

## **Trace elements in Libyan Cereals: Estimation of Daily Intakes of Copper, Iron, Manganese and Zinc**

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**Abstract:** Copper, iron, manganese and zinc contents of cereals that consume in Libya were determined and the intakes of these elements in Libyan population were estimated. Selected cereals including wheat and rice which are the most consumed by Libyan population were analysed. Cereal samples were digested by using microwave digestion method with nitric acid and H<sub>2</sub>O<sub>2</sub>. Concentrations of Cu, Fe, Mn and Zn were determined by ICP-MS instrument. Cu, Fe, Mn and Zn intakes were evaluated by estimation of cereal consumption in Libyan population and FAOSTAT data for the period from 1997 to 2007 was used. Intakes (mg/day) of these elements were Cu (2.2), Fe (17.1), Mn (15.8) and Zn (10) for all types of cereals in this study. Both RDA and PMTDI scales were calculated for Libyan cereals. The RDA of Cu and Mn exceeded by 1.5 and 2 fold, respectively. Importantly, Mn intake showed high level of Mn from cereals consumption; this demonstrates that Libyan population may exposure to high levels of Mn. The PMTDI % of Mn in this study was estimated to be 87.8% for all cereals. This value of Mn can be risk if it is added to concentrations of Mn from other food including vegetables, meat and tea infusion. More study should be done for estimating daily intakes of Cu and Mn in Libyan population for different type of foods. No deficiency of these elements was detected in the current study.

**Keywords:** Libyan cereals, trace elements, ICP-MS, daily intakes, RDA and PMTDI

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### **I. Introduction**

Copper (Cu), iron (Fe), manganese (Mn) and Zinc (Zn) are considered as essential metals, however, if the concentration of these elements are higher than their permissible limits, they may create toxic effects in human [1]. Cu, Fe, Mn and Zn are involved in the function of several enzymes and are essential for maintaining health throughout life [2,3]. Trace mineral deficiencies constitute the largest nutrition and health problem that affects populations in developed and developing countries. There is an increasing appreciation of the existence of multiple micronutrient deficiencies in developing countries.

Cu deficiency is less frequent and has been described mainly in premature infants and children recovering from malnutrition [4]. Cu deficiency may result from an inherited defect, such as Menke's syndrome; also some common clinical features include anaemia, neutropenia and bone abnormalities [5]. Nevertheless, it can be very toxic with high levels in food. Copper is mainly absorbed in the duodenum bound to specific proteins as Cu(II), however, absorption of copper is decreased when copper ingestion is high. Also the main route of copper excretion is via the bile, which is directly correlated with absorbed dose [5]. Food is the main source of Cu intake, whereas, cereals can be contributed a high amount of Cu for Libyan population.

Fe is an essential element in mankind and plays a vital role in the formation of haemoglobin, oxygen and electron transport in human body [6]. Fe deficiency is the most prevalent single nutritional deficiency in the world and is the main cause of anemia in infants, children, adolescents, and women of childbearing age [6,7]. Mn is an essential element for human health, exposure to high levels of Mn can induce neurological effects such as manganism which is characterised by movement disturbances similar to that observed in Parkinson's mitochondria, where it disrupts oxidative phosphorylation and increases the generation of reactive oxygen species (ROS) [8].

Zn is essential element that has many functions in the human body. The essentiality of Zn to humans as trace micronutrient is well documented [9-11]. In the adult human body there is 2 to 3 grams of Zn. More than 200 enzymes require Zn for their activity [9,10]. It is also necessary for a healthy immune system, and is of use in fighting skin problems such as acne, boils and sore throat; its ions are protective against free-radical injury [9]. Zn deficiency may be prevalent in developing countries, but it is under-recognized due to lack of sensitive biomarkers of Zn status [11]. Cereals including wheat and rice are the major sources in Libyan diet. Libyan population consume a high quantity of wheat from different types of food including bread, cakes and wheat derivatives. Many studies from different countries around the world have reported the quantities and daily intakes of trace elements in food including cereals [12-17]. On the other hands, a lack of studies of trace elements in the literature for Libyan food. Investigation of trace elements in Libyan food should be done for collecting more information and data base about Libyan diet. ICP-MS technique is widely used to determine trace elements in environmental samples including soil, water, biological samples, and it has high precision

compared with many other techniques [18]. In the current study ICP-MS technique was used to determine the concentrations of Cu, Fe, Mn and Zn elements in Libyan cereals. The purpose of this study was to measure Cu, Fe, Mn and Zn concentrations in Libyan cereals and to evaluate their daily intakes. Also the RDA (%) PMTDIs (%) of these elements in cereals were estimated in this study.

## II. Materials and methods

### 1.1 Sample collection

Selected cereals samples which are widely consumed in Libya were analysed. Cereals including wheat, Barley, rice and corn, were purchased from Libya during the months of August 2009 and June 2010. The products analysed in this study were mainly of Libyan origin except rice. Cereals samples were treated before digestion, ground using a coffee grinder and then kept for analysis.

### 1.2 Sample digestion

Cereals samples were digested using a microwave digester. A dry ground weight (0.3 - 0.5 g) of sample was mixed with 4 ml of 70% nitric acid (HNO<sub>3</sub>) (Romil-UpA™, Romil Ltd., Cambridge, UK) and 2 ml of hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) and then microwave digested for 40 minutes at a total pressure of 20 bars and a maximum temperature of 170°C (CEM, Microwave digestion MAR Xpress, Matthews, NC, USA). The digested solutions were evaporated to dryness and then diluted to 25 ml in volumetric flasks with ultra-pure water (Romil-UpS™, Romil Ltd., Cambridge, UK) prior to analysis.

### 1.3 Determination of toxic elements concentrations

Concentrations of trace elements (Cu, Fe, Mn and Zn) in the digested samples were determined by inductively coupled plasma mass spectrometry (ICP-MS). A Thermo-Fisher Scientific X-SeriesII instrument equipped with CCTED (collision cell technology with energy discrimination).

### 1.4 Quality control and standard reference material

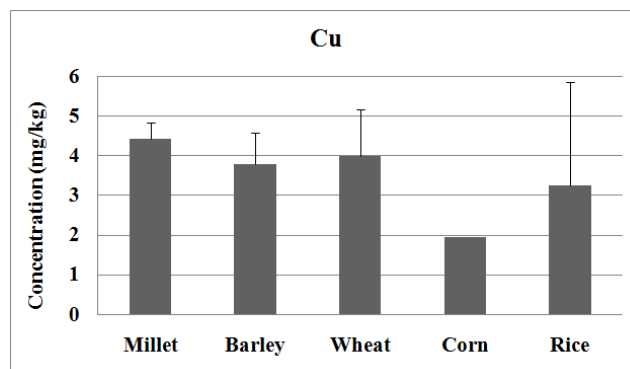
In this study, all the sample masses were measured to an accuracy of  $\pm 0.1$  mg. Elemental concentrations obtained by the ICP-MS technique were evaluated using certified reference material [Rice flour (NIES No. 10-b)] and were found to be in good agreement with the certified values [Cu ( $3.3 \pm 0.2$  mg/kg), Fe ( $13.4 \pm 0.9$  mg/kg), Mn ( $31.5 \pm 1.6$  mg/kg) and Zn ( $22.3 \pm 0.9$  mg/kg)]. The analytical procedure and the reliability of the digestion process of the samples were validated by analysis of all elements of standard reference material. The average recoveries of references material ranged from 83 to 96% for all measurement runs.

### 1.5 Estimation of risk assessments of toxic elements

In this study, the recommended daily allowances (RDA) of Cu, Fe, Mn and Zn from cereals intakes were estimated [19]. The Provisional Maximum Tolerable Daily Intake (PMTDI) which estimates the maximum daily intake of a trace element from individual cereal or more than one types of cereal and the unit that is used for this scale is mg of element per day. The PMTDIs of trace elements in cereals defined by Joint FAO/WHO Expert Committee on Food Additives (JECFA) and EVM [20,21]. The average adult body weight was assumed to be 70 kg for Libyan population.

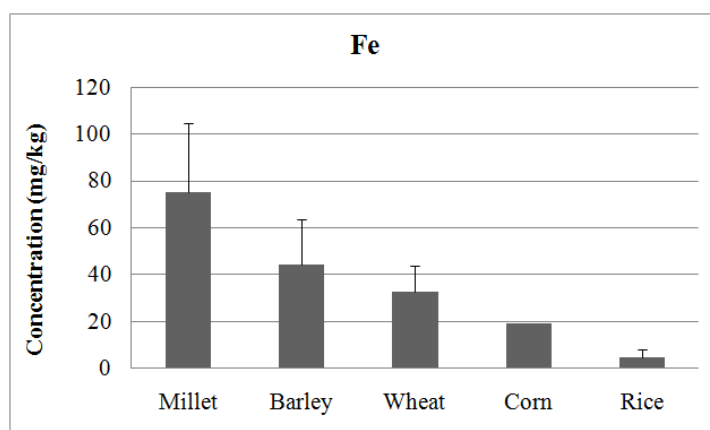
## III. Results and Discussion

ICP-MS determination of the concentrations of Cu, Fe, Mn and Zn in cereals including wheat, rice and barley are shown in figures (1 – 4). The highest concentrations of Cu and Mn were found in wheat (5.96 and 44.5 mg/kg, respectively). However, with regards to mean concentrations of Cu and Mn (mg/kg) were: wheat (3.99, 31.6), millet (4.42, 11.9), barley (3.78, 16), rice (3.25, 8.2) and corn (1.93, 8.2), respectively.

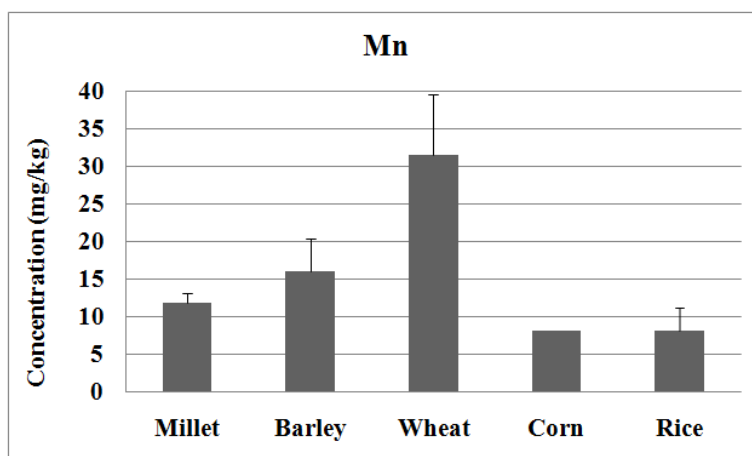


**Figure1:** Concentrations (mg/kg) of copper in Libyan Cereals (as mean and SD).

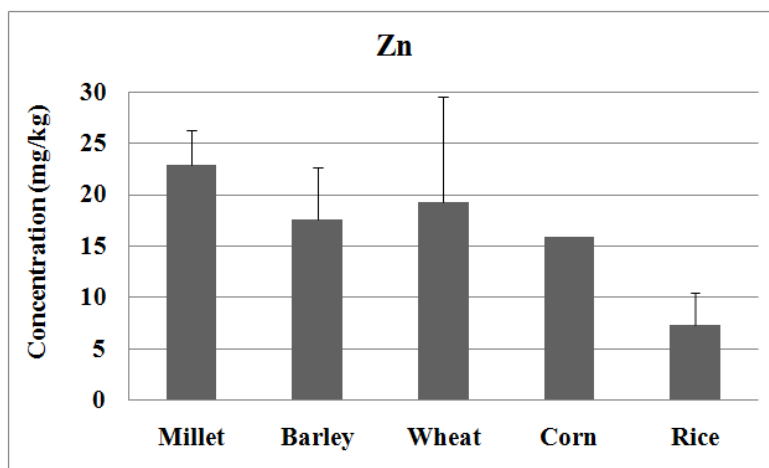
For wheat grains, the mean concentrations of Cu, Fe, Mn and Zn ( $\pm$  SD) were  $3.99 \pm 1.2$  mg/kg,  $32.7 \pm 11$  mg/kg,  $31.6 \pm 8$  mg/kg and  $19.2 \pm 10.3$  mg/kg, respectively (Fig. 1 - 4). On the other hands, the lowest levels of Cu and Mn elements among cereals were detected in corn grain (Fig. 1, 3). The mean concentrations of these elements were 1.93 and 8.2 mg/kg for Cu and Mn in corn grain, respectively (Fig. 1, 3). However, Fe and Zn levels in corn grain were 19.3 and 15.9 mg/kg (Fig. 2, 4). Millet grains has high concentrations of Fe and Zn compared with other cereals, mean concentrations were 75.2 and 22.9 mg/kg for Fe and Zn, respectively (Fig. 2, 4). Barley is another cereal that consume from Libyan population, the mean concentrations of Cu, Fe, Mn and Zn ( $\pm$  SD) were  $3.78 \pm 0.8$  mg/kg,  $44.1 \pm 19.5$  mg/kg,  $16 \pm 4.4$  mg/kg and  $17.5 \pm 5$  mg/kg, respectively (Fig. 1 - 4).



**Figure 2:** Concentrations (mg/kg) of iron in Libyan Cereals (as mean and SD).



**Figure 3:** Concentrations (mg/kg) of Manganese in Libyan Cereals (as mean and SD).



**Figure 4:** Concentrations (mg/kg) of Zinc in Libyan Cereals (as mean and SD).

Table 1 shows the daily intakes (DIs) of Cu, Fe, Mn and Zn elements (mean concentrations of elements were used). Generally, consumption of wheat can result in high daily intakes of these elements compared to consumption of other cereals. Wheat has the highest Mn intake (14.77 mg/day) for someone consuming 468 g of wheat (the weight quantity was adapted from FAOSTAT) [21], however, daily intakes of Cu (1.87 mg/day), Fe (15.31 mg/day) and Zn (8.99 mg/day) (table 1). Wheat can be the highest source of intake of these elements in Libyan population. Consumption of 60.3g of rice per day can reach the daily intake of Cu to be (0.2 mg/day) and Mn (50.49 mg/day), otherwise, daily intake of Fe and Zn were 0.29 and 0.44 mg/day respectively (table 1). In barley grain, daily intake of Cu was (0.13 mg/day) and Mn (0.54 mg/day), however, daily intake of Fe and Zn were 1.48 and 0.59 mg/day respectively (table 1). Corn grain has the lowest daily intake of trace elements, because it is low consumption by Libyan population (2.8g/day).

Wheat can be the major source of dietary intake of Cu, Fe, Mn and Zn. As already mentioned, average daily intake of wheat (468g of wheat/day, FAOSTAT) can provide an estimated Cu and Mn intake of 1.87mg/day (207.5% of RDA and 16.7% of PMTDI) and 14.77 mg/day (295% of RDA and **82% of PMTDI**), respectively (table 1 and 2). Mean concentrations of Cu and Mn content in Libyan wheat measured in the current study were 5.96 and 44.5 mg/kg, respectively (Fig. 1 and 3). It is very clear that Cu and Mn intakes from wheat exceeded the RDA, this demonstrates to the Libyan population consume high contents of Cu and Mn. Markedly, from our results in the present study, Mn intake from cereals was the highest exposure which may cause high risk to Libyan population.

**Table 1: Daily intake (mg/day) in cereals for Libyan population.**

| Elements | Wheat (n=4)<br>(468g/capita/day) <sup>a</sup> | Barley (n=6)<br>(33.6g/capita/day) | Rice (n=4)<br>(60.3g/capita/day) | Corn (n=1)<br>(2.8g/capita/day) | All cereals |
|----------|---|------------------------------------|----------------------------------|---------------------------------|-------------|
| Cu       | 1.87  | 0.13                               | 0.20                             | 0.01                            | 2.20        |
| Fe       | 15.31   | 1.48                               | 0.29                             | 0.05                            | 17.14       |
| Mn       | 14.77   | 0.54                               | 0.49                             | 0.02                            | 15.82       |
| Zn       | 8.99  | 0.59                               | 0.44                             | 0.04                            | 10.07       |

<sup>a</sup> Food supply quantity (g/capita/day) was adapted from FAOSTAT. Total mean concentrations were used to calculate daily intake.

Daily intakes of rice (60.3g of rice/day, FAOSTAT) can provide an estimated Cu and Mn intake of 0.20mg/day (21.8% of RDA and 1.75% of PMTDI) and 0.49mg/day (9.9% of RDA and 2.7% of PMTDI), respectively (table 1 and 2). High intake of wheat is clearly a major factor for the elevated exposure to Cu and Mn in the Libyan population. From the results, it is clear that wheat is the main source of Cu, Fe, Mn and Zn in Libyan population compared with other cereals.

**Table 2: The RDA<sup>a</sup>(%) and PMTDIs<sup>b</sup>(%) of Libyan cereals.**

| Cereals | Cu           |       | Fe    |       | Mn         |             | Zn   |       |
|---------|--------------|-------|-------|-------|------------|-------------|------|-------|
|         | RDA          | PMTDI | RDA   | PMTDI | RDA        | PMTDI       | RDA  | PMTDI |
| Wheat   | <b>207.5</b> | 16.7  | 102   | 34.8  | <b>295</b> | <b>82.0</b> | 60.0 | 18.4  |
| Rice    | 21.8         | 1.75  | 1.9   | 0.65  | 9.9        | 2.7         | 3.0  | 0.9   |
| Barley  | 14.1         | 1.13  | 9.9   | 3.37  | 10.7       | 3.0         | 4.0  | 1.2   |
| Corn    | 0.6          | 0.05  | 0.4   | 0.12  | 0.5        | 0.13        | 0.3  | 0.1   |
| Total   | <b>244</b>   | 19.6  | 114.2 | 38.9  | <b>316</b> | <b>87.8</b> | 67.3 | 20.6  |

<sup>a</sup> the RDA: The Recommended Daily Allowance [19]. <sup>b</sup>the PMTDI: The Provisional Maximum Tolerable Daily Intake, assuming the body weight for adult is 70kg. PMTDIs of trace elements in cereals defined by Joint FAO/WHO Expert Committee on Food Additives (JECFA) and EVM [20,21].

Cereals are a staple food for Libyan population and it can be the main source of micronutrients for them. The present study focused on concentrations of Cu, Fe, Mn and Zn in Libyan wheat and barley originating mainly from Libya and the others rice and corn which are imported from outside. Very little information is available in the literature about the trace element contents of Libyan cereals. Libyan population consume high quantities of wheat (468g of wheat per day) [22] and they may be exposed to high levels of trace elements from cereals consumption.

Some studies have been reported concentrations of trace elements including Cu and Mn elements in cereals and they have estimated total daily intakes of trace elements for foods including cereals [23-30]. Some heavy metals in selected cereals including wheat and barley from Ethiopia were determined [23]. Concentrations of Cu, Fe, Mn and Zn in the Ethiopian study were Cu (1.72 and 0.15 mg/kg), Fe (10.64 and 31.85 mg/kg), Mn (7.67 and 1.67) and Zn (8.54 and 3.85 mg/kg) for wheat and barley, respectively [23]. Compared to our results, the levels of elements in Ethiopian study lower than in the present study (see figures 1-4) except Fe concentration in barley was high in Ethiopian study. Spanish study has reported that the mean concentrations of Cu, Fe, Mn and Zn were detected in cereals, concentrations were Cu (3.2 mg/kg), Fe (21.9 mg/kg), Mn (7.8 mg/kg) and Zn (13.7 mg/kg), however, daily intakes of these elements were estimated to be 0.401, 2.744, 0.977

and 1.716 mg/day, respectively [24]. Compared to these, mean concentrations of these elements in the current study are higher for wheat; however, Cu concentration was similar. In contrast, daily intakes of these elements for cereals in the present study were high 6 fold, approximately, than that in Spanish study. Total daily intakes (TDs) of Spanish study were Cu (2.098 mg/day), Fe (13.16 mg/day), Mn (2.372 mg/day) and Zn (8.954 mg/day). Our results in the current study (see table 1) showed that the daily intakes of these elements exceeded the TDIs in Spanish study [24]. In Italian study, TDIs of Cu, Fe, Mn and Zn were estimated to be 1.14, 11.0, 1.38 and 12.0, respectively [25]. Compared to this data, it was also lower than our daily intakes of these elements in the present study for Libyan cereals. In some European countries, TDIs of Cu, Fe and Mn in food were estimated; TDIs in these studies were ranged Cu (1.4 to 1.6 mg/day), Fe (12.2 to 14.4 mg/day) and Zn (10.7 to 12.9 mg/kg) [26-30]. These data illustrated that our data in the present study were similar to the European studies, with the difference which is our data was for only cereals, however, TDIs of European studies were for all types of foods.

Evidently, RDA% of Cu and Mn in the current study demonstrates to that Libyan population expose to high levels of these elements (table 2). Daily intakes of Fe and Zn in our paper revealed that consumption of Libyan cereals does not reach to the risk or deficiency, however, Cu and Mn exceeded the RDA. Daily intake of Mn from consuming cereals in Libya was near to the PMTDI, and high level of Mn was detected from the present study. The mechanism underlying Mn induced toxicity in people exposed to high levels of Mn is poorly understood. However, it has been suggested that Mn plays a role in the generation of ROS that may result in neurotoxicity [31]. More recent studies have provided evidence suggesting that oxidative stress induced by Mn exposure can trigger apoptosis of neural stem cells [32].

We concluded that Libyan population may expose to high levels of Cu and Mn levels from cereals consumption which exceeded the RDA% of them, however, Fe and Zn were with the range of RDA%, also no deficiency were detected in this study. For this reason, more study should be done for total daily intakes of trace elements from consumption of different types of foods and to have complete sight of Libyan situation, also and to assess the risk of Cu and Mn amongst Libyan population. In summary, the results of this study suggest that the population in Libya is at risk of Cu and Mn overloads rather than deficiencies. Data generated on the cereals content of Cu, Fe, Mn and Zn will help to assess the nutrition status of these micronutrients.

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