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***Title: Evaluation of Fertilizer Value of
Human Urine in Aquaculture***

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1.2 Experiment - 2 Comparing the fertilizer values of fresh human urine under different treatment conditions

(i) The purpose of this experiment was to compare the fertilizer values of fresh human urine with that of cattle dung while keeping the nitrogen and phosphorus levels of mixed treatments similar to that of direct human urine fed treatment. Eighteen outdoor tanks (4500 l capacity) were allotted to six treatments in triplicate:

(a) Direct human urine under aerated and (b) non aerated conditions, (c) cattle dung alone, mixed treatment of cattle dung and human urine under (d) iso nitrogenous and (e) iso-phosphorus conditions, and (f) the control. Human urine was added at the rate of 0.01%. The collected fresh cattle dung was applied into the tanks @ 1.8 kg/ tank. The mixed treatment iso-phosphorus and iso-nitrogenous conditions was prepared by mixing cattle dung and human urine at the rate of 160 g CD + 333 ml urine and 315 g CD + 315 ml urine, respectively. The selected human urine or cattle dung was applied into the culture tanks at weekly intervals.

(iii) Twenty fry of fishes like common carp (2.5 g), rohu (4.5 g), mrigal (5.0 g) bata (1.5 g) punti (3 g) tilapia (2 g) and two freshwater prawn (1 g) were introduced into each culture tank following the numbers as: common carp – 4, rohu – 3, mrigal – 4, bata – 3, punti – 2, tilapia – 4 prawn – 2.

(ii) Samples of water were collected from each tank at different intervals for monitoring water quality parameters following the standard protocols described in the APHA (1995).

(iii) Water pH varied from 7.48 to 9.18 in different treatments employed. There was no marked differences of water pH among the treatments except that pH of cattle dung treatment remained always lower than the urine fed treatments.

(iv) The water of all treatments remained alkaline with carbonate alkalinity ranging from 0 to 18.0 mg/l. The amount of bicarbonate alkalinity was higher in the cattle dung treatment followed by the urine fed treatments where the differences were minor.

- (v) The electrical conductivity reflecting the total ionic strength of the treatment varied markedly in different treatments employed. The EC was maximum in the treatment added with cattle dung followed by treatments charged with human urine.
- (vi) The concentrations of DO remained well within the values recommended for fish culture. The overall mean values of DO ranged from 8.65 to 10.45 mg/l in different treatments.
- (vii) The concentration of ammonia-N was maximum in the direct urine fed treatment followed by cattle dung treatment, mixed treatment with iso-phosphorus condition, mixed treatment with iso-nitrogenous condition. Aeration resulted in significant reduction in the values of ammonia-N.
- (viii) There was a rising trend in the concentration of nitrite-N with cumulative increase in the dose of human urine in the treatment. There was no difference in the values of nitrite between the iso-nitrogenous and iso-phosphorus conditions. The values of nitrite were also considerably higher in the cattle dung treatment.
- (ix) As expected, aeration in the human urine caused the concentration of nitrate to be about 5 times higher than the values observed in urine fed treatment. The amount of nitrate was markedly higher in the iso-phosphorus treatment than in the iso-nitrogenous treatment condition.
- (ix) The concentration of phosphate ranged from 0.13 to 0.26 mg/l in different treatments employed. The amount of phosphate recorded in the mixed treatment with iso-nitrogenous condition was more than twice the concentration observed in human urine treatment.
- (x) The ratio of nitrate/phosphate was maximum in human urine aerated treatment followed by iso-phosphorus condition.
- (xi) The overall mean values of gross primary productivity of phytoplankton was maximum in the cattle dung treatment followed by human urine. No marked differences were found between iso-nitrogenous and iso-phosphorus treatment conditions.
- (xii) The net primary productivity of phytoplankton was maximum in human urine followed by cattle dung.
- (xii) The overall mean count of heterotrophic bacteria enumerated in cattle dung ($190 \times 10^3/\text{ml}$) treatment was the highest of all. This was followed by iso-nitrogenous condition, urine aerated treatment, iso-phosphorus conditions. Urine treatment showed the values comparable to control.
- (xiii) The counts of EMP media grown colonies with black pigment varied between 0 to 1570/ml in different treatments employed. The overall mean count of E. coli grown in EMB agar medium was maximum in the cattle dung treatment (680/ml) and minimal in the control (140/ml). There were no difference in the counts of EMB grown

E. coli among the three treatments with urine, urine and cattle dung maintaining the iso-phosphorus and iso-nitrogenous conditions.

(ix) The growth of fish was maximum in cattle dung treatment, followed by human urine fed treatment. No difference was found between iso-nitrogenous and iso-phosphorus conditions.

1.3 Experiment – 3 : Comparing the fertilizer value of stored and fresh urine charged with double strength at longer intervals

(i) The purpose of the experiment was to compare the fertilizer value of stored and fresh urine when applied in double strengths at longer intervals. Altogether 8 tanks were used for the study with 0.1% application of human urine. The treatments used were: (i) fresh human urine (ii) stored human urine and (iii) mixed treatment of fresh and stored human urine.

(ii) Twenty fry of fishes like common carp (2.5 g) , rohu (4.5 g), mrigal (5.0 g) bata (1.5 g) punti (3 g) tilapia (2 g) and two freshwater prawn (1 g) were introduced into each culture tank following the numbers as common carp – 4, rohu – 3, mrigal – 4, bata – 3, punti – 2, tilapia – 4, prawn – 2.

(iii) Samples of water were collected from each tank at different intervals for monitoring water quality parameters following the standard protocols described in the APHA (1995).

(iv) Water pH did not differ much from one treatment to the other with values ranging from 8.78 in fresh urine to 8.69 in stored urine treatments

(v) Total alkalinity was maximal in the mixed treatment followed by stored urine and fresh urine and control.

(vi) The values of EC were higher in the stored urine fed treatment followed by fresh or stored urine fed treatments showing no differences from each other.

(vii) The overall mean concentration of ammonia in the fresh urine fed treatment was markedly higher than that of stored urine fed treatment.

(viii) The amount of nitrite –N was considerably higher in fresh urine fed treatment compared to stored urine fed treatment.

(ix) The concentration on nitrate-N ranged from 0 to 0.746 mg/l in different treatments employed. The mean concentration was highest in fresh urine fed treatment and lowest in mixed treatment of fresh and stored urine, respectively.

(x) The overall mean concentration of phosphate observed in the stored urine fed treatment was significantly higher than that of fresh urine fed treatments.

(xi) The ratio for nitrate/phosphate was highest in stored urine fed treatment followed by fresh human urine treatment.

(xiii) There was no marked difference in the values of gross primary productivity of phytoplankton between the stored and fresh urine fed treatments.

(xiv) The mean count of heterotrophic bacteria (HB) was variable ranging from 4 to 50×10^3 /ml in different treatments. The count of HB in the stored urine treatment was nearly twice the count observed in fresh urine fed treatment.

(xv) The EMB media grown *E. coli* with black pigment ranged from 10 to 390/ml in different treatments.

(xvi). The fish growth observed in the fresh humane fed treatment was the highest of all the treatments. This was followed by mixed fresh and stored human urine treatment and the lowest in the control.

Detailed Report

2.0 Experiment -1: Comparing the fertilizer values of fresh and stored urine in aquatic production trials in three different doses

2.1 Experimental Design

The present experiment used 300 l outdoor tanks (PLATE 1A) to compare the fertilizer value of both stored urine and fresh human urine as well as its mixed combination for aquatic production. Fresh human urine was collected from the gents toilet of the Department of Zoology, Kalyani University used by the adult healthy male students aged between 22- 26 years. The human urine collected in the same manner and stored for about 8. 2 months was used as stored urine in the present study. Both the fresh and stored urine were used in three different concentrations of 0.25%, 0.50% and 1% . The mixed combination of fresh and stored urine (1:1) at 0.5 % was used in the study.



Plate -1A. An overview of urine-fed experimental units

Twenty four outdoor tanks were allotted for seven treatments and one control in triplicate (8 x 3 = 24). The concentrations used for fresh and stored human urine were 0.25%, 0.5% and 0.1%. A set of control in triplicate was also maintained (Treatment 7 x 3 and control – 3)

2.1.1 Protocol

The outdoor tanks were prepared ready for culture by thorough cleaning, sun drying and filling with ground water prior to their use in experiment. The culture tanks were allowed to stand for about 5 days for conditioning. Both fresh and stored urine were used in the experiment. Human urine collected from Gents toilet on the day was used as fresh urine and added to the tanks in three different test doses (0.25%, 0.5% and 0.1%) thrice during the trial period.

Fry of common carp (5 g), silver carp (5 g) and bata (2 g) were procured from the local fish farm and acclimated for 3 days under outdoor conditions. The fishes were then introduced into the culture tanks (12 fishes/tank) on April 1, 2008. The number of fishes introduced per tank was as follows: common carp – 5, silver carp – 5 and bata - 2. On the next two days, three of each silver carp and common carp died in the stored urine fed treatment and the lost fishes were replaced on the same day.

2.1.2 Collection

Samples of water were collected (PLATE 1B) from each of the outdoor tanks at different intervals for monitoring water quality parameters such as temperature, pH, free carbon dioxide, alkalinity, dissolved oxygen, phosphate, ammonia-N, nitrite- N, nitrate-N, phosphate-P and electrical conductivity. Standard methods described in the APHA (1995) were followed examination of water quality and nutrient parameters. The experiment was terminated after days when the available fishes were harvested and their live weights were recorded.

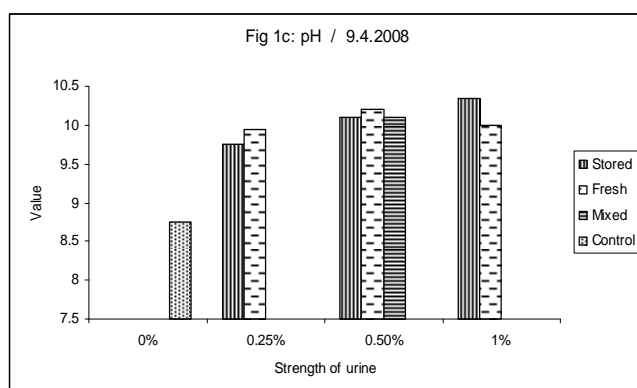
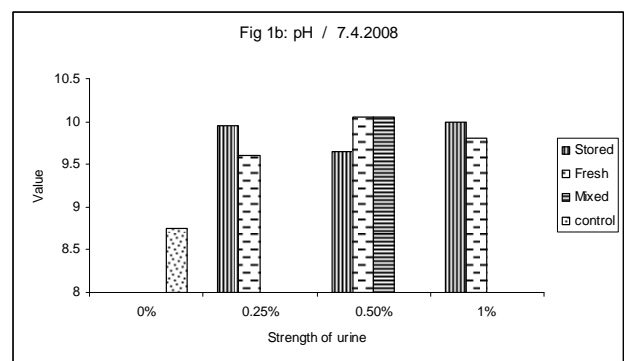
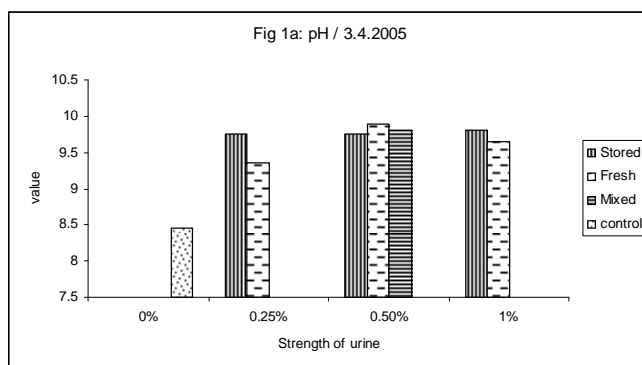


Plate -1B : Collection of sample and monitoring of water pH in urine fed treatments.

2.2 Results

2.2.1 Hydrogen-ion-concentration

Water pH remained alkaline in different urine fed treatments employed. Water pH of stored urine fed treatment remained little higher than that of fresh urine until day 56 of treatment after which a reverse trend was noticed when the values in the later exceeded the former. With increase in dose of human urine from 0.25 to 0.5%, no difference between fresh and stored urine was observed, but in the next higher dose (1%), the responses remained the same as that of 0.25% (Fig. 1) thereby indicating



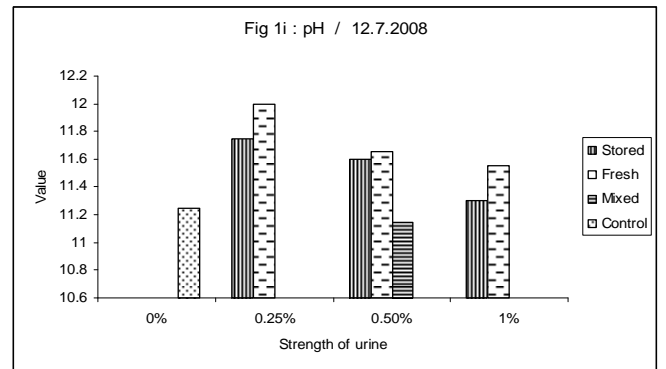
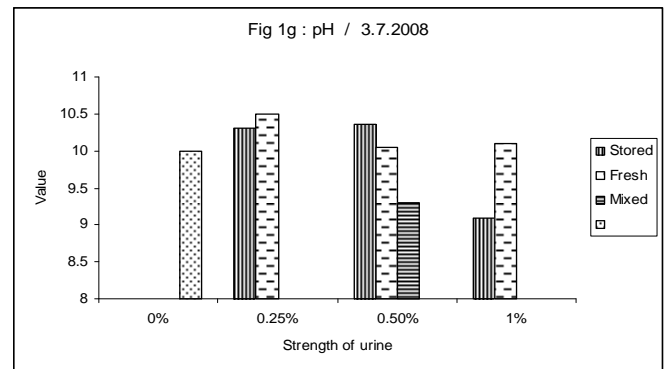
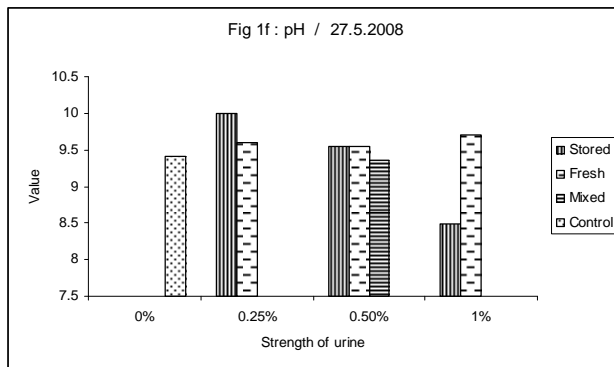
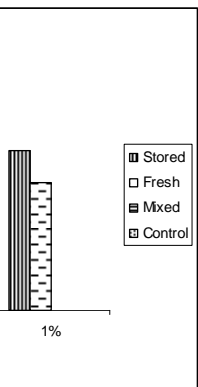
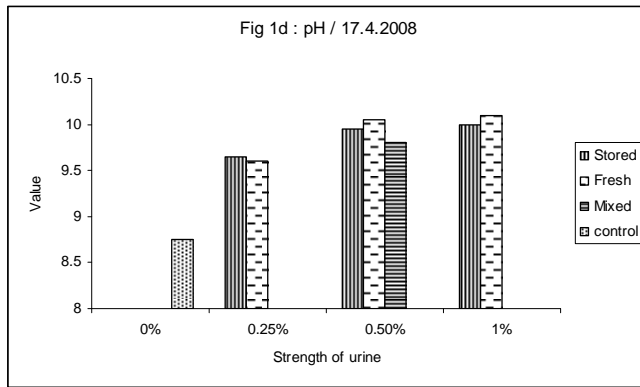


Fig 1. Values of water pH in different dates in urine fed treatments

2.2.2 Electrical conductivity (EC)

EC was higher in the fresh urine fed treatments compared to its counterparts with stored urine. Results were consistent in all the three doses of urine tested (Fig 2).

Measurement of EC everyday following application of second dose of urine in the treatment revealed a gradual rise in EC till day 13 followed by a downward trend. it appears that a period of 2 weeks may be allowed to complete the process of degradation of human urine under culture conditions employed (Fig. 2).

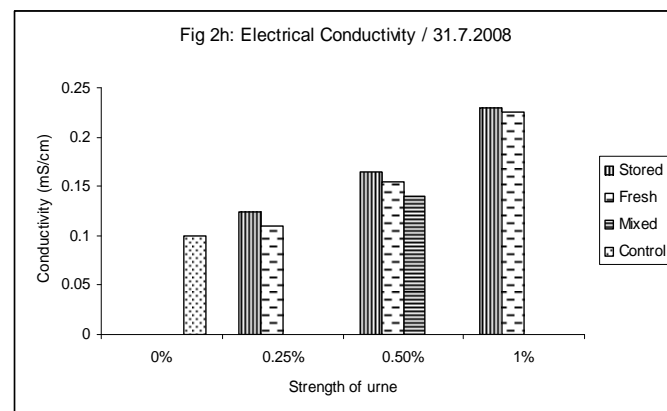
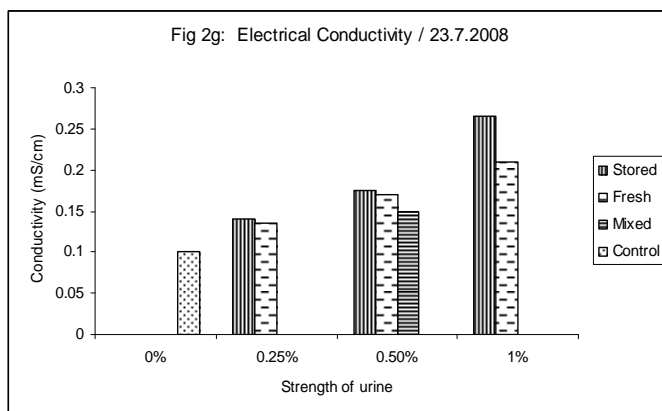
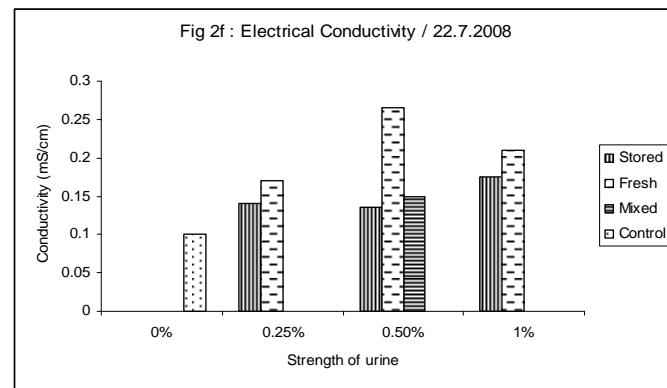
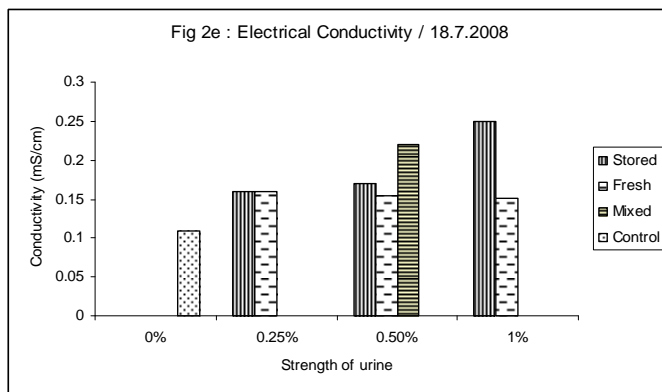
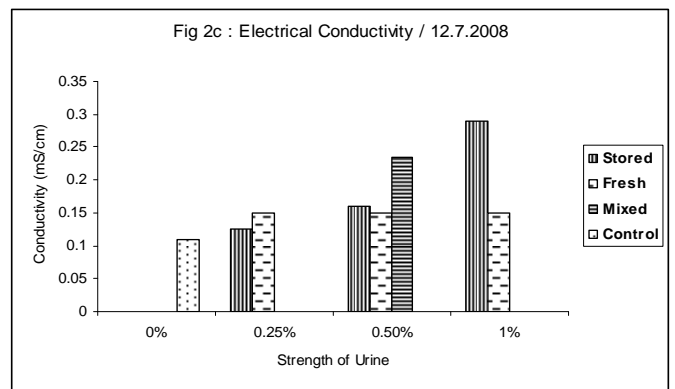
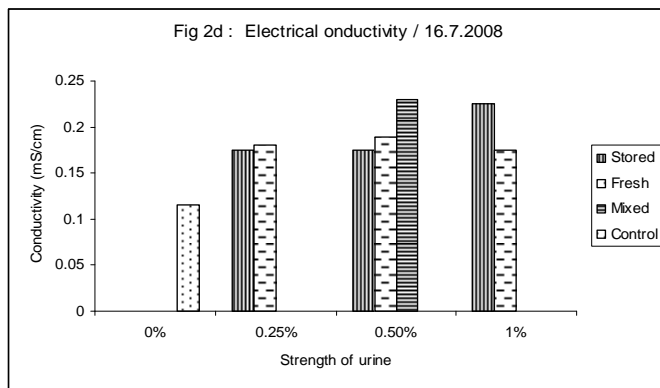
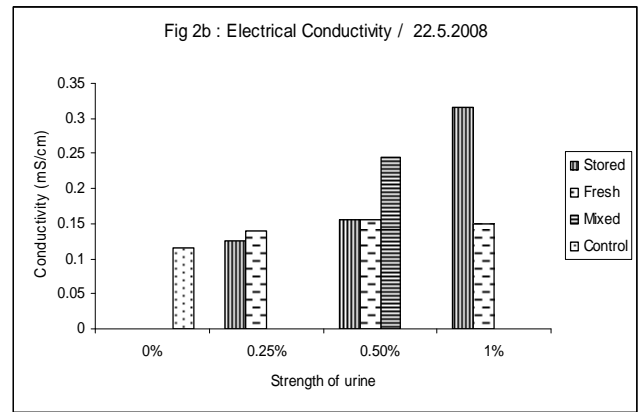
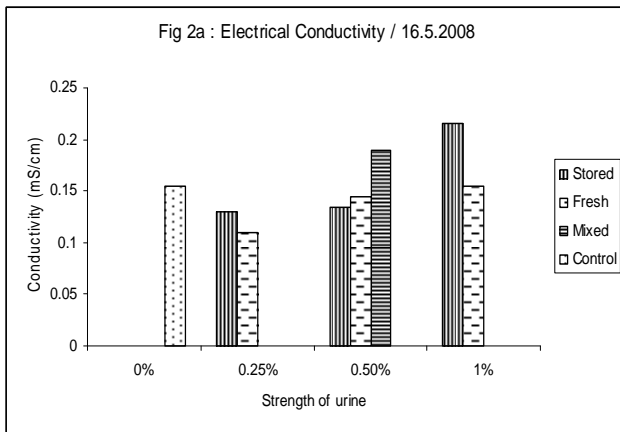


Fig 2. Variation of electrical conductivity in different dates in urine fed treatments.

2.2.3 Ammonium-N

The pattern of time scale changes in the concentration of ammonia-N of water was dose dependent in both fresh and stored urine fed treatments. The concentration was higher in fresh urine than that of stored urine. In the lowest dose of 0.25%, the concentration tended to rise from day 61 to day 119, but a declining trend was noticed in the higher dose of 0.5 and 1% during the same time scale (Fig. 3).

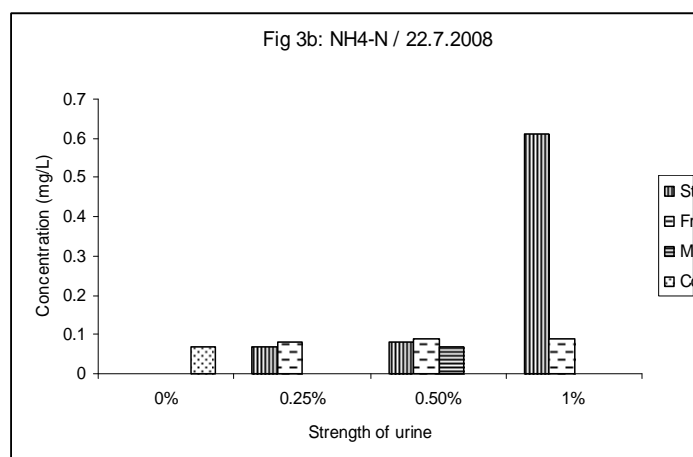
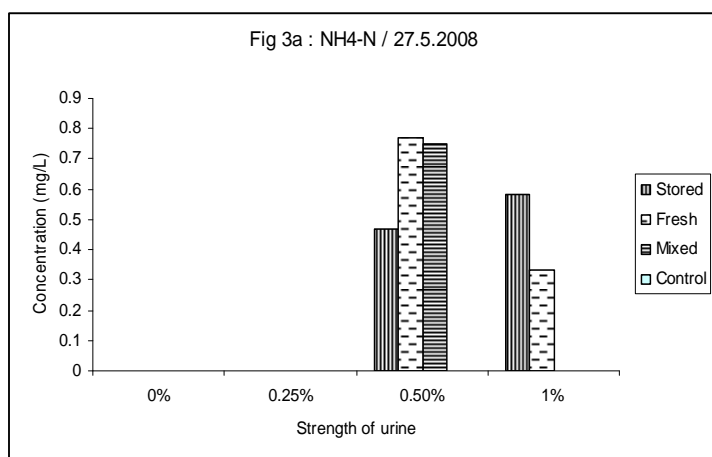


Fig 3. Variability of ammonia on two dates

2.2.4 Nitrite- N

The concentration of nitrite-N was markedly higher in stored urine than in fresh urine in all the three doses of urine tested. Differences between fresh and stored urine tended to become less as the culture period progressed at least in case of low and medium doses (Fig. 4).

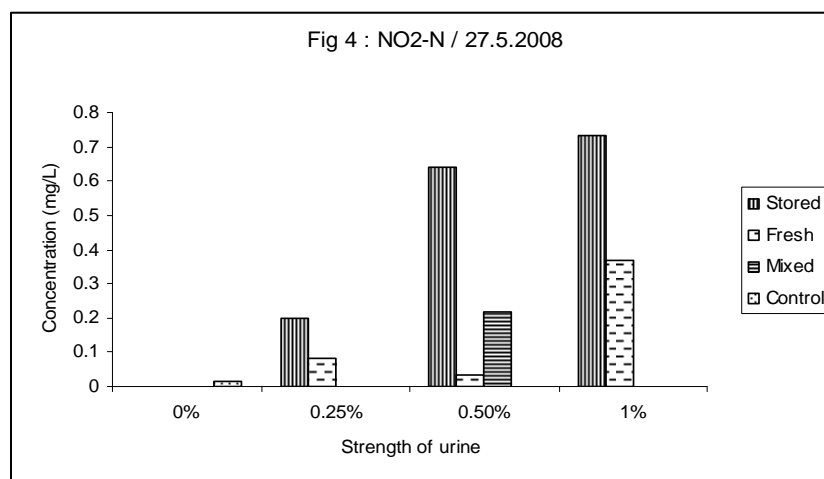


Fig 4. Variability of nitrite in urine-fed treatments

2.2.5 Nitrate-N

The dose dependent responses of nitrate were different between fresh and stored urine treatments. While there was steady rising trend of nitrate from 0.25% to 1% in the stored urine, the nitrate concentration increased from 0.25 to 0.50%, but showed sharp decline in concentration with further increase in urine dose. There was no difference in the concentration of nitrate between fresh and stored urine at 0.25% on day 10 of culture (Fig. 5).

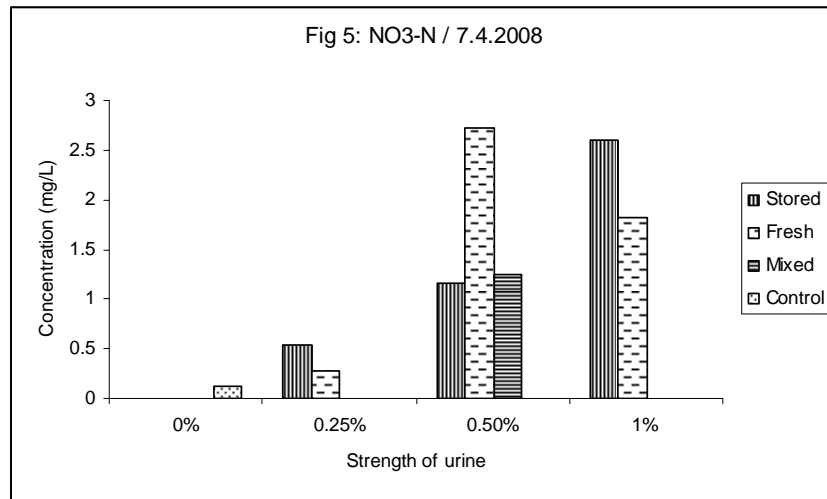
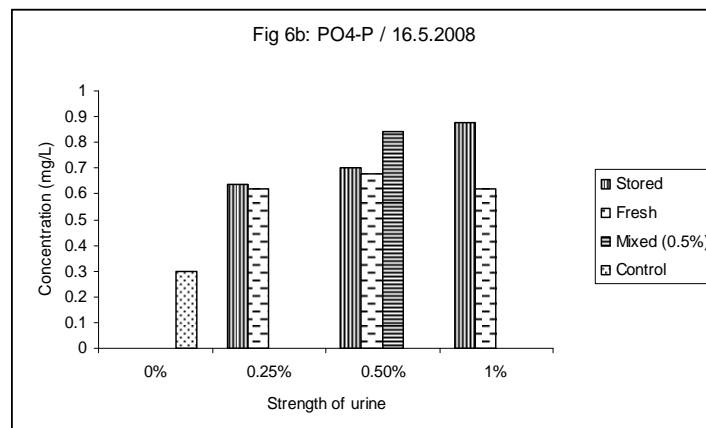
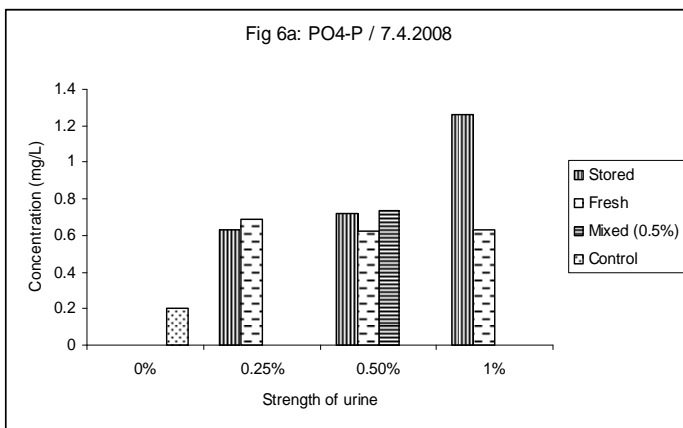


Fig 5. Variability of nitrate in urine-fed treatments

2.2.6 Phosphate

The time scale variability of phosphate remained same in both fresh and stored urine treatments though its concentration was lower in the former than the latter during early phase, but the values in the latter exceeded the former after day 56 of treatment. The situation was found to be true in all the three doses of urine tested (Fig. 6).



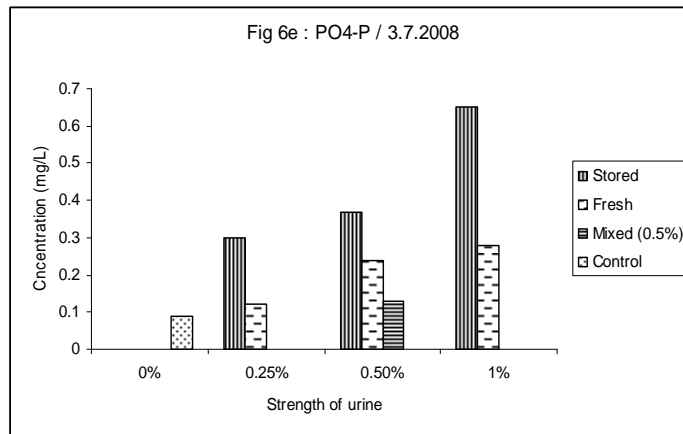
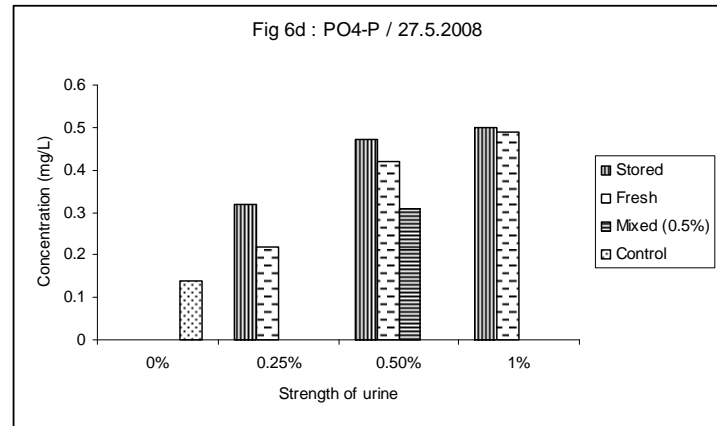
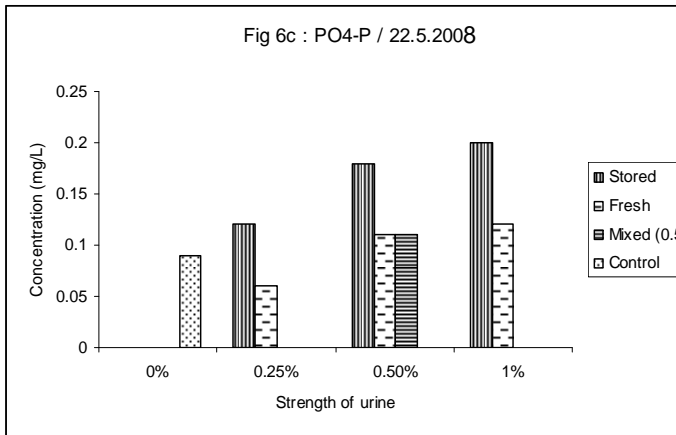


Fig 6. Variation of phosphate in different dates in urine fed treatments.

2.2.7 Nitrate-N/ phosphate-P

Though the N/P ratio of water did not differ between fresh and stored urine at 0.25% doses of human urine, the N/P ratio in the former remained higher than the later at 0.5%. The situation, however, changed at 1% with N/P ratio of stored urine being higher than the fresh urine. This shows a clear cut interaction between the dose and the age of urine (Fig. 7).

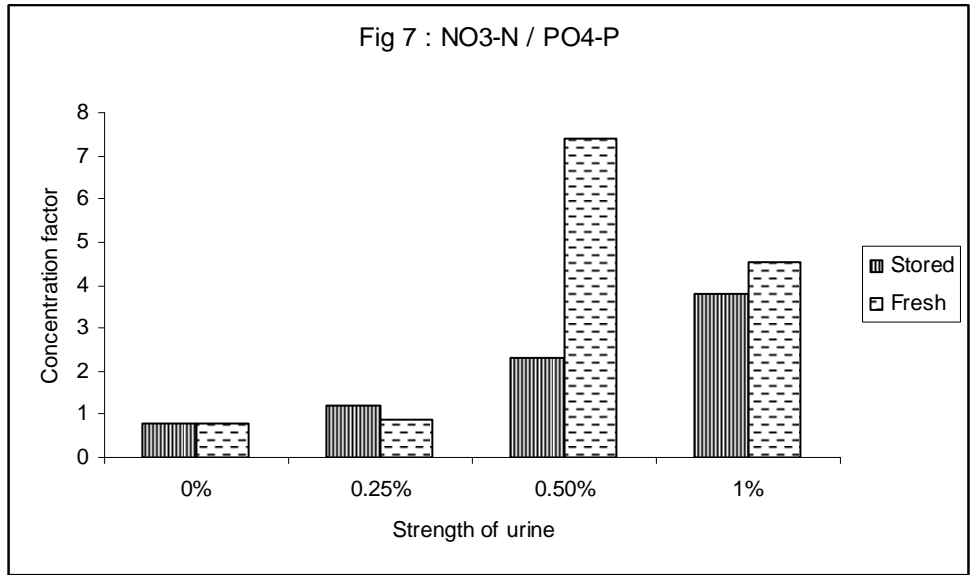


Fig 7. Variability of Nitrate-N/ phosphate-P in urine-fed treatments

2.2.8 Primary productivity of phytoplankton

The NPP tended to rise with increase in the dose of urine in fresh urine (PLATE -!C). Similar trend was also observed in case of stored urine till 0.5%, but a clear cut decline was observed at 1% dose of stored urine. This indicates that high dose of human urine was inhibitory to phytoplankton growth (Fig. 8) .



Plate -1C Urine fed treatment (1%) showing algal bloom

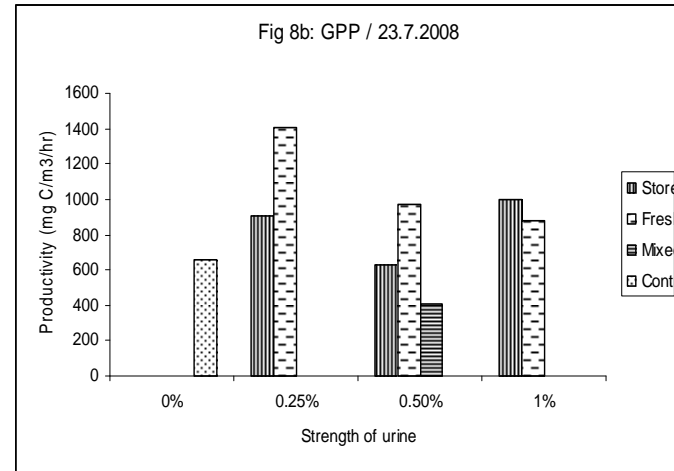
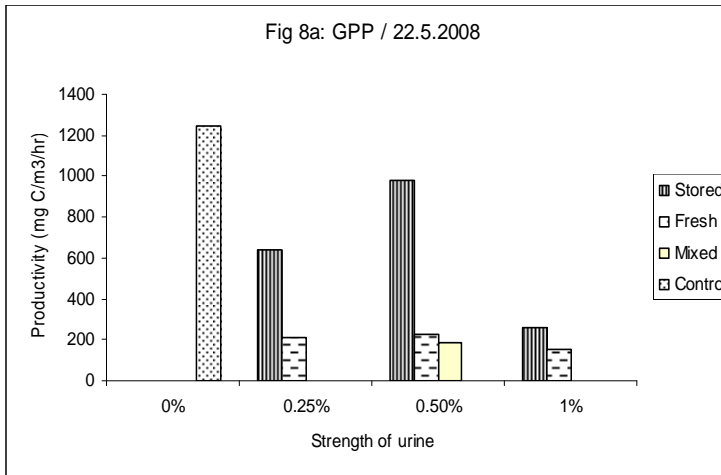


Fig 8. Variability of gross primary productivity of phytoplankton on two dates

2.2.9 Fish growth

The number of fishes harvested after the termination of culture experiment was quite low indicating high mortality in different treatments. However, there was also no indication of treatment specific mortality. This might be due to bird predation or loss due to some other factors not recorded so far.

2.3 Discussion

Dose and time dependent variability of phosphate were markedly different fresh and stored human urine. The amount of phosphate was higher in the fresh urine than in stored urine on some occasions especially during the early phase, but the superiority of the latter was maintained in majority of the cases especially during the later part of the study. Nevertheless, the overall mean concentration of phosphate hardly differed between fresh and stored urine especially in the higher doses. Thus there was clear cut interaction between time and age of urine examined.

Higher values of EC in the fresh urine was indicative of greater amount of nutrient levels than the stored urine. It is due to microbial degradation of stored urine over time. The variability of NPP was inversely related to the N/P ratio of water in different treatments. The lowest values of N/P ratio in the stored urine treatment (0.5%) induced the NPP and vice versa. Similar was the result with 1% urine fed treatment.

2.4 Conclusions

It is premature to conclude the growth performance of fish from the present experiment. Further, experiments are necessary for a definite conclusion.

3.0 Experiment - 2 Comparing the fertilizer values of fresh human urine under different treatment conditions.

3.1 Design

The purpose of the study was to compare the fertilizer value of human urine with that of cattle dung while keeping the nitrogen and phosphorus levels of mixed treatments similar to that of urine fed treatment. Eighteen outdoor tanks of 4500 l capacity (PLATE 2A) were used for the present study. The tanks were allotted to six treatments in triplicate. The treatments were (i) human urine under aerated and (ii) non aerated conditions, (iii) cattle dung alone, mixed treatment of cattle dung and human urine grouped as (iv) iso nitrogenous and (v) iso-phosphorus conditions, and (vi) control. The tanks filled with ground water without the input of fertilizer were used as controls. Iso-nitrogenous or iso-phosphorus conditions of mixed treatments were created by mixing both human urine and cattle dung in such proportions that nitrogen and phosphorus level of mixed treatment remained the same as that of human urine. The selected human urine or cattle dung was applied every week (PLATE 2 B – C). Human urine was added at the rate of 0.01% and cattle dung was applied at the rate of kg/ tank.



Plate – 2A : Overview of experimental unit



Plate – 2B : Measuring of urine sample



Plate – 2C : Application of urine

3.1.1 Protocol

The selected outdoor tanks were prepared for experiment by thorough cleaning, drying and then filling with ground water. The tanks were allowed to stand for few days for establishment. Human urine was added to the tank at the rate of 0.1% every week. Likewise, tanks allotted for other treatments were also added with cattle dung or cattle dung mixed with human urine.

Fry of common carp (2.5 g) , rohu (4.5 g), mrigal (5.0 g) bata (1.5 g) punti (3 g) tilapia (2 g) and freshwater prawn (1 g) were procured from local sources and acclimated for few days under outdoor conditions. The fishes were then introduced into the culture tanks on May 30, 2008. Twenty fish and 2 prawns were stocked in each tank following the numbers as common carp –4, rohu – 3, mrigal – 4, bata – 3, punti – 2, tilapia – 4 prawn – 2.

3.1.2 Collection

Samples of water were collected from each of the 21 experimental tanks every week for monitoring water quality parameters (PLATE 2 D), plankton (PLATE-2 E) and bacterial loads (PLATE 2 F) following the standard protocols as described in the APHA (1995). Live fishes and prawn were caught (PLATE 2 G - H) from each tank at periodical intervals for determination of their growth increments.



Plate – 2D : Analysis of water quality



Plate – 2E : Collection of plankton



Plate – 2F : Plating for HB and EMB bacterial load



Plate – 2G : Netting operation for recording fish growth



Plate – 2H : The harvested drawn in urine fed treatment

3. 2 Results

3.2.1 Hydrogen ion concentration

There was no marked differences of pH among all the urine fed treatments . The values were highly alkaline being ranging from 9.2 to 9.36. As a result of aeration, there has been slight decline in the values of pH (9.2) compared to direct urine fed treatment (9.36). The water pH in the cattle dung treatment without input of human urine remained considerably low (8.62) throughout (Fig 9). There was progressive rise in the values of pH over the culture period in the urine fed treatment.

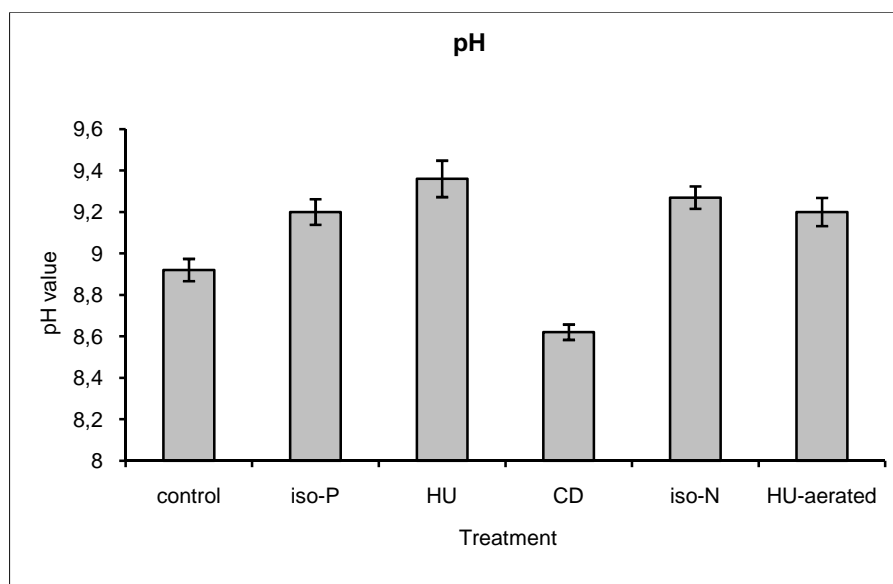


Fig. 9. Variability of water pH in different treatments

3.2.2 Total Alkalinity

Total alkalinity of water showed more than 4- fold variations ranging from 60 to 258 mg/l in different treatments employed. The mean concentration remained consistently high in the CD (136.04 mg/l) or CD-urine mixed treatment under isonitrogenous condition (129.83 mg/l). This was followed by mixed iso-phosphorus treatment (111.75 mg/l). Aeration resulted in reduction in the values of alkalinity to 100.41 mg/l which was even lower than the control(110.58 mg/l). In general, a declining trend was noticed over the culture period (Fig 10).

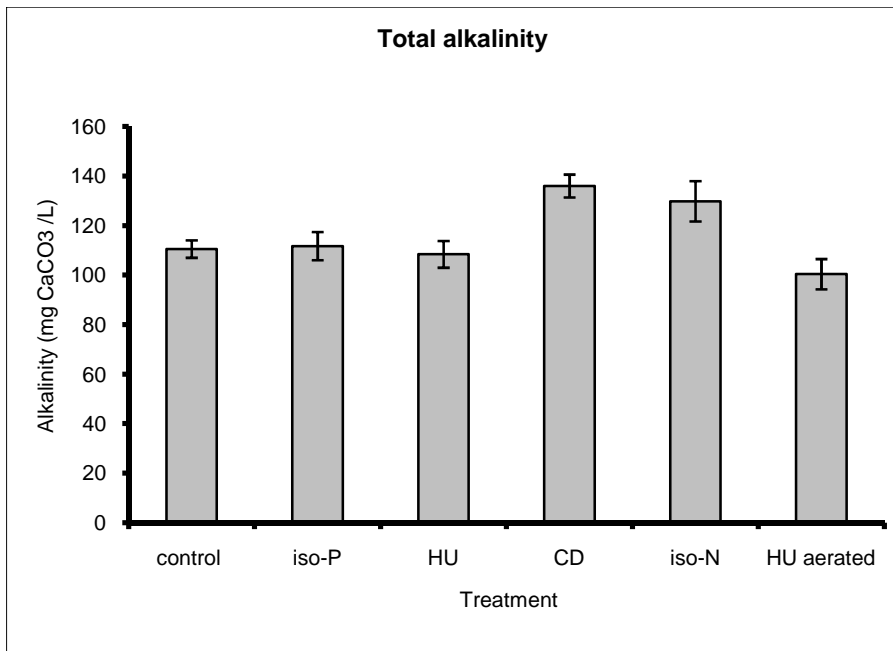


Fig. 10 . Variability of total alkalinity of water in different treatments employed

3.2.3 Electrical conductivity

The electrical conductivity reflecting the total ionic strength of the treatment ranged from 90 to 320 $\mu\text{S}/\text{cm}$ in different treatments employed. The mean values were highest in the CD (242.50 $\mu\text{S}/\text{cm}$) followed by iso-nitrogenous treatment created by mixing human urine and cattle dung (170.49 $\mu\text{S}/\text{cm}$), iso-phosphorus condition (166.10 $\mu\text{S}/\text{cm}$), aerated (154.15 $\mu\text{S}/\text{cm}$) or non aerated (152.56 $\mu\text{S}/\text{cm}$) urine fed treatment. The mean value in the control (133.64 $\mu\text{S}/\text{cm}$) was the lowest of all (Fig.11). This indicates that total ionic strength was moderate in urine fed treatment. There was a downward trend in the values of electrical conductivity over the culture period (Fig. 11).

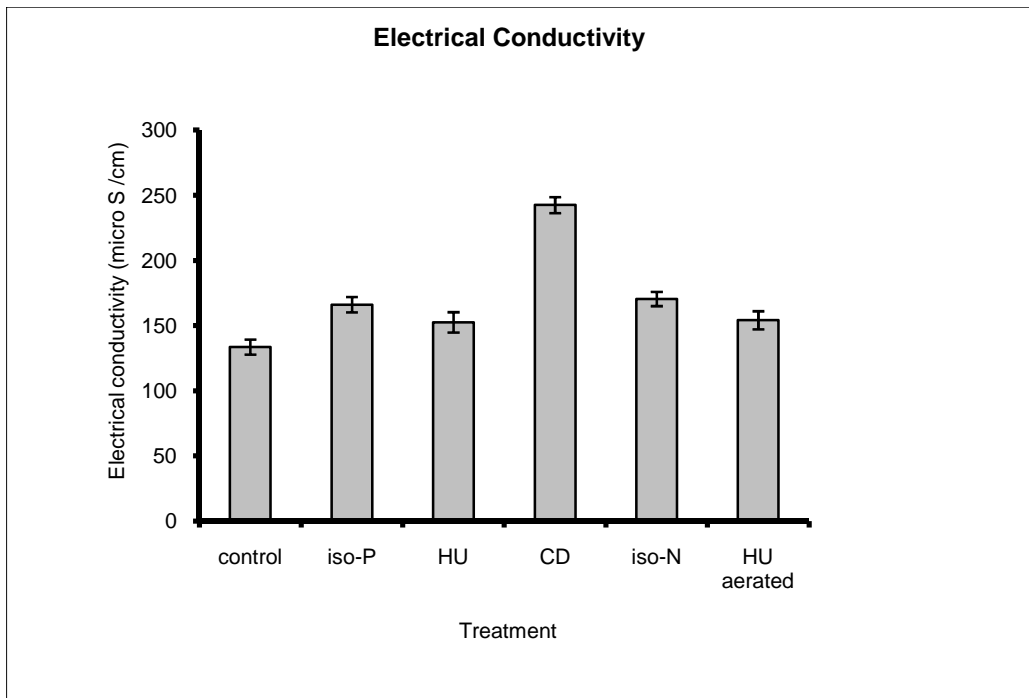


Fig. 11 : Variability of electrical conductivity of water in different treatments

3.2.4 Dissolved Oxygen

The concentrations of DO remained well within the values recommended for fish culture in all the treatments. The overall mean values ranged from 7.59 to 10.72 mg/l in different treatments employed (Fig.12). The mean concentration of DO did not differ much from one treatment to another. For example, DO level was almost similar in the CD treatment (10.72 mg/l) and aerated urine fed treatment (10.09 mg/l). Differences between the mixed treatment with iso-phosphorus (9.89 mg/l) and iso nitrogenous (9.36 mg/l) treatments are minor. No definite trend of variations was noticed during the culture period (Fig. 12).

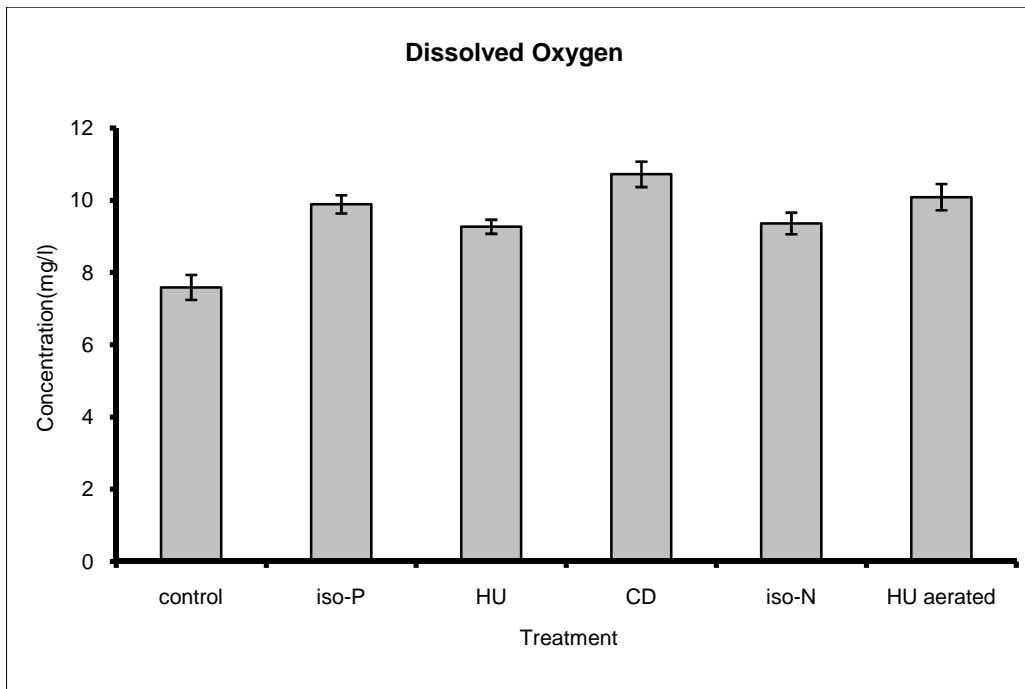


Fig. 12: Variability of Dissolved Oxygen of water in different treatments

3.2.5 Ammonium – N

The concentration of ammonia-N ranged from nil to 0.350 mg/l in different treatments employed. The concentration of ammonia-N was maximum in the direct urine fed treatment (0.159 mg/l) and minimum in the control (0.021 mg/l). Aeration in the urine fed treatment (0.103 mg/l) resulted in substantial reduction than direct urine fed treatment. The mean concentration of ammonia-N did not differ amongst the CD (0.0564 mg/l) and mixed treatment with iso-phosphorus (0.0567 mg/l) or iso-nitrogenous state (0.0564 mg/l) (Fig. 13). The concentration of ammonia tended to decline as the period of culture progressed.

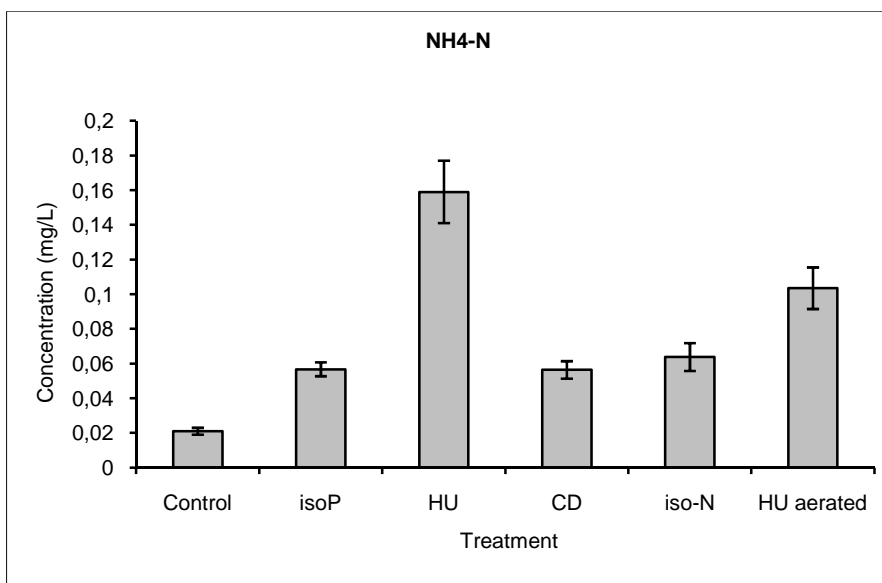


Fig. 13. Variability of ammonium concentration of water in different treatments

3.2.6 Nitrite –N

The variability in the concentration of nitrite-N was 3 orders of magnitude among the treatments ranging from 0.006 to 0.216 mg/l. As expected, aeration caused substantial rise in the mean concentration of nitrite (0.0939 mg/l) compared to its counterparts without aeration (0.0593 mg/l). There was hardly any difference in the values of nitrite between the iso-nitrogenous (0.0565 mg/l) and iso-phosphorus (0.0535 mg/l) conditions. The mean concentration of nitrite in the CD treatment (0.0664 mg/l) was higher than in the cattle dung treatment. (Fig. 14). Changes in the concentration of nitrite-N over the time were not clear cut.

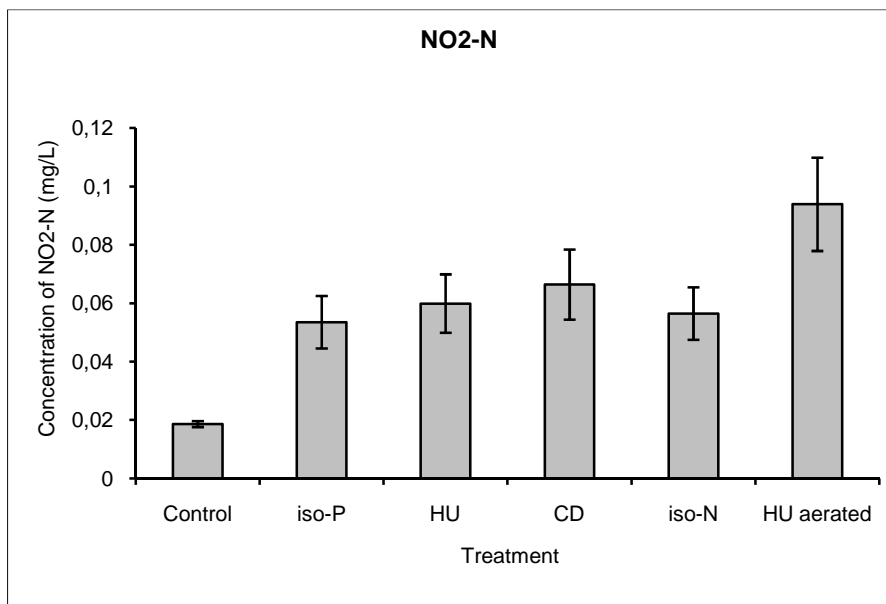


Fig. 14. Variability of nitrite-N concentration of water in different treatments

3.2.7 Nitrate – N

As expected, aeration in the human urine (0.230 mg/l) caused the mean concentration of nitrate to be 5 order of magnitude higher than the mean value observed in urine fed treatment (0.0456 mg/l). The amount of nitrate was markedly higher in the iso-nitrogenous treatment (0.053 mg/l) than in the iso-phosphorus treatment (0.0123 mg/l) (Fig. 15). There was no definite trend in the nitrate values during the culture period.

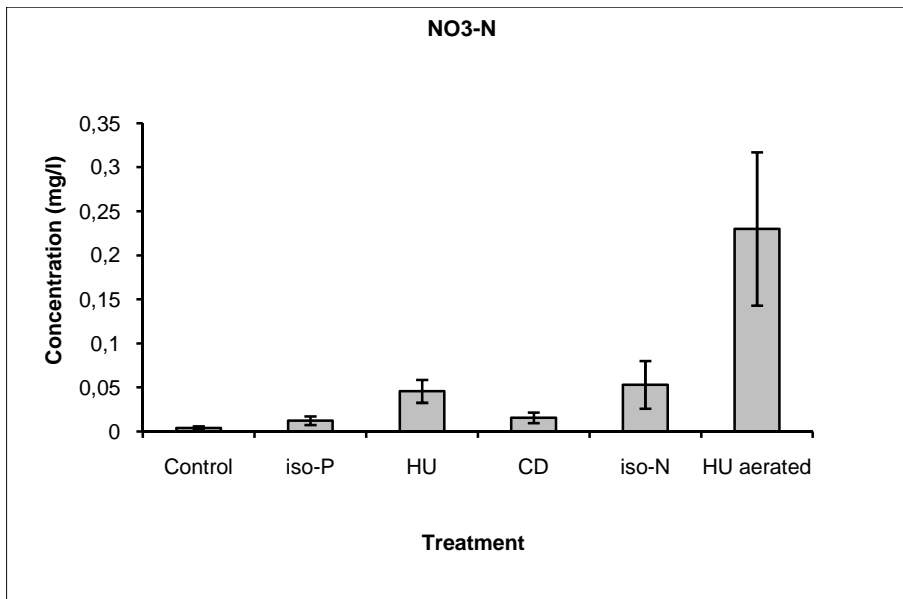


Fig. 15. Variability of nitrate concentration of water in different treatments

3.2.8 Phosphate – P

The concentration of phosphate showed more than one order of magnitude variations ranging from 0.04 to 0.54 mg/l in different treatments employed. The amount of phosphate recorded in the mixed treatment with iso-nitrogenous condition (0.219 mg/l) was higher than the concentration (0.137 mg/l) observed in human urine treatment. The values observed in the aerated human urine treatment (0.1672 mg/l) remained in between two treatments. There was no difference in the concentrations of phosphate in the remaining treatments being ranging from 0.077 mg/l to 0.152 mg/l (Fig 16)

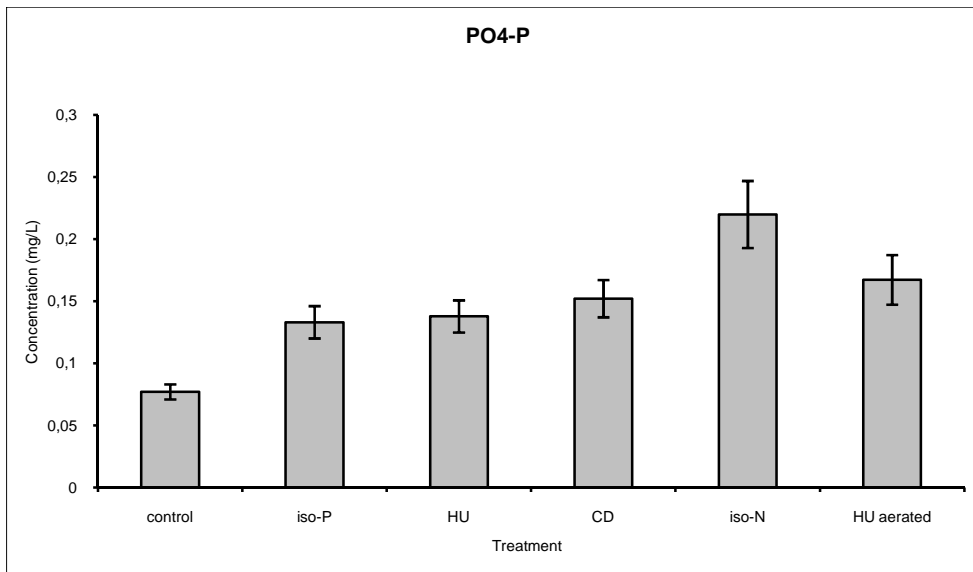


Fig. 16 Variability of phosphate concentration of water in different treatments

3.2.9 Nitrate-N/ Phosphate -P ratio

The ratio of nitrate/phosphate was maximum in human urine aerated treatment (1.3755) followed by direct human fed treatment (0.3311), iso nitrogenous condition (0.2411), CD (0.1019), iso-phosphorus state (0.0924) and control (0.0519) (Fig.17).

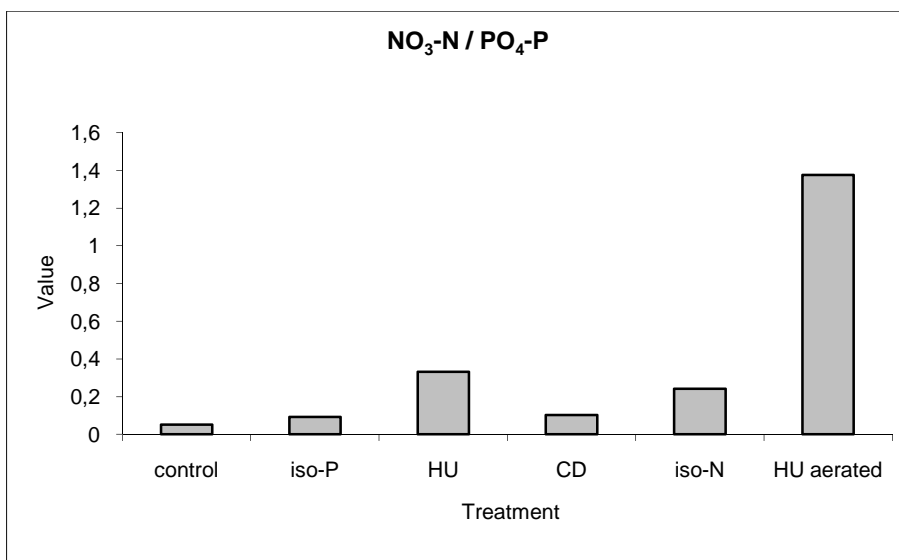


Fig. 17: Variability of nitrate -N phosphate-P ratio in different treatments

3.2.10 Primary productivity of phytoplankton

The overall mean values of GPP was maximum in the cattle dung treatment (751.20 mg C /m²/hr) followed by human urine (601.8 mg C /m²/hr), aerated human urine (525.5

mg C /m²/hr), mixed iso-nitrogenous condition (492.40 mg C /m²/hr), iso phosphorus (402.8 mg C /m²/hr) state and control (251.8 mg C /m²/hr) . (Fig.18) .

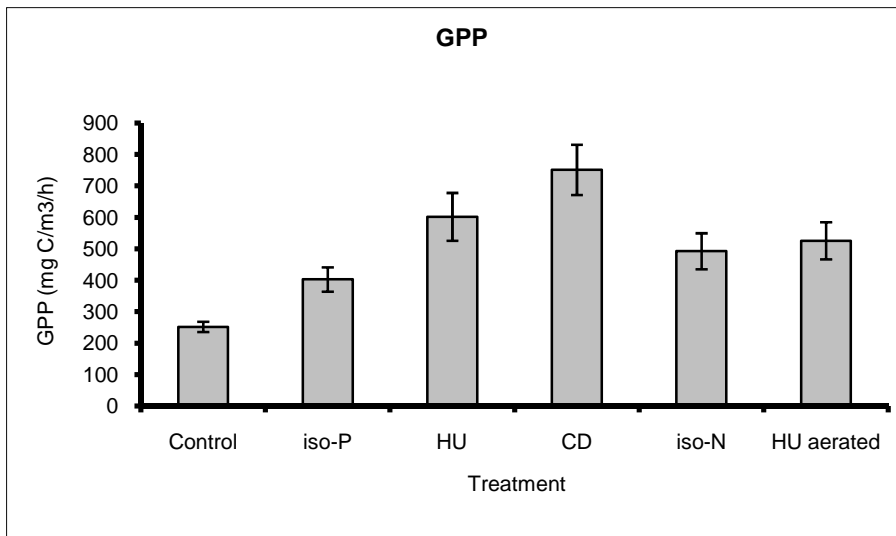


Fig. 18: Variability Gross Primary Productivity (GPP) in different treatments

The NPP was also maximum in the CD (544. 95 mg C /m²/hr) followed by human urine (498 mg C /m²/hr) and aerated urine (399 mg C /m²/hr) fed treatment (Fig. 19).

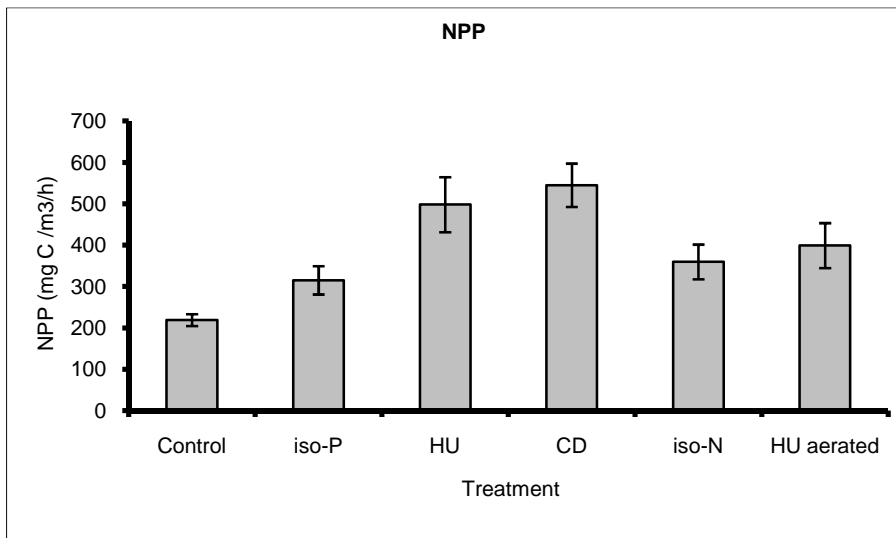


Fig. 19: Variability Net Primary Productivity (NPP) in different treatments

The increased values of CR in the cattle dung treatment (142.1 mg C /m²/hr) were due to greater abundance of heterotrophic bacteria in that system. The lowest value was recorded in the control (28.75 mg C /m²/hr) (Fig. 20).

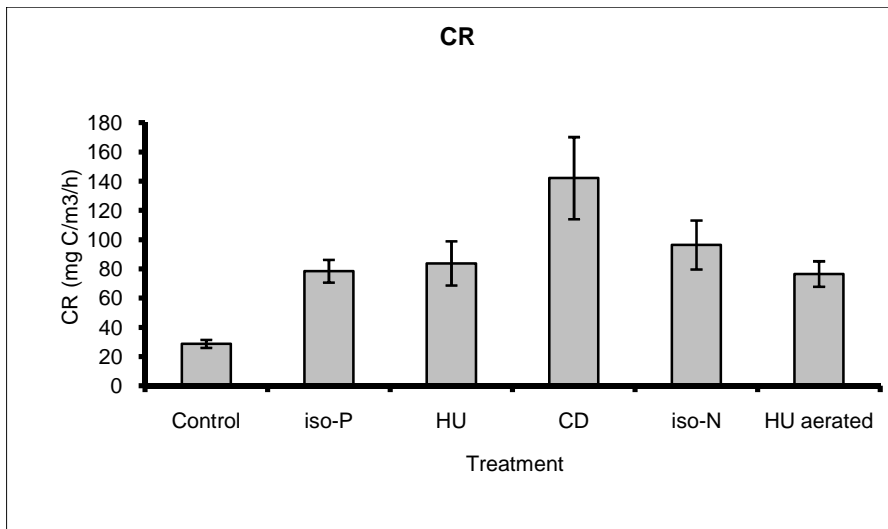


Fig. 20: Variability Community Respiration (CR) in different treatments

3.2.11 Bacterial loads

The overall mean count of heterotrophic bacteria enumerated in cattle dung ($110.69 \times 10^3/\text{ml}$) treatment was the highest of all. This was followed by iso nitrogenous condition $39.76 \times 10^3/\text{ml}$, urine aerated treatment ($25.69 \times 10^3/\text{ml}$), iso phosphorus conditions ($25.15 \times 10^3/\text{ml}$). The counts enumerated in the direct urine fed treatment ($15.23 \times 10^3/\text{ml}$) were almost twice that of control ($8.15 \times 10^3/\text{ml}$) (Fig. 21).

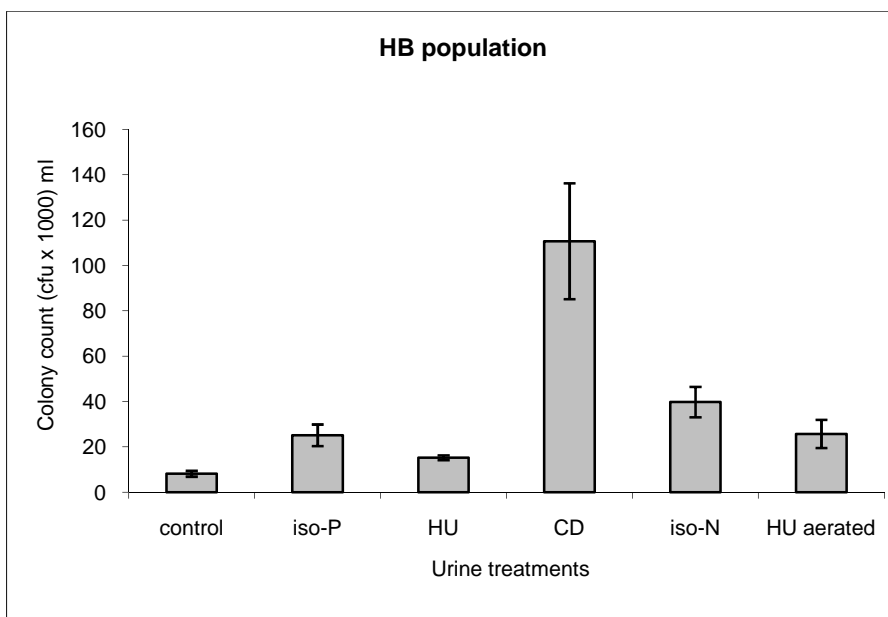


Fig.21 : The overall mean count of Heterotrophic Bacteria (HB) in different treatments employed.

The counts of EMP media grown colonies with black pigment varied between 3 to 208 /ml in different treatments employed. The overall mean count of *E. coli* grown in EMB agar medium was maximum in the cattle dung treatment (60/ml) followed by iso nitrogenous condition (39.85/ml) The counts recorded in the iso phosphorus condition (25.85 /ml) was higher than that of direct urine fed treatment (20.54/ml) and minimal in the control (10.76/ml). There were no difference in the counts of EMB grown *E. coli* between aerated urine treatment (11.54/ml) and the control (10.76/ml) (Fig.22).

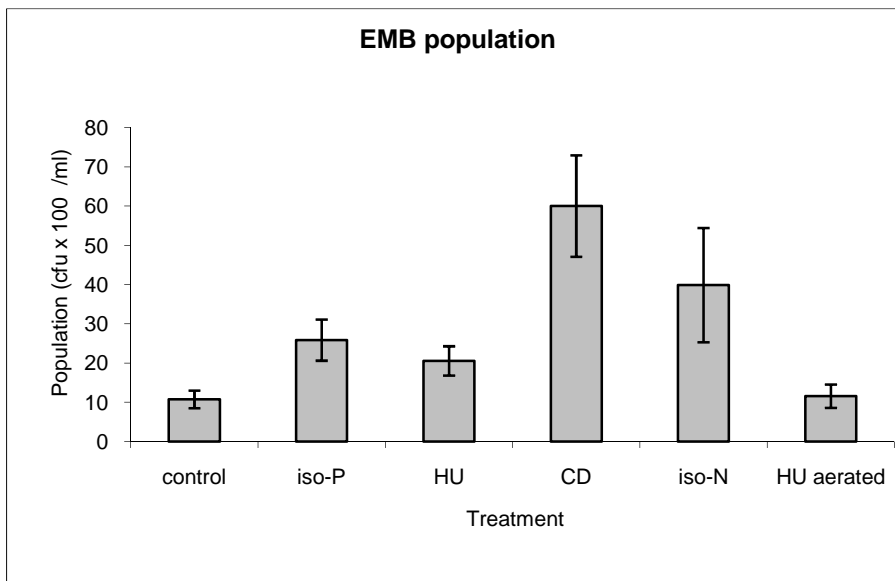


Fig 22: The overall mean count of EMB media grown colonies with black pigment in different treatments employed

3.2.12 Fish yield

The total fish yield in the culture tank was found to be maximum in the CD treatment (622 g/ tank) followed by mixed treatment with iso-nitrogenous condition (424 g/tank). The yield recorded in the direct urine fed treatment (319 g/tank) ranked third among all the treatments which was almost similar to that of treatment with iso-phosphorus (333 g/tank) state. The yield observed in the control (175 g/tank) was the lowest of all (Fig. 23).

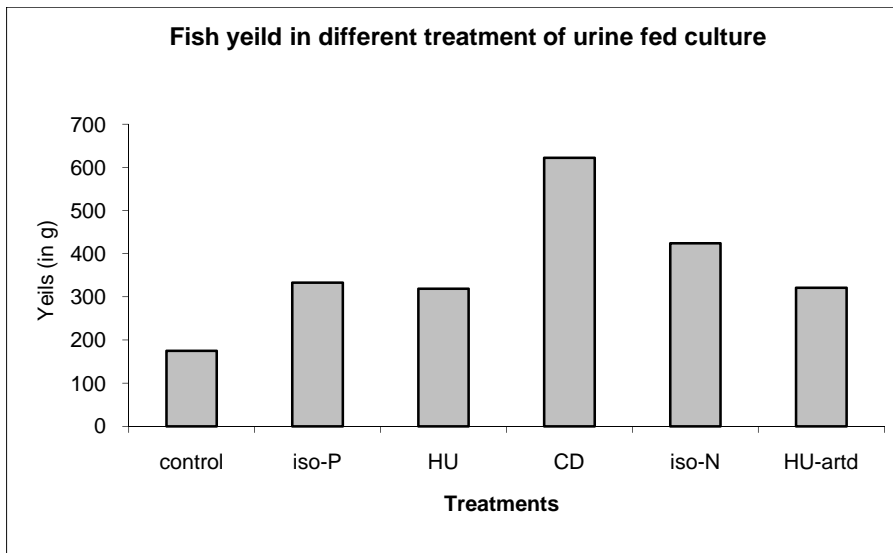


Fig. 23: Variability of fish weight in different treatments employed

3.2.13 Discussion

The results of the study indicated that fish culture is quite possible in the urine fed culture system. Its use in aquaculture is highly profitable because its direct discharge into the environment or through sewage would cause pollution in the environment. Because of its enormous nutrient potential, it has double benefit by the way of its bio conversion into fish wealth and at the same time preventing the pollution of the environment by avoiding direct discharge of human waste into the environment.

Of all the treatments tested, fish yield in the direct human urine fed treatment ranked second. The yield is less than CD, but significant higher than control. Though the yield was expected to be higher in the aerated urine fed treatment than the other urine fed treatments, the yield was up to the expectation due the less duration of aeration treatment.

It may be concluded that use of human urine in fish culture may be promoted taking proper care for avoiding health hazards of fish. The results of the present bacteriological study showed that the contamination load of E. coli in the culture tank with human urine was even less than the control. Though further bacteriological studies are highly essential to ascertain more details of the health risk, the overall assessment indicated positive results for its use in aquaculture in recommended doses.

4.0 Experiment -3: Comparing the fertilizer value of stored and fresh urine charged with double strength at longer intervals

4.1 Design

The next trial was conducted in the outdoor tanks (4500 l capacity) with a view to comparing the fertilizer value of stored and fresh urine when applied in double strengths at longer intervals. The properly cleaned and dried tanks were filled with ground water. Both fresh and stored urine was applied at 0.02% concentrations. The stored urine was months old. The treatments used were: (i) fresh human urine (ii) stored human urine and (iii) mixed treatment of fresh and stored human urine. Both fresh and stored urine was applied in the tanks every two weeks.

Fry of common carp (2.5 g), rohu (4.5 g), mrigal (5.0 g) bata (1.5 g) punti (3 g) tilapia (2 g) and freshwater prawn (1 g) were procured from local sources and were then introduced into the culture tanks.

4.1.1 Collection and examination procedures

Samples of water were collected from each of the 24 experimental tanks at regular intervals for monitoring water quality parameters following the standard protocols described in the APHA (1995). Standard protocols were also used to enumerate heterotrophic and E. coli populations. Live fishes from each tank were caught at periodical intervals for determination of their growth increments.

4.2 Results

4.2.1 Hydrogen ion concentration

Water pH did not differ much from one treatment to the other with values ranging from 8.65 in the control to 8.98 in the fresh urine fed treatment, which again did not differ from stored urine fed treatment (Fig. 24). The pH values in the fresh + mixed treatment was 8.54.

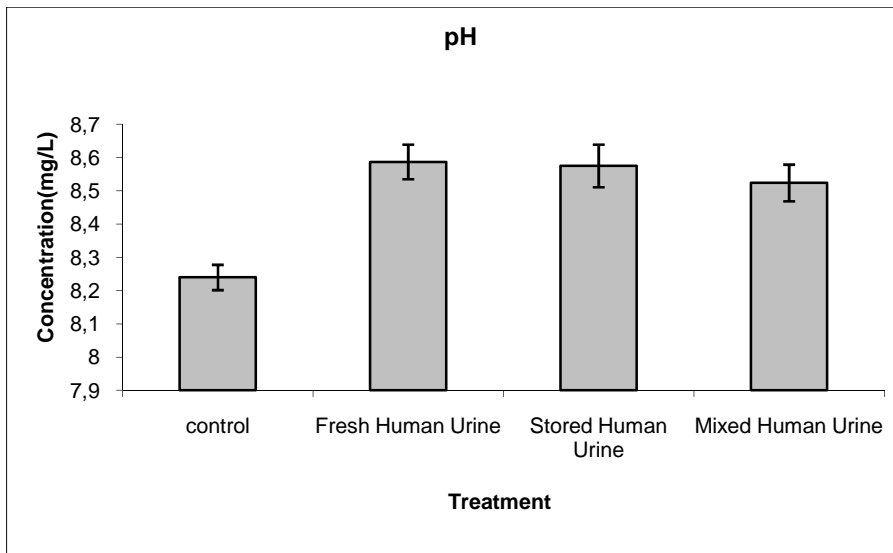


Fig. 24: Variability of pH of water in different treatments

4.2.2 Total alkalinity

Total alkalinity was maximal in the stored urine fed treatment (157.0 mg/l) followed by fresh urine fed treatment (132.66 mg/l), mixed treatment with fresh and stored urine (128.44 mg/l) and control (81.94 mg/l) (Fig.25).

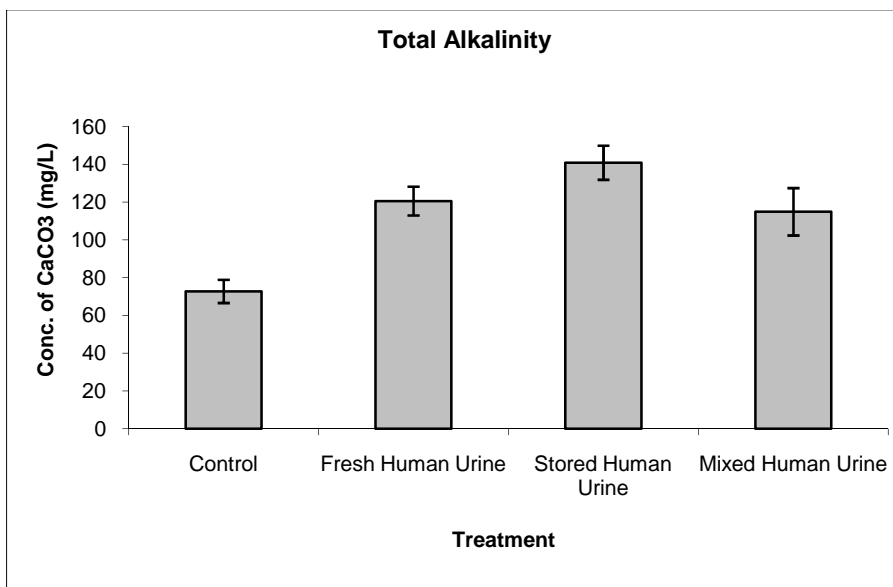


Fig. 25: Variability of total alkalinity of water in urine-fed treatments

4.2.3 Electrical conductivity

The values of EC were higher in the stored urine fed treatment (226.62 $\mu\text{S}/\text{cm}$) followed by fresh (203.80 $\mu\text{S}/\text{cm}$) or stored (200.27 $\mu\text{S}/\text{cm}$) urine fed treatments showing no differences from each other. The values in control remained the lowest (120 $\mu\text{S}/\text{cm}$) of all (Fig. 26).

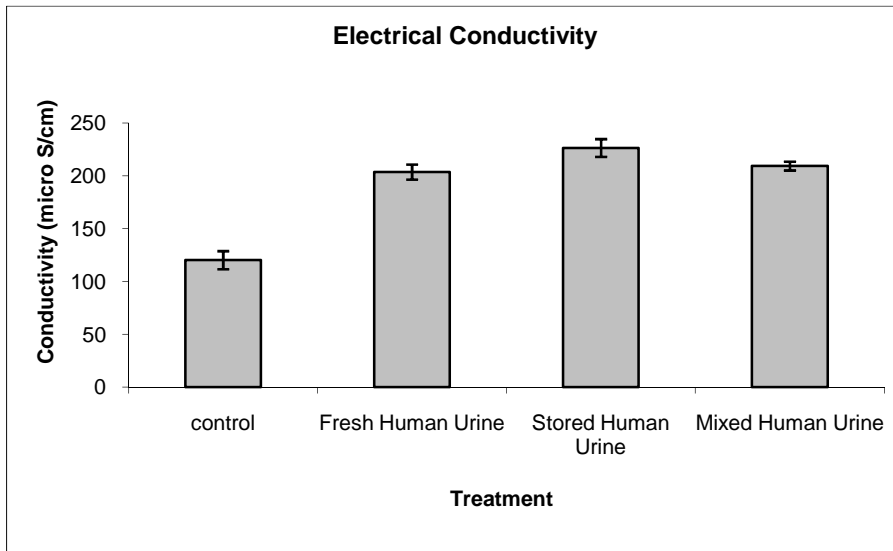


Fig. 26. Variability of electrical conductivity of water in urine-fed treatments

4.2. 4. Dissolved Oxygen

The concentration of dissolved oxygen of water remained quite well within the range for fish growth, being varying between 6.4 and 12.8 mg/l) in different treatments employed (Fig. 27).

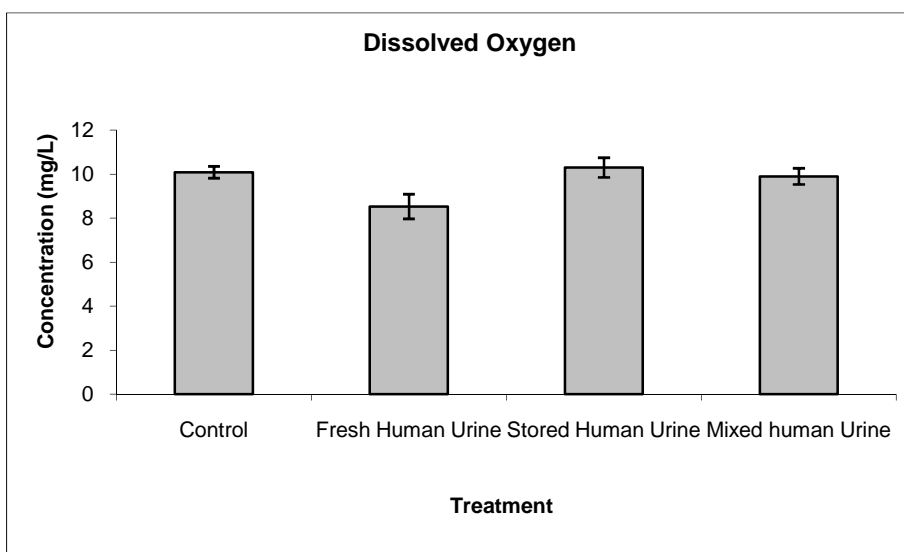


Fig. 27: Variability of dissolved oxygen of water in urine-fed treatments

4.2.5 Ammonium-N

The overall mean concentration of ammonia in the fresh urine fed treatment (0.2447 mg/l) was markedly higher than stored urine (0.080 mg/l) or mixed treatment with fresh and stored urine (0.0759 mg/l). The concentration observed in the control was the lowest (0.0247 mg/l) of all (Fig. 28).

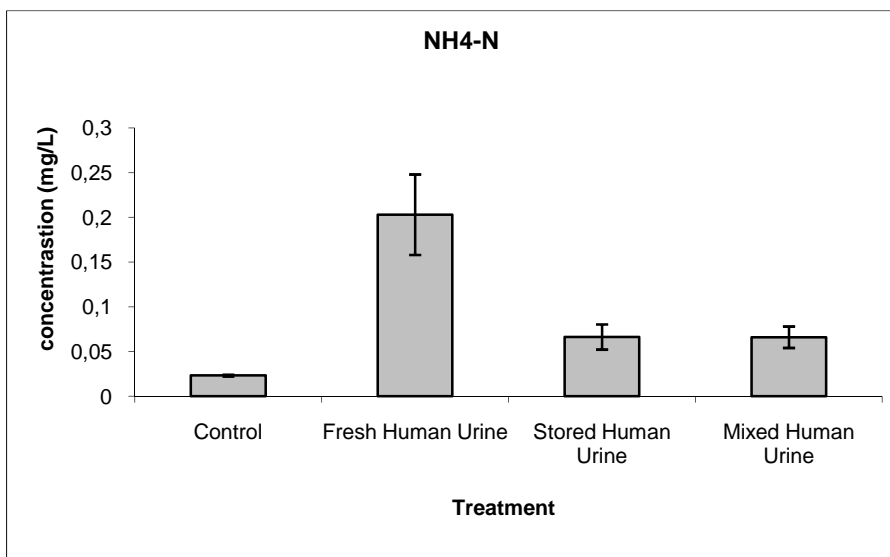


Fig. 28: Variability of ammonia concentration of water in urine-fed treatments

4.2.6 Nitrite - N

The amount of nitrite observed in the fresh urine fed treatment (0.0629 mg/l) was about 30% higher than that observed in stored urine fed treatment (0.0483 mg/l). The values recorded in the mixed treatment of fresh and stored urine (0.0412 mg/l) remained in between (Fig.29).

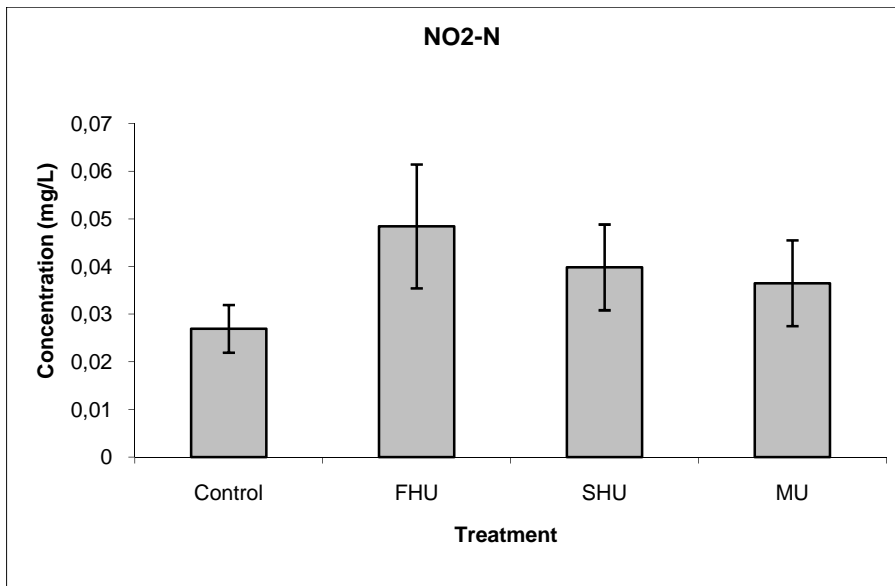


Fig. 29: Variability of nitrate concentration of water in urine-fed treatments

4.2.7 Nitrate – N

The concentration on nitrate ranged from 0 to 0.472 mg/l in different treatments employed. The mean concentration was 26% higher in the stored urine fed treatment (0.1333 mg/l) compared to fresh urine fed treatment (0.0365 mg/l) (Fig. 30).

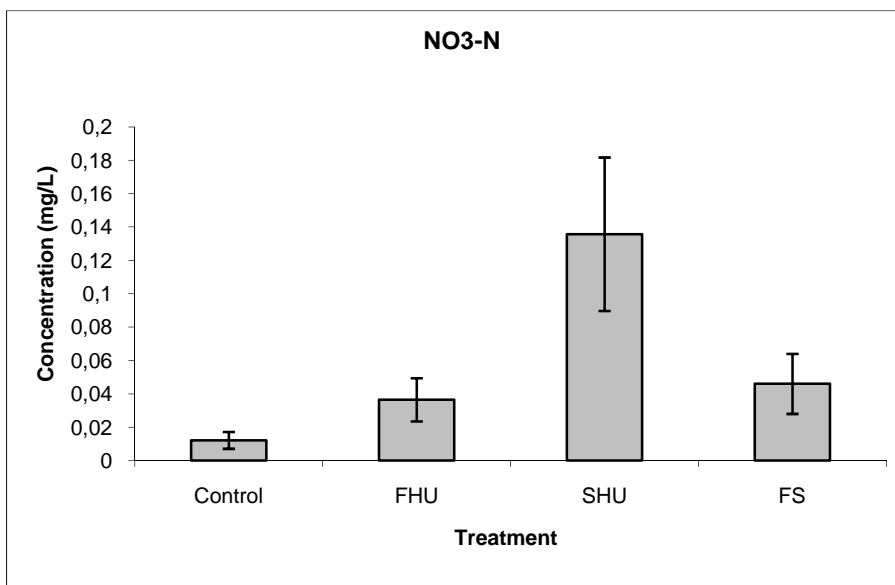


Fig. 30: Variability of nitrite concentration of water in urine-fed treatments

4.2.8 Phosphate –P

The concentration of phosphate ranged from 0.09 to 1.247 mg/l in different treatments employed. The overall mean concentration of phosphate observed in the stored urine fed treatment was as high as 157 % higher than that of fresh urine (0.1704 mg/l) fed treatments. The concentration observed in the mixed treatment (0.2813 mg/l) remained in between (Fig. 31).

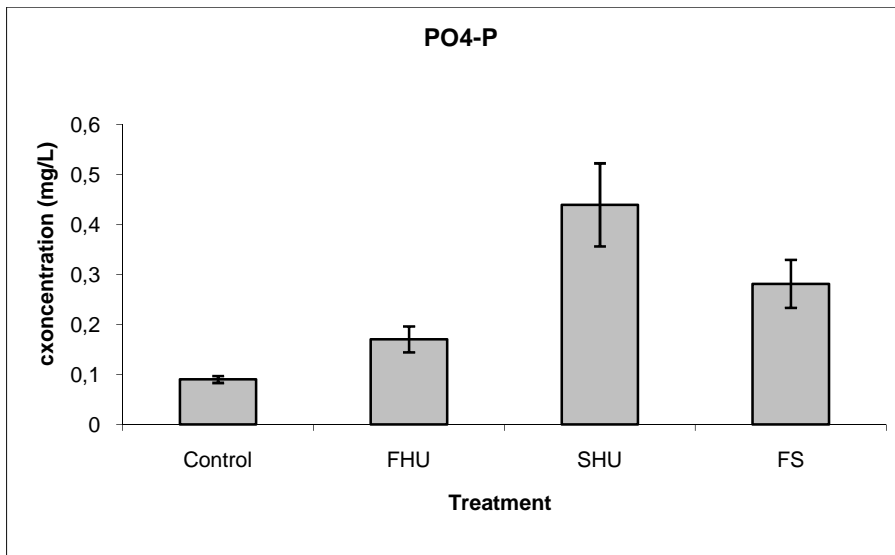


Fig. 31: Variability of phosphate concentration of water in urine-fed treatments

4.2.9 Nitrate-N/ Phosphate-P

The ratio for nitrate/phosphate was highest in stored urine (0.3034) fed treatment followed by fresh human urine (0.2142), mixed treatment (0.1635) (Fig.32)

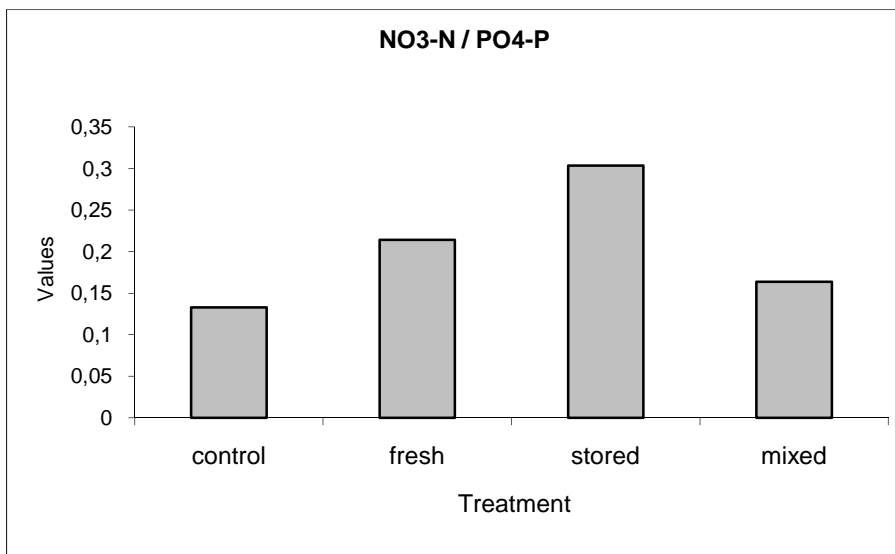


Fig. 32: Variability of nitrate-phosphate ratio in urine-fed treatments

4.3.0 Primary productivity of phytoplankton

There was clear cut difference in the values of gross primary productivity of Phytoplankton between the stored and fresh urine fed treatments. The values observed in the former was 66% higher than that of the later (303.25 mg C /m²/h). As expected, the values in the mixed treatment remained (353 mg C/m²/h) in between (Fig.33).The variability in the values of net primary productivity of phytoplankton among the treatments (0 – 520 mg C/m²/h) showed the trend similar to that of gross productivity of phytoplankton.

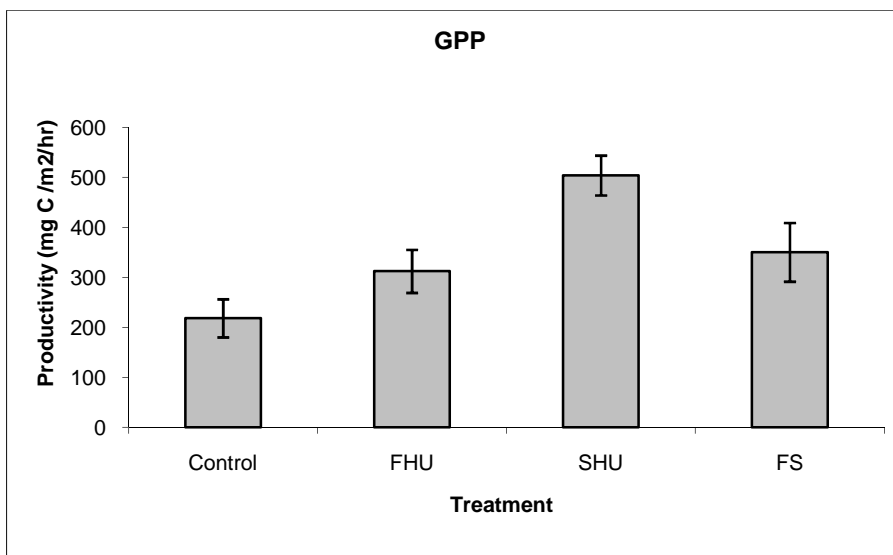


Fig.33: Variability of gross primary productivity of phytoplankton in urine-fed treatments

The CR was also higher in the stored urine fed treatment (64.66 mg C/m²/h) and lowest in the control (44.33 mg C/m²/h) (Fig 34).

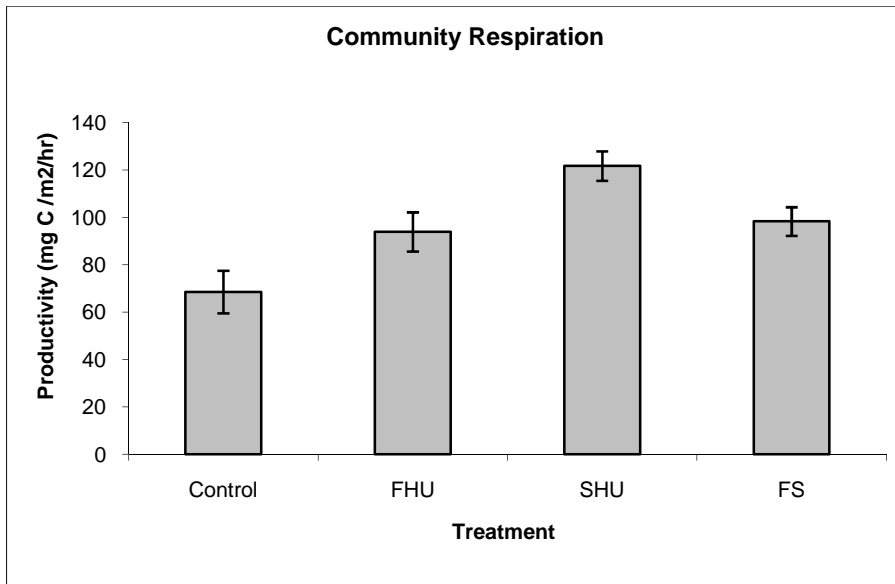


Fig.34: Variability of gross primary productivity of phytoplankton in urine-fed treatments

4.3.1 Bacterial load

The mean count of HB was variable ranging from 5 to 100×10^3 /ml in different treatments. As expected the count of HB in the stored urine treatment was as high as 58% higher than the count observed in fresh urine fed treatment. The counts in the mixed urine treatment (22×10^3 /ml) remained in between (Fig.35).

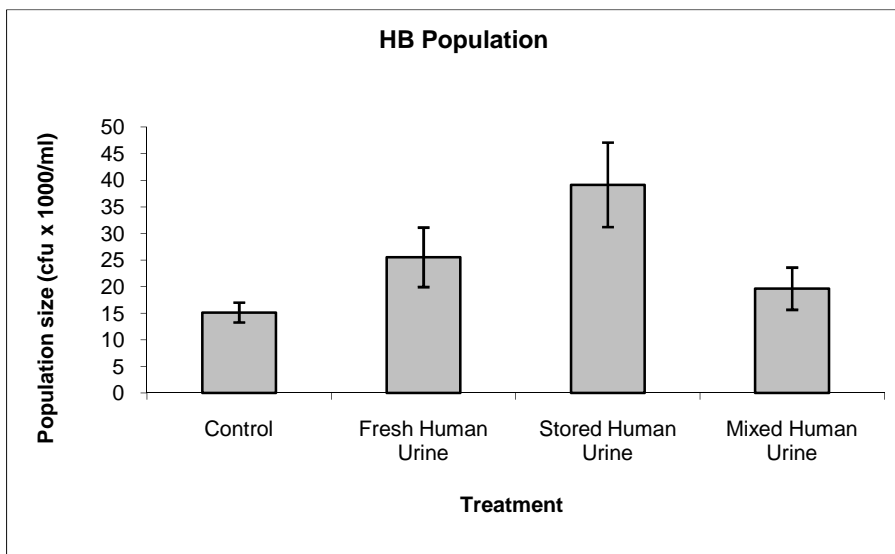


Fig. 35. Variability in the counts of heterotrophic bacterial population in different treatments employed.

The EMB media grown *E. coli* with black pigment ranged from 1 to 50/ml in different treatments employed (Fig 36). Among the treatments, mixed and fresh urine fed treatment showed the maximal and minimal counts of bacteria respectively.

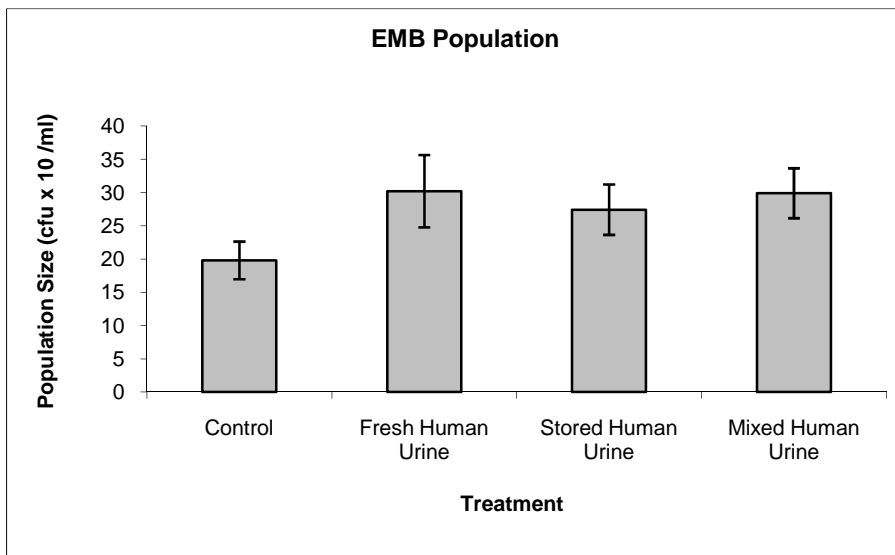


Fig. 36. Variability of EMB population in different treatments

4.3.2 Fish yield

Among the treatments, fish yield observed in the stored urine fed treatment (839 g/tank) was the highest of all. The fish yield in fresh human fed treatment was lower than stored urine fed treatments. The yield in the mixed fed treatment (664 g/tank) remained in between. Fish growth was lowest in the control (Fig. 37).

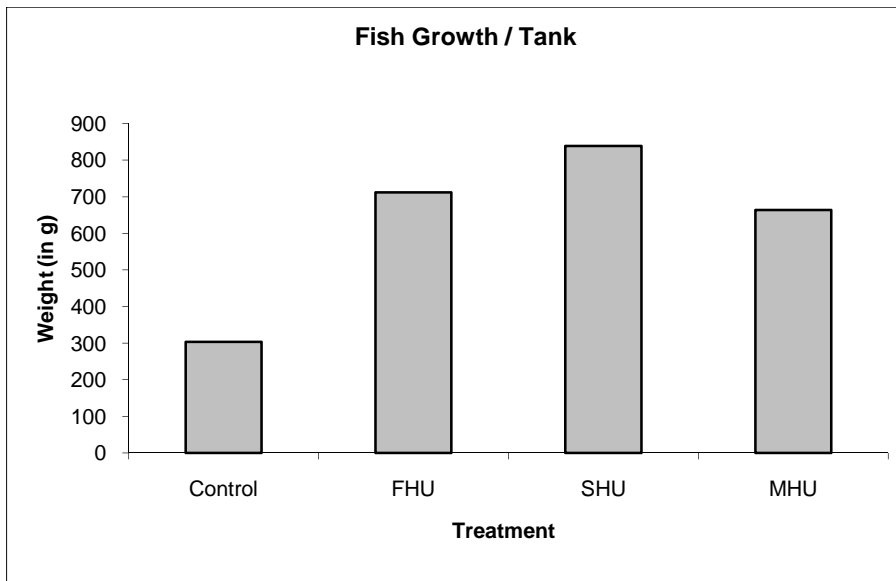


Fig 37: Responses of fish yield to urine-fed treatment

4.3.3 Discussion

It appears from the results of the study that double strength fresh urine was better than the single strength human urine because total fish yield in the former (712 g/tank) was at least 12 % higher than the latter (638 g/tank). The stored urine fed treatment contributed 20% higher fish yield (840 g/tank) than the fresh urine fed treatment within the culture period of 5 months only. This was attributed to high primary productivity of phytoplankton, high values of NO₃/PO₄ ratio. On the basis of the results, it can be safely stated that human urine can be used as a dependable source of fertilizer for the culture of carps. It is also noteworthy that freshwater prawn can even be grown in the human urine fed treatments. Further long term studies, are however, necessary for advancement of knowledge on the subject.

5.0 Limitations

Some of the parameters listed in the project have not been included as a regular monitoring parameter because of lengthy and expensive procedures. However, some of these will be determined at the time of final harvest of fish.



Plate – 3 A : Urine is being applied in a fish pond