for all other experiments including pH variation of urine with time, optimization of PA dose, shelf-life determination, and estimation of undecomposed urea. It was not possible to collect drained buffalo urine as the urine could not be drained on sandy floor of the shed and therefore fresh buffalo urine was used for all experiments including characterization of buffalo urine. Drained pig urine, collected from a discharge point of an underground drain pipe at a fishery 10m away from the piggery, was used for all experiments, since direct collection while urinating was not practical.

The laboratory experiments to see the effects of PA on pH of cattle urine were performed with 1L of drained cow urine, fresh buffalo urine and drained pig urine by adding 85%PA to optimize the dose of PA required to bring down the pH to 6.9. The field pilot experiments were carried out at the respective cattle-sheds at BKMF by pre-adding the optimized doses of 85%PA to 20L drained cow and pig urine and fresh buffalo urine in plastic containers.

The pH of urine was measured using a hand-held pH meter (Model-Hanna instruments HI98107 Phep pH meter) reproducible within ±0.1. Specific gravity, albumin, blood, and pus cell of cattle urine were analysed on a Siemens Biochemistry analyzer model Dimension XPAND Plus. Ammonium concentrations, $[NH_4^+]$, in cattle urine were determined using a handheld colorimeter ammonia high range checker (Model-Hanna instruments HI733). The elemental concentration of nitrogen in cattle urine was calculated from $[NH_4^+]$. Elemental phosphorous concentration was determined using ammonium molybdate spectrophotometric method at 700nm in a SHIMADZU UV-Visible spectrophotometer, model UV-2600i. The potassium ion concentrations were

measured using an atomic absorption spectrophotometer (AAS), model Analyst 200, Thermo ICE 3500 USA. Powder X-ray diffraction (p-XRD) spectra were recorded on a Bruker AXS (model D8 Focus, Germany) X-ray diffractometer with Cu-Kα radiation (λ =0.154nm) at 30kV and 30mA using a scanning rate of 0.05◦/s in 2θ ranges from 10◦ to 70◦. Fourier transform infrared (FTIR) spectra of precipitated samples were recorded on a Perkin Elmer Frontier MIR FIR spectrometer in KBr medium at room temperature in the region 4000-400cm^{-1}. Raman spectra were recorded on Raman spectrometer models - Renishaw Basis Series (514 Laser), and Horiba CPX100, LABRAM HR Revolution (633 Laser). Energy dispersive X-ray (EDX) spectra and scanning electron microscope (SEM) images were recorded on a scanning electron microscope (model JSM-6390LV, JEOL, JAPAN) at room temperature with an accelerating voltage of 15.0kV. Thermogravimetric analysis (TGA) and differential scanning calorimetry (DSC) graphs were recorded on a thermogravimetric analyser TGA-4000 (model Perkin Elmer, USA).

III. Results and Discussion

Effect of PA on pH of Cattle Urine

The average results of analysis of three samples of each cattle urine, collected in sterilized vials, is shown in Table S1 along with their N, P, and K values obtained by standard methods. Albumin, blood, and pus cell were found to be nil in all samples. The specific gravity of all three types of urine was almost same. The N-contents of cow, buffalo, and pig urine were found to be 3184mgL^{-1}, 4193mgL^{-1}, and 3882mgL^{-1}in cow, buffalo, and pig urine, respectively. The low P-content of 25mgL^{-1}, 46mgL^{-1} and 48mgL^{-1} of cow, buffalo, and pig urine may be attributed to lactating condition of most of the cattle. One needs to recognize that, ubiquitous presence of urease, varied extents of decomposition of urea after urination and varied extent of evaporation of ammonia from urine, mixing of urine with faeces and water in drained urine, etc., under normal practical conditions induce large errors in measurements of pH and other parameters like N, P, K, etc., of cattle urine (Safitri et al., 2019; Neglia et al., 2014; Zou et al., 2020; Dijkstra et al., 2013; Marumo et al., 2024).

The changes in the pH, for the different doses of 85%PA per litre of cattle urine, with time are shown in Fig.1 (Table S3).The initial pH of drained cow urine, fresh buffalo urine, and drained pig urine after collection as mentioned in the Materials and Methods section, were 8.8, 7.9 and 8.5, which increased after seven weeks to 9.4, 9.2 and 9.1, respectively. The dose of 85%PA was started with 1.5 mL per litre of the urine which was gradually increased to 4.2mL per litre of urine. The dose of PA required to bring down the pH of the urines to 6.9 were 3.2mL, 4.0mL and 2.8mL per litre of the urine, for drained cow urine, fresh buffalo urine, and drained pig urine, respectively. In all three cases, the pH of the urine increased gradually with time showing almost similar trends. The range of pH variation for doses from 1.5mL to 4.2mL per litre at any given time was much smaller in the case of the buffalo urine than the cow and pig urine. This can be attributed to purity of the absence of impurity and no decomposition of urea before treatment with PA for fresh buffalo urine. Formation of small amounts of precipitates, expected to be struvite, was observed after neutralization of all three cattle urines, which has been characterized thoroughly below (Prywer et al., 2015).

It may be noted that with 3.2mL dose of 85%PA per litre of cow urine, pH of the urine did not exceed 7.0 up to three weeks, which is very safe for crops. Three weeks of time is also enough to collect, store and transport the urine within a multipurpose (cattle cum agricultural) farm for use as a liquid fertilizer. The pH of cow urine with a pre-added PA dose of 3.2mL per litre of urine remained below 7.9 up to 56 weeks, which is only moderately alkaline and is suitable for fertiliser, indicating a shelf-life of one year. The pH increases above 8.5 if the dose of 85%PA is lower than 3.2mL per litre. The observed widening gap between the curves for PA doses of 3.2mL per litre and 3.0mL per litre may be due to incomplete inhibition below the dose of 3.2mL per litre. Similarly, the initial wide gap which narrows down with time, as observed between the curves for the control or without PA (black) and minimum dose of PA of 1.5mL per litre (red), may be attributed to a very low inhibition.

Similar trends in the variation of pH with time were observed for buffalo and pig urine. With pre-added doses of 4.0mL and 2.8 mL of 85%PA, the pH of buffalo and pig urine remained within 7.9 and 8.0, which are moderately alkaline and suitable for fertilizer, up to seven weeks. Thus, the doses of 3.2mL, 4.0mL and 2.8mL of 85%PA per litre of the cow urine, buffalo urine, and pig urine used in the present study giving liquid fertilizers with a shelf-life of at least seven weeks can be taken as the optimum doses, respectively.Though there are large errors in the quantitative estimations of pH and other characteristics of cattle urine, and hence in the optimised doses, that should not matter much as the required doses of PA can be easily optimized under any cattle-shed conditions, given the important qualitative information inferred in the present study.

Characterization of precipitates

Small quantities of precipitates, namely, 0.93g, 1.0g, and 0.45g obtained during neutralization of one litre of cow, buffalo and pig urine by PA have been characterized as described below (Also see Supporting Information 2).The p-XRD spectra of the precipitates obtained from PA-treatment of cow, buffalo and pig urine showed peaks at 2θ of 14.95 (1 0 1), 15.78 (0 0 2), 16.43 (0 1 1), 20.84 (1 1 1), 21.44 (0 1 2), 27.07 (1 0 3), 30.22 (2 0 2), 33.23 (0 2 2) and 33.60 (2 1 2) corresponding to orthorhombic magnesium ammonium phosphate hydrate phase, $MgNH_4PO_4.(H_2O)_6$ (struvite) with the respective h, k, l planes of reflection indicated within parenthesis (JCPDS card no-01-77-2303) as shown in Fig. 2. Detail p-XRD analysis of the precipitates obtained from cattle urine with PA is shown in Supporting Information 2.

The FTIR spectra of the precipitates obtained from PA-treatment of the cattle urine are presented in Fig.2 (See Table S2 in Supporting Information 2 for detail assignment of peaks). Common absorptions bands observed at 3276 and 2926cm^{-1} may be assigned to N-H stretching vibrations (Kirinovic et al., 2017).

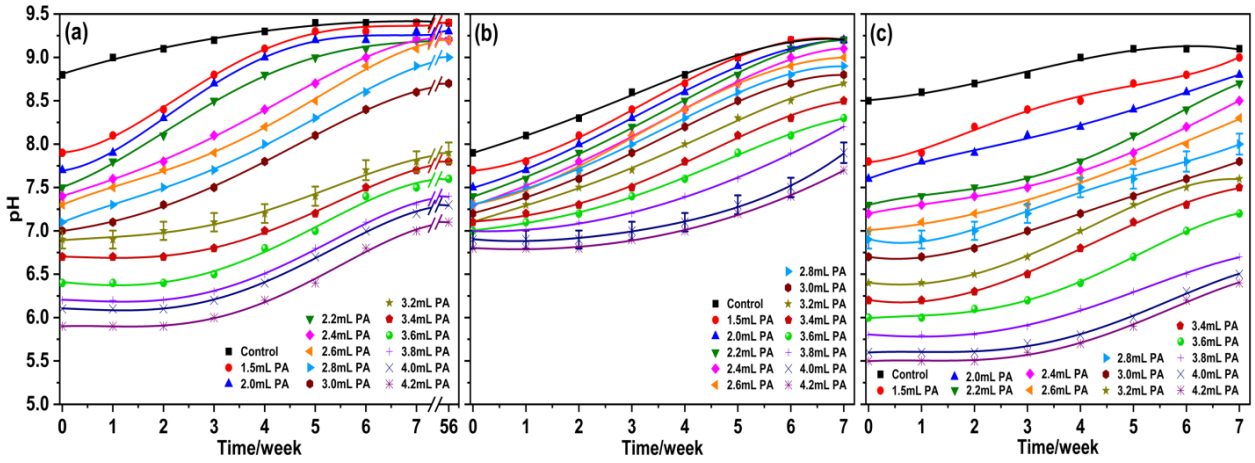

Fig. 1. pH from three independent experiments measured weekly from zero (initial) to fifty-six weeks in drained cow urine (a); and up to seven weeks in fresh buffalo urine (b); and drained pig urine (c); without (Control) and with pre-addition of varying quantities of 85%PA. Error bars are shown only in the optimum dose curves

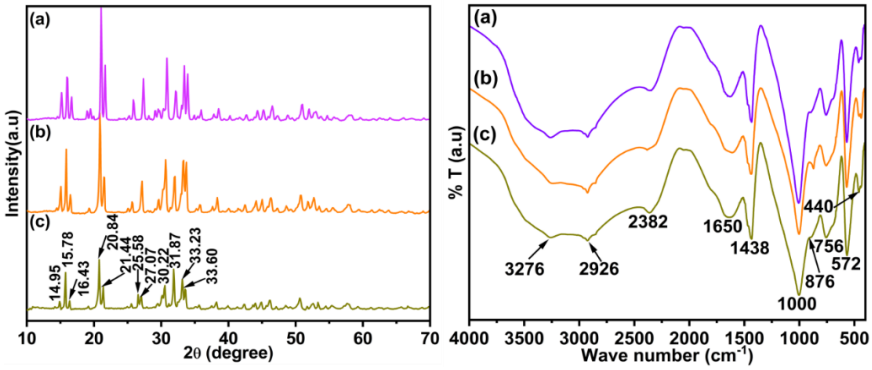

Fig. 2. p-XRDand FTIR spectra of the precipitates obtained from (a) cow, (b) buffalo, and (c) pig urine upon neutralization with H_3PO_4

Similarly, common bands at 2382 and 1000cm^{-1} may be assigned to water-PO_4^{3-} H-bonding and PO_4^{3-} antisymmetric stretching vibrations, respectively (Kirinovic et al., 2017). A sharp absorption peak at 1650cm^{-1} may be attributed to H-O-H deformation and NH_4^+ vibrations (Kirinovic et al., 2017). On the other hand, absorption bands observed at 1438 and 440cm^{-1}may be attributed to NH_4^+ asymmetric bending split by restrictive rotation and P–O bend (PO_4^{3-}) or Mg–O stretching vibrations respectively (Kirinovic et al., 2017; Sanghavi et al., 2020). Common absorption bands at 876 and 756cm^{-1} may be assigned to NH_4^+-water H-bonding and water-water H-bonding (Kirinovic et al., 2017) respectively. An absorption peak at 572cm^{-1} may be attributed to P–O bending of PO_4^{3-} or Mg–O stretching vibrations (Kirinovic et al., 2017; Sanghavi et al., 2020).Thus, the FTIR analysis of the precipitate supports the presence of P-O bond, N-H bond, Mg-O bond, NH_4^+ and PO_4^{3-} ions. Noisy pattern of Raman spectra was observed for the precipitates obtained from PA-treated cattle urines as shown in Figure S2B. A laser of 1.96eV (633nm) was used for Raman analysis of the precipitates as the precipitates were burnt on exposure to a higher energy excitation laser of 2.41eV (514nm), probably due to presence of organic substances.

SEM images of the precipitates as shown in Fig.3clearly indicates orthorhombic crystal morphology of the precipitates obtained from PA-treated cow, buffalo, and pig urines like that of struvite (Wena et al., 2018). The findings of EDX spectra of the precipitates obtained from cow, buffalo and pig urines showed significant presence Mg and no Ca. This again confirms predominant formation of orthorhombic $MgNH_4PO_4.(H_2O)_6$, *i.e.*, struvite.

Interestingly, the DSC curve of the precipitate obtained from PA-treated cow urine showed a peak at ~108°C (Fig. 4). This endothermic peak may be attributed to the loss of water of crystallization and release of ammonia (Chen et al., 2015). From the TGA curve, the mass loss of precipitates obtained from PA-treated cow urine was found to be 1.82mg, *i.e.*, 39.48% between ~86°C (onset) and ~117°C (end set). The mass loss of precipitates between ~86°C and ~117°C may be caused by the release of water and ammonia (Chen et al., 2015). The TGA and DSC of the precipitate obtained from PA-treated buffalo urine and pig urine were like those obtained with cow urine except for the position of the endothermic peak. The observed small shifts in the endothermic peak for struvite is reported and may be attributed to variation in organic impurities and factors like pH, temperature, presence of impurities and reaction time, affecting the precipitation (Huang et al., 2011).

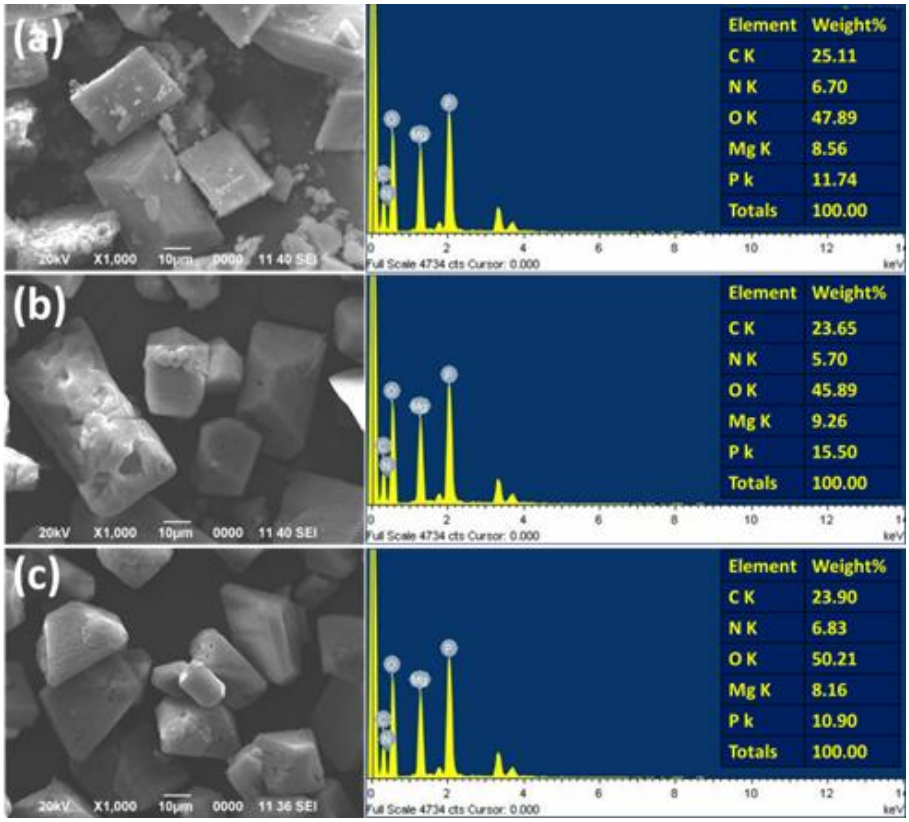

Fig.3. SEM images and corresponding EDX spectra of the precipitates obtained from (a) cow, (b) buffalo, and (c) pig urine upon neutralization with H_3PO_4

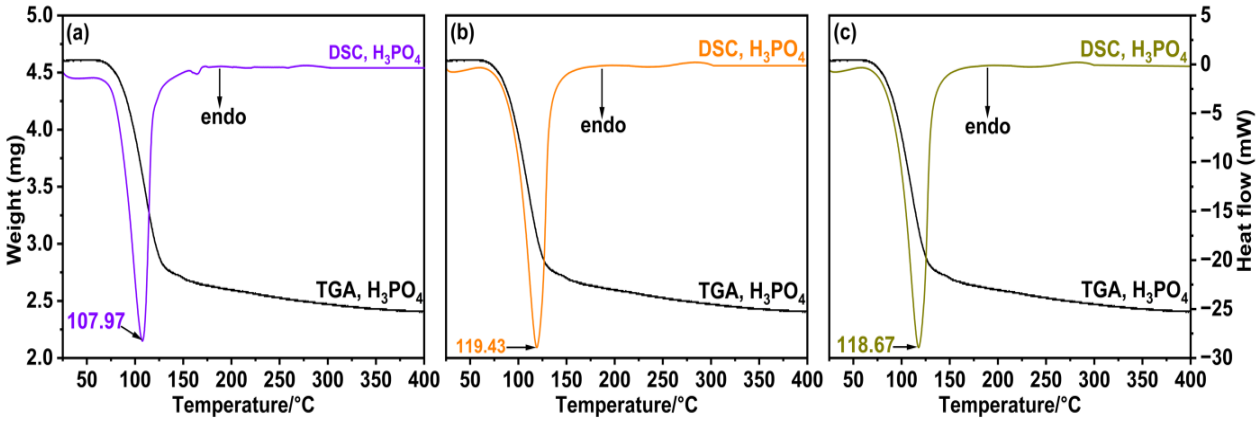

Fig. 4. TGA-DSC curve of the precipitates obtained from (a) cow, (b) buffalo, and (c) pig urine upon neutralization with H_3PO_4

Thus, the above detail analysis show that the precipitates obtained from PA-treated cow, buffalo, and pig urines are all struvite. These small quantities of solid struvite may be separated from the PA-treated cow, buffalo, and pig urines for application as solid N-P fertilizer. One may opt to ignore the small quantities (≤0.1%) of the solid struvite and apply it along with the liquid fertilizer as suspended solids.

Inhibition of urease by PA

In the absence of PA, the pH of cow urine is found to increase to 9.2 overnight. We observed a gradual increase in the pH of all samples of cow urine suggesting continuation of formation ammonia from decomposition of urea after mixing of the drained urine and PA (Fig.1). A slowdown of increase in the pH with increase in the dose of pre-added PA was also observed. The pre-added PA inhibits urease to retard further hydrolysis of the urea as soon as the urine reaches the container. The slow increase in the pH with time, *e.g.*, from pH 6.9 to pH 7.9, 7.9 and 8.0 in the cases of cow, buffalo and pig urine, observed on addition of optimised doses of 85%PA can be attributed to hydrolysis of urease though the uncatalyzed reaction which is much slower than the catalyzed one (Dutta & Saikia 2022).The gradual increase in the pH with time also indicates a varying degree of inhibition of the enzyme by phosphate group depending upon PA doses. Urease is reported to remain active

in acidic solutions with pH as low as 1.5 whereas here we have observed inhibition of urease at much higher or near neutral pH in the presence of PA (Stingl et al., 2022; Karplus et al., 1997). It is interesting to note other special utilities of PA like in food preservation and removal of fluoride from water (Nath & Dutta 2010; Gogoi et al., 2015). The inhibition of urease activity by phosphate group may take place through a mechanism like that of N-n-butyl thiophosphorictriamide and N-(n-butyl) phosphorictriamide (Kot et al., 2001; Hellström et al., 1999; Zaman et al., 2013).

Retention of N by PA as urea

PA also helps in retaining nitrogen of urine by inhibiting decomposition of urea. Ammonium ion ($[NH_4^+]$) concentrations measured after seven weeks in cow, buffalo, and pig urine without and with pre-addition of PA can be seen in Table 1. It may be mentioned here that the ammonium ion concentrations in untreated urine samples measured after seven weeks may be under-estimated as there must be some loss of ammonia due to evaporation as the lids were opened for measurement of pH. All treated cow, buffalo, and pig urine showed markedly lower $[NH_4^+]$ than the respective untreated urine samples. The amount of undecomposed urea remaining in the treated urine samples were calculated assuming that all urea had decomposed in the untreated samples in seven weeks, using equation(1):

$$\text{Undecomposed urea} = (\Delta\,[NH_4^+] \times M_{urea})/(M_{ammonium\ ion} \times 2) \quad \ldots\ldots\ldots\ldots(1)$$

where, $\Delta[NH_4^+] = ([NH_4^+]_0 - [NH_4^+]_{tr})$ is the difference between the concentrations of NH_4^+ ions in the untreated ($[NH_4^+]_0$) and the treated ($[NH_4^+]_{tr}$) urine samples in gL^{-1}. The results are shown in Table 1. The results indicate that large fractions of urea remained undecomposed in the treated samples. The amounts of remaining undecomposed urea were estimated as $4.25gL^{-1}$, $5.74gL^{-1}$, and $4.78gL^{-1}$, with 85%PA doses of 3.2mL, 4.0mL and 2.8mL per L of cow, buffalo, and pig urine, respectively. This also proves the inhibition of urea decomposition by urease in the presence of PA (Dutta & Saikia 2022).The decomposition of urea in the untreated samples might have taken place through catalyzed as well as uncatalyzed reactions.

TABLE 1

The pH and concentrations of NH_4^+ in $(\pm 0.2)gL^{-1}$, and in closed untreated (initial) and PA-treated cattle urine with the optimum dose of 85%PA per L of urine along with estimated undecomposed urea in $(\pm 0.2)gL^{-1}$, averages of three results, after seven weeks.

Urine type	Dose of PA/mL	Initial pH	Final pH	$[NH_4^+]$ /gL^{-1}	Undecomposed Urea/gL^{-1}
Cow urine	0.0	8.8	9.4	4.1	0.0
	3.2	6.9	7.9	1.6	4.25
Buffalo urine	0.0	7.9	8.8	5.4	0.0
	4.0	6.9	7.7	1.95	5.74
Pig urine	0.0	8.5	9.2	5.0	0.0
	2.8	6.9	7.8	2.13	4.78

Value addition in terms of odor, pH, N & P

It may be recalled that the pre-added optimum PA doses restricted the initial pH of collected cattle urine at 6.9, which rose to only 7.9-8.0 after seven weeks making the urine pH suitable for liquid fertilizer. Conversion of ammonia to less volatile ammonium and inhibition of further decomposition of urea also stop loss of N in the form of ammonia and hence stop the malodour of urine making its handling convenient. Moreover, the P-content of the cow, buffalo, and pig urine is also increased from $25mgL^{-1}$, $46mgL^{-1}$ and $48mgL^{-1}$ to $1.47gL^{-1}$, $1.87gL^{-1}$, and $1.33gL^{-1}$, respectively, making the process an atom efficient green process.

The recurring cost of the liquid fertilizer has been estimated to be ₹0.60, ₹0.75, and ₹$0.52L^{-1}$ for cow, buffalo, and pig urine, respectively, under the given conditions of the cattle-sheds and method of collection of urine considering the retail price of PA as ₹$100\ kg^{-1}$ in India. In fact, the recurring cost here is approximately zero if the utilization of the phosphate group is considered as a component of the fertilizer making the process atom efficient (Anastas & Warner, 2000). In addition to a neutral pH, absence of evaporation of malodourous ammonia and increased P-content, the water of urine also has a great value during water-scarce season and regions.

IV. Conclusion

The present work shows that pre-addition of PA can restrict the pH of the cattle urine from rising above 8.0 for at least seven weeks through neutralization of already formed ammonia in urine and inhibition of further hydrolysis of urea by urease. The optimized dose of 85%PA to bring down the pH from initial 8.8, 7.9 and 8.5 of cow, buffalo, and pig urine to 6.9 to restrict the pH within 8.0 for seven weeks, were found to be 3.2mL, 4.0mL, and 2.8mL per litre of urine, respectively. One also has an option to use the small quantity of struvite formed in the PA-treated cattle urines as a solid N-P fertilizer. The amounts of undecomposed urea remaining after seven weeks with the optimized PA doses have been estimated as $4.25gL^{-1}$, $5.74gL^{-1}$, and $4.74gL^{-1}$, for cow, buffalo, and pig urine, respectively. The decrease in evaporation of malodorous ammonia makes handling of the urine convenient as a liquid fertilizer in addition to decreasing the loss of nitrogen. Addition of PA also increases the P-content of P-deficient urine of lactating cattle making the process atom efficient green process. Thus, pre-addition of the optimum dose of PA to cattle urine can convert the urine into a convenient value-added liquid fertilizer at almost no cost through an atom efficient resource-oriented green process.

Acknowledgments

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APPENDIX A: SUPPLEMENTARY DATA

A Simple Resource-oriented Sanitation of Cattle Urine as a Value-added Liquid Fertilizer

Supporting Information - 1: Specific gravity, and N-P-K analysis in cow, buffalo, and pig urine

Table S1. Specific gravity (SG), and N-P-K values* of fresh cow urine, fresh buffalo urine and drained pig urine samples.

Cattle	SG	N/mgL^{-1}	P/mgL^{-1}	K/mgL^{-1}
Cow	1.023	3184	25	2940
Buffalo	1.023	4193	46	1428
Pig	1.020	3882	48	698

*Experimental error limits: SG (±0.013) and N-P-K within 5%.

Supporting Information - 2: Characterization of the precipitates obtained from cattle urine with phosphoric acid (PA)

The pictures of the precipitates obtained from PA-treatment of the cattle urines (Figure S2A) are presented below along with their PXRD, FTIR and Raman data.

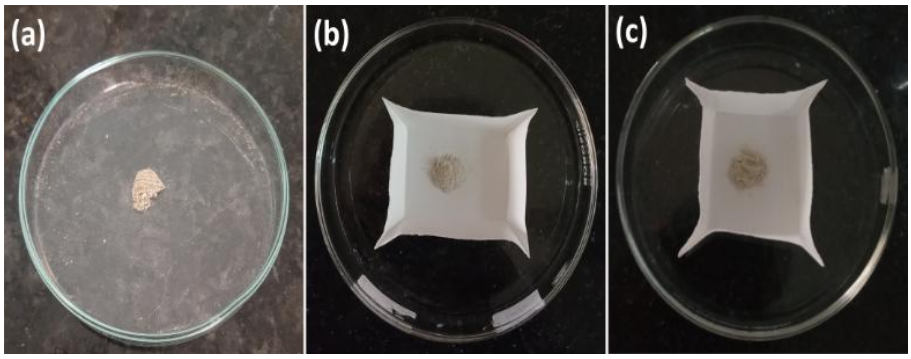

Figure S2A. Precipitates obtained from (a) cow, (b) buffalo, and (c) pig urine with H_3PO_4.

p-XRD analysis of the precipitates obtained from cattle urine with PA

Cow urine-H_3PO_4: 2θ = 14.97 (1 0 1), 15.77 (0 0 2), 16.43 (0 1 1), 20.85 (1 1 1), 21.42 (0 1 2), 25.65 (2 0 0), 27.04 (1 0 3), 30.22 (2 0 2), 31.87 (1 2 0), 33.23 (0 2 2) and 33.60 (2 1 2) - suggestive of orthorhombic magnesium ammonium phosphate hydrate- $MgNH_4PO_4(H_2O)_6$ (JCPDS card no- 77-2303).

Buffalo urine-H_3PO_4: 2θ = 14.95 (1 0 1), 15.79 (0 0 2), 16.43 (0 1 1), 20.85 (1 1 1), 21.44 (0 1 2), 25.58 (2 0 0), 27.07 (1 0 3), 30.22 (2 0 2), 31.87(1 2 0), 33.23 (0 2 2) and 33.60 (2 1 2) - suggestive of orthorhombic magnesium ammonium phosphate hydrate- $MgNH_4PO_4(H_2O)_6$ (JCPDS card no- 77-2303).

Pig urine-H_3PO_4: 2θ = 14.95 (1 0 1), 15.78 (0 0 2), 16.43 (0 1 1), 20.84 (1 1 1), 21.43 (0 1 2), 25.58 (2 0 0), 27.05 (1 0 3), 30.21 (2 0 2), 31.87(1 2 0), 33.23 (0 2 2) and 33.60 (2 1 2) - suggestive of orthorhombic magnesium ammonium phosphate hydrate- $MgNH_4PO_4(H_2O)_6$ (JCPDS card no- 77-2303).

FTIR analysis of the precipitates obtained from cattle urine with PA

Table S2. Summary of FTIR signals and assignments to various vibrational modes of precipitates obtained from from (a) cow, (b) buffalo, and (c) pig urine upon neutralization with H_3PO_4.

Signal	**Assigned IR active vibrations**	**Assigned to**	**References**
Bands at 3276 and 2926 cm^{-1}	N-H stretching vibrations	$MgNH_4PO_4(H_2O)_6$	Kirinovic et al., 2017
Band at 2382 cm^{-1}	Water-PO_4^{3-} H-bonding		Kirinovic et al., 2017
Peak at 1650 cm^{-1}	H-O-H deformation and NH_4^+ vibrations		Kirinovic et al., 2017
Peak at 1438 cm^{-1}	NH_4^+ asymmetric bending split by restrictive rotation		Kirinovic et al., 2017
Band 1000cm^{-1}	PO_4^{3-} antisymmetric stretching vibrations		Kirinovic et al., 2017
Band at 876 cm^{-1}	NH_4^+ -water H-bonding		Kirinovic et al., 2017
Band at 756 cm^{-1}	Water-water H-bonding		Kirinovic et al., 2017
Peak at 572 cm^{-1}	P–O bend (PO_4^{3-}) or Mg–O stretching vibrations		Kirinovic et al., 2017 Sanghavi et al., 2020
Peak at 440 cm^{-1}	P–O bend (PO_4^{3-}) or Mg–O stretching vibrations		Kirinovic et al., 2017 Sanghavi et al., 2020

Raman analysis of the precipitates obtained from cattle urine with PA

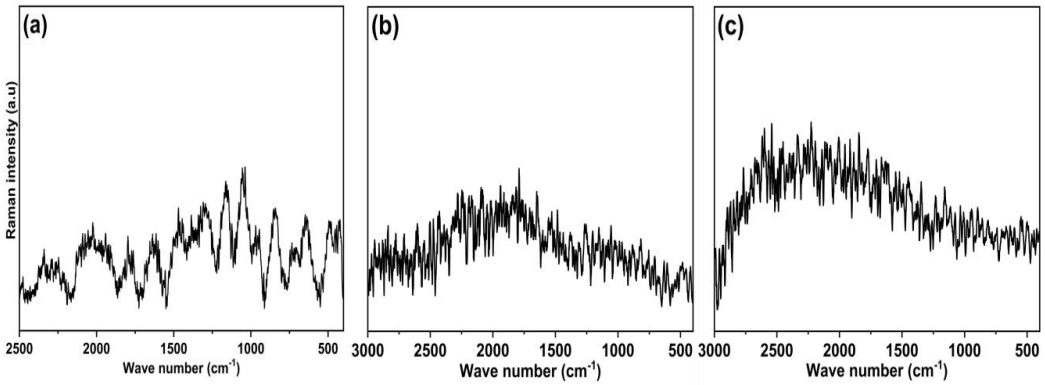

Figure S2B. Raman spectra of the precipitates obtained from (a) cow, (b) buffalo, and (c) pig urine upon neutralization with H_3PO_4 measured with laser excitation energy of 1.96eV (633nm)

Supporting Information - 3: Neutralization of cattle urine with PA

Table S3A. Variation of pH (±0.1, average value of three independent experiments) in treated cow urine with varying doses of 85% phosphoric acid (Initial pH of cow urine was 8.8)

Quantity of 85% PA pre-added per liter of urine	Initial pH of Cow urine after addition of PA	pH in treated cow urine after storing with time (week)							
		1	2	3	4	5	6	7	56
Control	8.8	9.0	9.1	9.2	9.3	9.4	9.4	9.4	9.4
1.5	7.9	8.1	8.4	8.8	9.1	9.3	9.3	9.4	9.4
2.0	7.7	7.9	8.3	8.7	9.0	9.2	9.2	9.3	9.3
2.2	7.5	7.8	8.1	8.5	8.8	9.0	9.1	9.2	9.2
2.4	7.4	7.6	7.8	8.1	8.4	8.7	9.0	9.2	9.2
2.6	7.3	7.5	7.7	7.9	8.2	8.5	8.9	9.1	9.2
2.8	7.1	7.3	7.5	7.7	8.0	8.3	8.6	8.9	9.0
3.0	7.0	7.1	7.3	7.5	7.8	8.1	8.4	8.6	8.7
3.2	6.9	6.9	7.0	7.1	7.2	7.4	7.7	7.8	7.9
3.4	6.7	6.7	6.7	6.8	7.0	7.2	7.5	7.7	7.8
3.6	6.4	6.4	6.4	6.5	6.8	7.0	7.4	7.5	7.6
3.8	6.2	6.2	6.2	6.3	6.5	6.8	7.1	7.3	7.4
4.0	6.1	6.1	6.1	6.2	6.4	6.7	7.0	7.2	7.3
4.2	5.9	5.9	5.9	6.0	6.2	6.4	6.8	7.0	7.1

Table S3B. Variation of pH (±0.1, average value of three independent experiments) in treated buffalo urine with varying doses of 85% phosphoric acid (Initial pH of buffalo urine was 7.9)

Quantity of 85% PA pre-added per liter of urine	Initial pH of buffalo urine after addition of PA	pH in treated buffalo urine after storing with time (week)						
		1	2	3	4	5	6	7
Control	7.9	8.1	8.3	8.6	8.8	9.0	9.2	9.2
1.5	7.7	7.8	8.1	8.4	8.7	9.0	9.2	9.2
2.0	7.5	7.7	8.0	8.3	8.6	8.9	9.1	9.2
2.2	7.4	7.6	7.9	8.2	8.5	8.8	9.1	9.2
2.4	7.3	7.5	7.8	8.1	8.4	8.7	9.0	9.1
2.6	7.3	7.5	7.7	8.1	8.4	8.7	8.9	9.0
2.8	7.3	7.5	7.7	8.0	8.3	8.6	8.8	8.9
3.0	7.2	7.4	7.6	7.9	8.2	8.5	8.7	8.8
3.2	7.1	7.3	7.5	7.7	8.0	8.3	8.5	8.7
3.4	7.1	7.2	7.3	7.5	7.8	8.1	8.3	8.5
3.6	7.0	7.1	7.2	7.4	7.6	7.9	8.1	8.3
3.8	7.0	7.0	7.1	7.2	7.4	7.6	7.9	8.2
4.0	6.9	6.9	6.9	7.0	7.1	7.3	7.5	7.9
4.2	6.8	6.8	6.8	6.9	7.0	7.2	7.4	7.7

Table S3C. Variation of pH (±0.1, average value of three independent experiments) in treated pig urine with varying doses of 85% phosphoric acid (Initial pH of pig urine was 8.5)

Quantity of 85% PA pre-added per liter of urine	Initial pH of pig urine after addition of PA	pH in treated pig urine after storing with time (week)						
		1	2	3	4	5	6	7
Control	8.5	8.6	8.7	8.8	9.0	9.1	9.1	9.1
1.5	7.8	7.9	8.2	8.4	8.5	8.7	8.8	9.0
2.0	7.6	7.8	7.9	8.1	8.2	8.4	8.6	8.8
2.2	7.3	7.4	7.5	7.6	7.8	8.1	8.4	8.7
2.4	7.2	7.3	7.4	7.5	7.7	7.9	8.2	8.5
2.6	7.0	7.1	7.2	7.3	7.6	7.8	8.0	8.3
2.8	6.9	6.9	7.0	7.2	7.5	7.6	7.8	8.0
3.0	6.7	6.7	6.8	7.0	7.2	7.4	7.6	7.8
3.2	6.4	6.4	6.5	6.7	7.0	7.3	7.5	7.6
3.4	6.2	6.2	6.3	6.5	6.8	7.1	7.3	7.5
3.6	6.0	6.0	6.1	6.2	6.4	6.7	7.0	7.2
3.8	5.8	5.8	5.8	5.9	6.1	6.3	6.5	6.7
4.0	5.6	5.6	5.6	5.7	5.8	6.0	6.3	6.5
4.2	5.5	5.5	5.5	5.6	5.7	5.9	6.2	6.4