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Research Article

Monthly variation and impact of pollution on the nutritional value of *Moringa oleifera* (Lam.) Leaves

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Abstract: *Moringa oleifera* Lam., commonly found tree in sub-Himalayan tracts of India, Pakistan, Bangladesh and Afghanistan has a high nutrition value. The leaves, flowers and immature pods of *Moringa* are used as a vegetable in many countries. The leaves are highly nutritious and medicinal in nature. They are a rich source of iron, calcium, vitamin A, vitamin C, proteins, and essential amino acids. Hence *Moringa* leaves can be a good source of protein for the vegetarians and the under-nourished population. Present investigation deals with the study of monthly variation in the nutritional value of *Moringa* leaves from the month of June 2015 to January 2016. Impact of urban sewage pollution and roadside vehicular pollution on the amount of reducing and total sugars, chlorophylls (a, b and total), proteins, vitamin-C and pH of the leaves, was also studied. Results revealed that the highest amounts of reducing sugars, total sugars, chlorophyll *b* and total chlorophyll during October 2015 whereas proteins, chlorophyll *a*, vitamin C and pH were highest during January 2016. Leaf samples collected from all the study sites exhibited minimum amounts of reducing sugars, chlorophylls (a, b and total) and pH during July 2015 whereas total sugars were lowest during December 2015. Proteins and vitamin C values were lowest during August and June 2015 respectively. Sewage and vehicular pollution showed an adverse effect on the nutritional contents of *Moringa oleifera* leaves. Of the two polluted sites, samples from near the sewage flow showed higher impact.

Keywords: *Moringa oleifera*, pollution, proteins, sugars, vitamin-C.

Introduction

Moringa oleifera Lam. is the most widely cultivated species of a monogeneric family Moringaceae. The tree is a native of Indian subcontinent and has become naturalized in the tropical and subtropical areas around the world (Farooq *et al.,* 2012). The tree ranges in height from 5 to 10 m (Morton, 1991). It grows best in dry sandy or loamy soil that is slightly alkaline (Abdul, 2007; Anjorin *et al.,* 2010); it is adaptable to various soil conditions ranging from 4.5 to 8.0 pH, but does not tolerate water logging, freezing or frosts conditions (Radovich, 2011). Reports suggest that Indians have been using it as a regular component of conventional eatables for nearly 5000 years (Anwar *et al.,* 2005; Anwar and Bhanger, 2003; D'Souza and Kulkarni, 1993). Trunk of the tree is soft, white corky and branches bear a gummy bark. Leaves are tripinnately compound. The flowers are white in color and three - winged seeds are scattered by the winds (Farooq *et al.,* 2012).

All parts of the tree are edible and have been consumed by humans since long. Fugile (2005) reported the various uses for M*oringa oleifera* which include: alley cropping (biomass production), animal forage (leaves and treated seed-cake), biogas (from leaves), domestic cleaning agent (crushed leaves), blue dye (wood), fencing (living trees), fertilizers (seed-cake), foliar nutrient (juice expressed from the leaves), green manure (from the leaves), gum (from tree trunk), honey and sugarcane juice-clarifier (powdered seeds), honey (flower nectar), medicine (all plant parts), ornamental plantings, biopesticides (soil incorporation of leaves to prevent seedling damping off), pulp (wood), rope (bark) and water purification (powdered seeds). The leaves possess remarkable nutritional and medicinal qualities (Mishra *et al.,* 2011; Singh *et al.,* 2012). They contain high amount of vitamin C, vitamin A, calcium and potassium. These leaves could be a great boon to people who do not get protein from meat. *Moringa oleifera* even contains two important amino acids arginine and histidine, important for infants (Duke, J.A. 1987, Fuglie, L.J. 1999, Oliveira, *et al.,* 1999, Babu, S.C. 2000, Lockett, *et al.,* 2000, Manzoor, *et al.,* 2007). As the leaves of *Moringa oleifera* have varied nutrients so it was decided to quantify some of them and highlight the best month/s when it is most nutritious for consumption. Along with this, effect of pollution (sewage and vehicular) was also analyzed.

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Material and Methods

Site selection and test sample collection:

The plant samples were collected from three different locations viz. least polluted area (termed as "Control"), near municipal sewage (polluted site -1) and along the roadside (polluted site-2). The leaf samples were collected every month on a fixed date. For the sake of uniformity of age of leaves, it was decided to collect the second compound leaf from the apex which was then immediately kept in a clean plastic bag to prevent leaf damage or loss of water. The samples were thereafter brought to the laboratory for all the biochemical analyses. The temperature (in °C) and relative humidity (in percentage) at the time of collection of sample were noted each time. The plant samples were gently washed with water to remove dust particles before analyses.

Biochemical analyses:

Reducing sugars: were estimated according to the method proposed by Folin and Wu (1929). The leaf sample was crushed in distilled water (DW) to which copper sulfate was added and boiled in water bath. This was followed by the addition of phosphomolybdic acid reagent and determination of optical density (O.D) at 663 nm with EQ-250 spectrophotometer.

Total sugars: were also estimated by Folin and Wu's method (1929). The leaf sample was crushed with DW, autoclaved with concentrated sulfuric acid and neutralized using sodium carbonate. The contents were filtered and used for the estimation of total sugars as given above for reducing sugars.

Estimation of proteins: Lowry's (1951) method was followed to estimate the amount of protein present in the sample. The plant material was crushed with DW, filtered through muslin cloth and diluted to 25 ml with DW. After adding reagent 'C' it was kept aside for a few minutes. Folin reagent was then added to this solution and O.D. was read at 420 nm by using spectrophotometer.

Estimation of chlorophylls: Chlorophylls *a*, *b* and total chlorophyll were estimated by the method proposed by Dr. Arnon (1949). Plant material was crushed with 80% acetone, centrifuged and diluted. O.D. was measured at 663 nm and 645 nm. Amounts of different chlorophylls were then calculated using Arnon's formula.

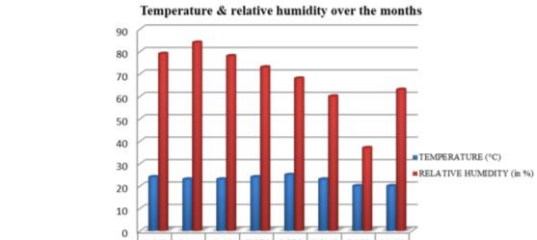
Vitamin C: Estimation of vitamin C was done by following Bessey and King's (1933) titration method. After keeping the leaf sample in metaphosphoric acid solution it was crushed using mortar and pestle. The extracted solution was titrated with an organic dye 2, 6-dichlorophenolindophenol (DCIP). DCIP serves as an indicator because excess DCIP turns the solution pink after passing through the end point (Bessey and king, *et al.,* 1933).

pH estimation: pH was determined according to the method proposed by Beckman (1934) using pH meter. 1% leaf extract was made by using fresh leaf material and distilled water. pH was then read using calibrated pH meter.

Results

Temperature and Relative Humidity:

Temperature (in °C) and Relative humidity (%age) records from June 2015 to January 2016 are as follows:

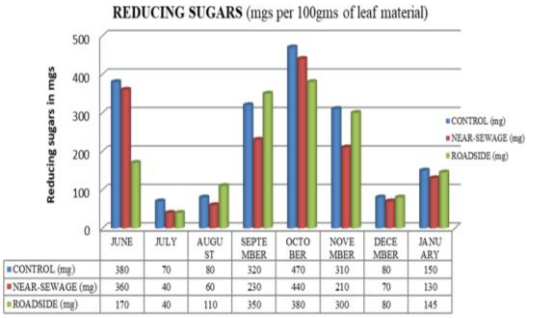


	JUNE	JULY	AUGUST	SEPTEMBER	OCTOBER	NOVEMBER	DECEMBER	JANUARY
TEMPERATURE (°C)	24	23	23	24	25	23	20	20
RELATIVE HUMIDITY (in %)	79	84	78	73	68	60	37	63

Figure 1. Temperature and relative humidity

Source: website of meteorological department

Reducing sugars:

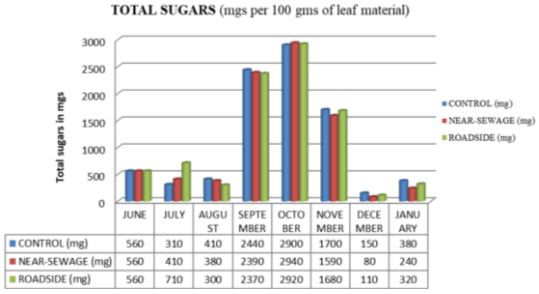


	JUNE	JULY	AUGUST	SEPTEMBER	OCTOBER	NOVEMBER	DECEMBER	JANUARY
CONTROL (mg)	380	70	80	320	470	310	80	150
NEAR-SEWAGE (mg)	360	40	60	230	440	210	70	130
ROADSIDE (mg)	170	40	110	350	380	300	80	145

Figure 2: Reducing sugars in *Moringa oleifera* leaves from June 2015 to January 2016

Reducing sugar content for all the sites (control, near-sewage and roadside) showed sudden decrease in July month as compared to June. From July onwards, all the three study sites showed gradual increase till October. After that the values began to drop till December and in January it showed slightly higher values than December. Maximum reducing sugar content was observed in the month of October and minimum in July in all the three sites i.e. control, near-sewage and roadside sites. Highest reducing sugar value (470mg/100gms of leaf sample) was observed in control plant in October month and the lowest value (40 mg/100 gms of leaf sample) was observed in near-sewage plant.

Total sugars:



	JUNE	JULY	AUGUST	SEPTEMBER	OCTOBER	NOVEMBER	DECEMBER	JANUARY
CONTROL (mg)	560	310	410	2440	2900	1700	150	380
NEAR-SEWAGE (mg)	560	410	380	2390	2940	1590	80	240
ROADSIDE (mg)	560	710	300	2370	2920	1680	110	320

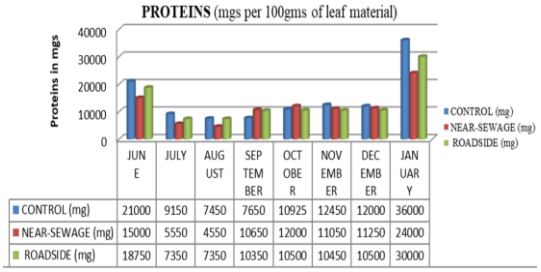
Figure 3: Total sugars in *Moringa oleifera* leaves from June 2015 to January 2016

Total sugars content for all the study sites i.e. control, near-sewage and roadside were low in the months of June, July and August as compared to those of September. The values showed gradual increase till October month and then began to decrease from November. In January, the values showed a slight increase from that of December.

The maximum total sugar content was observed in October and minimum in December, in all the three study sites i.e. control, near-sewage and roadside. The highest total sugar content i.e. 2.94 gms/100gms was observed in October in near-sewage sample as compared to the control and roadside sites while lowest total sugar i.e. 0.08 gms/100 gms in near-sewage sample in December.

The protein content for all the three study sites (control, near- sewage and roadside) showed a gradual decrease from June to August and a gradual increase from September to January. The maximum protein content was observed in January and minimum in August in all the three study sites. The highest protein recorded was 36gms/100gms in control sample in the month of January. The lowest protein content was found to be 4.55 gms/100 gms in August for the near-sewage sample.

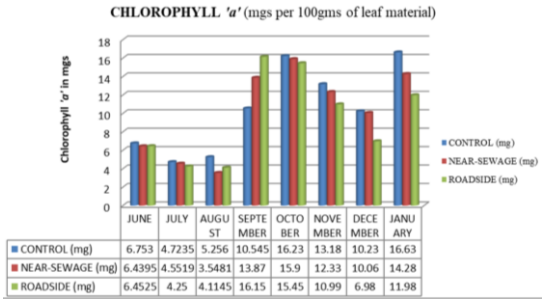
Proteins:



	JUNE	JULY	AUGUST	SEPTEMBER	OCTOBER	NOVEMBER	DECEMBER	JANUARY
CONTROL (mg)	21000	9150	7450	7650	10925	12450	12000	36000
NEAR-SEWAGE (mg)	15000	5550	4550	10650	12000	11050	11250	24000
ROADSIDE (mg)	18750	7350	7350	10350	10500	10450	10500	30000

Figure 4: Proteins in *Moringa oleifera* leaves from June 2015 to January 2016

Chlorophyll *'a'*:



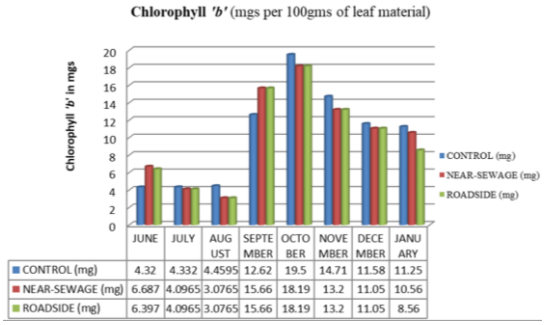
	JUNE	JULY	AUGUST	SEPTEMBER	OCTOBER	NOVEMBER	DECEMBER	JANUARY
CONTROL (mg)	6.753	4.7235	5.256	10.545	16.23	13.18	10.23	16.63
NEAR-SEWAGE (mg)	6.4395	4.5519	3.5481	13.87	15.9	12.33	10.06	14.28
ROADSIDE (mg)	6.4525	4.25	4.1145	16.15	15.45	10.99	6.98	11.98

Figure 5: Chlorophyll *'a'* in *Moringa oleifera* leaves from June 2015 to January 2016

The amount of chlorophyll 'a' in *Moringa oleifera* leaves of all the study sites (control, near-sewage and roadside) was low in July and August months as compared to June. All the sites showed gradual increase in chlorophyll 'a' content from August to October and afterwards, showed gradual decrease till December followed by an increase in January.

Moringa oleifera leaves from all the three study sites (control, near-sewage and roadside) showed maximum amounts of chlorophyll 'a' in October while minimum values in August in near-sewage and roadside plant samples. The control sample showed highest values (16.63mg/100gms) in January and the near-sewage sample showed lowest values (3.5481 mg/100 gms) in August.

Chlorophyll *'b':*



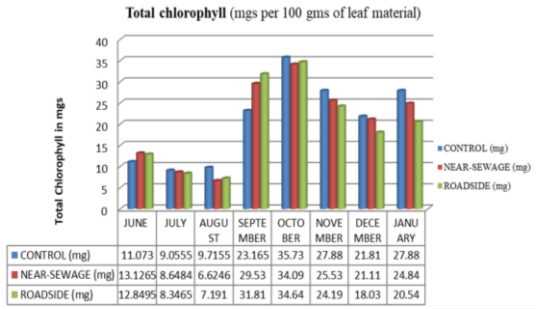
	JUNE	JULY	AUGUST	SEPTEMBER	OCTOBER	NOVEMBER	DECEMBER	JANUARY
CONTROL (mg)	4.32	4.332	4.4595	12.62	19.5	14.71	11.58	11.25
NEAR-SEWAGE (mg)	6.687	4.0965	3.0765	15.66	18.19	13.2	11.05	10.56
ROADSIDE (mg)	6.397	4.0965	3.0765	15.66	18.19	13.2	11.05	8.56

Figure 6: Chlorophyll *'b'* in *Moringa oleifera* leaves from June 2015 to January 2016

Chlorophyll '*b*' content in the leaf samples from all the three study sites (control, near-sewage and roadside) was low in July and August as compared to June. From August, all the sites showed gradual increase till October followed by a decrease thereafter till January.

Leaf samples from all the three study sites (control, near-sewage and roadside) showed highest values for chlorophyll '*b*' in October (except roadside sample) and lowest values in August. The maximum chlorophyll '*b*' content (19.5 mg/100 gms) was found in control plant in October month. The minimum chlorophyll '*b*' content (3.0765 mg/ 100 grams) was observed in August in near-sewage plant.

Total chlorophyll:

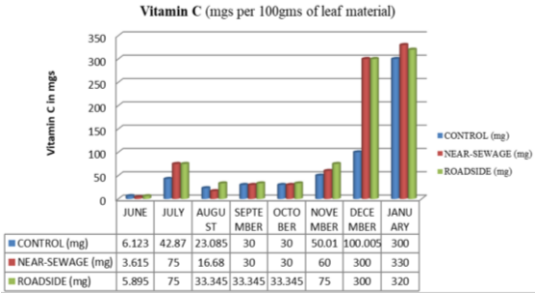


	JUNE	JULY	AUGUST	SEPTEMBER	OCTOBER	NOVEMBER	DECEMBER	JANUARY
CONTROL (mg)	11.073	9.0555	9.7155	23.165	35.73	27.88	21.81	27.88
NEAR-SEWAGE (mg)	13.1265	8.6484	6.6246	29.53	34.09	25.53	21.11	24.84
ROADSIDE (mg)	12.8495	8.3465	7.191	31.81	34.64	24.19	18.03	20.54

Figure 7: Total Chlorophyll in *Moringa oleifera* leaves from June 2015 to January 2016

Total chlorophyll content in the leaves from all the three study sites was high in June as compared to July and August. Gradual increase was observed from August to October followed by a decrease till December and increase in January. The highest amounts of total chlorophyll were observed in the leaf samples from all the study sites in October while lowest values were seen in the month of August. The maximum amount of total chlorophyll was recorded in control plant in October i.e. 35.73 mg/100 gms. The minimum value i.e. 6.6246 mgs/ 100 gms was found in near-sewage plant in the month of August.

Vitamin C:



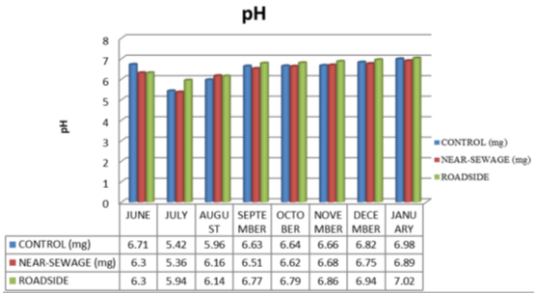
	JUNE	JULY	AUGUST	SEPTEMBER	OCTOBER	NOVEMBER	DECEMBER	JANUARY
CONTROL (mg)	6.123	42.87	23.085	30	30	50.01	100.005	300
NEAR-SEWAGE (mg)	3.615	75	16.68	30	30	60	300	330
ROADSIDE (mg)	5.895	75	33.345	33.345	33.345	75	300	320

Figure 8: Vitamin C in *Moringa oleifera* leaves from June 2015 to January 2016

Leaf samples from all the three study sites (control, near-sewage and roadside) showed high vitamin C content in the month of July as compared to June and August. From August onwards all the sites showed gradual increase till January. Test leaf samples from all the three study sites showed maximum vitamin C values in January and minimum in June. The highest value of vitamin C was found to be 330mgs/100gms in near-sewage sample in January whereas the lowest value of 3.615 mg/100 gms was also observed in near-sewage sample in June.

pH:

Moringa oleifera leaf samples from all the three study sites exhibited high pH values in the month of June as compared to July followed by a gradual increase from July to January. Leaf samples from all the three study sites (control, near-sewage and roadside) showed maximum pH values in January and minimum in July. The highest pH value (7.02) was found in roadside plant in January and the lowest (5.36) in near-sewage plant in July.



	JUNE	JULY	AUGUST	SEPTEMBER	OCTOBER	NOVEMBER	DECEMBER	JANUARY
CONTROL (mg)	6.71	5.42	5.96	6.63	6.64	6.66	6.82	6.98
NEAR-SEWAGE (mg)	6.3	5.36	6.16	6.51	6.62	6.68	6.75	6.89
ROADSIDE	6.3	5.94	6.14	6.77	6.79	6.86	6.94	7.02

Figure 9: pH in *Moringa oleifera* leaves from June 2015 to January 2016

Discussion

Reducing and Total Sugars: Sugars are found in the tissues of most plants. They are the primary source of energy for our body. Simple sugar acts as an immediate source of energy when metabolized. Sugars also enhance the flavour of different foods.

Although the test plants from all the three sites exhibited highest reducing sugar content in October but among the three sites, the maximum amount (0.47 gms/100 gms) of reducing sugar, was found in the leaves from control site. Similarly, plants from all the three sites showed minimum amounts of reducing sugar in July but among the three sites leaves from sewage and roadside showed lesser amount (0.04 gms/100 gms) of reducing sugar as compared to control site (Figure 2).

Although the total sugar content in plants from all the three sites was maximum in October, but among the three sites, leaves from the near-sewage site showed the highest amount (2.94 gms/100 gms) of total sugar. Similarly, plant samples from all the three sites exhibited minimum amounts of total sugars in December but amongst the three sites least amount (0.08 gms/100 gms) of total sugar was found in the sample collected from near-sewage site (Figure3).

Literature survey did not reveal any work reporting precise values for sugar analyses in *Moringa oleifera*. The present study showed that as the temperature increases, amount of sugars in leaves also increases. Similar observation was made by Han, *et al.,* 2013, he reported that with increasing temperature, soluble sugar content of leaves of lettuce seedling increased significantly.

Proteins: Proteins are the building blocks of bones, muscles, cartilage, skin and blood. They are thus required in large amounts. The present investigation showed that the leaves of *Moringa oleifera* are the great source of proteins and fresh leaf consumption can fulfil the human requirements. Although the protein content was

maximum in January for all the three sites but amongst the three sites, the leaves from control site showed the highest amounts (36 gms/100 grams) of proteins. Similarly, the minimum protein content during the year was found in August for all the three sites and amongst the three sites studied the leaves from the near-sewage site showed minimum amounts (4.5 gms/100 gms) of protein (Figure 4).

Protein estimation from *Moringa oleifera* Lam. Leaves was also done by many other researchers whose results are as follows: Campaore, *et al.,* (35.56 gms/100 grams), Mbaiguinam, *et al.,* 2014 (32.06 gms/100 gms), Oduro, *et al.,* (27.51 gms/100 gms) Abdulla, 2013 (27.2 gms/100 gms) Asnate, *et al., (*26.98gm/100gms), Kathryn A. (8.8 gms/100 gms). As compared to all the above given results, amounts of protein estimated in the current research work are higher. From the present investigation it was found that increase in temperature decreases the amount of proteins in leaves and *vice versa*. It was supported by Taiz and Zeiger, 2002 who reported that increasing temperature activates enzymatic activities. Temperatures higher than the optimum may cause many cell proteins to become unfolded, thereby leading to loss of proper structure and activity. This agrees with the findings of Modi (2007) who reported that cool environmental conditions are associated with high total protein in leafy vegetables while hot temperatures had a significant decrease in leaf protein content.

Chlorophyll '*a*': During the entire period of investigation it was found that chlorophyll '*a*' content was maximum (16.63 mg/100 gms) in the leaves during January, from the control site, amongst all the three sites studied. Similarly, minimum chlorophyll '*a*' content (3.5481 mg/100 gms) was observed during August in the sample collected from near-sewage site (Figure 5). Mbaiguinam *et al.,* 2014 reported 10.023 mgs/100 gms of vitamin C in the leaves of *Moringa oleifera* Lam. which lies between the maximum and minimum amounts estimated in the current research work.

Chlorophyll '*b*': Although the Chlorophyll '*b*' was maximum in October in all the three sites studied but among the three sites, control site showed maximum (19.5 mg/100 gms) chlorophyll '*b*' content. Similarly, chlorophyll 'b' content was minimum during the month of August (3.0765 mg/100 gms) in the leaves from near-sewage site among all the three sites (Figure 6). Mbaiguinam *et al.,* 2014, reported the chlorophyll '*b*' amount of 4.365 mgs/100 gms leaf material which happens to lie between the minimum and maximum of the current project.

Total chlorophyll: Chlorophyll is beneficial for humans as it is the precursor for haemoglobin and can increase the immunity to fight various diseases. Total chlorophyll estimated during the study period exhibited the maximum amount in October in all the three sites studied. But amongst the three sites, leaves from control site showed a maximum value (35.73 mg/100 gms). Similarly, the minimum amounts of total chlorophyll in the test samples was detected during the month of July for all the three sites but, amongst the three sites, leaves from near-sewage site showed minimum amount of total chlorophyll (6.6246 mgs/ 100 gms) (Figure 7). Literature survey did not reveal any work reporting precise values of total chlorophyll in *Moringa oleifera* Lam.

The present investigation revealed low chlorophyll content in rainy and warmer season while high contents in winters**.** It was supported by Chowdhury, *et al.,* 2003, who suggested that seasonal cycles of temperature, day lengths, rainfall, humidity and wind exert a pronounced control over the physiological and reproductive processes in plants. Seasonal environmental patterns of temperature, day length, humidity etc. provide plants with a pattern to which developmental changes are physiologically connected. An increase in the activity of chlorophyllase enzyme (responsible for enzymatic breakdown of chlorophyll by removing the phytol side chain) during summer and rainy season lowers the chlorophyll content; whereas decrease in the activity of chlorophyllase enzyme increases the chlorophyll content during winter season. Hence an increase in chlorophyllase activity always results in the lowering of chlorophyll content and *vice versa*. A higher activity of this enzyme ultimately leads to the reduced rate of photosynthesis resulting in poor growth and yield during the periods of environmental stresses.

Vitamin C: Vitamin C plays many functions in the human body viz., antioxidant agent, cofactor for enzymes etc. Vitamin C helps the body to make collagen, cartilage, ligaments and blood vessels. It is needed for healing wounds, and for repairing and maintaining bones and teeth. The study on leaves of *Moringa oleifera* showed that, they are a rich source of vitamin C. The daily requirement of vitamin C for adults ranges from 75 mg to 125 mg depending of activities (Mbaiguinam, *et al.,* 2014). Although the Vitamin C content was found to be maximum in month of January for all the three sites investigated, the near-sewage site had maximum vitamin C value (330 mgs/100 gms) among the three. Similarly, vitamin C content during the entire period of study was found to be minimum in June for all the sites but of the three sites, leaves from near-sewage site showed minimum values (3.615mgs/100gms) of vitamin C (Figure 8). Reports of vitamin C studies performed by other researchers in *Moringa oleifera* Lam. are as follows: Mbaiguinam, *et al.,* 2014, found 257

mgs/100 gms, Tamanna Talreja, 2011, reported 58.48 mg/100 gms, Iqbal, *et al.,* 2004, recorded 46 mg/100 gms, S. Iqbal, *et al.,* 2006, reported 40 mgs/100 gms and Mona Abdulla, 2013, found 17 mgs/100 gms. The current study showed that the amount of vitamin C increases with decrease in temperature.

pH: The present investigation revealed that lowest pH was 5.36 in the month of July from near-sewage area and highest pH was 7.02 in the month of January along the roadside area (Figure 9). (It should be noted that during rainy months of June to September 2015, Mumbai received scanty rainfall. It was merely 46mm during the month of July, 2015). Asante *et al.,* 2013 found pH of the leaves 8.35 and 8.71 collected from two different locations. As the pH of leaves increases protein content also increases. Current investigation also found a similar relationship between pH and protein.

Research by Diet. myfit (2012) indicates that there exists no recommended dietary pH for food substances, but the human body operates best within a pH range of 7.35 - 7.45. Therefore, the consumption of any food with a higher or lower pH value is an indication of it being alkaline or acidic to the human body.

Conclusion

The highlights of the current research work performed on *Moringa oleifera* are as follows: The plant growing in unpolluted condition showed highest amounts of reducing sugars, total sugars, protein, chlorophyll *a*, Chlorophyll *b* and total chlorophyll. The reducing and total sugars were found to be maximum in the month of October for *Moringa oleifera* plants growing in all the three study sites (with highest in control). Reducing sugars were minimum in July in all the three sites (with lowest in roadside and near-sewage site). Total sugars were minimum in December in all the three sites (with lowest in near-sewage site). As the temperature increases, amount of sugars (reducing and total) also increases. Proteins were maximum in January for all the three sites (with highest in control) and minimum in August for all the three sites (with lowest in near-sewage). Chlorophyll *'a'* was maximum in January for all the sites (with highest in control) and minimum in August for all the sites (with lowest in near-sewage). Chlorophyll *'b'* was maximum in October for all the three sites (with highest in control) and minimum in all the three sites (with lowest in control). Total chlorophyll was maximum in October in all the three samples (with highest in control site) and minimum in July for all the three sites (with lowest in near-sewage). Vitamin C was maximum in January for all the three sites (with highest in near-sewage) and minimum in June for all the three sites (with lowest in near-sewage). Vitamin C increases with decrease in temperature. pH was maximum in January for all the three study sites (with highest in roadside) and minimum in July (with lowest in near-sewage). (It should be noted that during rainy months of June to September 2015, Mumbai received scanty rainfall). Best month for high amounts of sugars, proteins and chlorophyll is October and for vitamin C is January. The findings revealed that *Moringa oleifera* leaves are a great and economic source of all the nutrients required for the well-being of humans. Pollution has a negative impact on their contents. Hence it is very important that awareness regarding this scientific finding should be spread to benefit the society.

Results at a glance:

	Control		Near-sewage		Roadside
Reducing sugar	✓ October	o	July	o	July
Total sugars	✓ October	o	December		
Protein	✓ January	o	August		
Chlorophyll 'a'	✓ January	o	August		
Chlorophyll 'b'	✓ October	o	August		
Total chlorophyll	✓ October	o	July		
Vitamin C	✓ January	o	June		
pH		o	July	✓	January

✓ Represents maximum values
o Represents minimum values

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