PROXIMATE COMPOSITION AND ELEMENTAL ASSESSMENT OF BARLEY (HORDEUM VULGARE L.)GRAINS USING CONVENTIONAL ANALYTICAL METHODS AND ATOMIC ABSORPTION SPECTROSCOPY

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ABSTRACT: Cereals are a significant source of energy, carbohydrates, protein, fat, and fiber. They also contain a range of micro-nutrients and bio-active components notably vitamins and minerals. Barley (Hordeum vulgare L.) is also important member of cereals family, as it remained the part of the human diet since historic times. The chemical and nutritional composition of barley (Hordeum vulgare L.) crops vary widely and depends on several factors, such as; environmental and soil conditions, variety, fertilizer management, and agricultural practices such as seeding rate. In This study evaluates the proximate composition and elemental profile of barley (Hordeum vulgare L.), five barley (Hordeum vulgare L.) grain samples were collected from different regions of Sindh, Pakistan, using standardized AOAC procedures and instrumental analysis. The results of this study showed that, Moisture content ranged from 9.76% to 11.65%, ash from 1.13% to 1.98%, crude protein from 11.21% to 12.13%, crude fat from 1.01% to 11.82%, crude fiber from 5.78% to 6.32%, and carbohydrates from 57.72% to 68.31%. Energy values ranged from 632.4 to 775.4 kcal/100 g. Elemental analysis using microwave-assisted acid digestion followed by atomic absorption spectroscopy (AAS) indicated very low concentrations of Pb (0.056–0.081 ppb), Cd (0.02–0.04 ppb), Cr (2.34–4.53 ppm), and Cu (0.02–0.08 ppm), all within internationally acceptable limits. The nutritional richness and negligible heavy-metal content demonstrate the safety and quality of barley cultivated in Sindh, reinforcing its suitability for food, feed, and industrial purposes.

Keywords: Barley; Proximate analysis; Heavy metals; AAS; Nutritional composition; Hordeum vulgare.

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INTRODUCTION

Barley (Hordeum vulgare L.) is one of the most ancient cereal crop cultivated throughout the World [1,2].Globally, European Union, Russian Federation European Union's produce the greatest quantities of barley [3].Barley is also cultivated in Pakistan [4].Pakistan is considered as a center of barley diversity since barley is a crop with a long history there. Pakistan is the country that produces the most barley. In Pakistan barley is the fourth most important cereal produced after maize, rice, and wheat [5]Barley types are predominantly categorized as food and malting barley, based on their uses; Food barley is commonly cultivated in stressed areas where soil erosion, occasional drought or frost limits the ability to grow other crops [6]. In Pakistan, barley plays an essential role in traditional diets and livestock feeding systems [7,8]. Since feed is the most significant production cost in the animal industry, appropriate processing should be applied to improve the efficiency of feeding and management. Grinding and milling are the most common physical processing methods applied to barley. For ruminants, it is better to roll barley rather than to grind it [9, 10]. The mature grain is typically composed of approximately 10−15% protein and 70-80% carbohydrate. [11] The variation in protein and carbohydrate is dependent on the variety cultivar and environmental factors. [12, 13].Nutritional evaluation through proximate analysis provides foundational data on moisture, ash, protein, fat, fiber, and carbohydrate content, which collectively determine the grain's dietary and industrial suitability. Additionally, the presence of heavy metals is a growing concern in cereal crops due to contamination from soil, fertilizers, and irrigation water. Trace metal accumulation in food crops can pose health risks; therefore, elemental profiling using reliable techniques such as atomic absorption spectroscopy (AAS) are vital for safety assessment.

This study aims to quantify the proximate composition and elemental concentrations of five barley

varieties collected from Sindh, Pakistan, using standardized methods to provide a comprehensive nutritional and safety evaluation.

MATERIALS AND METHODS

Reagents: During study All the chemicals which used during study were from E-Merk (Darmstadt, Germany).These chemicals includes; n-hexane, ethyl alcohol, sodium hydroxide, potassium hydroxide, HNO_3 and H_2O_2.

Collection of sample: Varieties of barley grain were collected from the different local Market in different areas of Sindh, Pakistan during the year of 2022.These are the five different verities of Barley Samples;

I. Sorab - 96 (SO-96)
II. Rakshan - 10 (RA -10)
III. Snobar -96 (SN-96)
IV. Awaran- 2992 (AW-2992)
V. Local Turbat (LT)

Proximate Analysis: Proximate composition was determined using AOCS standard procedures, including;

Moisture Content: Moisture content from barley grain was determined by using official AOCS method Ca 2c-25. Approximately, 15 g was weighed and placed in petri dish and heated in oven (Memmert, Schwabach, Germany) for 30 min at 130 ºC .

Ash Content: For ash content, 2g samples were burned in digital muffle furnace (Fenwal 550 single point) at average temperature about 600±15 0C for the duration of two hours according to (AOCS method Ba 5a-49-2013).

Protein Content: For determination of total protein content in oil sample by Kjeldahl method and CHNS analyzer were used by using conversion factor of nitrogen 6.25 AOCS (Aa 3-38).

Fiber Content: The crude fiber content was determined by the help of (AOCS method Ba 6-84 (AOCS 2013). Decaying oily substantial with weak base where as protein and starch with weak acid. After washing of remaining material put into the digital oven at the temperature about 105 ± 15 oC for time duration of twelve hours.

Carbohydrate Content: A large number of analytical techniques have been developed to measure the total concentration and type of carbohydrates present in foods. The carbohydrate content of a food can be determined by calculating the percent remaining after all the other components have been measured:
%carbohydrates = 100 - %moisture - %protein - %lipid - %mineral.

Energy (Kcal/g) Content: Food energy was derived by multiplying the percentages of crude protein and carbohydrates by 4.1 and crude fat by 9.3.
%Crude protein x 4.1
%Carbohydrates x4.1
%Crude fat x 9.3
+ (summation of Total) = Energy Kcal/g

Fat Content: For oil extraction from barley grain, standard AOCS 2013 (Aa 4- 38) method was used. High purity non polar (n-hexane) solvent was used to obtain maximum oil from barley grain then the extracted oil was refrigerated kept at (4oC) until for further analysis.

Elemental Analysis: Microwave-assisted digestion was performed prior to AAS (PerkinElmer double-beam) quantification of Lead (Pb) ,Cadmium (Cd) , Chromium (Cr)and Copper (Cu)

Triplicate of ash samples (0.5 g) of each barley grain then added (2:1) the content of freshly prepared mixture of HNO_3 and H_2O_2 in it. Samples were placed in microwave oven in near to dryness for 5-10 mints. In case the sample turned into brown or black color then, we repeated similar process by adding up the mixture of concentrated acid until the evolution of white fumes occurs. Digested samples were made up to 10 ml of 0.2 N HNO_3. The solutions were allowed to cool and filtered into a calibrated flask (100 mL) by whatman no. 42, and were diluted up to the mark. The analysis of digests samples by using electro thermal atomic absorption spectrophotometer and flame atomic absorption spectrometer for our selected metals analysis respectively.

Atomic Absorption Spectrometer: A Double beam Perkins-Elmer Atomic Absorption Spectrometer Model 700 (Norwalk, CT) was used for the determination of nickel and chromium which was set with HGA graphite furnace, pyro coated graphite tubes were also combined; an AS-800 auto sampler in addition to deuterium lamp is used for the correction of background system. (Perkin Elmer) Hollow cathode lamp as radiation source was used at its recommended current operating condition. All the instrumental operating conditions were used recommended by the company. All the modifier and standards or measured sample portion were moved into auto sampler cups, where in each case 10 µl of the volume for sample or standard and also the modifier volume were 10 µl injected into electro thermal graphite atomizer.

RESULTS AND DISCUSSION

The results of proximate composition and elemental profile of five selected varieties of barley (Hordeum vulgare L.) collected from different regions of Sindh, Pakistan, showed that, the moisture content of

barley grain was found to be 10.32%, 9.76%, 11.33%, 11.65%, and 10.77% for samples 1 to 5 respectively. The higher moisture content percentage was found in sample 4(Awaran-2992) while the lower percentage was observed in sample 2(Rakshan -10) as shown in Figure 1.1. Ash content refers to the amount of inorganic matter that remains after a sample has been completely combusted at high temperatures. It is typically expressed as a percentage by weight and used to determine the total mineral content of a substance. The results show that the ash content was found in the range 1.13-198%. Comparatively there are no major difference was observed sample 1 to 5. The results shows that the protein content was found 11.82%, 10.31%, 12.13%, 11.81%, and 11.21% for sample 1 to 5, whereas, the higher amount was found in sample 3(Snobar-96)as compared to other samples correspondingly. Figure 1.2shows protein content of barley varieties of grain samples. The results of crude fat was observed 1.12%, 1.1%, 1.01%, 1.11%, and 1.15% for sample 1 to 5, while the higher percentage was found in sample 5(Local Turbat) and lower percentage was found in sample 3(Snobar-96) respectively as shown in Figure 1.3. In present study the fiber content was found in the range 5.78-6.32% respectively. In the comparison the higher percentage was found to be in sample 2(Rakshan-10) as shown in Figure 1.4. The results of carbohydrate content in barley grain found 57.72%, 61.34%, 63.22%, 65.66% and 68.31% for sample 1 to 5 whereas the higher percentage content was shown in sample 5(Local Turbat) and lower percentage content was found in sample 1 (Sorab-96) as shown in Figure 1.5. Present study the results shows that energy was found to be 341.1%, 316.2%, 383.4%, 387.7%, and 382.9% for sample 1 to 5. In comparison, the higher percentage was observed in sample 4 (Awaran-2992) and lower was found in sample 2(Rakshan-10) Figure 1.6Shows energy content of barley varieties of grain samples. Table 4.1shows summarized physicochemical results of barley grain, and table 1.2 shows present study compared with reported value of Barley Verities. The elemental analysis confirms that the barley is safe for human and animal consumption. Levels of Pb and Cd were negligible, reflecting low environmental contamination in the cultivation zones. Cr and Cu values were also well below toxicological thresholds. Lead concentration was found to be in barley grain samples 0.081, 0.069, 0.067, 0.077, and 0.056 ppb for sample 1 to 5 respectively, as shown in Figure 1.7. The cadmium results show that the value was found to be at 0.04, 0.02, 0.04, 0.02 and 0.03 respectively. Whereas, comparison in barley grain samples is shown in Figure 1.8.

Figure 1.9 indicates the chromium results of barley grain. The results show that the chromium concentration was found between 3.69, 4.53, 2.98, 2.34, and 4.21 respectively. Figure1.10 indicates the results of copper the value shows that between 0.06, 0.07, 0.05, 0.08 and 0.02. Table shows 1.3 summarized results of elemental composition (Barley samples).

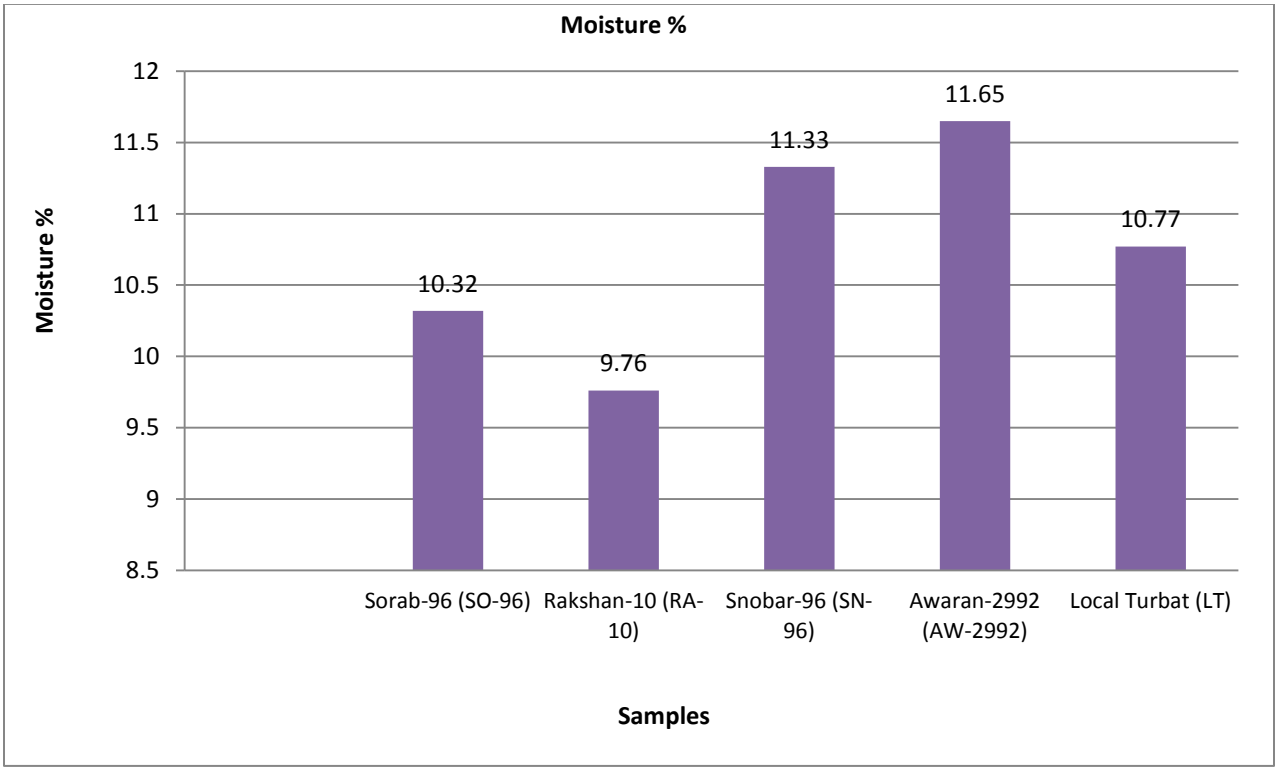

Figure 1.1 Moisture content of barley verities of grain samples;

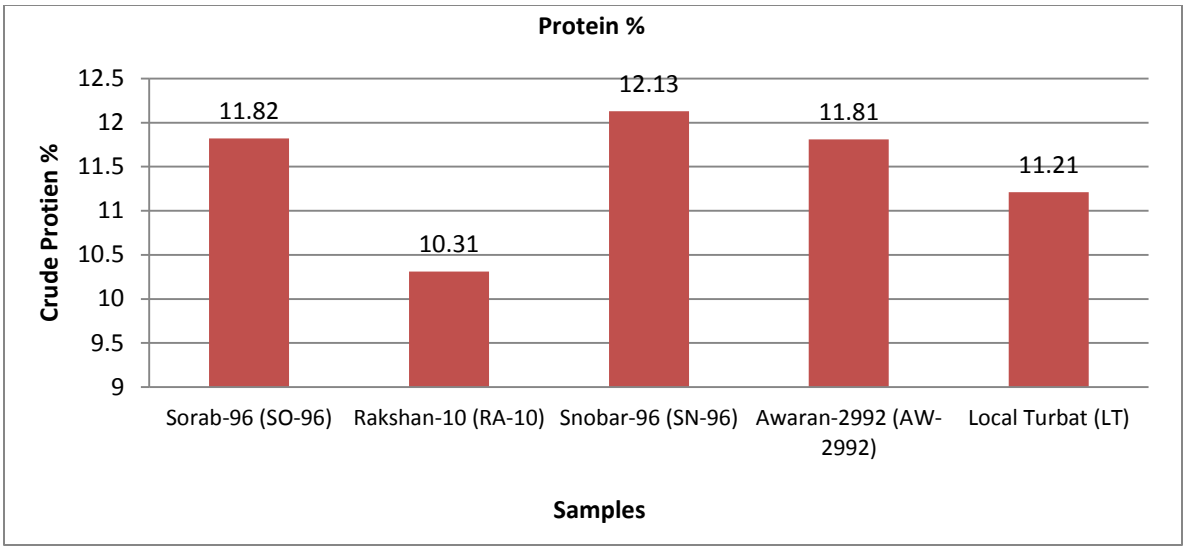

Figure 1.2 Protein content of barley varieties of grain samples

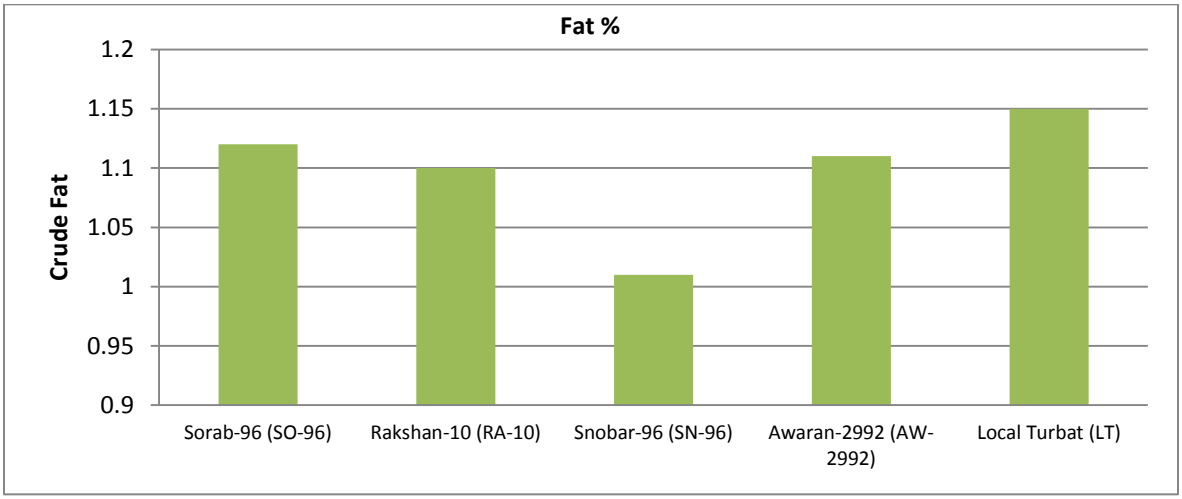

Figure 1.3 Fat content of barley varieties of grain samples

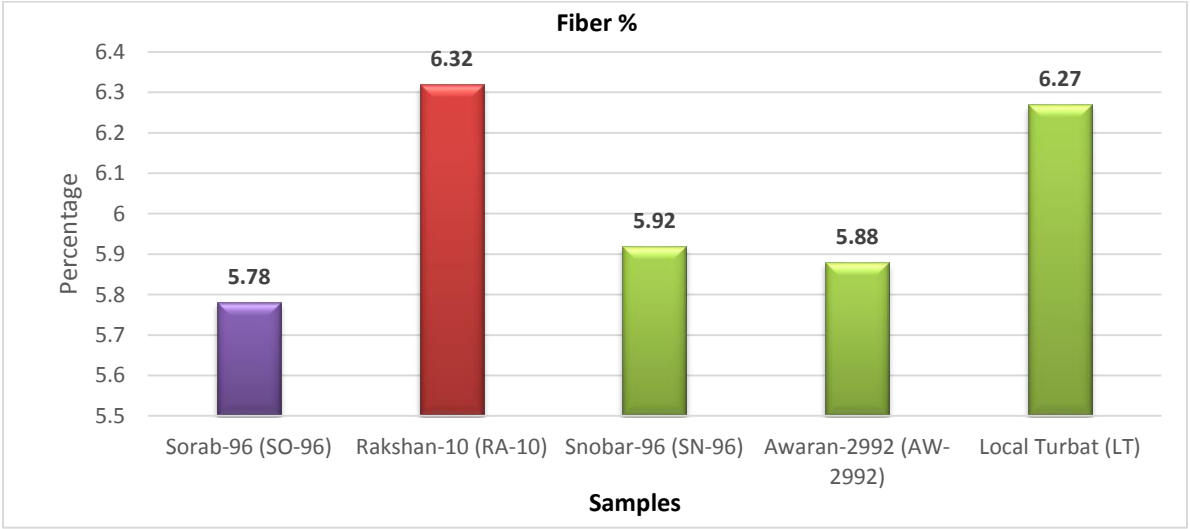

Figure 1.4 Crude Fiber content of barley varieties of grain samples

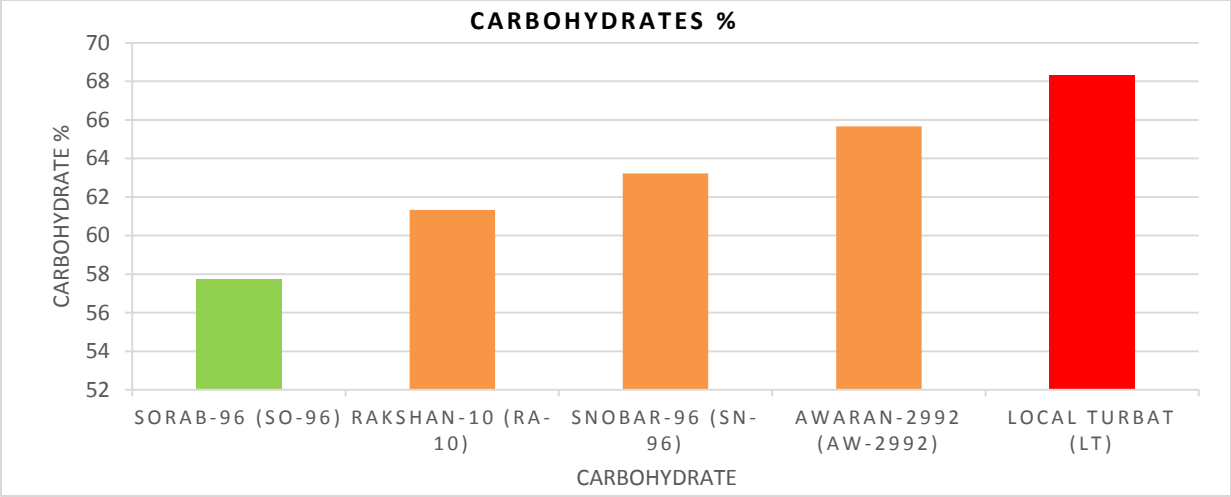

Figure 1.5 carbohydrates results of barley varieties of grain samples

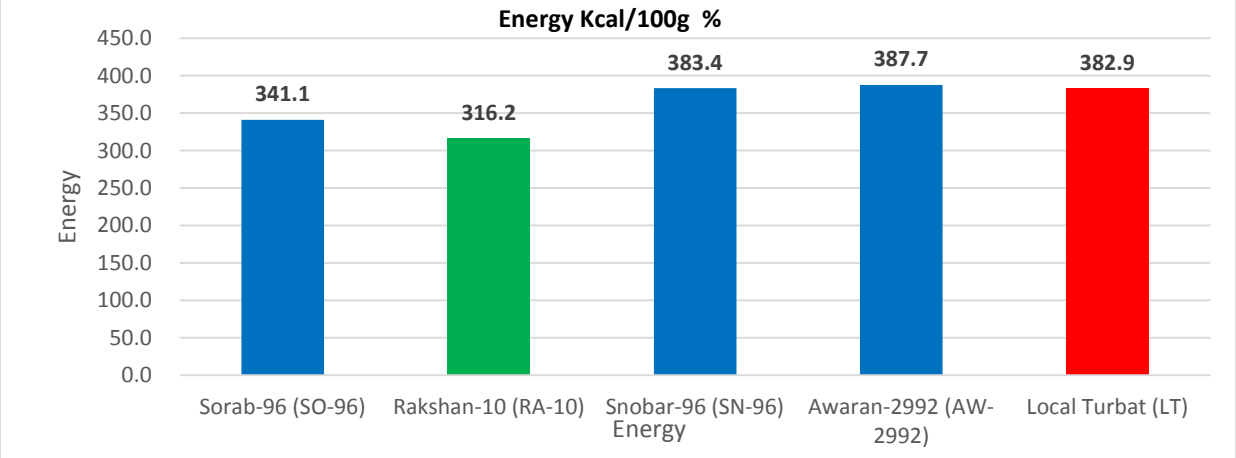

Figure 1.6 Energy results of barley varieties of grain samples

Table 1.1 summarized physicochemical results of barley grain

Parameters	Moisture%	Ash %	Protein %	fat%	fiber %	Carbohydrates	Energy Kcal/100g
Sample 1	10.32±0.81	1.98±0.12	11.82±0.42	1.12±0.11	5.78±0.43	57.72±1.25	341.13
Sample 2	9.76±0.72	1.65±0.11	10.31±0.31	1.10±0.12	6.32±0.45	61.34±1.45	316.21
Sample 3	11.33±0.91	1.33±0.21	12.13±0.45	1.01±0.15	5.92±0.34	63.22±1.33	383.43
Sample 4	11.65±0.77	1.21±0.15	11.81±0.41	1.11±0.13	5.88±0.51	65.66±1.42	387.72
Sample 5	10.77±0.78	1.13±0.16	11.21±0.32	1.15±0.12	6.27±0.37	68.31±1.56	382.88

Table 1.2 Present studies compared with reported value of Barley Verities

Parameters	Moisture%	Ash%	Crude Protein%	Crude fat%	Crude fiber%	Carbohydrates	Energy Kcal
Recommended Value-WHO	14-15%	2.36%	12.47%	2.67%	5.65%	76.84%	2400 Kcal/Day
Sample 1	10.32±0.81	1.98±0.12	11.82±0.42	1.12±0.11	5.78±0.43	57.72±1.25	341.13
Sample 2	9.76±0.72	1.65±0.11	10.31±0.31	1.10±0.12	6.32±0.45	61.34±1.45	316.21
Sample 3	11.33±0.91	1.33±0.21	12.13±0.45	1.01±0.15	5.92±0.34	63.22±1.33	383.43
Sample 4	11.65±0.77	1.21±0.15	11.81±0.41	1.11±0.13	5.88±0.51	65.66±1.42	387.72
Sample 5	10.77±0.78	1.13±0.16	11.21±0.32	1.15±0.12	6.27±0.37	68.31±1.56	382.88

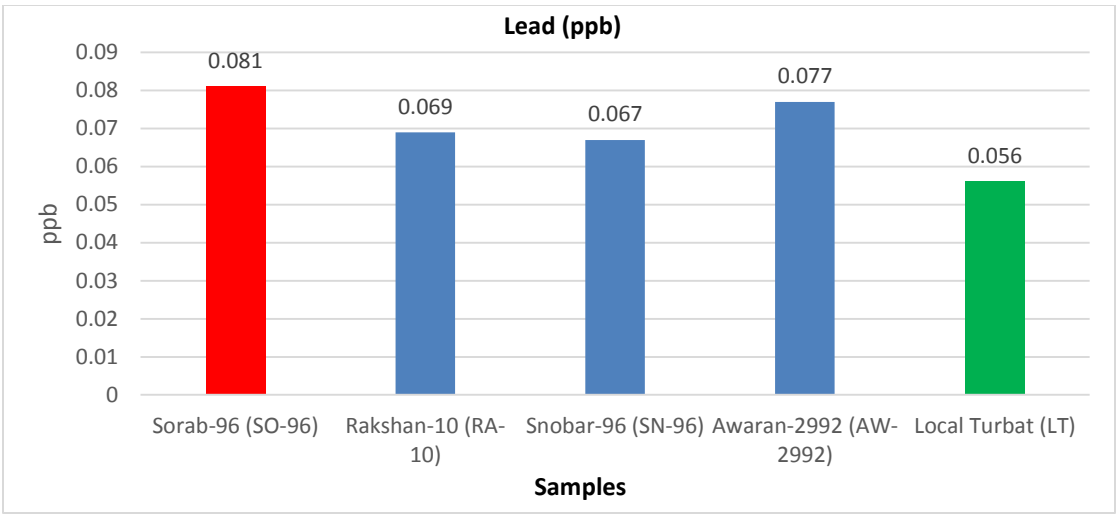

Figure 1.7 lead results of Barley grain samples

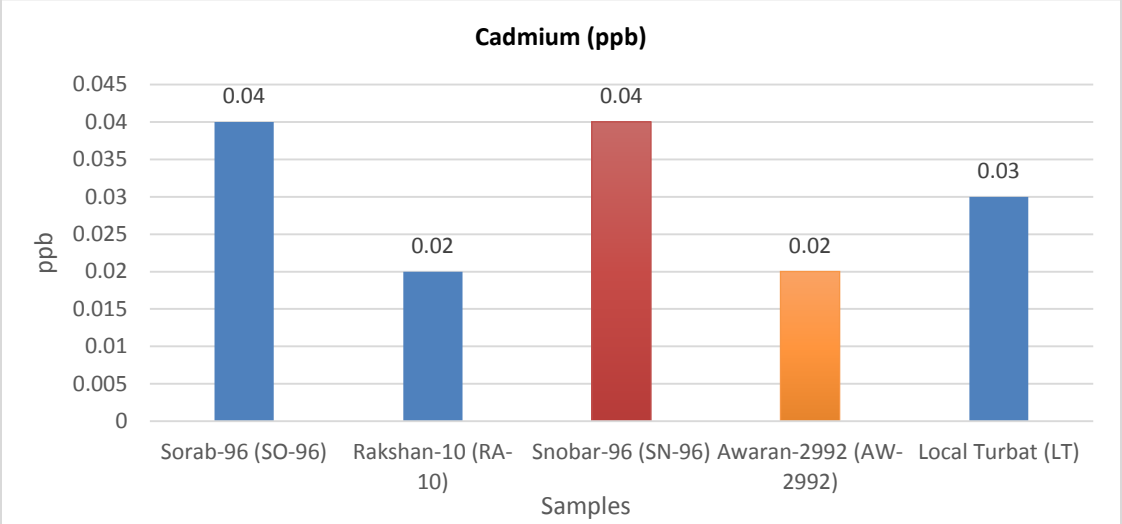

Figure 1.8 cadmium results of barley varieties of grain samples

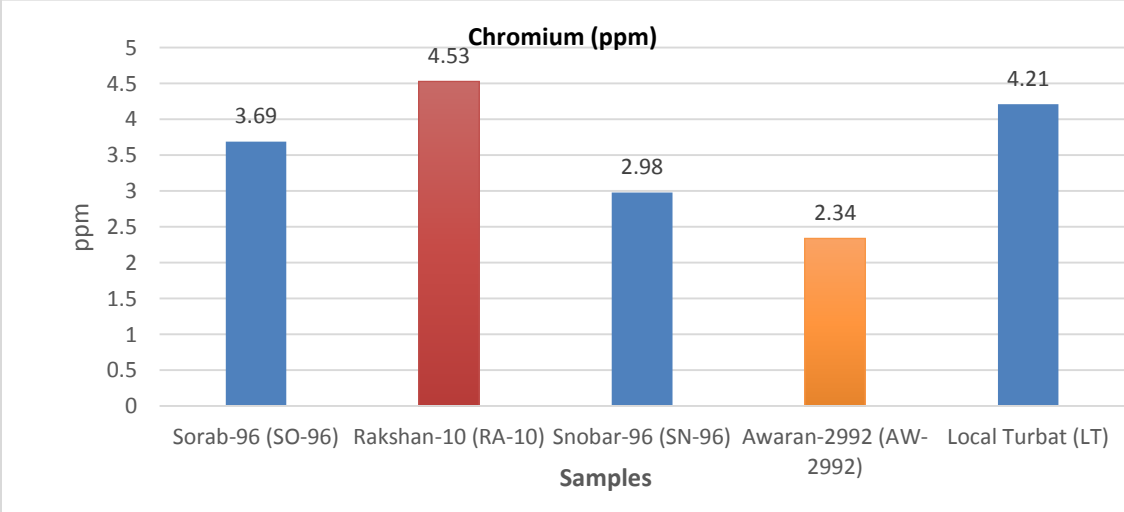

Figure 1.9 chromium results of barley verities of grain samples

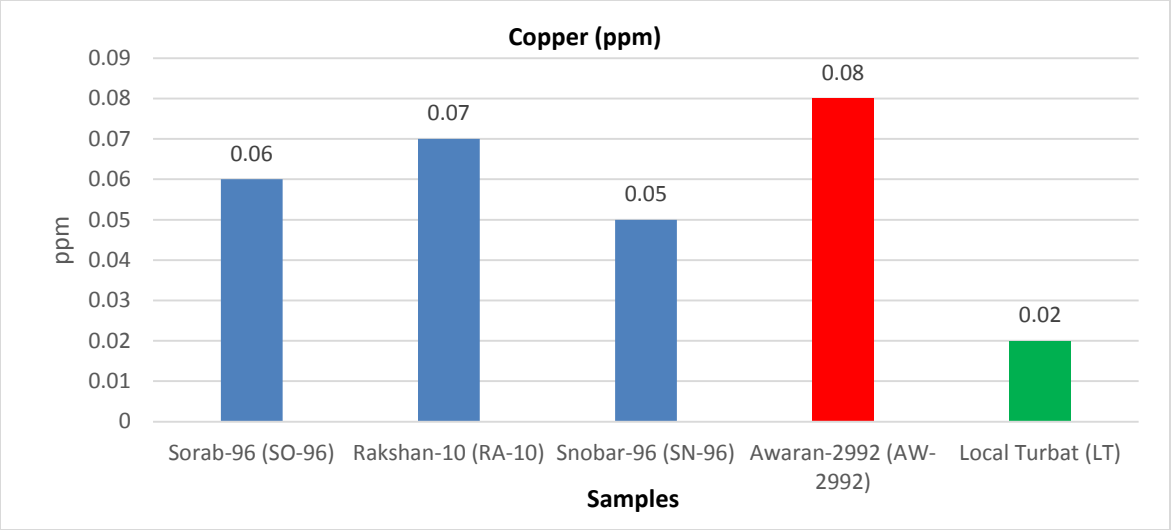

Figure 1.10 copper results of barley verities of grain samples

Table 1.3 Summarized results of elemental composition (Barley samples)

S. No	Lead	Cadmium	Chromium	Copper
1.	0.081 ppb	0.04 ppb	3.69 ppm	0.06 ppm
2.	0.069 ppb	0.02 ppb	4.53 ppm	0.07 ppm
3.	0.067 ppb	0.04 ppb	2.98 ppm	0.05 ppm
4.	0.077 ppb	0.02 ppb	2.34 ppm	0.08 ppm
5.	0.056ppb	0.03 ppb	4.21 ppm	0.02 ppm
Recommended Value	max: 1.5 ppm	Max: 0.05 ppb	0.05– 0.2mg	1-3 mg/day

Conclusion: Barley cultivated in Sindh exhibits favorable nutritional characteristics and minimal heavy-metal contamination. The grain meets international quality standards and remains a safe and valuable commodity for food, feed, and industrial uses.

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