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Determination of Bioaccumulation and Benefit-risk ratio of apple intake: A case study of Salooni, District Chamba, Himachal Pradesh

KEYWORDS: Heavy Metals, Atomic absorption spectroscopy, Bioconcentration factor, Hyperaccumulator, THQ, EDI

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ABSTRACT *This research was conducted to investigate the metal contamination in soil and three variety of apple i.e. Red, Royal and Golden, and also to evaluate the possible health risks to local population through food chain transfer in Salooni distt. Chamba (Himachal Pradesh). Contamination level of 9 heavy metals (Pb, Hg, As, Cd, Zn, Co, Ni, Mn, Fe and Cu) and 4 essential metals (K, Ca, Na and Mg) in soils and three variety of apple (Red, Royal and Golden) were measured. Results showed that in all the variety (red, royal and golden) of apple Pb concentration was found above the permissible limits of WHO/FAO whereas the concentration of Cd was found higher the permissible limits in royal apple. As found, HI is not higher than 1 in any sample so in general apple intake is not potentially hazardous for human health. Bioconcentration factor (BCF) from soils to vegetable plants and its total health quotient (THQ) were calculated accordingly. Our study also showed that different apple plant may have the potential for phytoremediation for different metals.*

Introduction

Consumption of fruits is essential for a diversified and nutritious diet. Many chronic diseases, such as cancer, cardiovascular diseases and other aging-related pathologies incidence can be significantly reduced by sufficient consumption of fruit and vegetables [1]. Polyphenols, carotenoids (pro-vitamin A), vitamins C and E present in fruits have antioxidant and can play as a free radical scavenger that prevents many diseases [1].

Urban food security in India is a matter of growing concern. Over the past few decades, in the focus of nutritional health point of view, chronic shortages of micronutrients, particularly iron and vitamin A is a point of great concern in India. Deficiency of these micronutrients can cause serious functional disorders particularly women and children [2]. In this context, it is particularly important to encourage the consumption of highly nutritious fruit and vegetable crops, but as the income elasticity of demand for these products tends to be high, there is a clear need to increase supply and maximize the nutritional quality of these products to benefit the poors.

Furthermore, several technologies like excavation of contaminated material and physical/chemical methods are available to remediate contaminated soils. However, these technologies are not very useful because of its high cost [3]. Phytoextraction i. e uptake of heavy metals through shoots and accumulate that heavy metals into plant shoots is one of the strategies of phytoremediation of metal contaminated soils. Phytoremediation can provide a cost-effective, long lasting and effective solution for remediation of contaminated soils [4]. It is one of the matter of great interest to search some native plants that are tolerant to heavy metals. Few studies have evaluated the phytoremediation potential of plants under field conditions [5, 6].

Although, pesticide and heavy metal contamination in food stuffs have been carried out for decades in most developed countries, [7, 8, 9] yet such studies are very few in India, with little information on toxic heavy metal contamination in fruit and their possible health risk assessment, though apple is the most important fruit for Indian people. The objectives of the present study, are primarily to find out the risk assessment of potential toxic heavy metals like Cd, As, Pb, Hg and Cr, in the surface soil and three variety of apple (Red, Royal and Golden) nearby Salooni Distt. Chamba (Himachal Pradesh) and also to assess their bioaccumulation factor.

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, 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, Royal and Golden apple) were collected from the orchid of the selected sub stations. In each farm, four sampling points were systematically choosed along crop planted rows at specified intervals of 20 cm 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. All the subsamples collected at each sampling station for each variety of apple were thoroughly mixed to form a composite sample. 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

Volume : 5 | Issue : 6 | June 2015 | ISSN - 2249-555X

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 samples

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.

2.3 Atomic Absorption Spetrophotometer determination

Concentration of heavy metals of interest was done by using a 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.

2.4 Calculation of Hazard Quotient for benefit-risk ratio

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

$$DM = [HM] .c \quad [1]$$

Where c (mg/kg) is the content of a metal in a studied fruit

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

$$EDI = DM/BW \quad [2]$$

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].

Risk of assuming a dose of heavy metal may be characterized using a total hazard quotient (THQ) [13], which can be represented as:

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

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.

To evaluate the potential risk to human health through more than one heavy metals, the hazard index (HI) can be used. [13] The HI is the sum of the hazard quotients, as described in equation 4. 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.

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

2.5 Statistical Analysis

Statistical analyses were done on the replicated values of the quantative results using the computer based SPSS (statistical) software version 16.0. Firstly, one way analysis of variance was conducted to compare the means of each heavy metals in three variety of apples and secondly, for each heavy metal, the mean for each variety of apple was compared with the WHO/FAO permissible limits.

Result and Discussion

3.1 Determination of heavy and essential metal ion concentration in soil samples

Table1. Heavy and essential metal concentrations (mg/kg) in different varieties of soil samples collected from Salooni, Himachal Pradesh

Metals	Mean	Standard Deviation	Range
Cu	1.9130	0.6133	1.21 - 2.37
Zn	5.8513	1.2830	4.45 - 6.98
Co	2.0163	1.4182	0.67 - 3.50
Ni	1.4640	0.9811	0.59 - 2.53
Mn	14.6673	5.6390	8.25 - 18.84
Fe	142.1453	117.2223	6.83 - 212.9
Pb	1.4947	0.8251	0.82 - 2.41
Cd	0.3700	0.5456	0.05 - 1.00
Hg	ND	ND	ND
Na	23.9967	6.5641	18.29 - 31.17
K	392.8233	478.8917	44.37 - 938.9
Ca	116.2667	0.6807	115.5 - 116.8
Mg	83.1633	42.8849	48.73 - 131.2

Table 1 shows the mean concentration of heavy metals of the 0-10 cm subsoil of the selected areas of Salooni. The investigation of the total content of the heavy metals in the soils was done from the sub surface soil since sub surface soil are better indicators of plant available metal uptake and not a major indicator of metallic burdens. The concentration of all the heavy metals in soil (mg/kg) in selected orchids of the study area are found much below than the permissible limit set by WHO [15]. The comparatively low soil trace levels might suggest that, perhaps, plant uptake of some of these metals under irrigation has been high to the extent that their levels in the soils has been significantly lowered.

It is important to note here that Fe shows remarkable change among all the study centers. Fe concentration was found the highest (212.9 mg/kg) at site 1, whereas its minimum concentration was found at site 3 (6.83 mg/kg). The higher standard deviation reveals higher variation in the heavy metal distributions from the point source emission to the adjacent areas. Among all the considered

Volume : 5 | Issue : 6 | June 2015 | ISSN - 2249-555X

heavy metals, the low standard deviation in Cu, Zn and Cd may be described to its continuous removal by vegetable grown in designated areas.

There is little variation in soil pH value in three sites (6.01 – 6.56) but, the pH value indicates the soils to be generally slightly acidic to neutral in reaction. Heavy metals and micronutrients uptake by plants depends on the soil pH so it is important to check the soil pH [16].

3. Result and Discussion

3.2 Heavy and Essential metal ion concentration in apple

The heavy metal concentrations (mg/kg) in three different variety of apple are listed in Table 2. Comparison of the mean values of heavy metal contents in apple of three verities with the maximum allowable limit set by WHO/FAO reveals that exceeding of these limits in the majority of the samples.

The Zn concentration was found higher the permissible limit in all the samples whereas its highest concentration was found in golden apple, which has 3 times higher concentration than the permissible limit. Co and Ni concentration was found little bit higher than the permissible limit in red and royal apple while in golden apple their concentrations are lower than the permissible limits. The concentration of Mn was found in the range of 0.485 – 1.476, that is much below than the permissible limit 5.0 mg/kg set by WHO/FAO. Results showed that only red apple have exceeding concentration of Fe among all the three variety of apple. The concentration of Cu and Cd was found above the permissible limits in golden and royal apple respectively. The highest concentration of Cu and Cd exceeded the permissible limits by approximately 3 and 2 times, respectively.

Cd is highly toxic to animals and humans making it of great concern in the environment [18] Moreover, with long term use of sewage sludge, heavy metals can accumulate to phytotoxic levels and results in reduced plant growth and/or enhanced metal concentration in plants, which consumed by animals then enter the food chain [19]. The higher concentration of Cd, Cu and Ni can become problematic at low pH value. Soil pH and organic matter are the most critical factors in controlling Cd availability and plant uptake. Low pH favours accumulation of Cd in the soil. [18]

Table 2: Heavy metal concentration (mg/kg) in different varieties of Apple sample collected from Salooni, Himachal Pradesh

Variety of Apple		Zn	Co	Ni	Mn	Fe	Cu	Cd	Pb	Hg	As
Red	Mean ±SD	**2.322** ± 1.20	**2.326** ± 0.968	**0.596** ± 0.175	0.485 ± 0.179	**6.28** ± 2.121	0.317 ± 0.097	0.079 ± 0.064	**1.127** ± 0.110	ND	ND
	Range	1.142 - 3.551	1.44 - 3.36	0.422 - 0.773	0.284 - 0.628	4.74 - 8.70	0.258 - 0.430	0.007 - 0.13	1.008 - 1.226		
Royal	Mean ± SD	**2.213** ± 0.222	**2.213** ± 0.222	**0.569** ± 0.179	1.164 ± 0.920	2.970 ± 1.81	0.180 ± 0.101	**0.340** ± 0.344	**1.579** ± 1.213	ND	ND
	Range	0.834 - 2.622	2.08 - 2.47	0.412 - 0.764	0.524 - 2.219	1.16 -4.78	0.0944 - 0.292	0.080 - 0.730	0.856 - 2.980		
Golden	Mean ± SD	**4.259** ± 2.608	1.369 ± 0.392	0.378 ± 0.021	1.476 ± 1.203	2.623 ± 0.565	**6.954** ± 6.143	0.179 ± 0.253	**1.751** ± 0.959	ND	ND
	Range	1.309 - 6.257	0.928 - 1.68	0.356 - 0.398	0.460 - 2.804	2.05 - 3.18	0.2245- 12.26	0.060 - 0.470	1.169 - 2.858		
Permissible level as per (FAO & WHO) 1.5		2.0		0.5	5	5	2	0.2	0.5	0.05	0.1

In each row, mean value with bold font is significantly different from the corresponding WHO/FAO permissible value, 2011.[17] Bold with underlined means are critical values for necessary intervention.

Pb is found above the permissible limits in all the studies samples of all the variety of apple. 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 high concentration of Pb may be attributed to crops located near roads of heavy traffic. The inhalation of airborne Pb from vehicle emissions is the main source of this element to humans and from direct atmosphere deposits on soils, water, and crops, constituting the gateway into the food chain [20]. It is gratifying to note that Hg and As were not detected in all the samples. These are highly toxic heavy metals, their presence in foods should be very negligible or in trace amount.

Table 3: Essential metal concentration (mg/kg) in different varieties of Apple sample collected from Salooni, Himachal Pradesh

Variety of Apple		K	Na	Mg
Red Royal	Mean ±SD	425.96 ± 37.90	19.44 ± 10.78	16.55 ± 4.20
	Range	386 - 461.4	7.0 - 26.07	11.8 0 - 19.79
Royal	Mean ± SD	520.86 ± 118.59	19.67 ± 2.029	17.67 ± 0.845
	Range	429.9 - 655	17.33 - 20.88	16.95 - 18.54
Golden	Mean ± SD	228.03 ± 110.73	9.71 ± 8.78	15.04 ± 2.46
	Range	150.8 - 354.9	4.33 - 19.85	12.31 - 17.09

Volume : 5 | Issue : 6 | June 2015 | ISSN - 2249-555X

Table 3 is showing the mean concentrations and range of essential metals (Na, K and Mg). It is clear from the results that K concentration is very much higher than Na and Mg in the studies samples. Whereas, it is important to note here that concentration of these three essential metals in found lesser in golden apple in comparison to red and royal. The concentration of K and Na is 2 times lesser and Mg little bit lesser in in golden apple as compared to red and royal.

3.3 Correlation Analysis

Table 4: Correlation coefficient of heavy metals in different varieties of Apple sample

	Zn	Co	Ni	Mn	Fe	Cu	Cd	Pb
Zn	1	-0.987	-0.987	0.709*	-0.533	0.999*	-0.180	0.678*
Co		1	0.999*	-0.810	0.657*	-0.992	0.025	-0.784
Ni			1	-0.813	0.662*	-0.991	0.020	-0.787
Mn				1	-0.974	0.730*	0.564*	0.999*
Fe					1	-0.557	-0.735	-0.983
Cu						1	-0.151	0.700*
Cd							1	0.599*
Pb								1

Statistical analysis was performed in order to elucidate the associations among metals in apples. Inter metal interactions may illustrate the sources and pathways of the metals present in apples. Correlation coefficients for the investigated metals are given in Table 4. The significant positive correlation is found between Zn/Mn, Zn/Pb, Co/Fe, Ni/Fe, Mn/Cu, Cu/Pb, Co/Ni, Mn/Pb and Zn/Cu. Cd is the only element which is not showing any strong correlation except Mn.

This strong correlation among metal-metal pair gives a clear indication of common sources of these metals [21], similar or nearly identical metal accumulation properties of fruits. [22] Zn/Cu, Co/Ni and Mn/Pb these metal pairs showed the highest correlations showing that these heavy metals had similar pollution levels and similar pollution sources.

3. 4 Accumulation of metals in Plants

Bioconcentration factor (BCF) or Plant concentration factor is a parameter used to describe the transfer of trace elements from soil to plant edible parts. In other words, we can say a plant's ability to accumulate metals from soils can be finding out by using the BCF, which may be defined as the ratio of metal concentration of plant to the corresponding soil. [23]

$$\mathbf{BCF = [X]_{plant}/[X]_{soil}}$$

Where, $[X]_{plant}$ and $[X]_{soil}$ represents the heavy metal concentration in extract of plants and soils respectively.

Table 5: Bio-concentration Factor (BCF) of Heavy as well as essential metals

Metals	Red Apple	Royal Apple	Golden Apple
Zn	0.396	0.378	0.727
Co	1.215	1.090	0.678
Ni	0.407	0.388	0.258
Mn	0.033	0.079	0.100
Fe	0.044	0.021	0.018
Pb	0.753	1.056	1.172
Cd	0.213	0.918	0.483
Cu	0.165	0.094	3.635
Na	0.810	0.819	0.404
K	1.080	1.320	0.580
Mg	0.199	0.212	0.18

Values>1 are in bold font.

By comparing BCF values, we can compare the ability of different plants in taking metals from soils. Plants exhibiting BCF values less than one are unsuitable for phytoextraction. A few plants were capable of accumulating heavy metals in the roots, but most of them had low BCF values, which shows the ability of heavy metal accumulation and translocation by plants [23].

The potential mobilization of heavy metal in red apple decreases as Co>Pb>Ni>Zn>Cd>Cu>Fe>Mn, in royal Co>Pb>Cd>Ni>Zn>Cu>Mn>Fe and in golden the sequence is Cu>Pb>Zn>Co>Cd>Ni>Mn>Fe and essential metals varies as K>Na>Mg in all types of apple. BCF value is greater than 1 for Co in red and royal apple and for Pb in royal and golden apple. An interesting observation is that red apple plant is efficient in taking up Co, royal in taking up Co and Pb and golden in taking up of Pb and Cu from soil.

It is important to note here that in our study Pb, Cu, and Co are the trace elements which are mainly transfer from soil to plants in the different variety of apple at studied sites and Zn, Cd, Ni, Mn and Fe are the showing least absorption in all the samples. Heavy metal-tolerant species with high BCF can be used for phytostabilization of contaminated site. According to our study red apple plant for Co, Royal apple plant for Co and Pb and golden apple plant for Pb and Cu can be used for phytostabilization of these particular sites. (Table 5)

Phytostabilization can be used to minimize migration of contaminants in soil [24]. This processes uses the ability of plant roots to exchange the environmental conditions via root edudates. Plants can immobilize heavy metals through absorption onto roots, or precipitation with rhizosphere. The process reduces metal mobility and leaching into ground water, and also reduces metal bioability for entry into the food chain. By using metal accumulator plant species for stabilizing contaminant in soil, particularly metals, it could also provide improved conditions for natural attenuation or stabilization of contaminants in the soil. However, more studies are needed in this particular field [25].

Volume : 5 | Issue : 6 | June 2015 | ISSN - 2249-555X

3.6 Hazard Quotient for benefit-risk ratio

Table 6: Estimation of daily intake of metal (mg/Kg) and target hazard quotient (THQ).

Variety of Apple		Zn	Co	Ni	Mn	Fe	Cu	Cd	Pb	Total
Red Apple	EDI	0.142	0.143	0.036	0.0298	0.386	0.0194	0.0048	0.069	0.83
	THQ	0.0047	0.0007	0.0018	0.0002	0.0007	0.0049	0.0097	0.0173	0.04
Royal Apple	EDI	0.136	0.136	0.034	0.0715	0.182	0.011	0.021	0.097	0.688
	THQ	0.0045	0.0006	0.0017	0.0005	0.0004	0.00276	0.0418	0.0243	0.076
Golden Apple	EDI	0.261	0.084	0.0232	0.09	0.161	0.427	0.011	0.107	1.164
	THQ	0.0087	0.0004	0.0012	0.0006	0.0003	0.0106	0.02202	0.0269	0.070
RfDo (mg/kg/day)		0.3	2	0.02	1.4	5	0.04	0.0005	0.004	8.7645

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 6). THQ in different variety of apple was found as in the order Pb> Cd> Zn>Ni>Cu>Mn>Fe. The THQ of each metal was less than 1, indicating that intake of apple does not pose to a considerable hazard. The maximum HI was found in Royal apple among the studies variety of apple so it can be said that red apple and golden apple are safer for human consumption in comparison to royal 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 laws analyzed individually, When all the heavy metals are considered, the potential risk could be multiplied. Hazard Index for all variety was found less 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 apple of Salooni, Himachal Pradesh, the main fruit of that area, potentially was very 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 estimate for the probability of an exposed population experiencing a reverse health effect. [26]. Many researches consider the risk estimation method reliable and it has been proven to be valid and useful.

Conclusion

The aim of this research was to provide as assessment of heavy metal contamination in three varieties of apples found in Salooni of Chamba district, Himachal Pradesh. Heavy metal concentration varied among the studied variety of apple, which reflects the differences in their uptake capabilities and their further translocation to edible potion of the plants. Hg and As was found unavailable in all the studied samples. Pb concentration was found above the permissible limit in all the three variety of apple whereas the highest concentration was found in golden apple which is three fold higher than the permissible limit of WHO/FAO. Concentration of Cd in royal apple and Cu in golden apple are also found in a critical situation. So we can conclude that golden apple is not suitable for human consumption in comparison of red and royal apple. Red apple is the most useful for its therapeutic value in respect of all studied locations. The concentration of all the heavy metals are also estimated in soil samples but their concentration in all the soil samples was not exceeding the maximum permissible limits.

In general, no THQ values were >1 through apple consumption indicating that no appearing health risk of eating the fruit in current scenario but may experience some adverse health effects in the future. Toxic heavy metal particularly Cd, Cr, Pb, Hg and As contamination may be reduced by doing organic agriculture with little use of agrochemicals in the fields producing apple, the staple fruit in Himachal Pradesh, India.

Furthermore, the study was also conducted to check plants growing on a contaminated site to determine their potential for metal accumulation. Among the three verities of all selected sites, red apple was considered as the most promising species for phytoextraction of Co, royal apple for Co and Pb and golden apple for Pb and Cu heavy metal. The phytoremediation potential of these plants species, especially for use in sub-tropical and tropical reasons need to be further investigated.

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