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| <b>Title</b>   | <b>Preliminary Studies on the Evaluation of Human Urine as Source of Nutrients for Vegetables in the Eastern Cape Province, South Africa</b>  |
| <b>Keywords</b>  | Urine, Spinach, Cabbage, Nutrients, Salinity  |
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| <b>Short CV for Introduction Purposes ( 100 words max)</b> | Pearson Mnkeni is an agriculturalist by training, and currently a professor of soil science at the University of Fort Hare. Before coming to South Africa in 1997, he served as an Associate Professor in Soil Science at the Sokoine University of Agriculture in Morogoro, Tanzania. He has undertaken a number of nationally and internationally funded research projects in Tanzania and South Africa focussing on the utilization of locally available resources for soil fertility improvement. His present research focus is in the area of organic wastes management for soil fertility improvement and environmental conservation in small-holder farming and urban agriculture. |
| <b>Photograph attached ( jpg)</b>                          |   |

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## Introduction

Urine is a valuable source of nutrients that has been used since ancient times to enhance the growth of plants, notably leafy vegetables (Jönsson *et al.*, 2004). The nutrients in urine are in ionic form and their plant availability has been found to compare very well with chemical fertilizers (Kirchmann and Pettersson, 1995; Richert-Stintzing *et al.*, 2001). Human urine is used to varying extents for crop fertilization in countries like Mexico, Germany, USA, Sweden, Denmark and Zimbabwe. However, there is little or no information on its use in South Africa. This has largely been due to the lack of the appropriate sanitation technology that enables the hygienic separation of urine and faecal matter at source. This situation has, however, changed with the recent introduction to South Africa of urine diversion (UD) toilets (Fig. 1). When a UD toilet is used the urine is diverted away from faeces via a specially designed pedestal (Fig. 1a) and directed to a storage container. The urine collected in this manner is considered sterile unless cross-contamination occurred and can thus be used as plant fertilizer without further treatment. The faeces are sanitized in the collection chamber of the toilet (Fig. 1b) through drying and the addition of lime or wood ash. The introduction of the UD toilets has created opportunities, not only for introducing safer sanitation in rural and peri-urban areas, but also the possibility of recycling human excreta in areas where these toilets have been installed.

Work on the fertilizer value of human faeces from ecological sanitation toilets was reported by Mnkeni and Austin (2004). However, work on the fertilizer value of human urine in South Africa remained to be done. The study reported herein was therefore carried out to provide preliminary indications of the fertilizer value of human urine in South Africa.

## Materials and Methods.

The urine used in this study was provided by male students at the University of Fort Hare Alice campus. The collection of the urine was done over a two weeks period in June 2004 using 25 l containers fitted with flexi-funnels that were placed near urinals at the student hostels. The urine was collected from the hostels every morning and stored in tightly capped containers in a cold room chamber maintained at a temperature of 26°C until ready for use. The experimental soil used in the tunnel house study was collected from the KwaSomgxada Community garden at Ntselamanzi location next to the University of Fort Hare in Alice. The site had been previously characterized for its fertility status and relevant fertilizer recommendations made for different crops by the Advisory Service of the Dohne Agricultural Development Institute, Stutterheim. Soil was sampled from the plough layer (0-15 cm), air dried, sieved through an 8mm sieve and 12 kg soil placed in 10 l pots for use in the trial.

The experimental protocol for the work done in Zimbabwe (Morgan, 2003) utilizing urine diluted at a ratio of 1:3 (urine: water) was followed. The treatments included 3 rates of the diluted urine achieved by different frequencies of application, namely once, twice and thrice per week; inorganic fertilizer N application using urea at the recommended rate for spinach (100 kg N/ha = 0.6g N/12 kg pot) and cabbage (200 kg N/ha = 1.2g N/12 kg pot), and a control treatment in which only tap water was used. The treatments were replicated four times and arranged in a randomized complete block design in the tunnel house. The inorganic fertilizer treatment was applied before the seedlings were transplanted on 12 August 2004. Two spinach seedlings were planted per pot, while only one seedling per pot was planted in the case of cabbage.

The application of the diluted urine commenced on 16 August 2004 and terminated on the 20 September 2004. A rate of 500 ml per pot was used for 3 weeks, but reduced to 250 ml due to cooler weather that resulted in reduced evapo-transpiration during the period. The diluted

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urine had a pH of 8.6 and an electrical conductivity (EC) of 2.0 mS/cm; while the tap water used had a pH of 7.0 and EC of 0.02 mS/cm. The N concentration of the diluted urine as determined on a Kjeltex Analyzer was 0.183%. Thus, the cumulative N application from urine applied once, twice and thrice per week translated to 3.7, 6.4 and 9.6 g N/12kg pot which was equivalent to 617, 1067 and 1600 kg N/ha, respectively. Regular watering of the pots was done during the trial with the addition of tap water as necessary to maintain the soil at approximately field capacity. Withered leaves from both crops that dropped before harvesting were recovered in labelled bags kept beside each pot.

The first harvesting of spinach was done on 28 September 2004 by cutting well developed leaves at the base of the plant leaving only the very young leaves for re-growth. Fresh weight of the leaves was taken; and thereafter thoroughly washed and oven dried at 65°C to a constant weight for dry matter determination. Daily watering (250 ml per pot) was continued; with no further addition of diluted urine. The spinach leaves re-growth together with the cabbage was harvested one month after the first spinach harvest on 28 October 2004. Both crops were harvested by cutting the leaves close to the soil surface followed by fresh and dry biomass determinations as described previously. A second re-growth was allowed for the spinach so regular watering of the pots with spinach was continued until the final harvesting on 30 November 2004.

Following the complete harvesting of the spinach and cabbage, soil in each pot was separately remixed after removing large stumps and large roots. Samples were then taken, air dried and ground to pass through a 2 mm sieve for laboratory analysis. Oven dried plant samples were then ground, digested and analyzed for total P, K, Ca, Mg and Na as described by Okalebo *et al.*, (2002). Total N in plant and soil samples was determined using a LECO Truspec C&N Analyzer (Anonymous, 2003). The extractable P, K, Ca, and Mg content in soil samples was extracted using the Ambic-1 procedure (Van der Merwe *et al.*, 1984). Statistical analysis of the data obtained was done using the MSTATC statistical package.

## **Results and Discussion**

### ***Effect of diluted human urine application on the performance of spinach***

Application of diluted urine once a week (3.7 g N/pot) resulted in the highest yields at the first harvest (Table 1). Higher rates of diluted urine application, twice (6.4 g N/pot) and thrice (9.6 g N/pot) a week, also resulted in significant yield increases relative to the control but the yields were much lower when compared to when the diluted urine was applied only once a week. By the second harvest the situation changed somewhat in that the highest yields were now recorded where urine was applied twice a week even though the urine application was terminated before the first harvest. However, diluted urine application three times a week (9.6 g N/pot) still resulted in the lowest yields relative to the other urine treatments. A similar pattern of response was observed at the third harvest except that at this stage there was a slight improvement in spinach yield from pots that received diluted urine three times a week. The observed increases in yields as a result of diluted urine application once or twice per week appeared to be largely related to the ability of the urine to supply nitrogen to the growing plants. This is reflected in increased tissue N levels of nitrogen in spinach at each harvest (Fig. 2). The recovery of added N was lower for urine when compared with urea (Table 2) possibly because of differences in method of application. Whereas the urea was incorporated in soil, the diluted urine was applied on the surface where a significant proportion could have been lost through volatilization. This is supported by observations that except for the highest rate of urine application, all treatments had similar levels of total N after harvesting (Table 5) implying that whatever N that was not taken up by plants was lost through volatilization.

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The depressive effects of higher rates of urine application on yield seemed to have been caused by higher salinity (electrical conductivity) levels in pots treated with higher levels of urine (Table 4). The high salinity levels in turn resulted in high tissue levels of sodium (Fig. 3). The severity of this effect decreased with each successive harvest as reflected by correspondingly lower tissue Na levels in the third harvest (Fig. 3) probably due to the lowering of Na in soil through crop removal at each successive harvest. The application of urine had an acidifying effect on soil (Table 4) but the magnitude of the effect was of such a level that it may not have affected the growth of the plants.

### ***Effects of diluted Human urine on cabbage growth***

Application of diluted urine had more or less the same effects on cabbage growth as observed for spinach. The application of diluted urine once a week (3.7 g N/pot) had the largest effect on cabbage growth (Table 3). The yield was increased by more than 5 times. However, higher rates of urine application had a depressing effect on yield even though they resulted in increased tissue nitrogen concentration (Fig. 5). The diluted urine application had little or no effect on the tissue P and K concentration (results not shown) but increased tissue levels of Na substantially (Fig. 5) possibly as a result of its effect to increase salinity in soil (Table 4). As observed for spinach, the application of urine had an acidifying effect on soil pH (Table 4). This was largely a result of the hydrolysis urea which is the main nitrogen bearing compound in urine. This effect may not have affected crop growth in this study because the soil used had a nearly neutral initial pH, however, it could have a negative effect on plant growth in soils that are already acidic to begin with.

### **Conclusions**

The results of this study revealed that urine diluted at a ratio of 1:3 (urine: water) is a good source of nutrients, particularly N, for spinach and cabbage when applied once a week for about a month. Thus if pathogenic tests prove a low risk of disease transmission, urine should be seriously considered for use where urine diversion toilets are in use in South Africa. Field trials are, however, recommended to establish optimum rates of application in areas where its use is envisaged. Higher rates of urine application tended to depress yields due to increased salinity in soils which in turn led to rather high levels of sodium in plant tissues. The use of human urine should, therefore, not be recommended for soils that are known to have salinity problems. Furthermore, the salinity status of soils that are regularly fertilized with urine should be monitored to guard against possible salt-build up.

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Table 1: Effects of diluted human urine on the leaf biomass yield of spinach at three successive harvests.

| Treatment                      |                    | Fresh leaf biomass yield (g/pot) |                 |                 |       | Dry leaf biomass yield (g/pot) |                 |                 |        |
|--------------------------------|--------------------|----------------------------------|-----------------|-----------------|-------|--------------------------------|-----------------|-----------------|--------|
| Frequency of urine application | Added N            | 1 <sup>st</sup>                  | 2 <sup>nd</sup> | 3 <sup>rd</sup> | Total | 1 <sup>st</sup>                | 2 <sup>nd</sup> | 3 <sup>rd</sup> | Total  |
|                                | Control            | 110c*                            | 61d             | 62b             | 234d  | 9.2 d                          | 4.2d            | 5.5 d           | 18.7 d |
|                                | 0.6g N/pot (Urea)  | 196c                             | 82d             | 69d             | 346c  | 20.2ab                         | 6.3cd           | 6.1 d           | 32.6 c |
| 1x per week                    | 3.7g N/pot (urine) | 273a                             | 320b            | 101b            | 695b  | 23.5a                          | 21.7b           | 10.8c           | 56.1 b |
| 2x per week                    | 6.4g N/pot (urine) | 167b                             | 451a            | 215a            | 833a  | 18.2b                          | 29.1a           | 22.5a           | 69.8 a |
| 3x per week                    | 9.6g N/pot (urine) | 98c                              | 120c            | 195a            | 413c  | 13.9c                          | 8.7c            | 16.6b           | 39.1 c |

\* Means in each column followed by the same letter or none at all are not significantly different from each other at  $p < 0.05$  according to the LSD test.

Table 2: Nitrogen uptake and recovery efficiency by spinach as affected by varying rates of diluted urine application.

| Treatment                      |                    | N uptake (mg/pot) per harvest. |                 |                 |        | N recovery efficiency (%) |
|--------------------------------|--------------------|--------------------------------|-----------------|-----------------|--------|---------------------------|
| Frequency of urine application | Added N            | 1 <sup>st</sup>                | 2 <sup>nd</sup> | 3 <sup>rd</sup> | Total  |                           |
|                                | Control            | 131 d*                         | 67 d            | 104 b           | 302 d  | -                         |
|                                | 0.6g N/pot (urea)  | 502 c                          | 102 d           | 111 b           | 716 c  | 69 a                      |
| 1x per week                    | 3.7g N/pot (urine) | 1194a                          | 666 b           | 203 b           | 2063 b | 48 b                      |
| 2x per week                    | 6.4g N/pot (urine) | 930 b                          | 1251a           | 779 a           | 2960 a | 42 b                      |
| 3x per week                    | 9.6g N/pot (urine) | 702 c                          | 514 c           | 885 a           | 2103 b | 19 c                      |

\* Means in each column followed by the same letter or none at all are not significantly different from each other at  $p < 0.05$  according to the LSD test.

Table 3: Effect of diluted human urine application on cabbage fresh and dry matter yield, tissue N concentration and N recovery efficiency.

| Treatment                      |                    | Fresh Wt. (g/pot) | Dry matter (g/pot) | Tissue N (%) | N uptake (mg/pot) | N recovery efficiency (%) |
|--------------------------------|--------------------|-------------------|--------------------|--------------|-------------------|---------------------------|
| Frequency of urine application | Added N            |                   |                    |              |                   |                           |
|                                | Control            | 153 c*            | 28 d               | 0.8 e        | 208 d             | -                         |
|                                | 1.2g N/pot (urea)  | 517 b             | 76 ab              | 1.1 d        | 868 c             | 55 a                      |
| 1x per week                    | 3.7g N/pot (urine) | 711 a             | 89 a               | 2.6 c        | 2292 b            | 56 a                      |
| 2x per week                    | 6.4g N/pot (urine) | 697 a             | 69 bc              | 4.0 b        | 2761 a            | 40 b                      |
| 3x per week                    | 9.6g N/pot (urine) | 640 a             | 57 c               | 5.5 a        | 3046 a            | 30 b                      |

\* Means in each column followed by the same letter or none at all are not significantly different from each other at  $p < 0.05$  according to the LSD test.

Table 4: Effect of added diluted human urine on soil pH and electrical conductivity after harvesting spinach and cabbage (mS/cm)

| Treatment                      |                    | Soil pH |         | Electrical conductivity (mS/cm) |         |
|--------------------------------|--------------------|---------|---------|---------------------------------|---------|
| Frequency of urine application | Added N            | Spinach | Cabbage | Spinach                         | Cabbage |
|                                | Control            | 6.5a*   | 6.5a    | 2.0b                            | 1.3d    |
|                                | 0.6g N/pot (urea)  | 6.3ab   | -       | 1.8b                            | -       |
|                                | 1.2g N/pot (urea)  | -       | 5.9bc   | -                               | 1.9d    |
| 1x per week                    | 3.7g N/pot (urine) | 5.7c    | 6.1b    | 1.4b                            | 5.8c    |
| 2x per week                    | 6.4g N/pot (urine) | 5.6c    | 5.8bc   | 1.7b                            | 10.2b   |
| 3x per week                    | 9.6g N/pot (urine) | 5.9bc   | 5.7c    | 17.7a                           | 18.8a   |

\* Means in each column followed by the same letter or none at all are not significantly different from each other at  $p < 0.05$  according to the LSD test.

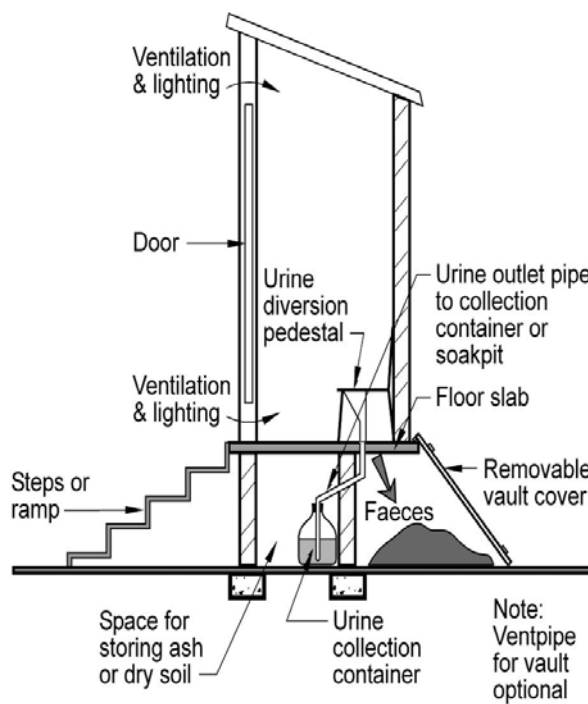
Table 5: Total N and Ambic-1 extractable P, K, Ca and Mg contents in soils after harvesting spinach (third harvest) and cabbage.

| Treatment        | Spinach |     |         |    |    | Cabbage |       |         |    |    |
|------------------|---------|-----|---------|----|----|---------|-------|---------|----|----|
|                  | N %     | P   | K mg/kg | Ca | Mg | N %     | P     | K mg/kg | Ca | Mg |
| Control          | 0.05b*  | 88  | 75      | 91 | 20 | 0.03b   | 80c   | 146     | 91 | 19 |
| 0.6g N/pot urea  | 0.05b   | 196 | 127     | 89 | 19 | -       | -     | -       | -  | -  |
| 1.2g N/pot urea  | -       | -   | -       | -  | -  | 0.03b   | 106bc | 132     | 88 | 19 |
| 3.7g N/pot urine | 0.05b   | 79  | 63      | 89 | 19 | 0.03b   | 77c   | 120     | 90 | 19 |
| 6.4g N/pot urine | 0.05b   | 187 | 102     | 84 | 18 | 0.03b   | 114ab | 133     | 89 | 20 |
| 9.6g N/pot urine | 0.07a   | 269 | 142     | 89 | 19 | 0.05a   | 145 a | 184     | 87 | 19 |

\* Means in each column followed by the same letter or none at all are not significantly different from each other at  $p < 0.05$  according to the LSD test.



(a)



(b)

Figure 1. Urine diversion pedestal (a) and schematic representation of a urine-diversion (“dry box”) toilet (b).

(Mnkeni and Austin, 2004).

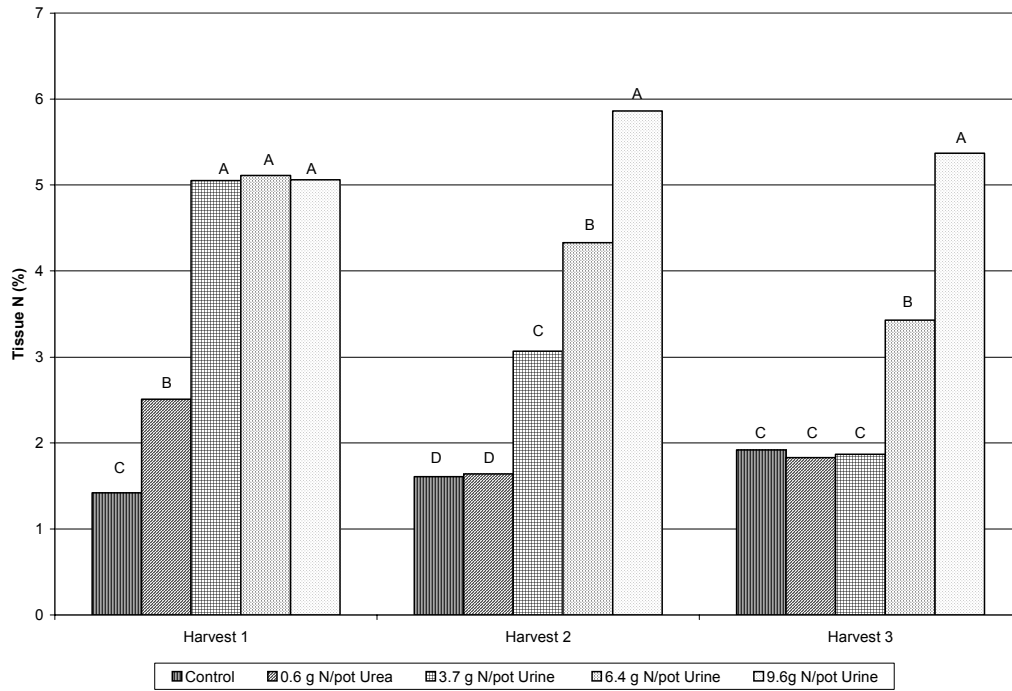


Figure 2: Effects of diluted human urine application on the tissue N concentration of spinach harvested at three different occasions. Bars within each harvest bearing different letters are not significantly different from each other according to the LSD test at  $p < 0.05$ .

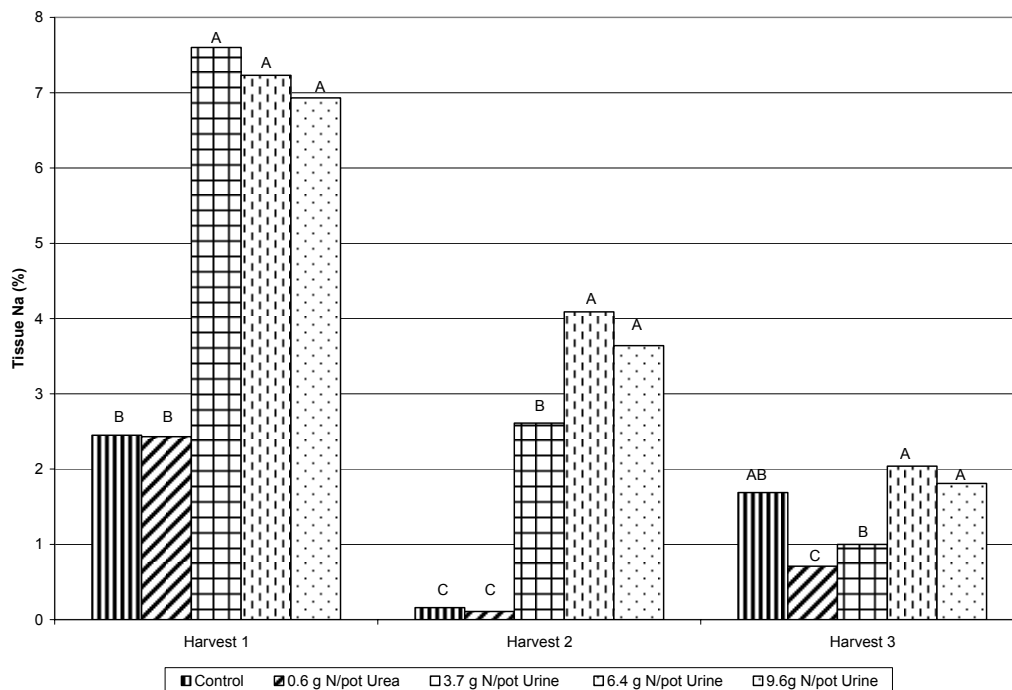


Figure 3: Effects of diluted human urine on tissue Na concentration of spinach harvested on three different occasions. Bars within each harvest bearing different letters are not significantly

different from each other according to the LSD test at  $p < 0.05$ .

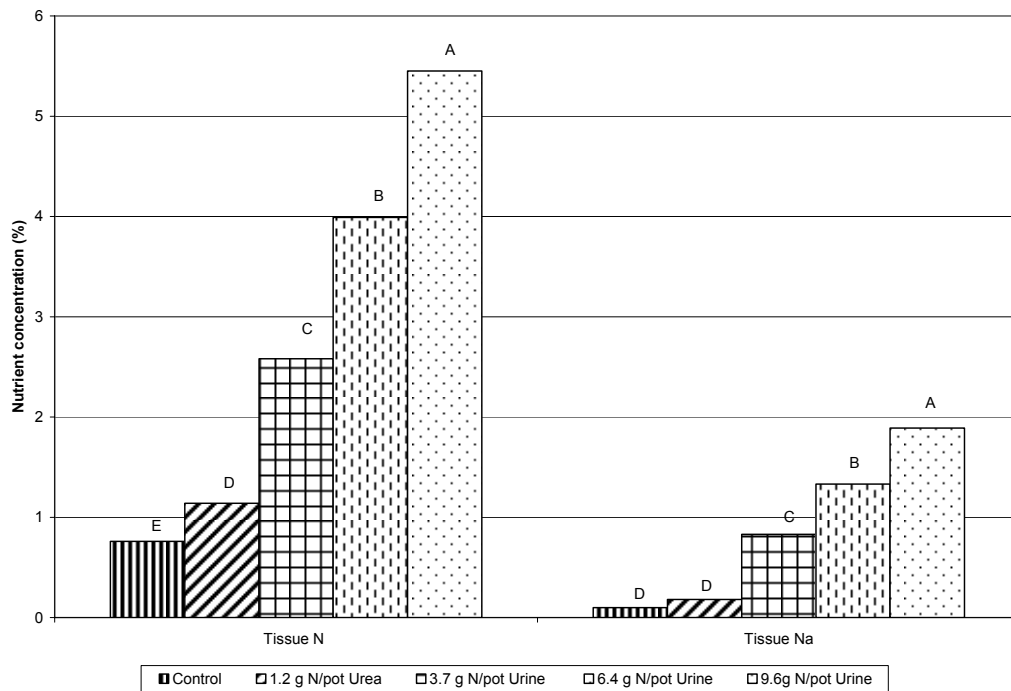


Figure 4: Effect of diluted human urine on cabbage tissue N and Na concentration. Bars within each harvest bearing different letters are not significantly different from each other according to the LSD test at  $p < 0.05$ .