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HEAVY METAL TOXICOLOGICAL STATUS OF WHEAT SAMPLES FROM DISTRICT SWABI, PAKISTAN

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ABSTRACT

Wheat is the primary food requirement of Pakistan. Wheat flour is used to make the bread that is nearly irreplaceable for almost everyone who use it as a daily staple. With an average 339-gram per capita consumption, it can potentially become a serious source of food-based toxicity if the wheat crop is contaminated. In order to establish a toxicology profile of wheat crop for heavy metal contamination, seventy-six samples of wheat grains were collected from the markets across the district Swabi, Pakistan and were analyzed for eight common toxic heavy metals including Nickle (Ni), Cadmium (Cd), Lead (Pb), Copper (Cu), Cobalt (Co), Iron (Fe), Zinc (Zn) and Chromium (Cr) by employing the flame atomic-absorption spectrophotometry. The mean concentration level of all metals was used to calculate dietary exposure by wheat consumption in the local residents. These values were in turn used to establish the toxicological status in terms of potential health risks by assessing (a) "Target-Hazard-Quotient (as THQ)", (b) "Health-Risk-Index (as HRI)", and (c) the percentage of metals to the corresponding reference toxicological standards i.e., "Provisional-Tolerable-Daily-Intake (as PTDI)", "Tolerable-Daily-Intake (as TDI)" and "Provisional-Maximum-Tolerable-Daily-Intake (as PMTDI)". Among eight metals, HRI and THQ values were found to be less than 1, *i.e*., for Cd, Cu, Zn and Cr while greater than 1 for Pb and Ni. The percentage contribution of dietary exposure to their respective toxicological reference values for Cu, Co, Zn and Cd were found to be less than 100% and greater than 100% for Fe, Pb and Ni.

Keywords: Dietary Exposures, Toxicological Risk Assessment, Heavy Metal Toxicity.

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INTRODUCTION

Pakistan is the seventh-largest wheat producer in the world with record 24.3 million metric ton production in 2019, only marginally lower than ~25.1 million metric ton in 2018 (FAOSTAT 2019). Wheat is dietary staple of Pakistan with an average daily consumption of about 339 grams per person which is one of the highest in the world (Raza, 2012). Wheat being the staple food of Pakistan is consumed in nearly every house in the country in the form of oven (*"tandoor"*) baked bread locally called *"roti", "nan"* or pan-backed *"chapati"*, therefore any contamination to this crop can directly impact the entire population. Hence it is very important and the need of the time to establish the toxicological status of this important crop of the country. This study is also important in the context of installation of industrial units near densely populated areas, unplanned expansions of cities and scarcity of related regulatory authorities in the country that can monitor the situation (Khan *et al.,* 2014). Industrial effluents from these industrial units near the population can contaminate not only land but freshwater resources and the sea (Abbas *et al.,* 2010). In addition to other organic contaminants, heavy metals are the major threat to the inland grown crops, especially wheat. To the best our research, no such study has been conducted to investigate the dietary exposure of heavy metals through wheat consumption and associated risks to the health of inhabitants of Swabi district of Pakistan. This study is definitely limited to district Swabi only, but is certainly a step forward in this capacity that undoubtedly concerns the entire population of the Pakistan.

The wheat crops in Swabi region are irrigated by rain water, tube wells, rivers or canal water, wheat plant can be exposed to heavy metal contamination not only from contaminated water supply but also from soil (Khan *et al.,* 2013). Earlier studies have shown different levels of heavy metal content in various parts of the plant body (Singh *et al.,* 2010a,b, Arif *et al.,* 2011), with the high quantities in roots than in areal parts. If the heavy metal stress intensifies by any mean (accounting for contamination from water or soil), the chances of uptake and translocation of these toxic metal from roots to aerial parts including grains will more likely to increase.

This study is carried out to evaluate the dietary exposure of heavy metals through consumption of wheat and associated health risks. This evaluation is based on (a) "Health-Risk-Index" (as HRI), (b) "Target-Hazard-Quotient" (as THQ) and (c) percent contribution of metals, by their reference toxicological values including "Tolerable-Daily-Intake" (as TDI), "Provisional-Tolerable-Daily-Intake" (as PTDI), "Provisional-Maximum-Tolerable-Daily-Intake" (as PMTDI), "Provisional-Tolerable-Weekly-Intake" (as PTWI), "Upper-Intake-Level" also called as "Upper-Limit" (as UL) and therefore any potential health risks to local inhabitants were estimated.

MATERIALS AND METHODS

Chemicals used and glassware pre-treatment: Analytical grade chemicals were used throughout the study and were bought from local supplier of BDH Laboratory Supplies, England. Chemicals includes H_2SO_4 98 %, $CdCl_2.H_2O$, HNO_3 65 % extra pure, $HClO_4$ about 70 %, $Pb(NO_3)_2$, $ZnSO_4.7H_2O$, $CuSO_4.5H_2O$, $Cr(NO_3)_3.9H_2O$, $(NH_4)_2Fe(SO_4)_2.6H_2O$, $NiSO_4.6H_2O$, $CoSO_4.7H_2O$. Prior to use, the required glassware was soaked overnight in acid bath of freshly prepared 30 % HNO_3 solution, washed first with ordinary tap water followed by rinsing with double distilled and finally rinsed with de-ionized distilled water.

Sample collection and wet-digestion: Samples of whole wheat grain with a minimum weight of 500 g were collected from markets scattered across the district in late 2019, in figure 1, the study area is shown on the map. The extraneous matter was first removed from all the samples followed by sealing in clean polythene bags and kept frozen until analysis. Samples were subjected to wet-digestion (wet mineralization) method in triplicates according to reported procedures (Tuzen *et al.,* 2004). Blank solution was prepared after digestion for every ten samples.

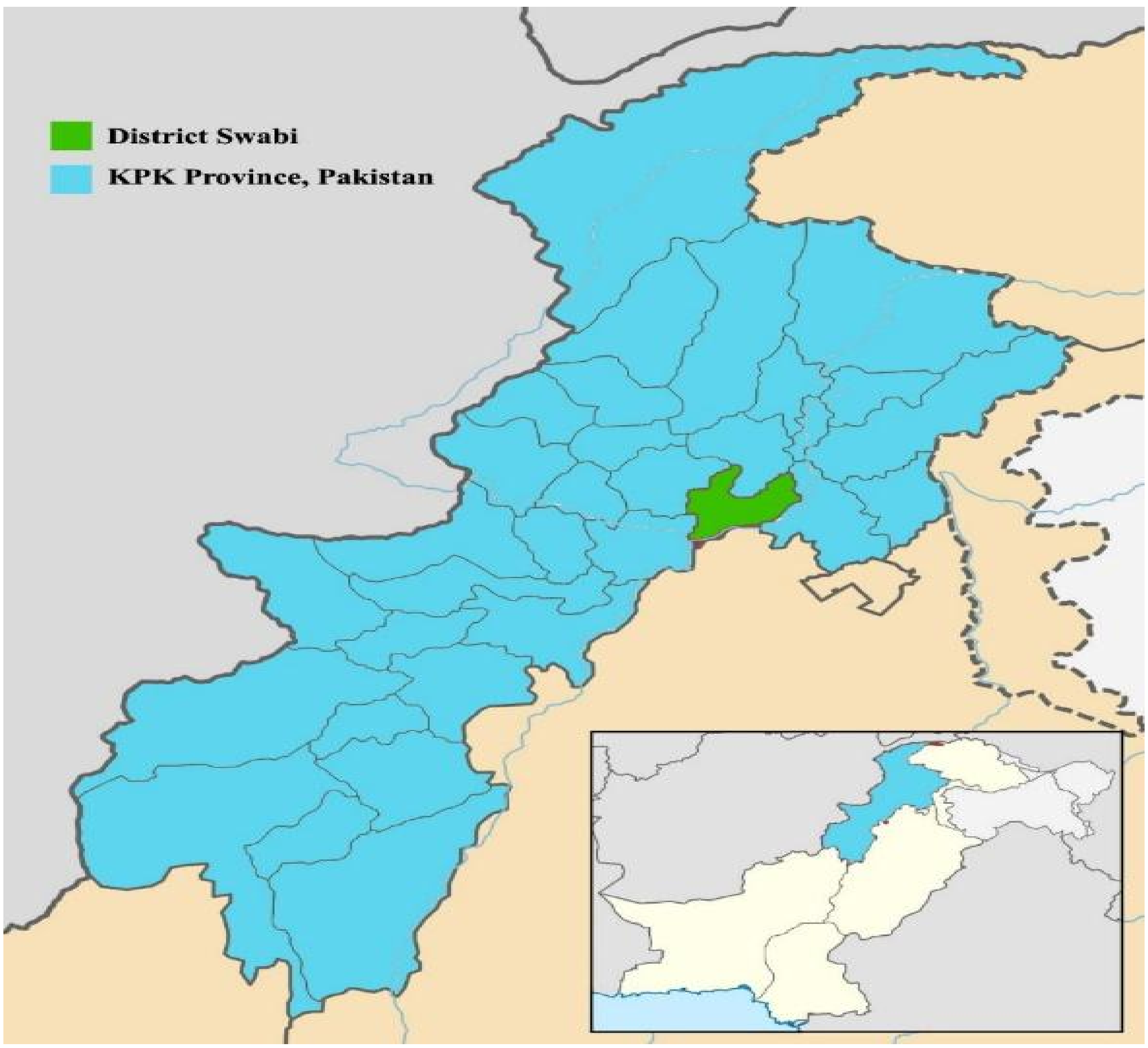

Figure 1: Study area, district Swabi, KPK Pakistan

Standard preparation and analysis of samples: Stock standard solutions of selected metals were prepared from metal-salts in 5 M HNO_3 solution. For each metal, eight working standards were prepared by serial dilution method. Shimadzu AA-7000 equipped with air/acetylene atomizer and metal-specific cathode lamps was used for quantitative analysis of samples. The average body weight of 60 Kg (Eqani *et al.,* 2013, Alamdar *et al.,* 2017) and daily wheat consumption of 339g per day have been used (Radiation 2000, Akhtar *et al.,* 2011, Khan *et al.,* 2020).

RESULTS

Mean concentration level of metals in samples are shown in Table 1. The Dietary exposure of metals was calculated as ADI, calculated by the following equation (Cui *et al.,* 2004, Fu *et al.,* 2008, Singh *et al.,* 2010b):

$$A = \frac{C_H \times C_A}{W_A} \text{ ……………………..(Eq. \# 1)}$$

Where: C_{HM}, C_{AD} and W_{AB} represent mean concentration level of metals (mg/kg or μg/g), average consumption per day in grams (wheat) and average body weight (in kg) respectively. The average body weight for this district was assumed as 60 kg. Samples for which metals were not detected, a value that equals to half the limit of detection (LOD) was used for calculation (Shrivastava *et al.,* 2011).

Table 1 – Concentration level of heavy metals in fresh weight of wheat grain and dietary exposure.

Metals	Mean ± SD (mg/kg)	Range (mg/kg)		Average Daily Intake (ADI)	
		Min	Max	(10^{-3} mg/kg/day)	(mg/person/day)
Cd	0.17±0.1	ND	0.41	0.82	0.05
Pb	0.97±0.2	ND	1.18	4.93	0.38
Ni	10.63±1.3	5.74	14.47	52.16	3.36
Co	0.11±0.1	ND	0.18	0.56	0.04
Cu	6.67±1.1	4.36	10.19	34.33	2.24
Zn	30.21±2.3	16.44	42.45	153.13	9.93
Fe (Total)	204.39±4.6	126.63	298.36	1033.81	67.22
Cr (Total)	8.51±1.9	4.46	12.11	42.50	2.76

"Risk to Human Health" (RHH) was calculated in terms of these three factors i.e., (a) "Health Risk Index" (HRI), (b) "Target Hazard Quotient" (THQ) and (c) percent contribution of dietary exposure or ADI of heavy metals to their respective reference toxicological values. HRI of all metals was figured out by the following relationship as described by (Granero *et al.,* 2002, Cui *et al.,* 2004, Fu *et al.,* 2008, Singh *et al.,* 2010b):

$$H = \frac{A}{R} \text{ ……………………..(Eq. \# 2)}$$

where ADI and R_{FD} represents the "Average Daily Intake" and "Oral Reference Dose" or "Reference Dose" of metals respectively. R_{FD} is defined as safe level of exposure of a metal for a life time or it is the maximum tolerable risk to individuals by daily ingestion exposure (mg/kg/day). R_{FD} for Cd is 1 x 10^{-3} mg/kg/day (Byerlee *et al.,* 1994, Granero *et al.,* 2002, Singh *et al.,* 2010b, Wang *et al.,* 2011); for Pb it is 0.004 mg/kg/day (EPA 1997), for Ni it is 0.02 mg/kg/day, for Cu it is 4 x 10^{-2} mg/kg/day (Wang *et al.,* 2011), for Zn it is 0.3 mg/kg/day (Singh *et al.,* 2010)[b] and for Cr the value is 1.5 mg/kg/day (Byerlee *et al.,* 1994, Epa 1997, Granero *et al.,* 2002, Singh *et al.,* 2010b , Wang *et al.,* 2011). HRI under a value of 1 is assumed as safe while when it is more than 1 it is considered as not safe (EPA 2002). In this study, HRI was found to be less than 1 for Cd, Cu, Zn and Cr and greater than 1 for Pb and Ni. THQ was calculated by the following relationship (Wang *et al.,* 2011):

$$T = \frac{E \times E \times M \times F \times 1^{-3}}{W \times R \times T} \text{……………..(Eq. \# 3)}$$

where ED is duration of exposure (70 years), EF is exposure frequency (as 365 days); FIR is the food ingestion rate (g/person/day) (i.e., food item being tested e.g., wheat); MC is the concentration of metal being analysed in that food sample (in mg/kg); RFD is oral reference dose (as mg/kg/day); WAB is averaged body weight (in kg) and the TA is averaged time of exposure (for non-carcinogens) (as 365 days). Years of exposure were supposed to be 70. For THQ with a value lower than 1, it suggests that the population exposed may be assumed as safe (Wang *et al.,* 2011), for THQ ≤ 1, the adverse health impacts are unlikely, while in the case of THQ > 1 however, the situation is with concerns of potential non-carcinogenic health risks (Al-Saleh *et al.,* 1999). We found THQ for Cd, Cu, Zn and Cr to be less than 1 which is good news and no risks of toxicities from these metals. On the other hand, though, THQ for Pb & Ni were found more than 1 which signifies that the population exposed may be at the risk of somewhat Pb and Ni toxicity.

DISCUSSION

Several organizations have recognised reference toxicological values to address metal toxicities, in terms of several toxicity level monitoring parameters. For example, "Tolerable-Daily-Intake" (as TDI), "Provisional-Tolerable-Daily-Intake" (as PTDI), "Provisional-Maximum-Tolerable-Daily-Intake" (as PMTDI), "Provisional-Tolerable-Weekly-Intake" (as PTWI) and "Upper Limit" (as UL), as shown in Table 2. These are upper limits of exposure levels used as comparison for contaminants (i.e., heavy metals) consumed for a lifetime period without significant risk for a given study.

The percent contribution of the dietary exposure or ADI of metals to their corresponding TDI, PTDI, PMTDI or UL was calculated and was found to be lower than 100% for Co, Cd, Cu and Zn that indicated the dietary exposure of these metals is safe and health risks can be safely ruled out. However, the percentage contribution of dietary exposure or ADI of Pb, Ni and Fe to their corresponding TDI, PTDI, PMTDI or UL were found greater than 100% suggesting health risks cannot be ruled out.

Table 2 – Reference toxicological values.

Metal	Category	Concentration (by body weight)	References
Cd	PTDI/TDI	1 μg/kg/day	(Fao 1989, Van Koten-Vermeulen *et al.,* 1993, Pagán-Rodríguez *et al.,* 2007, Fu *et al.,* 2008, Nasreddine *et al.,* 2010)
Pb	PTWI	25 μg/kg/week	(Webb *et al.,* 2003, Kwoczek *et al.,* 2006, Nasreddine *et al.,* 2010)
Co	TDI	1.6–8 μg/kg/day	(Arnich *et al.,* 2012)
Cu	PMTDI	0.5 mg/kg/day	(Nasreddine *et al.,* 2010)
Zn	PMTDI	1 mg/kg/day	(Nasreddine *et al.,* 2010)
Fe	PMTDI	0.8 mg/kg/day	(Fao 1989)
Cr	---	---	---

P=Provisional, T=Tolerable, D=Daily, I=Intake, W=Weekly, M=Maximum

Table 3 – Health risk assessment to exposed population.

Metals	HRI	THQ	% Contribution to Ref. Values	Deduction
Cd	0.82	0.82	81 %	Exposure considered as "safe" and risks could safely be ruled out
Pb	1.24	1.24	141.7 %	Exposed cannot be considered "safe" risks cannot be ruled out
Ni	2.61	2.61	237 %	Exposure considered to be "unsafe" and risks cannot be ruled out
Co	---	---	7.6 %	*Correlation cannot be concluded*
Cu	0.86	0.86	6.8 %	Exposure considered as "safe" and risks could safely be ruled out
Zn	0.51	0.51	15.3 %	Exposure considered as "safe" and risks could safely be ruled out
Fe	---	---	129.2 %	*Correlation cannot be concluded*
Cr	0.02	0.02	----	Exposure considered as "safe" and risks could safely be ruled out

Uncertainties and exceptions may occur for two reasons, firstly, more than 70 % wheat is milled to make whole-wheat flour that is used to bake flat breads and (Rehman *et al.,* 2006) milling process cause some of Fe and Zn with some other minerals to be lost (Zhang *et al.,* 2010), secondly the absorption of these metals also depends on other food components (Karami *et al.,* 2009). For example, *Phytate*, an anti-nutrient compound present in wheat in the form a complex that keeps the absorption of micronutrients especially Zn and Fe within certain limits (Sandström *et al.,* 1987, Yang *et al.,* 2007, Fang *et al.,* 2008, Tang *et al.,* 2008, Karami *et al.,* 2009). So, the actual supply of Fe and other metals for this fact (e.g., Pb, Ni and Cr) from whole-wheat flour may be somewhat less than our results.

Conclusion: In this study we analysed wheat samples from district Swabi of KPK, Pakistan, to establish a toxicological profile for metal-toxicity through its consumption. The results indicated that for Co, Cd, Cu and Zn the dietary exposure is safe and the associated health risks can be safely ruled out while for Pb, Ni and Fe, the non-carcinogenic health risks could not be ruled out, but considering uncertainties, facts and observations from similar studies, as discussed earlier, the toxicological profile of wheat in terms of metal toxicities from this district is not too precarious.

Appendix 1 – FAOSTAT statistical data, accessed on 1-15-2021.

DOMAIN	AREA CODE	AREA	ELEMENT	ITEM	YEAR	UNIT	VALUE
Crops	165	Pakistan	Production	Wheat	2018	tons	25076149
Crops	165	Pakistan	Production	Wheat	2019	tons	24348983

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