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Research Article

Atomic Absorption Spectroscopic Determination of Few Major and Trace Elements in Nature's Finest Medicine (Wheatgrass Juice Powder) *Triticum aestivum* L. and their Possible Correlation With Reported Therapeutic Activities: Part-I

*Sharma Vivek, Sharma Navdeep, Bano Anisha, Kumar Sanjeev, Dhaliwal Harcharan Singh

Akal School of Biotechnology, Eternal University, Baru Sahib-173101 (H.P.) India

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ABSTRACT

The young wheat plant (*Triticum aestivum* L.) belongs to the family Poaceae is referred as wheatgrass. It provides all the important nutrients for a healthy and rejuvenating body and acts as an efficient source of energy. The extensive research on wheatgrass is already mentioned in Ayurveda, different herbal system of medicines and different pharmacopoeias of the world. It possesses number of pharmaceutical properties, in which some have already been known and many more are still to be identified. In the present study the major and trace element contents like (Zn, Cu, Mn, Fe, Mg, K and Ni) were first time comparatively analysed in the three samples viz.: Wheat Grass Juice Powder (WGJP), Wheatgrass Fibre Powder (WGFP) and Dry Wheatgrass Powder (DWGP) of *Triticum aestivum* L. from Northern India with the help of Atomic Absorption Spectrophotometer (AAS). The main purpose of the exploration is to document confirmation and spreading the benefits of wheatgrass to daily users, practitioners and also for the researchers worldwide by correlating the properties of these elements with reported therapeutic activities.

Keywords: Wheatgrass, *Triticum aestivum* L., Poaceae, AAS, Lyophilization, Toxicity, Enzymes, Vitamins, Nutrients, Trace Elements.

INTRODUCTION

Triticum aestivum L. belongs to the family Poaceae, a green herb found worldwide. Plant is believed to have multifarious pharmacological activities in addition to its numerous nutritional values. It is already well reported in different research papers that both fresh juice or dried powder of wheatgrass provide chlorophyll, amino acids, minerals, vitamins and enzymes and considered as powerhouse for human body. It is well reported by researchers worldwide that many low income families of the developing countries rely on simple diet of staple food crops such as wheat, maize and rice and for a healthy and rejuvenating body, green crops could be very useful in providing nutrients like vitamins, proteins, minerals and antioxidants with numerous health benefits.[1-6] It is also referred that 50 pounds of wheatgrass is equal to 450 pounds of garden vegetables.[7] In one of the research article, it has been referred that, in Asia and Europe, wheatgrass based products consumed in the form of juices, powders and extracts for healthy growth of human body. Wheatgrass has been identified as a complete food providing all the nutrients than provided by any other food. Wheatgrass juice provides manifold health and wellness benefits by curing problems related with digestive system and anaemia. Wheatgrass acts as a blood purifier and strengthen the immune system. Anti-carcinogenetic activity has already been reported for wheatgrass juice.[8] It is well known and reported widely that, wheat possesses anti-mutagenicity and antioxidant activity because of redox enzymes including catalase, peroxidise and other antioxidant compounds such as phenolic acids, alkyresorcinols and amino phenols.[9, 10] The products of wheatgrass also reported to cure many dreadful diseases such as thalassemia and distal ulcerative colitis.[11, 12] The research work by Bar-Sela and co-workers reported that the breast cancer patients who drank wheatgrass juice daily showed a decreased need for blood and bone marrow building medications during chemotherapy, without diminishing the effects of the therapy.[13] Cytotoxic effects of commercial wheatgrass and fibre towards human acute promyelocytic leukemia cells are also reported recently.[14] A very little information has been available on the trace element and heavy metal profile of the wheatgrass and its different products such as wheatgrass powder, wheatgrass juice, etc. Still there are massive research lacunas in the same field because lyophilized wheatgrass juice powder has not been studied previously and first time studied in our lab. Therefore, the present study was designed to estimate the major and trace elements in wheat grass (Wheatgrass Juice Powder (WGJP), Wheatgrass Fibre Powder (WGFP) and Dry Wheatgrass Powder (DWGP) through spectrophotometry (AAS) and to make it accepted

*Author for correspondence: E-mail: vivek03sharma@rediffmail.com

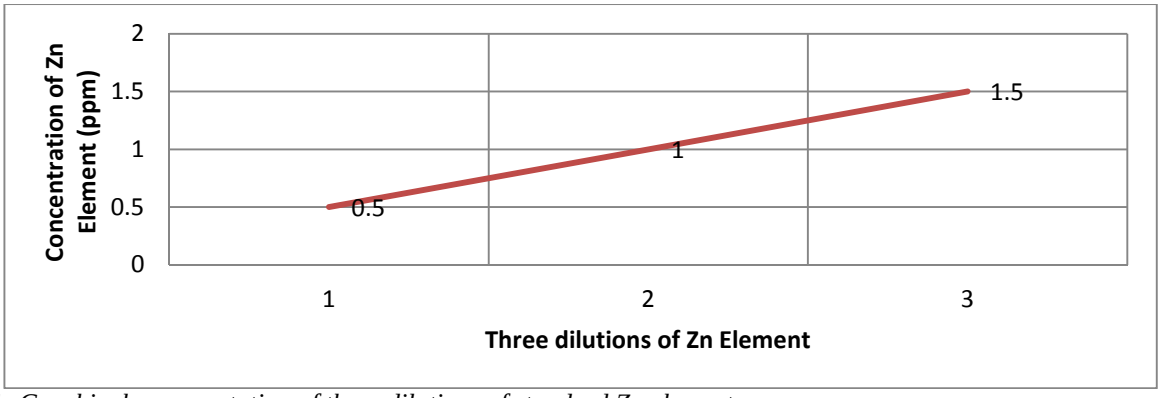

Fig. 1: Graphical representation of three dilutions of standard Zn element.

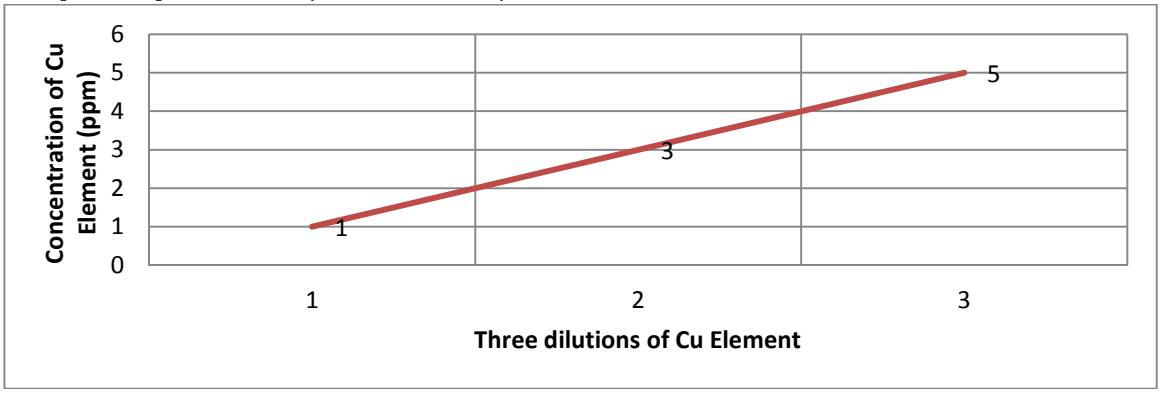

Fig. 2: Graphical representation of three dilutions of standard Cu element.

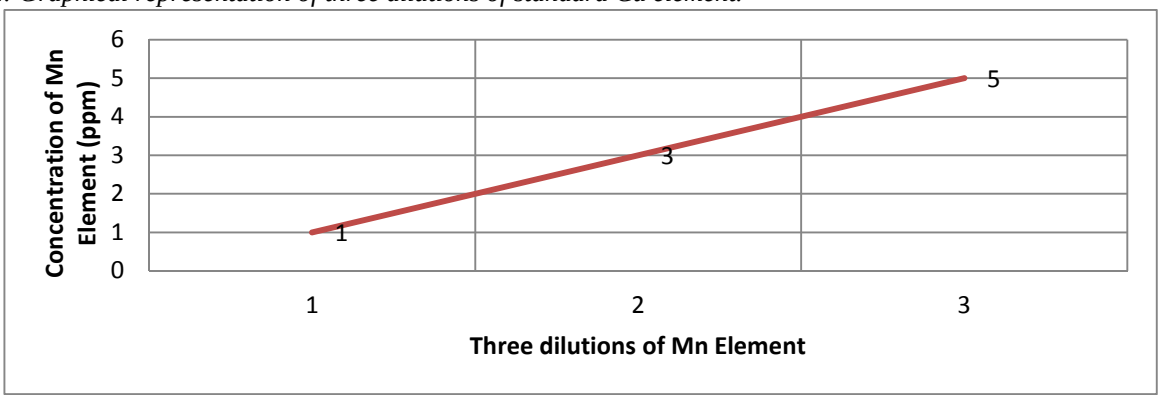

Fig. 3: Graphical representation of three dilutions of standard Mn element.

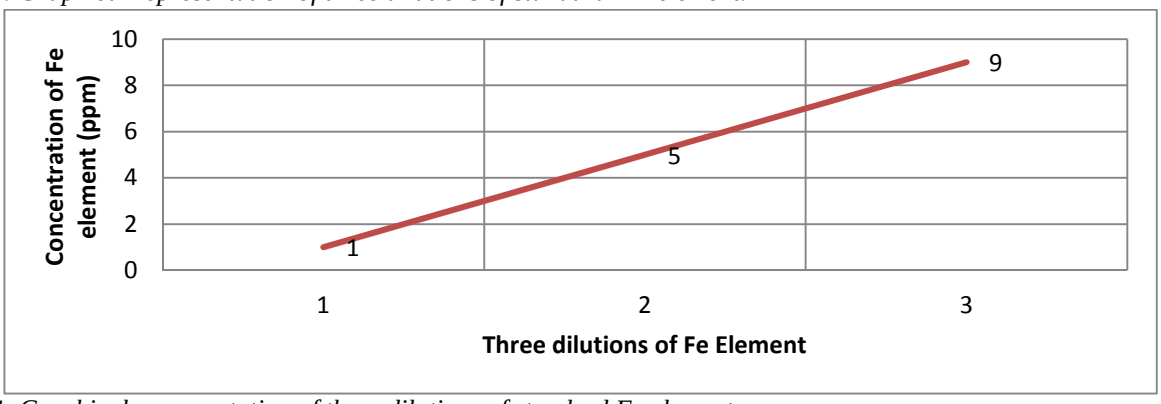

Fig. 4: Graphical representation of three dilutions of standard Fe element.

worldwide. This research paper has been carried out to show all these major and trace elements associated with these wheatgrass samples. We know that, wheatgrass is not significantly popular due to very less convincing research on its different indoor or outdoor growing conditions, low characteristic features corresponding to its taste, aroma, colour, poor shelf-life and its cost. Our main objective for future study is to improve the different indoor or outdoor growing conditions for wheat grass and also to optimize its taste,

aroma and shelf-life of wheatgrass juice powder for supreme recognition worldwide without modification in its originality.

MATERIALS AND METHODS

Plant Material: The samples of (*Triticum aestivum* L.), Wheatgrass Juice Powder (WGJP), Wheatgrass Fibre Powder (WGFP) and Dry Wheatgrass Powder (DWGP) were taken for determination of major and minor elements through atomic absorption spectrophotometer analysis. The samples of *Triticum aestivum* L. were collected from agricultural experimental farms, prepared in Biotechnology Laboratory of Eternal University, Himachal Pradesh.

Preparation of Samples: The collected plant materials were washed thoroughly with running tap water and followed by washing with deionised autoclaved water to remove the dust particles and possible parasites. The samples like Wheatgrass Juice Powder, Wheatgrass Fibre Powder and Dry Wheatgrass Powder were freeze dried/ lyophilized to prepare powder and all these powdered samples were stored in closed air tight bottles for further experimentation. 200mg of above said samples were digested with 5ml of nitric acid and 2ml HF at 250°C, ramp time was 30 minutes and hold time was 25 minutes until the volume reduced to 2ml in digester. The digested samples were dissolved in double distilled water, filtered and the volume was made up to 25ml for each sample and used for determination of different elements. The acidity of 0.1% of nitric acid was maintained in all the sample solutions. Blank was also prepared without adding the samples for better precise analysis. The dilutions of sample solutions were done to keep the concentration of different elements within linear range of absorbance. The complete digestions for different materials were achieved with minor modifications into the standard procedure as recommended by the Royal Committee of Experts.[15]

Preparation of Standard Solution: Standards of different elements such as: Zn, Cu, Mn, Fe, Mg, K and Ni were

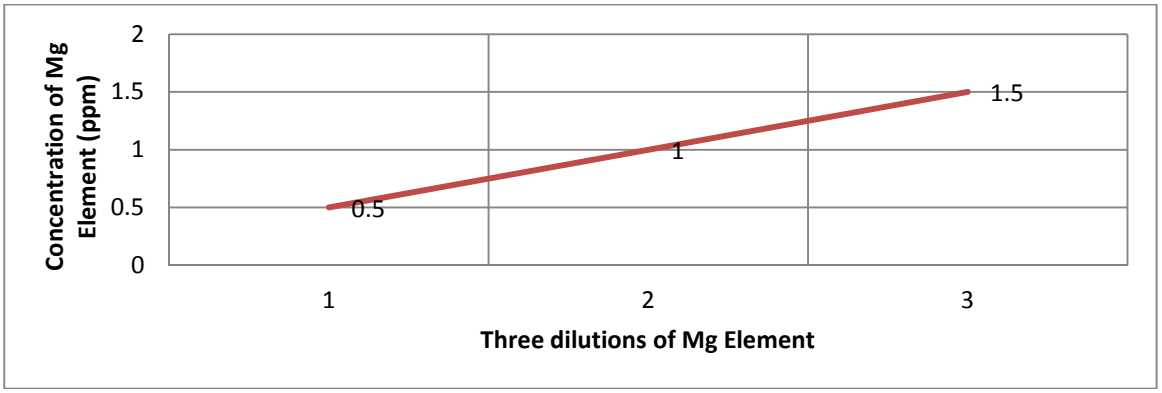

Fig. 5: Graphical representation of three dilutions of standard Mg element.

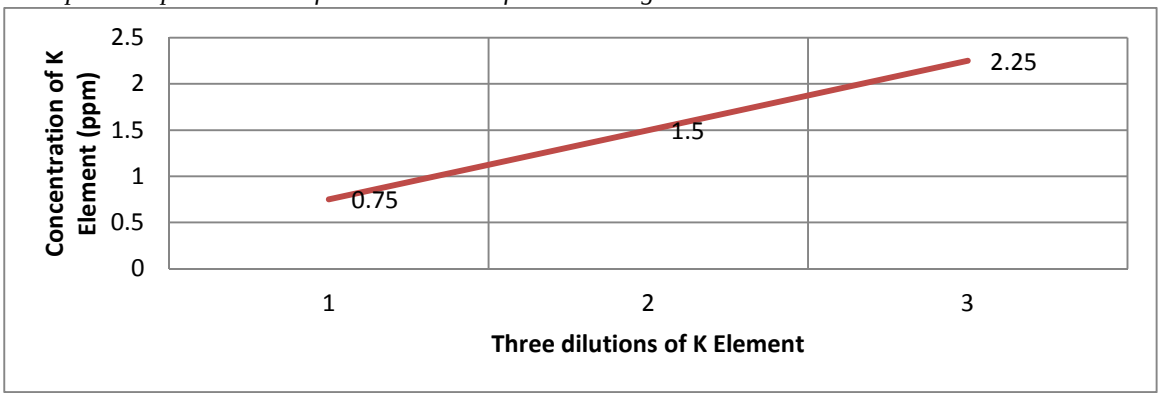

Fig. 6: Graphical representation of three dilutions of standard K element.

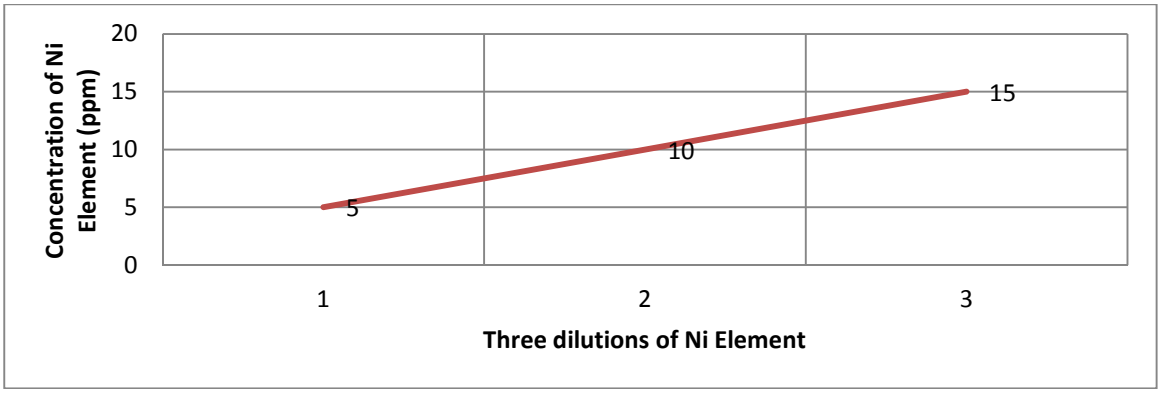

Fig. 7: Graphical representation of three dilutions of standard Ni element.

procured from Merck, Germany and were used as reference analytes for quantitative estimation of major/minor elements as well as accurate calibration and quality assurance of each analyte. The standard stock solutions (1000 ppm) were diluted to obtain working standard solutions ranging from 1 ppm to 50 ppm and stored at 4°C for further use. The final acid concentration maintained in all standard solutions was 0.1%.

Zinc (Zn) Standard Solution: Zinc (Zn) standard solution was prepared from stock solution (1000 ppm). Standard solutions of concentration 0.5, 1.0 and 1.5 ppm were prepared and analysed. The absorption of standard solutions were measured at 213.9 nm using hallow cathode lamp as a light source & air acetylene flame source on atomic absorption spectrophotometer (Figure 1).

Copper (Cu) Standard Solution: Copper standard solution was prepared from stock solution (1000 ppm). Standard solutions of concentration 1.0, 3.0 and 5.0 ppm were prepared and analysed. The absorption of standard solutions were measured at 324.8 nm using hallow cathode lamp as a light source & air acetylene flame source on atomic absorption spectrophotometer (Figure 2).

Manganese (Mn) Standard Solution: Manganese (Mn) standard solution was prepared from stock solution (1000 ppm). Standard solutions of concentration 1.0, 3.0 and 5.0 ppm were prepared and analysed. The absorption of standard solutions were measured at 279.5 nm using hallow cathode lamp as a light source & air acetylene flame source on atomic absorption spectrophotometer (Figure 3).

Iron (Fe) Standard Solution: Iron (Fe) standard solution was prepared from stock solution (1000 ppm). Standard solutions of concentration 1.0, 5.0 and 9.0 ppm were prepared and analysed. The absorption of standard solutions were measured at 248.3 nm using hallow cathode lamp as a light source & air acetylene flame source on atomic absorption spectrophotometer (Figure 4).

Magnesium (Mg) Standard Solution: Magnesium (Mg) standard solution was prepared from stock solution (1000 ppm). Standard solutions of concentration 0.5, 1.0 and 1.5 ppm were prepared and analysed. The absorption of standard solutions were measured at 285.2 nm using hallow cathode lamp as a light source & air acetylene flame source on atomic absorption spectrophotometer (Figure 5).

Potassium (K) Standard Solution: Potassium (K) standard solution was prepared from stock solution (1000 ppm). Standard solutions of concentration 0.75, 1.50 and 2.25 ppm were prepared and analysed. The absorption of standard solutions were measured at 766.5 nm using hallow cathode lamp as a light source & air acetylene flame source on atomic absorption spectrophotometer (Figure 6).

Nickel (Ni) Standard Solution: Nickel (Ni) standard solution was prepared from stock solution (1000 ppm). Standard solutions of concentration 5.0, 10.0 and 15.0 ppm were prepared and analysed. The absorption of standard solutions were measured 232.0 nm using hallow cathode lamp as a light source & air acetylene flame source on atomic absorption spectrophotometer (Figure 7).

Instrumentation: Samples were freeze dried with the help of Lyophilizer made of Christ Alpha 1-4 LD plus. Antonpaar microwave Digestion System Multiwave ECO was used for digestion of samples. AAS made of Agilent Technologies 240FSAA, Fast Sequential Atomic Absorption Spectrophotometer equipped with SIPS power supply, nitrous burner, spray chamber assemblies, SPS-3 auto sampler, VGA-77 continuous flow vapour generation system for the determination of Hg and hydride forming elements, air compressor-oil free 381/min @ 8bar, HI TECH SFA20 mounting rack, SpectrAA software and worksheet AA software with version 5.2 PRO data processor were used for different element analysis in samples. The fuel used was air/acetylene. All parameters were set and followed strictly according to the manufacturer's instructions using flame atomization technique (Table 1). All samples and standards were analysed in triplicate using flame atomic absorption spectrophotometer for precision and accuracy of the results.

RESULTS AND DISCUSSION

Herbal or alternative medicines are gaining popularity and scientific research about wheatgrass as a "functional food" is becoming more available and popular as a research topic. It is found to be used as treatment for minor ailments and serious life threatening issues as well as a preventive dietary supplement. The major and trace element analysis in different samples of wheatgrass has been extensively studied and correlated with the possible therapeutic activities. Trace elements or essential elements are necessary for multiple physiological and metabolic reactions involved in maintaining good health.[16] In the present study the major and trace element contents like (Zn, Cu, Mn, Fe, Mg, K and Ni) were first time comparatively analysed in the three samples such as: Wheatgrass Juice Powder (WGJP), Wheatgrass Fibre Powder (WGFP) and Dry Wheatgrass Powder (DWGP) of *Triticum aestivum* L. from Northern India with the help of Atomic Absorption Spectrophotometer (AAS). As per the reported results**, t**he concentration of Zinc (Zn) in formulated Wheatgrass Juice Powder (WGJP), Wheatgrass Fibre Powder (WGFP) and Dry Wheatgrass Powder (DWGP) are 94.24, 30.84 and 30.48 ppm. Whereas, the concentration of Copper (Cu) in all three samples is 18.04, 7.63 and 11.46 ppm. In literature, it is clearly mentioned that, Zinc (Zn) is helpful in the treatment of prostate gland disorders and nourishes hair. Sub normal plasma Zn level has already been reported in patients with atherosclerosis.[17] The presence of Zn and Cu in the plant is correlated with its anticancer property, as both elements are required in growth and proliferation of normal cells. Zn concentration decreases in cancer patients, whereas, Cu concentration increases.[18] It is believed that low concentration of plasma Zn in cancer patients is due to the increased requirement of Zn by cancer tissues. This seems to be sensible because of the fact that tumour cells have high rate of DNA synthesis and most of the enzymes involved in nucleic acid synthesis are Zn dependent.[19] Copper deficiency in human may results in hypochromic

Table 1: Instrumental conditions required for flame atomization during whole analysis.

Conditions	Zn	Cu	Mn	Fe	Mg	K	Ni
Lamp Current (mA)	5.0	4.0	5.0	5.0	4.0	5.0	4.0
Wave Length (nm)	213.9	324.8	279.5	248.3	285.2	766.5	232.0
Slit Width (nm)	1.0	0.5	0.2	0.2	0.5	1.0	0.2
Oxidant	Air	Air	Air	Air	Air	Air	Air
Oxidant Pressure (L/min)	13.50	13.50	13.50	13.50	13.50	13.50	13.50
Fuel	Air/C_2H_2	Air/C_2H_2	Air/C_2H_2	Air/C_2H_2	Air/C_2H_2	Air/C_2H_2	Air/C_2H_2
Fuel Pressure (L/min)	2.0	2.0	2.0	2.0	2.0	2.0	2.0
Burner Height (mm)	13.5	13.5	13.5	13.5	5.0	13.5	5.0
Calculation Mode	Integral	Integral	Integral	Integral	Integral	Integral	Integral
Calculation Time (s)	5.0	5.0	5.0	5.0	5.0	5.0	5.0
Conc. Units	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L
Instrument Type	Flame	Flame	Flame	Flame	Flame	Flame	Flame
Instrument Mode	Absorbance	Absorbance	Absorbance	Absorbance	Absorbance	Absorbance	Absorbance
Calibration Mode	Concentration	Concentration	Concentration	Concentration	Concentration	Concentration	Concentration
Replicates Standard	3	3	3	3	3	3	3
Replicates Samples	3	3	3	3	3	3	3
Measurement Type	5.0s	5.0s	5.0s	5.0s	5.0s	5.0s	5.0s
Pre-Read Delay	10s	10s	10s	10s	10s	10s	10s
Measurement Mode	Integrate	Integrate	Integrate	Integrate	Integrate	Integrate	Integrate

microcytic anaemia, neutropenia and bone changes.[20] Copper along with Fe is needed for neurovascular system, maintenance of body pigmentation and play an important role in anaemia.[21] As per the results, it is clear that the concentration of Zn and Cu is higher (94.24 and 18.04 ppm) in Wheatgrass Juice Powder (WGJP) as compared to other samples. So, it is strongly indicated that high concentration of Zn and Cu are beneficial for human beings to fight against various ailments. The concentration of Manganese (Mn) in all three samples is 85.92, 23.50 and 54.25 ppm. It is reported that Manganese (Mn) has antioxidant properties and plays a role in the prevention of toxic oxygen forms. It also plays part in the degenerative process in the aging. It also acts as a catalyst in the synthesis of fatty acids, cholesterol and mucopolysaccharides.[22] As per the reported results, concentration of Mn is higher in Wheatgrass Juice Powder and will surely have higher antioxidant properties than other two samples. Concentration of Fe is 254.59, 39.02 and 106.63 ppm in all three wheatgrass samples. It is well reported and universally accepted that, iron is the essential element for life, iron deficiency creates shortage of haemoglobin in blood, it is helpful in pregnancy, for excessive sweating, pale complexion, laziness, lethargy and insomnia. Iron is the structural or core component in haemoglobin and other heme proteins, many of which are enzymes of the Krebs cycle. Iron deficiency also causes anaemia.[23] According to the study, Fe is found in two oxidative states ferrous and ferric, through the intestinal track only ferrous state is absorbed and ferric state is reduced by the food constitutes.[24] The daily intake limit is 8-11 mg/day. Ferric deficiency is a global problem especially in Asian countries.[25] It has been reported that about 60 % of the world population is Fe deficient. Fe also shows some psychological symptoms as reduced attentiveness and loss of concentration.[26] The concentration of Mg is 1.87, 0.54 and 1.96 ppm, in three samples. As we know that Mg deficiency results in increased excitability of the nervous system. Mg plays an important role in nervous system stability, muscles contraction and protein synthesis. [27, 28] Green leafy vegetables, fruits, cereals, legumes, sea foods are the richest source of magnesium.[29] It has been reported that about 60% of Mg is found in the skeletal muscles and reduced intake of Mg is compensated by skeletal muscles, so the risk of osteoporosis is increased.[30] It has been studied that Mg deficiency directly reduces the cell formation.[31] The reported results depicted the higher concentration of Mg in Dry Wheatgrass Powder (DWGP) of *Triticum aestivum* L. The concentration of K is 23.70, 13.53 and 22.95 ppm in all three samples. Maximum amount of K is reported in Wheatgrass Juice Powder

Table 2. The average concentration (ppm) of major and trace elements found in different samples: (Wheatgrass Juice Powder, Wheatgrass Fibre Powder, Dry Wheatgrass Powder. (*n*=3)

S. No.	Sample Name	Sample Code	Zn	Cu	Mn	Fe	Mg	K	Ni
1.	Wheatgrass Juice Powder	WGJP	94.24	18.04	85.92	254.59	1.87	23.70	ND
2.	Wheatgrass Fibre Powder	WGFP	30.84	7.63	23.50	39.02	0.54	13.53	ND
3.	Dry Wheatgrass Powder	DWGP	30.48	11.46	54.25	106.63	1.96	22.95	ND

(WGJP). K is one of the essential element of human diet, play important role in cellular mechanism, as cofactor it catalysis the conversion of ADP to ATP, and deals with the excitability of the nerve impulses. Beside other role, K has an important function in carbohydrates and protein metabolism. It is required in very less amount and plays an important role to maintain the body fluid osmotic pressure and acid-base balance. According to the Institute of Medicine of the National Academies of Science, Food and Nutrition Board (2004-2006), the daily recommended amount of K for an adult is 4700 mg/day.[32] The proper K intake can also stable the blood pressure and reduces the stroke risk.[33] Whereas, Nickel (Ni) is not detected in all three samples of wheatgrass. In 1984 FAO/ WHO set permissible limit for Ni is 1.683 ppm. Ni plays an important role in the production of insulin.[34] Nickel compounds bind a variety of enzymes, including hepatic microsomal enzymes, as well as enzymes that catalyze carbohydrate metabolism and transport of ions across membranes. Nickel was thought to be essential for plants and some domestic animals but not considered to be a metal of biological importance. But after discovery of urease, it is consider as an element of biological importance. Lungs are adversely affected by Ni and it is identified as a suspected carcinogen. Nickel causes skin disorder known as nickel-eczema.[35-37] All results are given in Table 2. The amount of trace elements in Wheat Grass Juice Powder (WGJP), Wheatgrass Fibre Powder (WGFP) and Dry Wheatgrass Powder (DWGP) of *Triticum aestivum* L. are well represented (Figure 8, 9, 10).

In a recent survey, controlled clinical trials have been conducted to study the therapeutic effect of wheatgrass. [38] As we know that wheatgrass juice is a rich source of vitamin A, C, E, and B complex, including B_{12}. It contains multitudes of minerals like calcium, phosphorus, magnesium, alkaline earth metals, potassium, zinc, boron and molybdenum. It is also called as green blood, contains all the important nutrients required by the body and considered as complex food. [39] Wheatgrass therapy is also recommended for patients suffering from chronic diseases like asthma, atherosclerosis, parkinson's disease, joint pains, TB, constipation, hypertension, diabetes, bronchitis, insomnia, eczema, sterility, haemorrhage, obesity and flatulence.[40, 41] It is also used in the treatment of cancer. Chlorophyll is the basis of all plant life, wheatgrass is highly oxygenated due to higher content of chlorophyll in it. Chlorophyll is anti-bacterial, rebuilt the blood stream, RBC count can be normalized within 4-5 days of the administration of chlorophyll, wheatgrass juice is superior detoxification agent as compared to other agents. There are few reports revealing that chlorophyll reduces the blood sugar level in the body. Wheatgrass juice prevents the tooth decay, skin problems like eczema or psoriasis, it prevents the hair from greying. It is also called as the concentrated sun power and increases the function of heart, affects the vascular system, intestine, the uterus and the lungs. As per the detailed discussion on the basis of reported results, it is highly recommended that the presence of major, trace elements and toxic heavy metals in different herbal products should be properly checked before regular use with the help of recent analytical techniques for protection of human health. As we know that heavy metals also play a great role in the metabolic functioning of the human beings. Heavy metals are classified in two main categories i.e. essential and toxic heavy metals. Essential heavy metals (Cu, Zn Cr, Fe and Co) are required in very trace quantities for the proper functioning of enzyme systems, haemoglobin formation, and vitamin synthesis in human beings and for the growth and development and photosynthesis in plants. Metabolic disturbances are encountered in case of both deficiency and excess of these essential metals. On the other hand toxic metals like Pb, Cd, As, and Hg are not required by the body and they produce lethal effects when consumpted even at very low concentrations.[42-44] Heavy metal contamination in herbal and synthetic drugs can cause serious health hazards such as injury of kidney, renal failure, and liver damage.[45] Physiological concentration of trace elements must always be maintained for proper maintenance of cellular functions in animals. However, the normal concentration of trace elements in different cells mainly depends on the dietary concentration, absorption, and homeostatic control mechanism of the body.[46] Unfortunately less attention is paid towards the legislation and quality control parameters of herbal drug industry as compare to allopathic system of medicines. FAO/WHO has highlighted this critical issue and strongly recommends heavy metal analysis in the herbal medicines along with other necessary biological, chemical, and environmental analysis in their guidelines and also documented the dietary allowances, absorption, elimination, and toxic profiles of heavy metals.[47-50]

CONCLUSION

It is concluded that studies regarding metals in herbal preparations are useful for mankind. The plant based food and food products are the major source of nutrients. The results of this research could be useful to make this product more popular and acceptable by the consumers. Our study regarding the analysis of these elements reveals that the major and trace elements have significant role in combating a variety of human ailments and diseases. Further research on these elements along with heavy metals is yet to be carried out. Most of the herbal drugs are

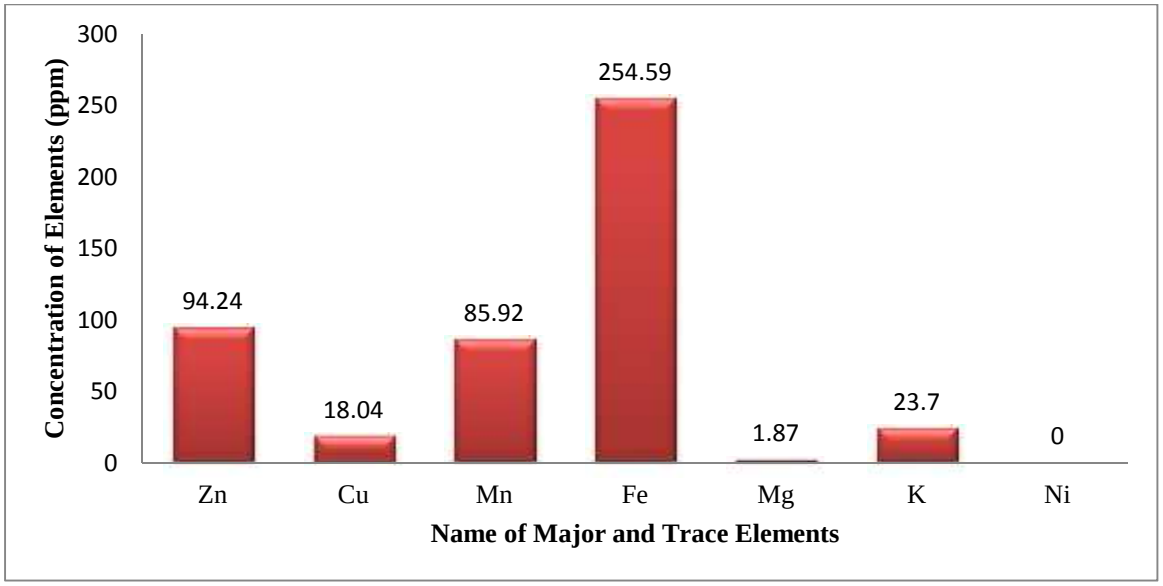

Fig. 8: Graphical representation of data showing amount (ppm) of major and trace elements in WGJP sample.

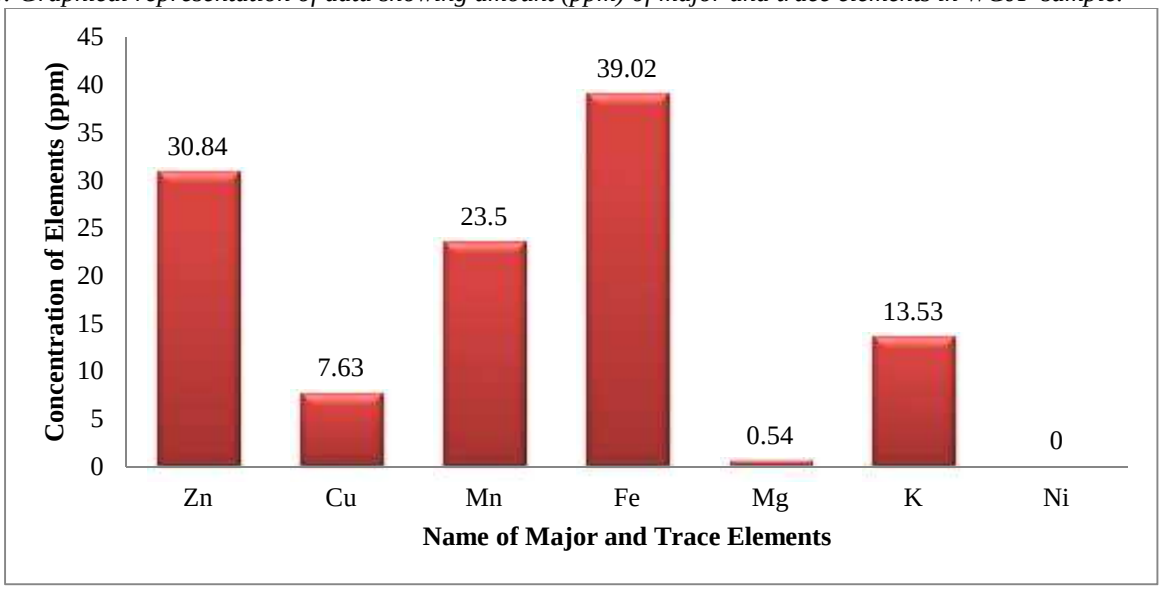

Fig. 9: Graphical representation of data showing amount (ppm) of major and trace elements in WGFP sample.

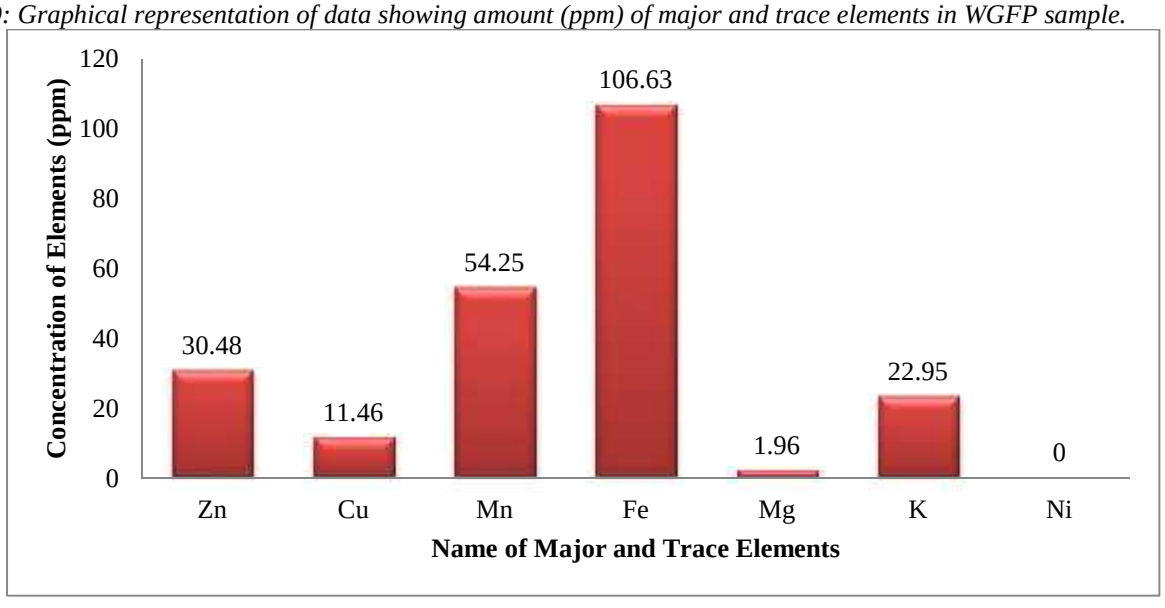

Fig. 10: Graphical representation of data showing amount (ppm) of major and trace elements DWGP in sample.

being sold in the markets were found toxic and unsafe for human consumption due to presence of heavy metals in them. Our main aim is to develop this health drink at low cost to the people, with development of the flavours and to improve the taste. We are also engaged to increase the self-life of this product. Our next target is to study the different activities of wheatgrass and to analyse the potency of this product at different levels. In the present study the major and trace element contents were first time analysed in the Wheatgrass Juice Powder (WGJP), Wheatgrass Fibre Powder (WGFP) and Dry Wheatgrass Powder (DWGP) of *Triticum aestivum* L. from Northern India with the help of Atomic Absorption Spectrophotometer (AAS).

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