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Risk Evaluation and Biological Utilization of Some Micronutrients Due to Heavy Metals Resulted from Fuels Used in Baladi Bread Baking

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Abstract: Baladi bread is cornerstone of food subsidy program, as main source energy. Current study aimed to evaluate the effect of using different sources of fuel on concentration of heavy metals (HMs) in bread collected from different fuel sources from Egypt as well as biological effects of HMs on some micronutrients. Representative Baladi bread samples baked from different fuel sources (Wood, Solar and Condensed natural Gas (CNG)) were collected for approximate composition and analysis of Aluminum (Al), Arsenic (As), Lead (Pb), Mercury (Hg), Nickle (Ni), Chromium (Cr) and Cadmium (Cd). Risk evaluation was performed among different Egyptian regions. Biological experiment on (control, heavy metal mixtures (HMMs) and 1/10LD_{50}) male albino-rats were performed for 12 weeks. Ferritin, iron (Fe) and zinc (Zn) levels were determined. Wood fuel resulted in the highest value for all HMs in bread samples followed by solar, while CNG showed only presence of Al. Rural areas showed higher hazard index (HI) compared with urban. Statistical correlation between increased HMs in serum with some micronutrient deficiencies were proved. Wood fuel is the worst method in baking bread. Long-term; low-level exposure to HMMs induced toxic effects.

Key words: Baladi bread · Daily Intake · Heavy metals · Hazard Index · Ferritin · Risk assessment · Zinc deficiency

INTRODUCTION

Egypt is the biggest wheat importer in the world and ordered as eighth and seventh country consumed wheat and corn in the world, 21 and 665 thousand metrics ton respectively have been imported in 2021/2022 according to principal Importing Countries of Wheat Flour and Products 2021/2022 Statista, n.d. [1]. Baladi bread represent the primary source of carbohydrates and energy; it's the cornerstone of food subsidy program in Egypt; therefore, it has been implemented through two subprograms; (Baladi bread flour among rural area and ration cards for baked Baladi bread). It aims to stabilize the availability of essential foods for the majority of vulnerable population [2]. According to financial statement of the fiscal year 2015/2016, based on Ministry of Finance, food subsidy cost 37.7 Billion LE (which represent 59.8% from the budget of social safety net program) with 71 million ration cards and 82.2 million Baladi bread for beneficiaries in 2016 versus 56 million in 2013 [3]. Egyptians, consume an average between 180- 210 Kg of bread annually, or about 2.44 loaves per day, mostly of the subsidized Baladi bread [4].

Bakeries produce Baladi bread differ in their usage of fuels (solar +oil, solar + mazot, mazot, solar, high quality solar and CNG) [5]. Fumes exhaust of fuels and oil derivatives contains numerous contaminants, including heavy metals (HMs), which classified to inside emission such as CO, SO_2, NO_x, C_xH_x and outside emission such as benzene, toluene, xylene and trichloroethylene therefore, causing hazards impact to human health as they are toxic even at very low concentrations. Fuels except CNG emitted high amounts of emissions either inside or outside resulted from incomplete combustion of fuels [5, 6]. On contrast, CNG bakeries showed reduction by more than 60-90% in inside and outside emission respectively compared with other fuels [5].

HMs such as Arsenic (As), Cadmium (Cd), Chromium (Cr), Lead (Pb) and Mercury (Hg) are classified as the most toxic metals, there presence with permutations and

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combinations had an effect on the concentration distribution of some macronutrients such as Calcium (Ca), Magnesium (Mg), Copper (Cu), Zinc (Zn) in mice and there was also a joint effect [7]. Also it had destructive effects on the functional properties of micronutrients such as Fe, Cu and Zn as they impedes the utilization of micronutrients that play an important biological roles in hormonal balance and as co-enzymes contribute in many physiological and pathological processes and oxidative metabolism [8]. Therefore, HMs molecules transfer into the cell membrane and interact with cell components as mitochondria and DNA, as an important target site of these ions, thereby damaging the cell division mechanism inducing cell modulation, carcinogenesis, or apoptosis [6].

As one of the most abundant and ubiquitous trace metals, aluminum (Al) is known for its toxicity for human health [9]. Al has been extensively used in cooking utensils and food contact materials, aluminum ammonium sulfate (E523) as food additives in baking powder and recently Al_2O_3 is used as additive in diesel fuel to improve engine performance and ignition characteristics. Exceeding the normal average daily intake for adults from Al (1-10 mg) have been reported in accumulative researches; exclusively: (1) Al ion displace some biological ions such as Ca, Fe, Zn, Cu and Mg from their binding sites, also (2) broke the balance of some micronutrients (Zn, Fe, Cu) resulting in lipid peroxidation, (3) Al is connected with anemia, osteomalacia and neurologic syndrome and proposed to cause oxidative damage, neuro-toxication that induce Alzheimer, rather than alterations in skeletal, hemopoietic and respiratory systems and reproductive toxicity [9-14].

Risk evaluation defined as: a scientifically based process consisting of the following steps: (i) hazard identification, (ii) hazard characterization, (iii) exposure assessment and (iv) risk characterization [15]. After specifying the qualitative and quantitative risk factors from that exposure to different HMs and their cons with micronutrients, their risks could be assessed and consequently identify the range of prevalence, symptoms and vulnerable groups of that studied hazards.

World health organization (WHO) classified Egypt as early transition nutrition country with moderate prevalence of overweight and obesity, high moderate levels of micronutrient deficiencies/inadequacies and anemia reached up to 47% among children, adolescents and women from all aged groups [16]. Moreover El-Asheer *et al.* [17] found that, 41.2% and 47% of the toddlers one to three years of age (boys were more affected compared with girls in the same age), suffering from low serum ferritin level and low transferrin saturation respectively, which classified as sever health problems facing Egypt according to WHO [16]. According to Egypt Demographic Health Survey (EDHS) [18], rural children were more likely to be anemic than urban children (29% and 23%, respectively). Children 9-12 months were found to have the highest prevalence of anemia at 49.2%, whereas those between 48 months and 59 months had the lowest prevalence at 16.1% [18]. Nine percent of Egyptians are at risk with zinc deficiency [19].

The present study aimed to determine the effect of bakeries fuel on HMs concentrations in Baladi bread for hazard risk evaluation as well as the effect of HMMs on utilization and performance of micronutrients.

MATERIALS AND METHODS

Materials

Bread Samples: Baladi bread baked by three Different types of fuels, were purchased from different governorates at 3 intervals times/ week each and categorised according to type of fuel used as follow:

- Wood Baladi bread were baked in primitive clay oven and heated with hardwood fuels from Fayoum governorate, Egypt.
- Bread baked using compressed natural gas (CNG): subsidized Baladi bread collected from Giza governorate (three districts); Dahshour- 6th October district, Aloumrania and El-Remaia, Giza, Egypt.
- Bread baked using solar heating (Solar): subsidies Baladi bread collected from El- Sharqia governorate, Egypt.

Bread samples from each fuel sources of each time intervals were mixed to make a representative sample, dehydrated at 60°C overnight and cooled then kept in coded plastics bags till analysis.

Methods

Determination of Approximate Analysis and Heavy Metals in Bread:

Gross chemical Analysis: the chemical analysis has been done in the Regional Center for Food and Feed (RCFF) - Agricultural Research Center. Approximate analysis including moisture, total protein, fat, ash and crude fiber were carried out according to the methods described by A.O.A.C. [20]. Carbohydrate (CHO) content was calculated by difference. Energy value (kcal/100 g dm) = (protein in g × 4) + (CHO in g × 4) + (fat in g × 9).

Minerals determination: Concentrations of Al, Pb, As, Hg, Cu, Ni, Cr, Co and Cd were determined in the bread [21]. The samples were treated with mixture of concentrated Nitric and Perchloric acids to get rid of any organic matter. Analysis was carried out using an Inductively Coupled Plasma Optical Emission Spectrometer (ICP-OES) – (Optima 2000 DV- Perkin Elmer, Germany) at RCFF. Standard stock solutions of HMs and Al (1000 ppm) from Sigma- Aldrich were diluted to serial concentrations for building the calibration curve for each element. Two replicates were used to get the average concentration of each heavy metal as mg/kg.

Calculating the Risk Evaluation of HMs in Bread: Bread consumption data for Egyptians was obtained from CAPMAS [22], these data was published as number of loaves consumed by household. Therefore, the following equations have been applied to obtain daily consumption/kg/ per capita:

- Bread consumed (quantity) ×average weight of loaf (kg) = weight of bread consumed (Kg) /sample population/year.
- Number of household of population/ total sample size = factor.
- (Weight of bread consumed × factor)/ 365 = bread consumed (kg) / capita / day.

Estimated Dietary HMs Intake (EDI): The daily intake of HMs was calculated to evaluate the average daily concentration of each metal into the body system of a specified body weight of a consumer $EDI = \frac{F \times C}{bw}$ [23, 24].

"EDI" = estimate daily intake (mg/kg b.w. /day), "F" = amount of daily bread consumed (kg /person/ day), "C" = concertation of HMs determined (mg/kg /day), "b.w." body weight. The mean b.w. for Egyptian women aged 15-59 years old was 77.1 Kg and for men was 79.1 Kg with calculated average of 78.1 kg [25].

Hazard Quotient (HQ): Values were calculated by using the determined concentrations of seven metals according to AliEsfahani *et al.* [10]. Oral Reference Doses (ORD) for Cr, Co, Ni, Cu, As and Cd are 3×10^{-3}, 3×10^{-4}, 4×10^{-2}, 2×10^{-2}, 3×10^{-4} and 1×10^{-3} mg/kg/day, respectively [24]. ORD for Al (1.43×10^{-1} mg/kg/day), Hg (0.71×10^{-3} mg/kg/day) was taken from the study conducted by Ziola- Frankowska *et al*. [26] and ORD for Pb (4×10^{-3} mg/kg/day) [27].

Hazard Index (HI): Consumers are exposed to more than one heavy metal on dietary basis. Consequentially, the risks posed by all HMs on human health reduces the error in risk evaluation. HI was calculated according to the formula: $HI = \Sigma\ THQ$.

If HI is <1: there is no health concern, 1<HI<5: a low level of health concern, 10<HI<100: health concern is significant and should be monitored systematically [24].

Biological Experiment

HMMs Preparation: Based on the highest results obtained from the determination of HMs content in different bread samples which obtained by wood, a HMMs dose was prepared to be added to basal diet of rats. Compounds used on preparation are Aluminum Chloride ($AlCl_3$), Analytical Reagent (AR) ≥ 99.0%; Lead Acetate ($Pb(C_2H_3O_2)_2$), AR ≥ 99.5%; Sodium Arsenate (Na_3AsO_4); Mercuric Chloride ($HgCl_2$), AR ≥ 99.5%; Nickle Pure (Ni); Potassium dichromate (K_2Cr2O_7), AR ≥ 99.8%; and Cadmium dichloride ($CdCl_2$) AR ≥ 99.0%, all AR were purchased from Merck (Darmstadt, Germany). HMMs constituents were prepared according to their molecular proportion as well as preparing of 1/10 part of LD_{50} dosage of same metal and added to diet as indicator of long consumption duration. The components of the salts used, atomic mass for elements and molar mass for salts used were presented in Table (1).

Animals and Treatments: Eighteen male Sprague - Dawley Albino (SDA) rats weighing about 160±10g supplied and housed by Food Technology Research Institute (FTRI), Agricultural Research Center, Giza, Egypt. All rats kept under stress-free, clean, animal-friendly conditions in stainless steel cages (6 rats/ cage), environmental conditions [(relative humidity = 60%± 10%), (room temperature 12-h light/dark cycle, 22±20°C)]. Rats have free access to water and basal diet throughout accommodation period (2 week) and experimental period (12 weeks). Basal diet was formulated according to guidelines of National research council [32]. Meanwhile experimental protocol conforms to the recommendations of the European Union regarding animal experimentation (directive of the European Counsel 86/609/ec). Rats were randomly divided into three groups (n = 6) as follow: GI (control): received basal diet. GII (bHMMs): rat received HMMs /kg diet at dose level represent the concentration and content of HMs in bread baked by wood (Table 1), GIII (1/10 LD_{50}): rats received 1/10 of LD_{50}/kg b.w. of HMMs in diet (Table 1). At the end of the experiment, SDA rats were starved for 12 hours from

Table 1: Concentration of (HMMs) in the experimental basal diet

Salt	Salt molar mass (g/mol)	Element atomic mass (u)	Salt quantity (mg/kg b.w./ diet[1])	1/10 (LD_{50}) mg/kg b.w. diet
Aluminum Chloride	133.34	26.986	208.563	40[(2)]
Lead Acetate	325.29	207.2	0.644	200[(3)]
Sodium Arsenate	207.89	74.92	2.741	10[(4)]
Mercuric Chloride	271.52	200.592	0.218	5.5[(3)]
Nickle Pure	58.693	58.693	0.091	3.82[(5)]
Potassium dichromate	294.18	51.996	3.423	9.6[(3)]
Cadmium dichloride	183.314	112.41	0.006	15.8[(3)]

(1) salt quantity for rats' basal diet |
(2-5) References for calculating 1/10 LD_{50} [28-31]

water and basal diet. At last, samples were collected from rat's eye (from retro-orbital venous plexus) under CO_2 anesthesia in 2 tubes, 1st tube contains heparin (anticoagulant) to obtain whole blood which divided in 2 aliquot to determine hematological parameters and elements, 2nd tube contain no anticoagulant to obtain serum which separated by centrifuging (HETTICH, universal 16, German) at 5000 rpm for10 min at 4°C, then collected into sterilized tubes and stored at -20°C for biochemical parameters.

Hematopoietic Analysis: The red blood cell count (RBCs), white blood cell count (WBCs), hemoglobin concentration (Hb), mean corpuscular hemoglobin concentration (MCHC), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), Hematocrit (HCT) and Platelets (PLT) were determined by Automated Hematology analyzer 5(XT-2000 i,sysmex corporation, KOPE, JAPAN).

Minerals and HMs in Blood: Concentrations of Al, Pb, As, Ni, Cr, Zn and Fe were performed for blood samples as same as described in bread.

Serum Ferritin: Serum ferritin level in rats were quantitated using rat ELISA kits anti-ferritin, (San Francisco, CA, USA) [33].

Statistical Analysis: Results introduced in form of mean± Standard Error (S.E.). Data statistically analyzed utilizing computer Duncan institute program and the least significant difference test (LSD) at the 0.05% level of probability. Statistical analysis of variance (ANOVA) for risk evaluation was performed using Duncan $P< 0.05$ [34].

RESULTS AND DISCUSSION

Gross Chemical Analysis: Table (2) present the approximate analysis of bread samples collected from different bakeries using various fuel sources; and revealed that wood was the highest DM weight followed by CNG and the lowest showed for solar (88.73, 88.22 and 87.29g/100g DM, respectively). Regarding moisture content, bread baked on solar was the highest content followed by CNG and the lowest was for wood fuel (12.71, 11.78 and 11.27 g/100g DM, respectively). CNG bread recorded the lowest crude fat and fiber contents vs. solar bread (0.61 vs. 1.04) and (0.28 vs. 0.66) g/100 g DM respectively. Egyptian Organization for Standardization Control and Quality (EOSQC) [35] published basic requirements and states that total solid material not decreased than 62% from total loaf weight. The moisture, protein, ash and CHO were in the range contents of Saleh [5], while fat and fiber were less content in current study compared with Saleh [5] 1.65-1.90 and 1.44-1.76g/ 100 gm DM, respectively for subsidized bread from the same studied governorates.

Heavy Metals Contents: HMs are a bio-accumulative structure, low concentration of them is hard to metabolize and excrete after ingestion, there hazards refer to the chronic effects in all body organs. Data in table (3) present HMs content in bread samples. CNG recorded zero HMs content except for Al (0.124 mg/kg DM). In contrast, wood bread showed the highest Al, Pb, As, Hg and Cd contents (42, 0.41, 0.988, 0.163 and 0.003 mg/kg DM respectively), followed by solar bread which has Al (21.55), Pb (0.51), As (0.41), Hg (0.105), Ni (0.095), Cr (0.605) and Cd (0.003) mg/kg DM. HMs content in bread samples was discussed according to the Egyptian Organization for Standardization and Quality Control (EOSQC) [35] No. 1419/2006, 3/9 which refer to the limitation of HMs in bread produced to the EOSQC No. 2360 concerns the maximum limitation of heavy metals in Egyptian foods. Hassan *et al*. [36] recorded that HMs concentration (mg/Kg) in Baladi bread was 0.00 for (Hg, Cd and Pb), 0.001 for As, 0.12 for Ni and 3.56 for Cu. Al mean values were found to be upper the maximum value (0.14 mg/kg DM) according to FAO/ WHO [37].

Table 2: Gross chemical analysis of Baladi bread baked by different fuel sources

Analysis (g/ 100 g dry matter)	WOOD	CNG	Solar	$LSD_{0.05}$
Dry matter weigh (DM) (g)	88.73^{a} ±0.14	88.22^{ab} ±0.2	87.29^{b} ±0.29	6.77
Moisture	11.27^{ab} ±0.14	11.78^{b} ±0.20	12.71^{a} ±0.29	6.77
Crude Fat	0.92^{a} ±0.02	0.61^{b} ±0.16	1.04^{a} ±0.06	0.39
Crude Fiber	0.37^{b} ±0.02	0.28^{c} ±0.12	0.66^{a} ±0.03	0.89
Crude Protein	14.32^{a} ±0.0.6	13.04^{a} ±0.46	13.64^{a} ±0.39	2.79
Ash	1.70^{a} ±0.24	1.32^{a} ±0.14	1.6^{a} ±0.15	0.78
CHO	82.68^{b} ±0.28	84.76^{a} ±0.25	83.05^{b} ±0.47	2.53
Energy (Kcal /100g DM)	396.33^{a} ±0.91	396.65^{a} ±1.06	396.13^{a} ±0.91	4.64

Within the same row, various superscript letters indicate significant differences (Duncan, P<0.05). Values are means of 3 replicates

Table 3: HMs contents of in Bread samples

HMs content (mg/kg DM)	WOOD	CNG	Solar	$LSD_{0.05}$	Reference values (mg/kg) DM
Aluminum (Al)	42.000^{a} ±0.85	0.124^{c} ±0.01	21.550^{b} ±0.21	2.15	0.14(2)
Lead (Pb)	0.410^{a} ±0.01	0.000^{b} ±0.00	0.410^{a} ±0.02	0.05	0.31(1)
Arsenic (As)	0.988^{a} ±0.01	0.000^{c} ±0.00	0.496^{b} ±0.02	0.04	0.38(1)
Mercury (Hg)	0.163^{a} ±0.01	0.000^{c} ±0.00	0.105^{b} ±0.00	0.01	
Copper (Cu)	0.000	0.000	0.000		
Nickle (Ni)	0.091^{a} ±0.00	0.000^{b} ±0.00	0.095^{a} ±0.00	0.01	0.77(3)
Chromium (Cr)	0.527^{b} ±0.00	0.000^{c} ±0.00	0.605^{a} ±0.00	0.02	
Cobalt (Co)	0.000	0.000	0.000		
Cadmium (Cd)	0.003^{a} ±0.00	0.000^{c} ±0.00	0.003^{a} ±0.00	0.00	0.31(1)

(Mean ±S.E.) of 3 replicates. Within the same row, various superscript letters indicate significant differences (Duncan, P (<0.05))

(1) Maximum value [41]; (2) Toxicological guidance value [37]; (3) Nickle Reference exposure level [42].

High Al content in all studied samples was agreed with several studies [5,10,38]. The possible explanations for increased Al content are: (1): most of household wives and bakers ferment dough in Al utensils and/or use very old machines where the fermentation outputs CO_2, ethanol, organic acid, aroma compounds and other metabolites [39], react with Al metal in utensils and resulted in immigration of Al atoms to the dough. (2): presence of high amount of Al in bread raw materials wheat flour, water, baker's yeast ranged 1725- 4485, 89.6-381, 22453µg /kg respectively in some areas in Giza and Cairo governorates [5]. And (3): Acrylamide formation involves Maillard-type reactions which can be aggravated by Al transferred from cook-ware and utensils into the food [40]. Saleh [5] determined and ordered heavy metal contents descendingly in Baladi bread samples were Al > Pb > Ni > Cd>As >Hg in studied governorate districts and that raw materials as wheat flour, water, yeast have variable percentage of HMs.

Risk Evaluation

Edible Daily Intake (EDI) And Hazard Quotient (HQ): Food safety has always been a concern in many studies which have investigated HMs detection and health risk evaluation by focusing on wheat flour and bread [10, 15, 24, 43]. According to the CAPMAS [22] the mean daily consumption from subsidized Baladi bread for urban, rural and total Egyptians were 250, 270 and 260 g/capita/day respectively. Table (4) compared the means of (EDI) and (HQ) for HMs in subsidized Baladi bread baked using different types of fuels in rural, urban and total Egyptian regions. Statistical analysis showed that mean values of EDI and HQ for subsidized Baladi bread baked on CNG were the lowest (Al, Pb, As, Hg, Ni, Cr and Cd) EDI and HQ when compared with other studied bread baked fuels (wood and solar).

Al: The descending order of mean values of EDI and HQ for Al in bread samples baked by different types of fuels were woods> solar> CNG. Statistical analysis among regions showed very high significant difference between EDI for Al in the three fuel sources, as subsidized bread baked on wood in rural and total area were higher EDI and HQ. There were no significant differences ($P \leq 0.05$) between EDI for Al in CNG and solar in the studied regions.

Pb, Cr and Cd: EDI and HQ showed no significant statistical differences for Pb, Cr and Cd for subsidized bread baked using wood and solar in tabulated regions.

As: mean values of EDI and HQ for subsidized bread baked on wood fuel showed higher significances in As value compared with solar fuel, while the statistical analysis didn't show differences between the regions.

Table 4: Mean EDI (mg×10^3/kg bw/day)

Fuel	Region	Al	Pb	As	Hg	Ni	Cr	Cd
Wood	Urban	136.983^{b}	1.347^{a}	3.221^{a}	0.533^{ab}	0.295^{ab}	1.717^{a}	0.010^{a}
Wood	Rural	146.082^{a}	1.437^{a}	3.435^{a}	0.568^{a}	0.315^{a}	1.831^{a}	0.011^{a}
Wood	Total	142.197^{a}	1.399^{a}	3.344^{a}	0.553^{a}	0.307^{a}	1.783^{a}	0.011^{a}
CNG	Urban	0.404^{d}	0.000^{b}	0.000^{c}	0.000^{b}	0.000^{b}	0.000^{b}	0.000^{b}
CNG	Rural	0.431^{d}	0.000^{b}	0.000^{c}	0.000^{b}	0.000^{b}	0.000^{b}	0.000^{b}
CNG	Total	0.420^{d}	0.000^{b}	0.000^{c}	0.000^{b}	0.000^{b}	0.000^{b}	0.000^{b}
Solar	Urban	70.296^{C}	1.337^{a}	1.617^{b}	0.343^{ab}	0.310^{ab}	1.974^{a}	0.011^{a}
Solar	Rural	74.965^{C}	1.426^{a}	1.724^{b}	0.366^{ab}	0.331^{a}	2.105^{a}	0.012^{a}
Solar	Total	72.972^{C}	1.388^{a}	1.678^{b}	0.357^{ab}	0.322^{a}	2.049^{a}	0.012^{a}
				Mean HQ (g×10^3/kg bw/day)				
Fuel	Region	Al	Pb	As	Hg	Ni	Cr	Cd
Wood	Urban	957.926^{b}	336.860^{a}	10736.527^{a}	750.453^{ab}	7.384^{ab}	572.431^{a}	10.219^{a}
Wood	Rural	1021.551^{a}	359.234^{a}	11449.640^{a}	800.298^{a}	7.874^{a}	610.452^{a}	10.898^{a}
Wood	Total	994.386^{a}	349.681^{a}	11145.176^{a}	779.017^{a}	7.665^{a}	594.219^{a}	10.608^{a}
CNG	Urban	2.828^{d}	0.000^{b}	0.000^{c}	0.000^{b}	0.000^{b}	0.000^{b}	0.000^{b}
CNG	Rural	3.016^{d}	0.000^{b}	0.000^{c}	0.000^{b}	0.000^{b}	0.000^{b}	0.000^{b}
CNG	Total	2.936^{d}	0.000b	0.000^{c}	0.000^{b}	0.000^{b}	0.000^{b}	0.000^{b}
Solar	Urban	491.583^{c}	334.196^{a}	5389.101^{b}	483.714^{ab}	7.752^{ab}	657.955^{a}	11.220^{a}
Solar	Rural	524.234^{c}	356.393^{a}	5747.042^{b}	515.842^{ab}	8.267^{a}	701.656^{a}	11.965^{a}
Solar	Total	510.294^{c}	346.916^{a}	5594.218^{b}	502.125^{ab}	8.047^{a}	682.998^{a}	11.647^{a}
LSD Fuel		0.0027	0.0001	0.0001	0.0001	0.0000	0.0000	0.0000
LSD region		0.0027	0.0001	0.0001	0.0001	0.0000	0.0000	0.0000
LSD Fuel× Region		0.0049	0.00055	0.00055	0.00055	0.00055	0.00055	0.00055
			Provisional Tolerable Daily Intake (PTDI)(mg×10^3/kg bw/day)*					
		143000	3570	2100	180	22000	3300	360

Means (n=2) within the same column with different superscript are significantly different ($P \leq 0.05$).

* Reference values [24]

Table 5: Mean HI for different fuel used for baking Baladi bread on different region

Fuel	Region	Mean HI
Wood	Urban	13.37^{b}
Wood	Rural	14.26^{a}
Wood	Total	13.88^{ab}
CNG	Urban	0.003^{d}
CNG	Rural	0.003^{d}
CNG	Total	0.003^{d}
Solar	Urban	7.376^{C}
Solar	Rural	7.865^{C}
Solar	Total	7.656^{C}
LSD Fuel× region		0.000083

Within the same column, various superscript letters indicate significant differences (Duncan, $P < 0.05$)

Hg: the mean values of EDI and HQ for Hg were higher in subsidized Baladi bread baked on wood rather than solar. On the other hand, EDI and HQ for Hg from Baladi bread baked on wood in urban regions are lower than rural and total regions. Statistical analysis didn't show any significant change in EDI and HQ for Hg in Baladi bread baked on solar.

Ni: EDI and HQ for Ni in Baladi bread showed no differences between solar and wood fuels. Also, urban regions were lower mean value of EDI and HQ for Ni in Baladi bread rather than rural and total region.

HI: Human have been exposed to large amount of HMS from different surrounding environment. Data on Table (5) & Fig. (1) present mean HI for daily consumption of subsidized Baladi bread from different fuel in different regions. Statistical analysis presents very high significant differences in HI between different baked fuels. CNG showed the lowest mean HI was <1 which means no health concern. Baladi bread produced using solar and wood fuels the HI ranged from 7.376 to 7.865 and from 13.37 to 14.26 respectively meaning a significant health concern (10<HI<100) [24].

Data from Table (6), Fig (2) showed that 78.6% of governmental bakeries using solar versus 21.71% using CNG, the distribution of solar bakeries is higher in Upper (81.61%), Lower (85%) and border (86.11%) governorates compared with Metropolitan governorates. Only in Metropolitan governorates the distribution of

Table 6: Distribution of Solar and Natural gas bakeries among Egyptian Governorates

Region	Number of active bakeries	Number, (%) of bakeries using solar	Number, (%) of bakeries using CNG
Metropolitan	3480, (11.86%)	1506, (43.28%)	1974, (56.72%)
Lower Egypt	12781, (43.55%)	10430, (81.61%)	2351, (18.39%)
Upper Egypt	12510, (42.62%)	10634, (85.0%)	1966, (15.72%)
Border Areas	583, (1.99%)	502, (86.11%)	81, (13.89%)
Total	29354	23072, (78.60%)	6372, (21.71%)

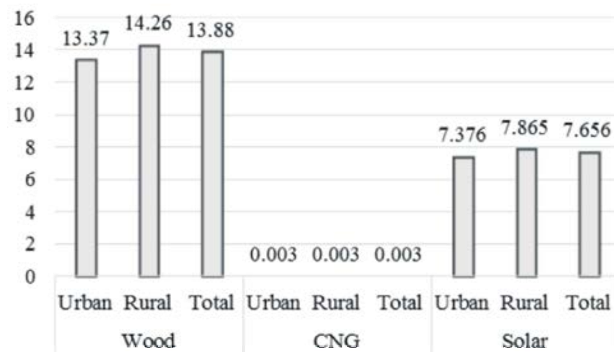

Fig. 1: Mean HI for different fuel used for baking HI baladi bread on different region

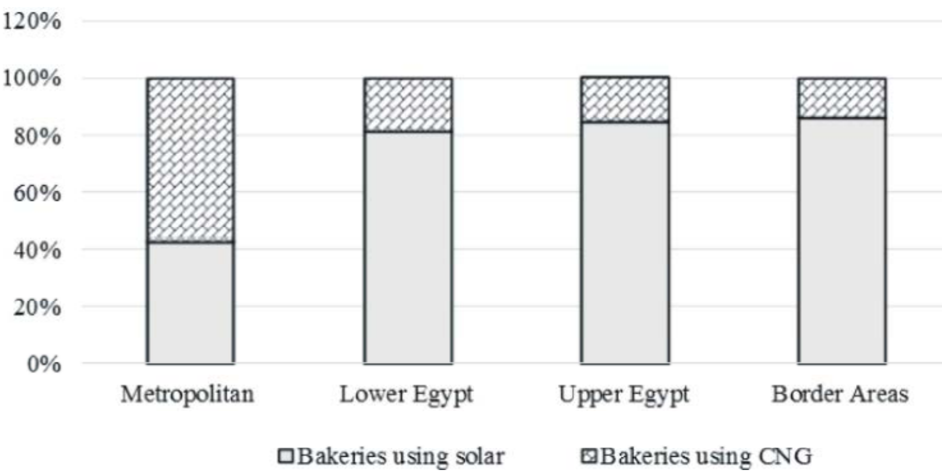

Fig. 2: The Percentage of CNG and solar bakeries in Egypt

Table 7: Hematological parameters (Mean ±S.E.) of rat fed HMMs diet

Parameters	GI control	GII bHMMs	GIII 1/10LD$_{50}$	LSD $_{0.05}$
RBCs (10^6/μL)	8.57^a ±0.04	7.47^b ±0.06	7.09^c ±0.04	0.14
WBCs(10^3/μL)	11.93^a ±0.62	7.98^b ±0.60	5.63^c ±0.40	1.69
Hb(g/dl)	11.98^a ±0.36	9.83^b ±0.45	7.59^c ±0.43	1.28
HCT(L/L)	35.46^a ±0.59	28.41^b ±0.45	24.29^c ±0.54	0.85
MCVs (fL)	54.24^a ±0.68	47.14^b ±0.63	41.62^c ±0.72	2.09
MCH (pg)	18.86^a ±0.35	17.12^b ±0.23	15.36^c ±0.31	0.94
MCHC (g/dl)	39.48^a ±0.30	37.26^b ±0.25	35.46^c ±0.29	0.86
PLT (10^3/μL)	622.00^a±14.10	506.60^b±12.27	420.40^c ±11.84	39.44

Within the same row, various superscript letters indicate significant differences (Duncan, *P* <0.05).

Table 8: HMs in blood samples of rat fed HMMs diet

Groups parameters	Al (mg/L)	Pb (mg/L)	As (mg//L)	Ni (mg//L)	Cr (mg//L)
GI: control	0.00^c ±0.00	0.00^c ±0.00	0.00^c ±0.00	0.00^b ±0.00	0.18^c ±0.01
GII: bHMMs	21.27^b ±3.48	1.37^b ±0.15	0.30±0.01^b	0.00^b ±0.00	0.42^b ±0.01
GIII: 1/10 LD$_{50}$	80.08^a ±4.55	7.04^a ±0.50	2.01^a ±0.03	1.16^a ±0.07	2.91^a ±0.10
LSD 0.05	11.40	1.04	0.07	0.13	0.19

Mean ±S.E. of three replicates, Within the same column, various superscript letters indicate significant differences (Duncan, *P <0.05*).

Table 9: Micronutrients in rat blood of rat fed HMMs diet

Rat groups	Ferritin (ng/ml)	Zn (mg/L)	Fe (µg/dl)
GI: Control	130.22^{a} ±7.77	6.81^{a} ±0.34	122.16^{a} ±2.81
GII: bHMMs	78.88^{b} ±6.37	4.18^{b} ±0.31	96.73^{b} ±3.36
GIII: 1/10 LD_{50}	35.38^{c} ±6.26	2.62^{c} ±0.32	71.10^{c} ±3.48
$LSD_{0.05}$	21.09	0.99	9.97

Mean ±S.E. of three replicates, Within the same column, various superscript letters indicate significant differences (Duncan, P <0.05)

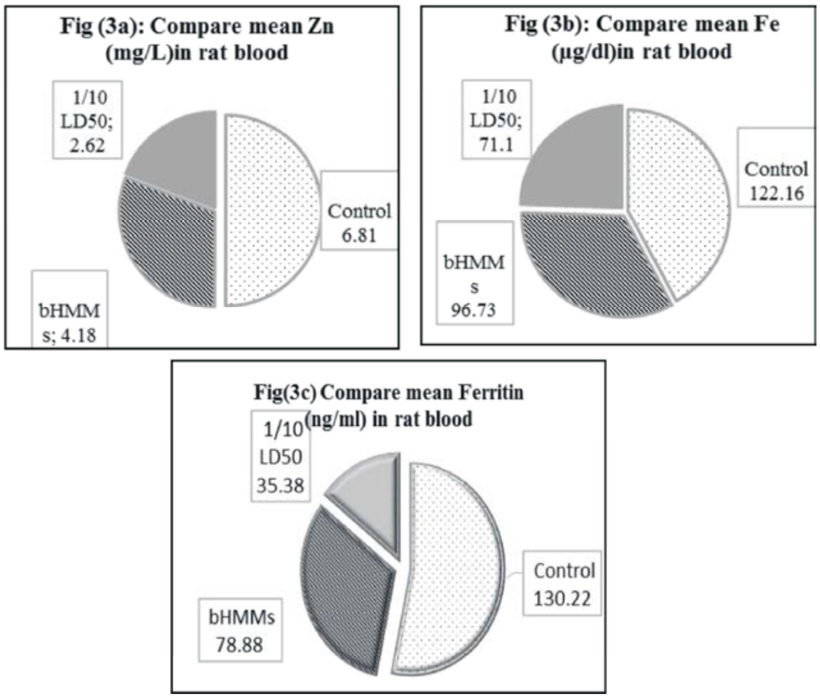

Fig. 3: Comparing of Zn, Fe, Ferritin in treated rats with control

CNG is higher than solar fuels 56.72% and 43.28% respectively, meanwhile in the rest of governorates the distribution of solar bakeries are higher than CNG [44]. When we take in account such distribution in Tables (5,6) it could be concluded that, Upper and lower governorates are in risk of health concern according to the HI as they are depending on using solar fuel rather than CNG. On contrast with metropolitan governorates the presence of CNG percentage was (65.7%) higher than urban and rural regions and according to the HI metropolitan governorates was <1, allow them to be away from health risk.

Biochemical Analysis

Hematology Analysis: The effects of HMMs are depend not only on the individual components of the mixture but also on the combined effect of its composition [29]. SDA rats were subsequently fed for 12 weeks on highest HMMs in Baladi bread and the 1/10 LD_{50} of HMMs as reflection of long-term consumption. Hematological parameters result in blood of control and treated rats in Table (7) and revealed that, administration of rats with both dose of HMMs (GII, GIII) resulted in highly significant reduction of all hematological parameters comparing with control (GI) (P<0.05).

These changes were more pronounced with administrations of 1/10 LD_{50} of HMMs (GIII). Results were agreed with Su *et al*. [7] who reported adverse effect on HMs on hemopiotiec system of rats after 34 days of administrations such dramatic reduction in RBCs, Hb, MCV, MCH and MCHC levels in our experimental models were strongly refer to increasing MCV levels with no globulin causing iron deficiency anemia (IDA).

It could be concluded that, interactions between metals directed the results towards the negative impact on hematological parameters by which chronic Al, sodium dichromate exposure affects the heme group through lowering RBCs, MCH, MCV and transferrin (Tf) saturation rate resulting in reduction of serum iron concentration levels and coagulation disorders and other hemorrhagic disturbances causing microcytic anemia [45,46].

HMs In Blood Samples: Data presented in table (8) showed higher Al, Pb, As, Ni and Cr concentration in blood of rats of GII and GIII comparing with control GI. It worthy to note that administration of rats with 1/10 of LD_{50} of HMs resulted in highly significant accumulation of HMs in blood which refer to possible toxic effect of HMs after consistent prolonged administration.

Therefore, long exposure to HMMs doses through daily consumption or in another words the accumulative effect of contaminants can severely affects the hematologic, hepatic, renal and neuro system behavioral function [7]. From Table (8) it can also be concluded that, HMMs shows highly positive linear relationship between its components [24]. Combination of HMMs exert synergized toxic effect by co-exposure due to possible competition among HMMs on blood parameters [7]. Al plays an important role on the incidence of anemia, osteomalacia and neurologic syndrome [13].

Micronutrients in Rat Blood: As shown in Table (9), Fig. (3), the values of Ferritin, Zn and Fe are highly and very highly significantly decreased in GII and GIII respectively compared with GI "control. Fe is an essential trace element that's very important to make healthy blood cells, it carries oxygen to body tissues. Ferritin, a blood protein that contains iron, is directly related to the stored amount of iron in the body.

In general, reduced ferritin levels in blood serum indicates iron deficiency which is supported by data in table (7) where results showed reduction in Hb, MCV, MCH and MCHC parameters that cause anemia. A significant Zinc deficiency was induced by HMMs. Zinc is a basic micronutrient needed for important biological processes, proper immune system and it plays a vital role in cell division, cell growth and necessary for several enzymatic reactions. GIII (1/10 LD_{50}) is one of the important indicators to predict the effect of increased exposure to certain hazardous substance as a consequence of long-term consumption. So, it was observed from the (GIII) 1/10 LD_{50} values obtained in table (9), that increased duration of contaminates consumption can leads to anemia.

Accumulative research extended to explain the hazard effects of Al on Fe; the most positive explanation agreed with current study are: (1) Al able to interfere with Fe hemostasis and impedes cellular metabolism, (2) able to create labile Fe pool and interact with membrane lipids resulting on increase oxidative stress on the cells and (3) presence of inverse correlation between serum Al and serum Fe therefore, Al intoxication affect Fe metabolism and (4) Al ion replace Fe and Mg ions resulting in decreasing Fe^{+2} to ferritin. Free radicals released from biological complexes by Al catalyze hydrogen peroxide decomposition to hydroxyl radical lead to peroxidation [12]. This was supported by studies on rats, which revealed that iron deficiency can leads to a decrease in brain Fe and ferritin concentrations and increase in (Tf) concentration with an increased rate of iron uptake from the plasma pool [46,47]. Long-term consumption of HMMs compartmentalized into body cells and tissues, binding to proteins and nucleic acids, cause mutations, mimic hormones thereby disrupting the endocrine and cellular functions, destroying macromolecules, reproductive system and eventually lead to cancer [48]. It seems to be that Al had a dominant toxic effect as it represent the highest element in our study.

CONCLUSION

Current study was conducted as an attempt to evaluate the hazards impact on human health and the effects on some micronutrient parameters from HMs on the daily and long-term consumption of Baladi bread baked using three different types of fuels wood, compressed natural gas (CNG) and solar from some Egyptian governorates.

The HMs analyzed in bread samples (Al, Pb, As, Hg, Cu, Ni, Cr, Co, Cd), Al>As> Pb were the highest toxicant detected especially in bread samples baked on wood followed by solar, while CNG recorded zero HMMs contents in bread samples except for Al. A positive correlation between the prevalence of wood and solar fuels in Upper and rural bakeries and EDI, HQ and HI of HMs were found. CNG has lowest mean value (HI<1) which means no health concern, while solar and wood fuels have HI ranged 10<HI<100 which reflects significant health concern.

The biological experiment with HMMs and 1/10LD_{50} as an indicator for long term consumption compared with control group proved negative correlation between HMMs and hematopoietic parameters (RBCs, Hb, MCV, MCH, MCHC) and positive correlation with ferritin deficiency, different types of anemia and zinc deficiencies. Finally, to maintain safety of human health, it is recommended to transform toward using CNG especially in rural and Upper governorates.

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