British Journal of Applied Science & Technology
14(2): 1-11, 2016, Article no.BJAST.23454
ISSN: 2231-0843, NLM ID: 101664541

SCIENCEDOMAIN *international*
www.sciencedomain.org

Quick Release Nitrogenous Fertilizer from Human Hair

Md. Mominur Rahman[1*], Kazi Bayzid Kabir[1], Md. Masudur Rahman[1] and Zannatul Ferdous[1]

[1]Department of Chemical Engineering, Bangladesh University of Engineering and Technology (BUET), Dhaka-1000, Bangladesh.

Authors' contributions

This work was carried out in collaboration between all authors. Author Md. Mominur Rahman designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript and managed literature searches. Authors KBK, MMR, ZF managed the analyses of the study and literature searches. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/BJAST/2016/23454
Editor(s):
(1) Verlicchi Paola, Department of Engineering, University of Ferrara, Via Saragat 1, Ferrara, Italy.
Reviewers:
(1) Yongchun Zhu, Shenyang Normal University, China.
(2) A. Veerareddy, Defence Research Development Laboratory, Hyderabad, India.
Complete Peer review History: http://sciencedomain.org/review-history/12917

Original Research Article

Received 1st December 2015
Accepted 29th December 2015
Published 7th January 2016

ABSTRACT

Liquid nitrogenous fertilizer was synthesized using waste human hair as the raw material. Potassium hydroxide (KOH) and Tetramethylammonium hydroxide (TMAH) were used as the non-recoverable solvents for diffusion controlled reductive cleavage of hair protein. Performance of these solvents was analyzed and compared both individually and for different combinations. Experiments showed that KOH was better for dissolving hair and extraction of nitrogen in the forms of ammonium and nitrate ions compared to TMAH. Thereafter a vegetation pot experiment was established to investigate the performance of the produced liquid fertilizers compared to the commercial Nitrogen-Phosphorus-Potassium, NPK (15-15-15), fertilizer on the growth and yield of a short cycle crop, spinach (*Spinacia oleracea* L). The study design consisted of four treatments viz. no fertilization (T1), KOH based liquid fertilizer from human hair (T2), TMAH based liquid fertilizer from human hair (T3) and commercial NPK (T4). Each treatment had four replicate pots and was also done in parallel. The different treatments were added to the pot based on the total nitrogen requirement of 50 kg/ha for the spinach following broadcasting method. Plant physiology

**Corresponding author: E-mail: mrrahman@che.buet.ac.bd;*

including plant height, number of the leaves, fresh and dry weight of the edible part of the harvested plant after 28 days suggested that the performance of the synthesized fertilizers as a nitrogen source were better than the commercial NPK fertilizer. These outcomes encourage such novel use of waste human hair.

Keywords: Human hair; liquid nitrogenous fertilizer; saolvent; Spinacia oleracea L; treatment.

1. INTRODUCTION

Human hair is a complex tissue consisting of several morphological components, each consisting of several different chemical species. The presence of trace elements in hair is the reason behind its forensic importance in replacing blood and urine analyses [1-3], skin disease diagnostics, drug identification and tracking human migration patterns by isotopic correlation [4]. The physical properties of hair have interested researchers for different applications such as adsorption for utilisation in effluent treatment [5,6] and arsenic removal from drinking water [7]. Hair, a potential human waste, contains up to 17% nitrogen by weight within the complex protein structure [8,9] which, if transferable, could be used as a nitrogen source. Several attempts have been made to use hair as a slow-release source of nitrogen, assuming disintegration and mineralization within a timescale of months [10,11]. To the authors' knowledge, preparation of a quick-release nitrogenous fertilizer from human hair has never been reported in open literature.

The main problem with synthesizing a quick-release nitrogenous fertilizer from human hair is disintegrating the hair. Human hair has a high cystine content (1400-1500 micromoles/g hair) [9] accounting for the inertness of the material. Cystine is an amino acid characterised by a disulphide bond, which provides strong intramolecular and intermolecular cross-links [12].

Cleavage of the disulphide bond is extremely pH dependent. High and low pH enhances decomposition by different mechanisms [9]. At a pH above 10, moving boundary kinetics or diffusion of alkaline substances into the hair control the reaction rate. In addition, the bond-strengthening effects of macro and micro molecules present in the soil are enhanced under alkaline conditions.

When selecting the hair solvent, other potentially advantageous properties should be considered in conjunction with pH. For instance, KOH contains potassium (K), a macro element essential for plant life. Sodium hydroxide, although less expensive, is not particularly beneficial as the plant requirement for Na^+ is low and an excess of Na^+ ions has an adverse effect on plant physiology [13].

The use of aqueous solutions of TMAH to dissolve hair and other biological materials for analytical purposes has been reported [14,3,15]. TMAH could also be a potential nitrogen source, contributing to the total nitrogen content of the liquid fertilizer, and is therefore included in this study for comparison.

This paper reports the synthesis of nitrogenous fertilizers from human hair using aqueous solutions of KOH and TMAH as solvents. The scope of this study also included demonstration of the effect of the synthesized fertilizers on spinach (*Spinacia oleracea* L) in comparison with that of a commercial NPK (15-15-15) fertilizer. The synthesized fertilizers using KOH and TMAH as the solvent were found to enhance plant growth and yield in comparison to the commercial product.

2. MATERIALS AND METHODS

2.1 Fertilizer Synthesis

Potassium hydroxide pellets and TMAH 25% (w/v in water) were used to prepare solvent solutions of different concentrations. A single bulk sample of collected hair was used for all tests.

The composition of the collected human hair sample was determined using a Perkin Elmer 2400 Series II CHNS/O analyser.

To prepare the fertilizer, a measured amount of dry, water-washed human hair was placed in a solvent solution. The mixture was stirred for 5 minutes and then rested for 5 minutes to avoid excessive foaming. The cycle was continued until the hair was completely dissolved. The resultant solution was passed through a 200–mesh Tyler series sieve to remove any undissolved hair. This process was repeated for increased amount of hair up to the saturation point of the hair solution. This process was then repeated for the various solvent solutions of interest.

The prepared liquid fertilizer samples were then analysed for pH, density, viscosity and ion concentration of nitrate and ammonium. pH was measured using a HACH pH meter and density was measured using a Mettler Toledo DA-100M density meter. A model 35 FANN viscometer was used to measure the dynamic viscosity and ion concentrations were measured using a HACH DR/4000 spectrophotometer (USA).

2.2 Vegetation Pot Experiment

The main objective of the pot experiment was to identify the performance of synthesized nitrogenous fertilizers from human hair compared to the commercial synthetic fertilizer. Four treatments were included in the experiment, viz. no fertilization (T1), synthesized fertilizer from human hair with KOH (T2), synthesized fertilizer from human hair with TMAH (T3) and commercial NPK 15-15-15 (T4). A short cycle crop, spinach (*Spinacia oleracea* L) was selected for this study. The study design was set up such that it was a randomized trial with a total of four different treatments and each treatment had four replicate pots and was also done in parallel, amounting to eight pots per treatment. This gives a total of 32 pots.

The soil was selected based on the most common soil present in the agricultural areas of the particular region in which the selected crop is grown. The top layer of the soil, (i.e., the first 0–10 cm) was excavated from the identified site and any unwanted objects such as vegetation, stones, debris were discarded during excavation. After transporting to the laboratory, any soil clogs remaining were separated or broken down using a wooden mallet. The soil was then dried at room temperature, after which it was passed through a 6mm sieve. A composite soil sample from each treatment was mixed thoroughly and crushed gently to allow it to pass through a 4 mm sieve. A sub-sample was then ground again and passed through a 2 mm sieve. The resulting sample (< 2 mm) was then analyzed for different properties of the soil. Soil texture was determined following sedimentation method [16]. Moisture content was determined using oven dry method at 105℃ up to constant weight [17]. Soil to water ratio 1:2.5 was maintained to measure the soil pH [18]. Total nitrogen (N) was determined by adding Total Kjeldahl nitrogen (TKN) [19] and available nitrogen in the form of nitrate-nitrogen (NO_3-N). NO_3-N was measured in 1:10 2 M potassium chloride (KCl) extract [20,21] using ultraviolet visible (UV) spectrophotometer (Cadmium reduction method and measured at a wavelength 400 nm). Bray-P1 test protocol was followed in determining extractable phosphorus (P) in soil [22]. Soil sample was extracted by Bray-1 solution and filtered. The filtrate was then analyzed for extractable P using UV Spectrophotometer at a wavelength 890 nm (Ascorbic Acid Method). Potassium was extracted from the soil by mixing 1N ammonium acetate solution at pH 7 [23]. The available K was then measured by analyzing the filtered extract on an atomic absorption spectrometer (AAS). Cation exchange capacity (CEC) of the soil was determined by the barium chloride ($BaCl_2$) compulsive exchange method [24]. Water holding capacity of the soil was examined by water saturation method [25].

The pot trials were carried out in 2-litre capacity pots with 20.32 cm opening diameter. All the pots were filled with experimental soil and fitted with a tray placed at the bottom of the pots to capture any excess water escaping from the pots. The water was added to all the pots until they comply with the field capacity levels and was allowed to settle for 48 hours. Different treatments were applied to the pots on January 12, 2015 using broadcasting method according to the recommended dose 50 kg N/ha for spinach [26]. Then the pots were allowed to stand for 5 days before seedling transplantation.

The seedlings were grown from seeds in the same greenhouse in which the pot trials were conducted using the same soil. Seeds were sown on December 27, 2014. After 14 days of seed germination, the seedlings were transplanted into the experimental pots with a single plant per pot on January 17, 2015. The pots were regularly watered to maintain their water content within the field capacity levels throughout the duration of the experiment to ensure maximum growth of the crop. Any water that percolated through the pot and into the tray was collected and used to water the same pot in order to minimize the nutrient loss via leaching. The pots were moved around once in a week in a randomized manner to minimize any climate effects.

The number of leaves and stem height of each of the plants grown using all the treatments were recorded at 0, 1, 2, 3, 4 weeks after transplantation. The spinach plants were harvested after 28 days (4 weeks) and after harvesting a number of parameters were measured: Fresh and dry weight and moisture content of the edible part of the plant (dried at 105℃ to constant weight).

3. RESULTS AND DISCUSSION

3.1 Preparation of the Fertilizer

The hair sample contained 46.3% carbon, 6.7% hydrogen, 14.8% nitrogen and 3.1% sulphur.

Two potential solvent solutions were investigated; KOH and TMAH. Unlike KOH, TMAH can contribute to the total nitrogen content of the resultant liquid fertilizer. KOH and TMAH dissolved hair at concentrations as low as 2% w/v. The time required to complete the dissolution process is inversely related to the concentration. The dissolution performance of KOH was invariably superior to that of TMAH, as shown in Fig. 1. For 10% KOH and 10% TMAH the dissolution process was complete after 1 and 1.5 hrs respectively.

Combined use of solvents was also considered. The dissolution results show that the solubility of hair in the 10% KOH solution decreased on addition of TMAH (Fig. 2). The concave shape in the plot was the result of reaction between solvents before reacting with hair. Mixing the solvents in any proportion reduced their effectiveness, with minimum dissolution of hair at ~70% TMAH solution in KOH solution.

Additional experiments were performed in which the hair was added to the solvent gradually. The results show that this method decreases the maximum dissolved amount (Fig. 3). This is consistent with the fact that the reaction of hair proteins with bases is strongly dependent on pH and each subsequent addition of hair results in a decrease in solution pH.

Large scale dissolution experiments were carried out for both solutions. The linear relationships between the volume of solvent and mass of the hair dissolved are shown in Fig. 4. KOH dissolves more hair compared to TMAH. The quantity of hair dissolved can be expressed as

$$W_{KOH} = 0.9985V_{KOH}$$
$$W_{TMAH} = 0.7557V_{TMAH}$$

where W is the weight (kg) of hair and V is the volume (L) of the solvent.

The densities of the prepared fertilizer solutions (KOH and TMAH based) were 2097-2115 kg/m^3 and 1739-1748 kg/m^3 respectively. The dynamic viscosity was determined as 6.2-6.4 mPa.s for the KOH solution and 6.7-6.8 mPa.s for the TMAH solution. The pH of the final solution in both cases was found to be between 7.5 and 8.

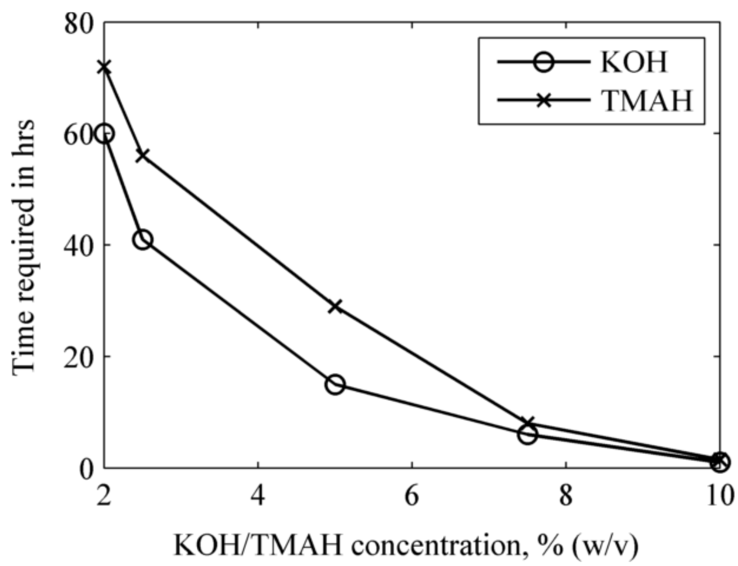

Fig. 1. Time required for dissolving hair in aqueous solvent of KOH/TMAH

Ammonium (NH_4^+) ion and Nitrate (NO_3^-) ion concentrations of the synthesized fertilizers were determined and are shown in Table 1. With 10% KOH as the solvent, approximately 79% of N was extracted from hair as NH_4^+ and 18% as NO_3^-. For the equivalent TMAH solution, the contribution of hair to NH_4^+ and NO_3^- ions could not be determined since the solvent solution itself contains nitrogen. However, the total nitrogen content was lower than that for KOH due to the lower dissolution of hair in TMAH 6.

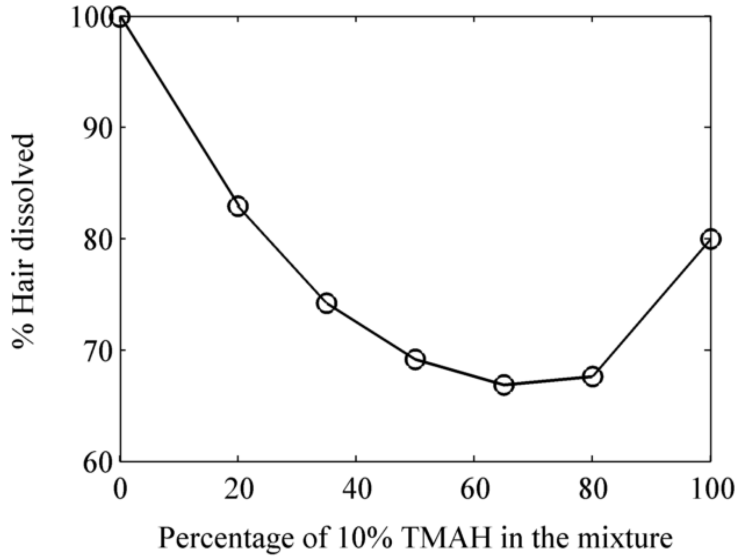

Fig. 2. Variation of hair solubility in solvent mixtures (quantity of TMAH is shown in x-axis; the rest is KOH)

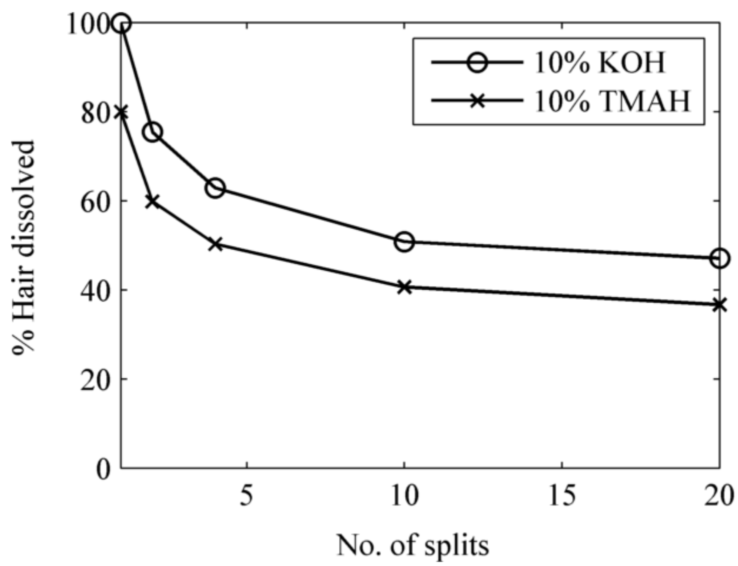

Fig. 3. Variation in solubility due to gradual addition of hair to KOH (upper curve) and TMAH solvents

Table 1. Nitrogen content in the synthesized liquid fertilizer

Solvent	g NH_4 / L	g NH_4-N / L	g NO_3/ L	g NO_3-N / L
10% KOH	150.17	116.80	117.82	26.60
10% TMAH	101.79	89.07	89.40	20.19

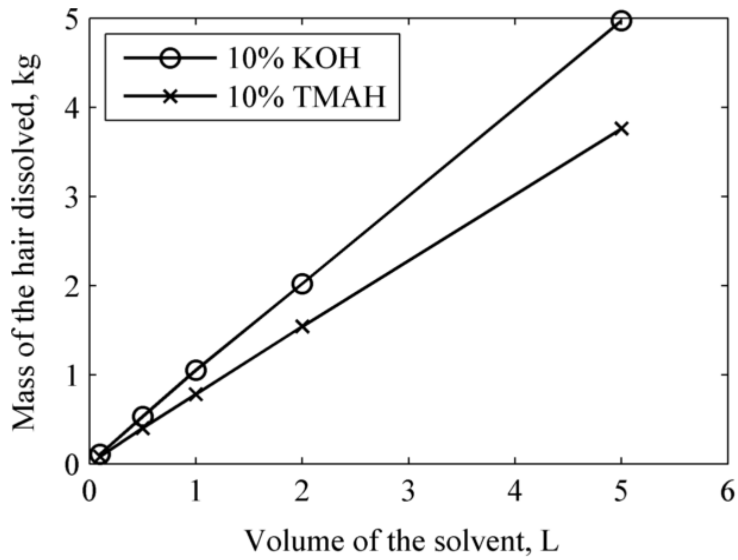

Fig. 4. Relationship between volume of solvent and mass of hair dissolved

Considering the quantity of hair dissolved, concentrations of the ions in the product fertilizer and cost of the solvents 10% KOH is preferable compared to the 10% TMAH. However, the performances of the synthesized fertilizers were then compared with commercial NPK (15-15-15) fertilizer through pot trial on spinach.

3.2 Performance of Synthesized Fertilizers

Sedimentation result shows that the soil for pot experiment contains 65% sand, 15% silt and 20% clay which categorize the soil texture as Sandy Loam. The soil was slightly acidic in nature with a pH of 6.8 which was suitable for the synthesized fertilizers as they are slightly basic in nature. Physico-chemical properties of the soil are shown in Table 2.

Leaves count on each of the plants of different treatments at 0, 1, 2, 3, and 4 weeks revealed that there appeared to be no significant differences in number of leaves on the plant grown using different treatments till the end of first week. However, there appeared a significant difference in leaf count at the end of the pot trial session. Plants grown using no fertilizer (T1) and KOH based fertilizer from human hair (T2) showed the least (13±1.19) and the highest (17±2.07) number of leaves respectively at the end of fourth week (Fig. 5).

Table 2. Physico-chemical properties of the soil for vegetation pot experiment

Parameters	Value	Methods
Moisture (%)	8.85	Oven dry method at 105°C up to constant weight [17]
pH (Soil:Water :: 1:2.5)	6.80	Glass electrode pH meter [18]
Total N (% dw*)	0.12	Kjeldahl and NO_3-N by Cadmium reduction method using UV spectrophotometer [21,19,20]
NO_3-N (mg/Kg dw*)	7.70	Cadmium reduction method using UV spectrophotometer
Available P (mg/Kg dw*)	12.6	Bray-P1 and Ascorbic acid method using UV spectrophotometer [22]
Available K (mg/Kg dw*)	13.3	Ammonium acetate method using Atomic Absorption Spectrophotometer [23]
CEC (meq/100 g)	10.74	$BaCl_2$ compulsive exchange method [24]
Field Capacity (% dw*)	16.91	Water saturation method [25]

**Dry Weight Basis (dw)*

Treatment T2 contributed the highest positive change in leaf count (565%) whereas this was the lowest for treatment T1 (346%) during the vegetation pot experiment (Fig. 6).

An inverse relationship between the leaf count and stem height of grown spinach was observed using different treatments. Though this result is not vivid from the weekly changes in stem height of plants grown using different treatments (Fig. 7), overall changes in stem height manifest it quickly (Fig. 8). The highest overall changes in stem heights were observed for treatment T1 and the lowest overall changes were for treatment T2. The higher change in stem height of spinach might be related with the nutrient deficiency of the soil.

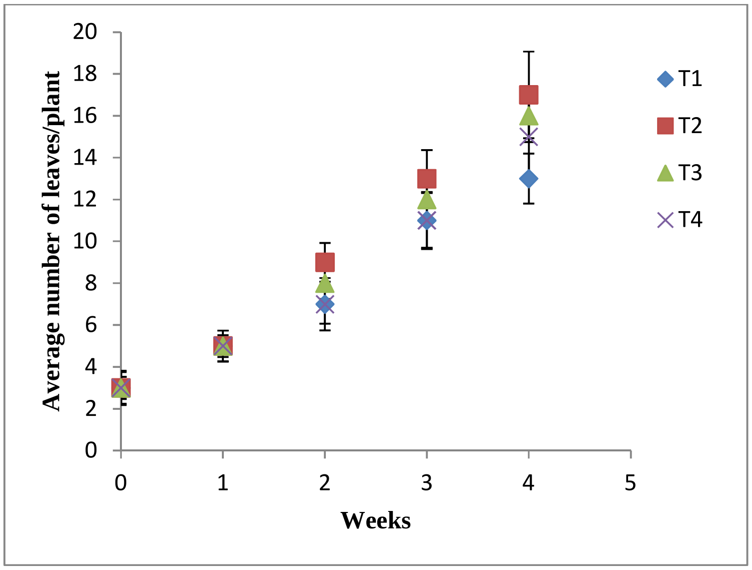

Fig. 5. Weekly average leaf count of the grown spinach using different treatments

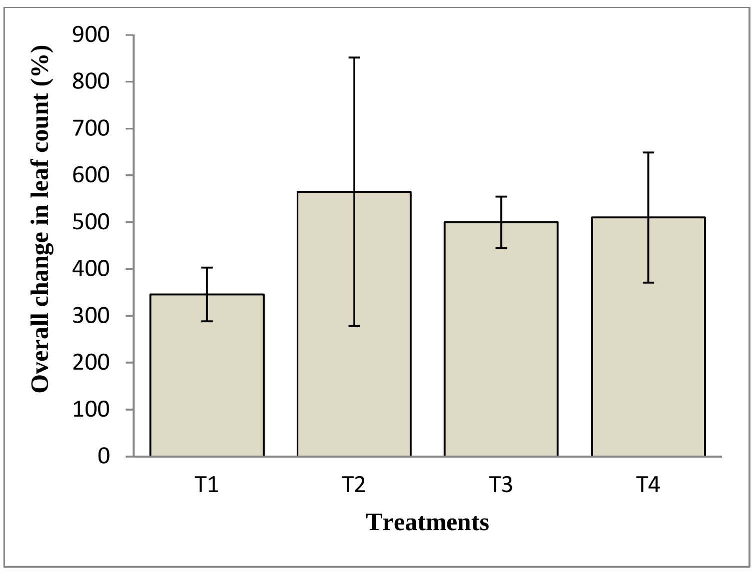

Fig. 6. Overall changes in leaf count for different treatments

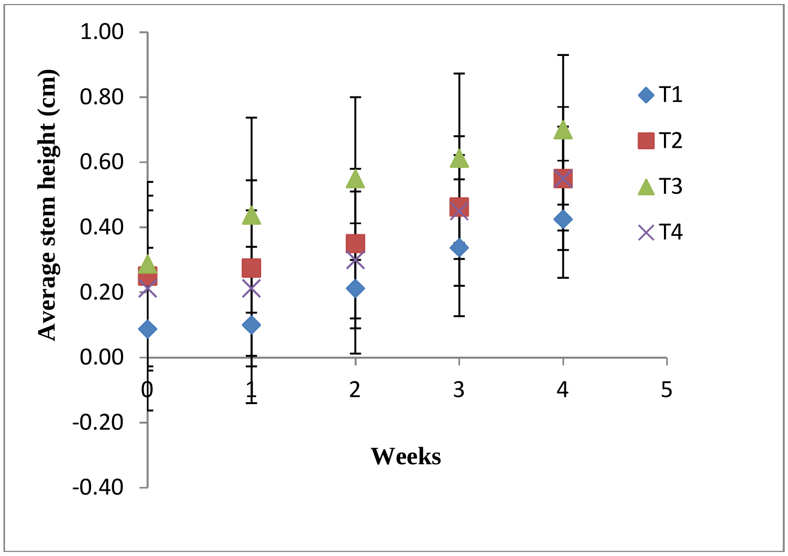

Fig. 7. Weekly average stem height of the grown spinch using different treatments

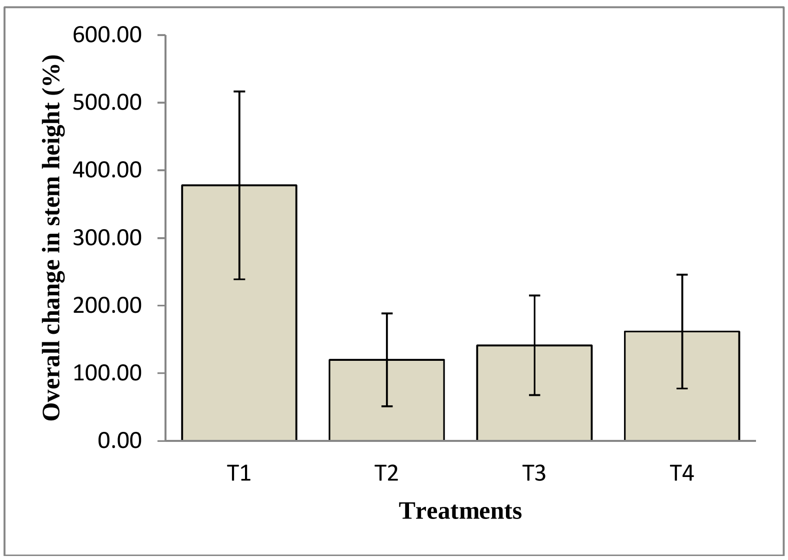

Fig. 8. Overall changes in stem height for different treatments

However comparison of the dry weight of the edible part of the plants after harvesting (Fig. 9) there appears to be a significant difference between different treatments. The lowest average weight (<2 g/plant) was seen for the plants grown without any additional nutrients (T1) which is what it would be expected. The results for the TMAH based liquid fertilizer from human hair (T3) and KOH based liquid fertilizer from human hair (T2) showed remarkable differences compared to the commercial NPK (T4). The average dry masses of the edible part of the grown spinach were 5.12 g/plant for T3 and 5.79 g/plant for T2 which were much higher than 3.83 g/plant for the commercial NPK (T4). Improved performances were seen with T2 and T3 compared to control T1 and T4 which was due to readily available nitrogen in the synthesized fertilizers T2 and T3 (Table 1). It was further manifested that the T2 (KOH based synthesized fertilizer from human hair) showed the best performance on the dry mass basis of the grown spinach.

Fig. 10 shows the average NO_3 concentrations in the edible part of the harvested spinach using different treatments. The concentrations of nitrate varied between treatments. As expected the concentrations in the edible part of the plant for those grown without addition of fertilizer (T1) was very low. There was a significant difference in nitrate concentrations in the plants grown on NPK (T4) and TMAH based fertilizer (T3) compared to KOH based fertilizer (T2) as the nitrogen concentration in the form of nitrate was in the highest amount in T2 (Table 2). Though the nitrate concentration in the edible part of the grown spinach using T2 was the highest (2289 mg/kg green mass) among the treatments, it was below the upper limit of the nitrate concentration (2500-3000 mg/kg) in green spinach set by European Union [27].

Since the only nitrogen source was the synthetic fertilizer added to the soil during the vegetation pot trial, the physical growth and enhanced nitrate content in the grown spinach can be attributed to the effect of this synthesized fertilizer. These results and observations are direct consequences of the presence of nitrogen. The results of some other studies showed that high levels of readily available nitrogen increased leaf number and vegetative growth of plants thus increasing the photosynthetic capacity which resulted higher dry matter in the grown plants [28].

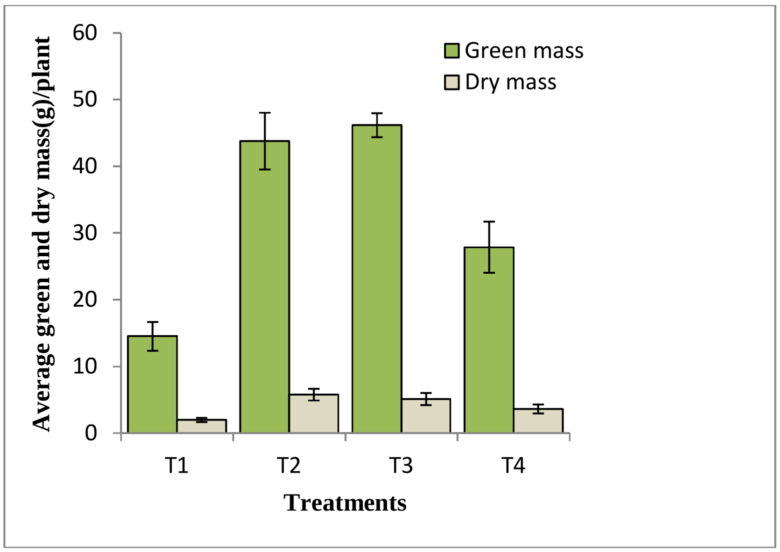

Fig. 9. Average green and dry mass of the hearvested spinach using different treatments

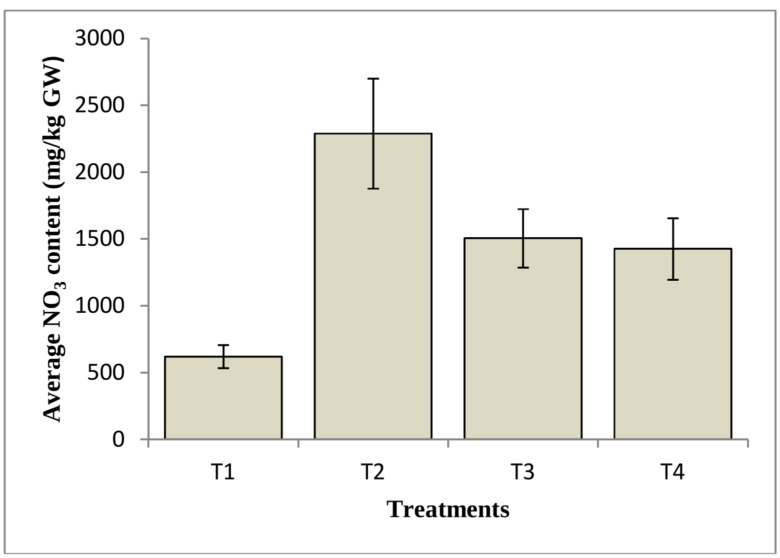

Fig. 10. Average nitrate concentrations in the grown spinach using different treatments

3.3 Practical Considerations and Implications

A survey was conducted by the authors to estimate the amount of hair available from barbers' shops around Dhaka city. The calculations showed that a total of approximately 20 tonnes of waste human hair can be collected per month from the city, containing approximately 3 tonnes of nitrogen. Development of a feasible collection strategy for will therefore be the next big challenge.

The synthetic fertilizer analysis showed that the nitrogen is available in suitable forms for plant uptake. The final pH of the solution was slightly basic, implying that the liquid fertilizer can be effectively used for acidic soils. The soil pH levels in 45 districts of Bangladesh are acidic, while in the other 19 districts the soil pH ranges from acidic to slightly basic (Mia et al. 2005). Therefore, the synthesized fertilizer could be used throughout the country.

4. CONCLUSION

The synthesis of a quick-release nitrogenous fertilizer from waste human hair incorporating such simple methodology either remains unexplored or has not yet been reported. The synthesized fertilizers were found to be an effective nitrogen source for plant growth enhancement. Hence this study forms the basis for further work on the effects of such fertilizer on plant physiology. Future commercialization of this process would largely depend on the development of a feasible collection strategy for waste hair.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Bencze K. What contribution can be made to biological monitoring by hair analysis. Fresenius J Anal Chem. 1990;338(1):58-61.
2. Pozebon D, Dressler VL, Curtius AJ. Análise de cabelo: Uma revisão dos procedimentos para a determinação de elementos traço e aplicações. Química Nova. 1999;22:838-46.
3. Ribeiro AS, Curtius AJ, Pozebon D. Determination of As, Cd, Ni and Pb in human hair by electrothermal atomic absorption spectrometry after sample treatment with tetramethylammonium hydroxide. Microchem J. 2000;64(1):105-10.
4. Ehleringer JR, Bowen GJ, Chesson LA, West AG, Podlesak DW, Cerling TE. Hydrogen and oxygen isotope ratios in human hair are related to geography. Proceedings of the National Academy of Sciences. 2008;105(8):2788-93.
5. Krishnan S, Cancilla A, Jervis R. Industrial wastewater treatment for toxic heavy metals using natural materials as adsorbants. J Radioan Nucl Ch. 1987; 110(2):373-8.
6. Krishnan S, Cancilla A, Jervis R. Waste water treatment for heavy metal toxins using plant and hair as adsorbents. Sci Total Environ. 1988;68:267-73.
7. Wasiuddin N, Tango M, Islam M. A novel method for arsenic removal at low concentrations. Energ Source. 2002; 24(11):1031-41.
8. Mitu AM. Damage assessment of human hair by electrophoretical analysis of hair proteins (PhD Thesis): Bibliothek der RWTH Aachen; 2004.
9. Robbins CR. Chemical and Physical Behavior of Human Hair. Springer Berlin Heidelberg; 2012.
10. Zheljazkov VD. Assessment of wool waste and hair waste as soil amendment and nutrient source. J Environ Qual. 2005; 34(6):2310-7.
11. Zheljazkov VD, Silva JL, Patel M, Stojanovic J, Lu Y, Kim T et al. Human hair as a nutrient source for horticultural crops. HortTechnology. 2008;18(4):592-6.
12. Hilterhausbong S, Zahn H. Contributions to the chemistry of human hair. 1. Analyses of cystine, cysteine and cystine oxides in untreated human hair. Int J Cosmet Sci. 1987;9(3):101-10.
13. Volkmar K, Hu Y, Steppuhn H. Physiological responses of plants to salinity: A review. Can J Plant Sci. 1998;78(1):19-27.
14. Pozebon D, Dressler VL, Curtius AJ. Determination of volatile elements in biological materials by isotopic dilution ETV-ICP-MS after dissolution with tetramethylammonium hydroxide or acid digestion. Talanta. 2000;51(5):903-11.

15. Rodrigues JL, Nunes JA, Batista BL, De Souza SS, Barbosa Jr F. A fast method for the determination of 16 elements in hair samples by inductively coupled plasma mass spectrometry (ICP-MS) with tetramethylammonium hydroxide solubilization at room temperature. J Anal Atom Spectrom. 2008;23(7):992-6.
16. Whiting D, Card A, Wilson C, Reeder J. Estimating soil texture. Colorado master gardener program, Colorado State University Extension; 2014.
17. Rayment GE, Lyons DJ. Soil chemical methods: Australasia. CSIRO Publishing; 2011.
18. Blakemore LC, Searle PL, Daly BK. Methods for chemical analysis of soils. NZ Soil Bureau, Department of Scientific and Industrial Research; 1987.
19. Kane PF. AOAC Method 978.02. Nitrogen (total) in Fertilizers. In: Cunniff P, editor. Official Methods of Analysis of AOAC International. 16th ed. Gaithersburg: AOAC International; 1998.
20. Rynk R, Service NRAE. On-farm Composting Handbook. Northeast Regional Agricultural Engineering Service; 1992.
21. Barrington S, Choinière D, Trigui M, Knight W. Effect of carbon source on compost nitrogen and carbon losses. Bioresour Technol. 2002;83(3):189-94.
22. Bray RH, Kurtz lT. Determination of total, organic and available forms of phosphorus in soils. Soil Sci. 1945;59(1):39-46.
23. Carson PL. Recommended potassium test. In: Dahnke WC, editor. Recommended chemical soil test procedures for the North Central Region. N.D. Agric. Exp. Stn., Fargo, N.D; 1975.
24. Gillman G, Sumpter E. Modification to the compulsive exchange method for measuring exchange characteristics of soils. Soil Res. 1986;24(1):61-6.
25. Harding D, Ross D. Some factors in low-temperature storage influencing the mineralisable-nitrogen of soils. J Sci Food Agr. 1964;15(12):829-34.
26. Jones CL, Maness NO, Stone ML, Solie JB, Zavodny D, editors. Variable rate nitrogen application on row crop spinach. ASAE Annual International Meeting; 2005; Tampa.
27. EC. Food science and techniques. Reports of the Scientific Committee for Food (38th series). Luxembourg: Office for Official Publications of the European Communities; 1997.
28. Allen EJ, Morgan DG. A quantitative analysis of the effects of nitrogen on the growth, development and yield of oilseed rape. J Agr Sci. 1972;78(02):315-24.

Peer-review history:
The peer review history for this paper can be accessed here:
http://sciencedomain.org/review-history/12917