Health Risk Assessment by Consumption of Heavy Metal Contaminated Apples of Salooni, District Chamba, Himachal Pradesh

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Abstract- **In the present study, the atomic absorption spectroscopy (AAS) was employed for the determination of heavy metal concentration in three (Red, Royal and Golden) verities of apples growing in Salooni, District Chamba, Himachal Pradesh, India. An assessment of risk involved due to consumption of contaminated food also calculated. Accumulation of heavy metals especially Pb was found higher than the permissible limits of WHO/FAO standards in all varities of apples. As calculated, THQ was found >1 for Pb and Cd in all the studies samples. The overall study suggests that apples in the area were contaminated by the assayed metals and its consumption can cause potential health risks to consumers. Correlation analysis between heavy metals of soil and apples was also done and result showed that these heavy metals in fruits are not accumulated through soil.**

I. INTRODUCTION

Apples constitute a frequent component in the diet of the Polish population. They are readily eaten in large quantities due to their tasty properties and the mineral salts and vitamins present. That being so, apples should systematically and thoroughly undergo quality tests. Such tests should cover the content of heavy metals, in particular lead, cadmium, arsenic and mercury. These elements may be the cause of serious deseases. Factors that may influence the content of trace elements in apples include the properties attributable to a particular variety of apples, air pollution, agro-technical treatment or specific soil characteristics [1, 2]. Agricultural regions usually feature smaller concentrations of these elements, compared to urbanized areas or areas located in the vicinity of industrial plants [3].

Heavy metal pollution of agricultural soil and fruits/vegetables is one of the most severe ecological problems on a world scale and also in India. The food chain contamination is the major pathway of heavy metal exposure for humans [4, 5]. The consumption of fruits/vegetables is one of the most important pathways for trace metals that harm human health [6].

Several methods have been proposed for estimation of the potential risks to human health of heavy metals in fruits/vegetables. The risks may be divided into carcinogenic and non-carcinogenic effects. Risk assessment is one of fastest method which is needed to evaluate the impact of the hazards on human health. Current non-cancer risk assessment methods do not provide quantitative estimate of the probability of experiencing non-cancer effects from contaminant exposure. These methods are typically based on the Target Hazard Quotients (THQ) [7, 8].

Although, part of this study has been published in Indian Journal of Applied Sciences [9] but due to some mistake some results has been misinterpreted. We herein again report the risk assessment of potential toxic heavy metals—Cd, As, Pb, and Cr, in the soil and three variety (Red, Royal and Golden) of apple of cultivated area nearby Salooni, Chamba (Himachal Pradesh).

II. MATERIAL AND METHODS

2.1 Sample collection

This study was carried out in the Salooni sub-tehsil of Chamba, Himachal Pradesh, India. Salooni is one of the places which are famous for red, royal and golden variety of apple. There are 1972.80 ha area in Salooni, where apple of different variety is grown. In Salooni area three sub sites namely, Chandroon, Narohi-I and Narohi-II cited as Site 1, 2 and 3 throughout the paper, are choosed for the study. Fresh apple samples of three different varieties (Red Apple, Royal Apple and Golden apple) were collected from the orchid of the selected sub stations. In each farm, four sampling points were systematically selected along crop planted rows at specified intervals of 20 feet from each other. Apple sample of three varieties (red, royal and golden) were collected from these four different locations to provide replicate samples of each plant. Soil samples from the rooting zone (0-10 cm) were also taken from each sample location.

2.2 Sample preparation and treatment

2.2.1 Sample preparation of Apple sample

The fruit samples were rinsed with distilled water, peeled, sliced to obtain the edible portion for analysis. The samples were washed by the procedures as for food preparation to remove any surface deposits. [10] Immediately after slicing samples were oven dried at 150 ^{0}C for 28 hours. The dried samples were then grounded to fine powder and passed through 2 mm sieve which further kept in polythene bags for acid digestion.

1.0 g of the samples were weighed and digested with the help of a mixture of 5 ml of HCl, 2 ml of conc. H_2SO_4 and 20 ml of conc. HNO_3 in a conical flask under a fume hood. The content was mixed and heated gently at 180-220 °C for about 30 min on a hot plate. The content was continuously heated till dense white fumes appear. The sample was then finally heated strongly for 30 min and then allowed to cool before making up to the mark in 50 ml volumetric flask. All reagents used were of analytical grade.

2.2.2 Sample preparation of soil sample

The collected soil samples were air-dried and sieved into fine powder. Well-mixed samples of 2 g each were taken in 250 ml glass beakers and digested with 8 ml of aqua regia for 2 hours. After evaporation to near dryness, all the samples were dissolved by 10 ml of 2% nitric acid, filtered and then diluted to 50 ml with distilled water.

2.2.3 Atomic Absorption Spetrophotometer determination

Concentration of heavy metals of interest was done by using an Atomic Absorption Spectrophotometer, fitted with a specific lamp of particular metal using appropriate drift blanks, of thermo spectronic-vision 32 software V1.25. All calculations have been performed to double precision as defined by ANSI/IEEE STD 754-1985.

2.3 Calculation of Hazard Quotient for benefit-risk ratio

Consuming fruits, a person will obtain a dose of heavy metal (DM) (mg/kg/day), which can be defined as:

$$\mathbf{DM = [HM] .c} \qquad [1]$$

Where c (mg/kg) is the content of a metal in a studied fruit and [HM] is the calculated concentrations of Heavy metals.

Average daily intake (EDI) of metals can be calculated as:

$$\mathbf{EDI = DM/BW} \qquad [2]$$

Where, BW is the body weight. The average body weight was taken as 70 kg for adults [11]. Daily apple consumption rate for adult residents was an average of 1.35 kg per year [12].

In this study, the health risks associated with heavy metals ingested through three different varieties of apples consumption were assessed using the Target Hazard Quotient (THQ). The THQ has been recognized as a useful parameter for evaluation of risk associated with the consumption of metal contaminated food crops. The THQ index can be defined as the ratio of determined dose of a pollutant to the reference dose (RfD) (mg/kg b. w day) [13], which can be represented as:

$$\mathbf{THQ = \frac{EF \times ED \times FI \times MC}{R_fD \times BW \times AT} \times 10^{-3}} \qquad [3]$$

Where EF is the exposure frequency (365 days/year), ED is the exposure duration (70 years), FI is the food ingestion (g/person/day), [MC] is the metal concentration in fruits (mg/kg), RfD (mg/kg/day) is a reference dose, defined as the maximum tolerable daily intake of specific metal that does not result in any deleterious health effects, BW is the average body weight. AT is average time (365 days/year x number of exposure years, assuming 70 years in this study). The oral reference dose was taken as 0.3, 2.0, 0.02, 1.4, 5.0, 0.04, 0.0005 and 0.004 mg/kg/day for Zn, Co, Ni, Mn, Fe, Cu, Cd and Pb respectively [14].

If the THQ is less than 1, obvious adverse effects are experienced by the exposed population of that particular area. If the THQ is equal to or higher than 1, there is a potential health risk, and related interventions and protective measures should be taken. Conversely, an exposed population of concern will experience health risk if the dose is equal to or greater than the RfD. THQ is calculated by the method provided in the United States EPA Region III risk-based concentration table. [15] The dose calculations were carried out using standard assumptions from an integrated United States EPA risk analysis.

Hazard Index

To evaluate the potential risk to human health, through more than one heavy metals, the hazard index (HI) can be used. [13] Furthermore, HI is the sum of hazard quotients (HQs) for substances that affect the same target organ or organ system. Because different pollutants can cause similar adverse health effects, it is often appropriate to combine HQs associated with different substances. EPA has drafted revisions to the national guidelines on mixtures that support combining the effects of different substances in specific and limited ways. Ideally, HI should be combined for pollutants that cause adverse effects by the same toxic mechanism. However, because detailed information on toxic mechanisms was not available for most of the substances EPA aggregates the effects when they affect the same target organ regardless of the mechanism.

It assumes that the magnitude of the adverse effect will be proportional to the sum of the multiple metal exposures. It also assumes similar working mechanisms that linearly affect the target organ.

$$\mathbf{HI = \sum HQ = HQ_{Zn} + HQ_{Co} + HQ_{Ni} + HQ_{Mn} + HQ_{Fe} + HQ_{Cu} + HQ_{Cd} + HQ_{Pb}} \quad \textbf{[4]}$$

III. RESULT AND DISCUSSION

3.1 Heavy metal concentration in Apples (Red, Royal and Golden)

Figure 1 and 2 are showing the concentrations of heavy metals in three varieties of apples in comparison to WHO standards. As already discussed in the paper published in Indian Journal of Applied Research [9], the alarming concentration of Pb was found in all the studies samples of all the varieties of apples. The highest concentration of Pb in red, royal and golden apple was found above the permissible limits by 2, 3 and 4 times respectively. The concentration of Cobalt and Nickel was found little bit higher than the permissible limit in red and royal apples yet in golden apple its concentration was found within the limits of WHO/FAO. (Figure 1) The concentration of cadmium was found within the range for Red variety, but in golden and royal varieties Cd concentration was found more than the limits provided by WHO/FAO.

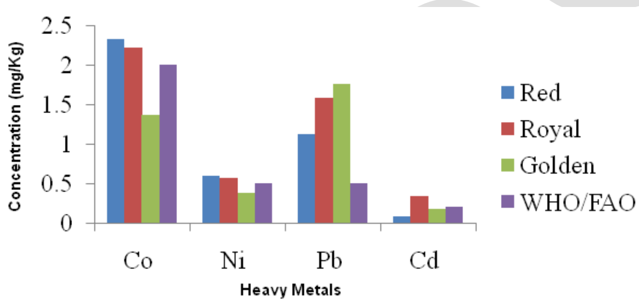


Figure 1: Heavy Metal Concentration (Co, Ni, Pb and Cd) in mg/kg in comparison to WHO/FAO standards

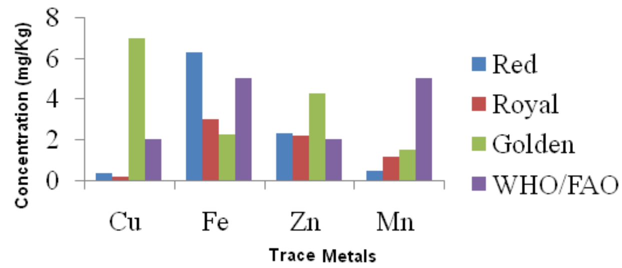


Figure 2: Trace Metal Concentration (Cu, Fe, Zn and Mn) in mg/kg in comparison to WHO/FAO standards

Zn concentration was found higher the permissible limit in all the samples whereas its highest concentration was found in golden apple. Cu concentration was found above the permissible limits in golden variety of apple. Fe was found above the permissible limit in Red variety of apple. Mn in all samples was found well within the limits.

3.2 Heavy metal concentration in soil samples

The concentration of all metals was found much below than the permissible limits of WHO/FAO in all the studies samples. [9]

3.3 Correlation Analysis between soil and apple samples in Salooni

Statistical analysis was performed in order to elucidate the associations between heavy metals in different varieties of apples and soil and to identify if the various concentrations of heavy metals in the soil had an impact on the fruits. Inter metal interactions may illustrate the sources and pathways of the metals present in apples. [16, 17]

Correlation coefficients for the investigated metals are given in Table 1. The significant positive correlation is found only in Zn, Co and Fe in royal variety of apples and soil sample while other heavy metals showing no correlation between soil and apples. It gives a clear indication that soil is not the key factor of higher concentration of heavy metals in apples yet it may be due to some anthropogenic activities of humans or environmental pollution.

Table 1: Correlation coefficient of heavy metals in different varieties of Apple samples with soil

Verity of Apples	Zn	Cu	Co	Ni	Mn	Fe	Pb	Cd
Red	-0.67415	0.370061	-0.46314	0.330097	-0.11499	-0.98337	-0.0996	-0.97437
Royal	**0.726941**	-0.99212	**0.897553**	-0.93571	-0.95678	**0.8525**	-0.7173	-0.33597
Golden	-0.61666	-0.85359	-0.14131	0.465791	-0.99219	-0.86648	-0.68254	-0.58039

3.4 Hazard Quotient for benefit-risk ratio

In order to observe the health risk of any pollutant, it is very important to estimate the level of exposure. One important aspect of the estimation is by the evaluation of the daily metal intake. The estimated daily intake (EDI) of heavy metals depends on both the metal concentration in food crops and the amount of consumption of the respective food crop. The EDI is widely used to describe safe levels of metallic intake through food consumed. It also combines data on the levels of heavy metals in foodstuff with quantities of food consumed on the daily basis. Some assumptions listed in Table 2 are taken during this study for the health risk calculations.

Table 2: Assumption for THQ calculation

S. N.	Assumption for THQ calculation	Reference
1	Ingested dose is equal tothe absorbed Pollutant dose	[18]
2	The average body weight for Adults Assumed to be 70 Kg	[19]
3	Average lifetime is 70 years	[19]

The accumulation of heavy metals in the edible parts of fruits could have a direct impact on the health of nearby inhabitants. Therefore, fruits could be a concern to the local residents. The estimated daily intake of metal (EDI) and target hazard quotients (THQ) were used to assess the human health risk associated with trace metal contamination of different variety of apple grown in the study area (Table 3). THQ was found as in the order Pb> Cd> Zn>Ni>Cu>Mn>Fe in red apple, Cd>Pb>Ni>Zn>Cu>Co>Mn>Fe in royal apple and Pb>Cd>Cu>Ni>Zn>Mn>Co>Fe in golden apple. (Figure 4)

Furthermore, it is important to note here that Pb and Cd is the major contributor for health risk to the local recipient of Salooni, Chamba district. Pb, the most risk contributor account for 57.94 % for red apple, 35.23 % for royal apple and 43.43 % for golden apple of total THQ. (Table 4)

Cd is the second most risk contributor to human health having 32.24 % for Red apple, 61.017 % for royal apple and 35.71% for golden apple of the total THQ. An important observation is that the percentage of THQ for Cd is 2 fold higher in royal apple than in red and golden apple. (Table 4)

Table 3: Estimation of daily intake of metal (mg/Kg) and target hazard quotient (THQ).

Variety of Apple		Zn	Co	Ni	Mn	Fe	Cu	Cd	Pb
Red Apple	**EDI**	0.142	0.143	0.036	0.030	0.386	0.019	0.005	0.069
	THQ	0.473	0.071	**1.800**	0.021	0.077	0.485	**9.600**	**17.250**
Royal Apple	**EDI**	0.136	0.136	0.034	0.072	0.182	0.011	0.021	0.097
	THQ	0.453	0.068	**1.700**	0.051	0.036	0.275	**42.000**	**24.250**
Golden Apple	**EDI**	0.261	0.084	0.023	0.090	0.161	0.427	0.011	0.107
	THQ	0.870	0.042	**1.160**	0.064	0.032	**10.675**	**22.000**	**26.750**
RfDo (mg/kg/day)		**0.3**	**2**	**0.02**	**1.4**	**5**	**0.04**	**0.0005**	**0.004**

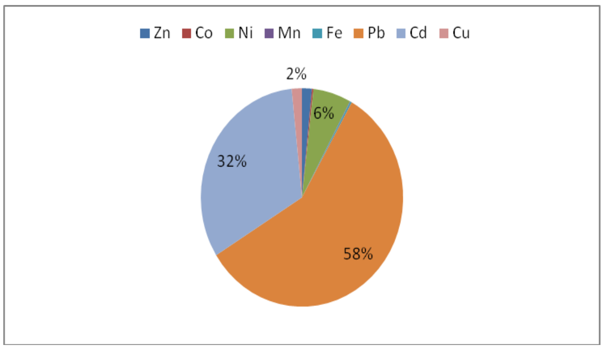
Table 5: Showing % of metal contribution to THQ

Variety of Apple	Zn	Co	Ni	Mn	Fe	Pb	Cd	Cu
Red Apple	1.580	0.238	6.046	0.070	0.258	57.940	32.240	1.629
Royal Apple	0.660	0.099	2.469	0.074	0.052	35.230	61.017	0.399
Golden Apple	1.412	0.068	1.883	0.103	0.052	43.430	35.718	17.330

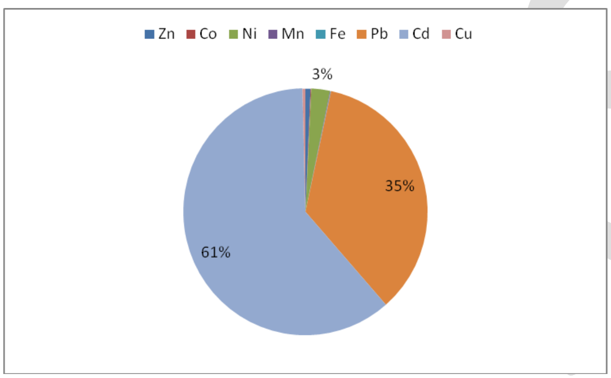
Ni is the third most risk contributor showing 6.046 % of THQ in case of red apple, 2.469 % in royal apple and 1.883 % of THQ in golden apple. (Table 4)

The THQ of Zn, Co, Mn, Fe and Cu is generally less than 1, suggesting that people would not experience significant health risks from the intake of individual metals through apple consumption. Moreover, a significant change is observed in THQ of Cu. In golden apple THQ for Cu is significantly higher than red and royal apple.

The THQ of Pb, Cd and Ni is very much greater than 1 in all the three types of apples whereas THQ of Pb, Cd and Ni is highest in golden, royal and red apple respectively. These results indicate that people would experience significant health risk from the consumption of individual metals through contaminated apple from the selected region. However, the health risk associated with royal and golden apple is higher than red apple among the three varieties of apples.



(A)



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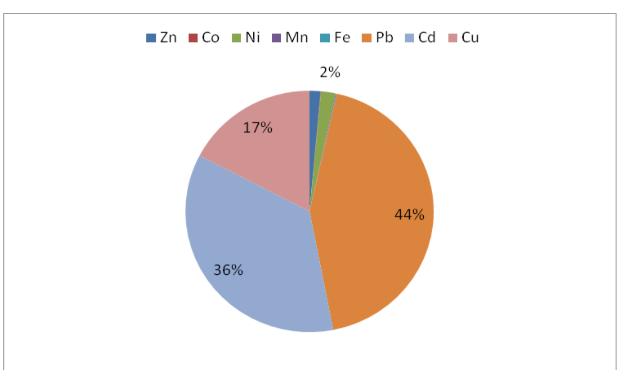

(C)

Figure 3: Pictorial Representation of % of THQ of different heavy metals in (A) Red Apple (B) Royal Apple (C) Golden apple

When the hazard index (HI) exceed 1.0, there is concern for potential health effect. Even though there was no apparent risk when each metal analyzed individually. When all the heavy metals are considered, the potential risk could be multiplied. Hazard Index for all variety was found greater than 1. Thus, using the derived hazard quotients, HQ, for benefit –risk ratio of heavy metal contents, it was found that in general intake of apples of Salooni, Himachal Pradesh, the main fruit of that area, potentially was not useful for human health.

Although, the HQ based risk assessment method provides an indication of the risk level due to exposure of pollutants. However, it does not provide a quantitative estimation for the probability of an exposed population experiencing a reverse health effect [20]. Many researchers consider the risk estimation method reliable and it has been proven to be valid and useful. However, this HQ method consider only exposure to heavy metals via consumption of vegetables, without taking into account other vias like dermal contact, soil ingestion, and other factors such as the presence of agrochemicals and herbicide molecules.

IV. CONCLUSIONS

1. The average content of heavy metals in apples from orchards located in Salooni, Chamba district, Himachal Pradesh proved that the concentration of Pb was found greater than the permissible limits in all the three variety of apples, whereas the highest concentration was found in golden apple which is three fold higher than the permissible limit of WHO/FAO. The Concentration of cadmium in royal apple and Cu in golden apple were also found in a critical situation.
2. Pearson's correlation analysis confirmed that extended concentration than permissible limits of Pb and Cd in apple samples is not due to soil but environmental pollution or some anthropogenic activity of humans may be responsible of exceeding concentrations of these heavy metals.
3. THQ calculations conclude that Pb was found the major risk contributor in all types of apples followed by Cd, the second major risk contributor. The highest value of THQ for Pb was found in golden variety of apple whereas for Cd it was found in royal variety of apple.
4. On the basis of results obtained we can conclude that Red apple is the most useful for its therapeutic value in respect of all studied locations.

As concluded that Pb and Cd were above the recommended legal limits established by FAO/WHO, they must be eaten in moderation due to possible hazard and risks derived from metal ingestion. Moreover, different remediation measures should be taken promptly to remove excising metal contamination of the study area.

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