COMPARATIVE STUDY OF HEAVY METALS IN GRAPES IRRIGATED BY CLEAN AND DIRTY WATER IN QUETTA CITY, BALOCHISTAN (PAKISTAN)

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ABSTRACT: *The main objective of this work is to present concentrations of several heavy metals in grape samples irrigated by dirty waters and clean waters collected at Spain Road and Qambrani road of Quetta city, respectively. The determination of magnitudes of heavy metalloids such as lead, zinc, copper, iron, etc. was executed with the application of A.A.S (atomic-absorption-spectrophotometry-(PerkinElmer Corp.—Model_5100-PC). After analysis of the grapes samples, it was found that proportions of these metals were in the subsequent direction $Cd^+<Fe^+<Cu^+<Zn^+<MnO<Pb^+$ and the proportion of the heavy metalloids are in discordance with the customs and criterion of W.H.O. This analytical research reveals that customers of municipal city and clients are at bigger menace for procuring novel fruits with greater extents of heavy metalloids with lawfully tolerable restrictions. These foodstuff supplies are recounted to be polluted with noxious and health perilous substances. Further, the present literature emphasizes the harmfulness proportion of heavy metalloids amid the public in non-rural regions and the degree of metallic contagion in grapes.*

Key Words; Heavy Metals, Atomic Absorption Spectroscopy and water contamination in Fruits of grapes.

1. INTRODUCTION

Pakistan, a land that is fruited with whole seasonal flavors and grown landscape. Bulks of various fruited items have been growing here. These citrus fruits are accepted and liked greatly in domestic, native as well in overseas. Pakistan is blessed with the fecund territory, appropriate environs, and virtuous climatic statuses. Pakistan is one of those country lands in a sphere where entirely 4 seasonal spells ensue. For that reason, it yields abundant fruits and vegetation and exports these fruits to nearly sixty countries.

Pakistan’s largest province Balochistan covers an area of347,190 sq. km making43.6% of the entire Pakistan area. It possesses 29districts and the lowest populace compared to other provinces’ populaces. This region situates betwixt the latitude 24Ø55and32˚.04-North and longitude 60˚.45and70˚.17-East. The increment is observed in the province's entire populace yearly at an average development and progression percentage of 2.42 in comparison with the national average percentage of 2.61 [1].

Balochistan is recognized as the fruit creel of Pakistan. It is blessed with an idiosyncratic atmosphere where productions of diverse actualities of fruits are achieved. For this reason, the title of “fruit garden of Pakistan” has been given to this province which is capable of fabricating cherries,berries&appricots in bulk amounts while moderate quantities have been fabricating of peach, pomegranate, apricot, apple and dates. In Balochistan developing fruit trees are greater than the area of 1, 49, 726ha which harvests about 0.9M tonnes per annum [2]. Grapes are scrumptious fruits grown in many districts of Qta such as Pishin, Kalat, Zhob, Loralai and Mastong dists. Kishmishi and Sundarkhani are eminent for producing diversities of grapes. In the highlands of Balochistan, the production of Apricots, Plums and Peaches are rampant. Huge quantities of coal, gas, gold and different minerals are found in this province [3].

A grape is considered as a fruited drupe of the evanescent wooded lianas of the botanic genre “*Vitis*”. This fruit can be consumed fresh as table-grapes/ Vlaams-Brabant or they can be applied for production wineries, candied fruits, nectar, compotes, grape nuts residues, currants, balsamic and grape-nuts oleaginous. Grapes are non-crucial sort of fruit, usually transpiring in clumps.

The farming of the tame grape was initiated 600-800 decades earlier in the adjacent areas of the East [4]. Most primitive domesticated microbes such as “Yeast-(Saccharomyces cerevisiae)" occurs organically on the outer epidermal layer of grapes, taking people to the novelty of winery liquors. The antediluvian paleontological factual indication for a prevailing situation of winery and liquor production in anthropoid civilization dates back 6000 BC in Georgia [5-6]. The primogenital recognized wineries were found in Armenia 4000BC. The city of Shiraz was well-known for the production of fewer but finest and optimum wineries liquors in the Arabian region. Therefore recommendation of Syrah red wine was given next to Persian city “Shiraz” where the grapefruits were put in an application to produce Shirazi-winery [7].

In the Northern region of the U.S.A, intuitive grapes associated with several types of the genera “*Vitis*” burgeon in the wild throughout the continent, also as a dietetic constituent of numerous Intuitive Americans, but were well-thought-out by European colonials to be inappropriate for winery liquors. *Vitis-vinifera* cultivars were traded in for that objective.

Three varieties of wineberries (Kikishmishi, Haitha and native-black) were estimated beneath infuser irrigation(inundation) systematic mechanism. By the last year, ingrained florae were interlined at the distance of 5feet and rows12 feet wide. Subsequently, 6 yrs of orchard farming utmost production of fruited items was chronicled in native-black (3535kg/ha) tracked by Haitha with a productive output of 3025kg/ha and Kishmishi had formed 2910kg/ha. Exertions were applied too to estimate the prospective probability of formation of greater compression in grape groves. The fruit of grapes are given in Figure-1

Figure-1 The fruits of Grapes

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Heavy metals are not biodegradable and have the potential for accumulation in the different body organs leading to unwanted side effects [8-9]. Production and trade units such as industries and vehicles emanate metallic constituents which can possibly be assembled on the vegetable exteriors during their making and manufacturing, conveyance and trading. Cd, Pb and Hg are lethal metalloids due to their capacity to cover long distant areas while transportation in the air [10]. Contribution of swift and chaotic non-rural/metropolitans trade and industrialized units have been made for the increment in the intensity of heavy metalloids in the metropolitan environs of industrially emerging states such as Egypt [11-13].

Heavy metals are widely dispersed in the environment. Heavy metal contamination of the food items is one of the most important aspects of food quality assurance (Marshall F *et al.*, 2003). Heavy metals are among the major contaminants of the food supply and may consider the most important problem to our environment [14]. They get into the food chain and transpire in diverse quantities in anthropoid nutrition [15]. Recently, [16-17] have reported that atmospheric deposition can significantly elevate the levels of heavy metals contamination in vegetables commonly sold in the markets of Varanasi, India. These food commodities are reported to be contaminated with toxic and health-hazardous chemicals. The uptake of heavy metals in vegetables is influenced by some factors such as climate, atmospheric depositions, concentrations of heavy metals in soil, nature of the soil and the degree of maturity of the plants at the time of harvest [18-19]. Excessively found pollutants in fruit and vegetables are pesticide residues, crop contaminants such as aflatoxins, patulin, ochratoxin, etc., and heavy metals. Moreover, direct aspiration of the fruit juices and milk can cause flame fluctuations and accumulation of solid deposits on the burner head [20].

Effluence in foodstuff due to metallurgies is a severe risk to the health of living beings. When these metalloids are consumed they deliver severe impacts on cellular tissues. In human's nutritional kinds of stuff usually, levels of toxic metalloids do not exceed up to 5-10%. However, fewer metalloids demonstrate lethal impacts in comparatively small amounts and besides their proportion in cellular tissues progressively rises as a result of the accretion procedure [21]. The excessive content of these metals in food is associated with a number of diseases such as cardiovascular, kidney, nervous as well as bone diseases [22-23]. Abnormal ingestion causes neurological anomalies, hepatic and renal disturbances [24]. Dietary intake of heavy metals causes cancers, tumors, a mutation in genetic material & teratogenesis [25-26].

Curative and therapeutic florae are being used for consumption globally. Such floras are also an essential basis of mineral deposits and reserves for pharmacological production units. On the other hand, these florae contain metalloids in wide-ranging quantities. Several therapeutic herbs applied for the formulation of these remedies can depict a health menace due to the existence of lethal components like heavy metalloids. The harmfulness of heavy metalloids relies on the biological structure of elements. Heavy metalloids are perilous in the shape of their cations and are vastly toxic when paired up with the short chains of carbon atoms.

At this time there is rising sentience that toxins and poisons for instance metallurgies and alloys discovered in topsoil, H_2O and air are ensuring a solemn effect on anthropoid healthiness. Heavy metalloids get via food-pipe and pollute plants, therefore eventually terminate in the anthropological body. As a consequence, the determination of heavy metalloids' quantity in organic samples is very imperative [27-28].

Reciprocations betwixt metallic ores and constituents in topsoil, aqua and atmosphere are influenced by several biochemical aspects. These biochemical aspects regulate the exodus of contaminants. The absorptivity of metallic ores from H_2O on particles of soil is the utmost significant biochemical aspect that controls the kinetic perimeter of metalloids in topsoil [29]. Florae engross substantial metalloids from the loam predominantly via roots. Roots consume biochemical substances from the soil very effectively [30].

These metallic components can deliver lethal impacts to humanoid health, specifically Cadmium Cd^{2+} and Lead (Pb^{2+}), as these are insignificant components. Cd^{2+}, existent predominantly as Cd^{2+} in the earth, is peripatetic and travels from the topsoil into the florae. These floras fluctuate in their absorption of Cd^{2+}. The absorbency/assimilation of Cd^{2+} is quicker than Pb^{2+}. A persistent vulnerability to Cd^{2+} can develop renal dysfunction, ossein malformations, and circulatory complications [29&31]. Pb^{2+} salinities do not dissolve well in H_2O because of the p.H of the earth; for that reason, Pb^{2+} is less peripatetic. Consequently, Pb^{2+} accrues on the superficial layers of the topsoil. Pb^{2+} can develop neural dysfunctions (particularly in kids) and cardiac functions [32].

Even though copper(Cu+) is a fundamental constituent of metalloids, but lethal effects can be transferred to anthropoids and faunas if its proportion surpasses the permissible confines [33]. Ferrous (iron) is a vital component for anthropoids and faunas for the reason that it is a significant factor of hemoglobin/haem. An excessive amount of ferrous in florae, H2O and soil is due to the assimilation of metalloids from the surroundings. Excessive consumption of Fe_2 possibly develop mutilations in the digestive tract, spewing, diarrhea, hepatic mutilations, alimentary and arthralgia, maceration, enervate, parched and hankering, carcinoma, cardiac maladies, rheumatism, osteopetrosis, ketoacidosis and several psychiatrical illnesses, hepatic cirrhosis, exorbitant dermal coloration, feebleness. Moreover, deficiency of ferrous compounds leads digestive complications [34].

Zinc (ZnO/Zn^{+2}) is one more indispensable metallurgic alloy for hominids, faunae and florae. For the metabolization of nearly 300 catalysts of the body, for the structural formation and normal functioning of cell membranes Zn^{+2} is required. This alloy is very indispensable for the progressive growth of collagenous, elastic cartilage and fibrous tissues. It's also vital for dental formation, ossein and dermal features. It performs an imperative part in the absorptivity of Ca^+ into the osseous tissues and delivers impacts on the operations of somatotropins. A discrepancy of Zn^{+2} in the body is infrequent [35].

Manganese-(II)/ MnO are chemical compounds in several biosynthetic catalysts for enzymatic operations in developed organisms; they are crucial in the purification operations concerning superoxide radically polymerizable. As a result, that metallurgy is a necessity as oligo-elements for entire biotic creatures. MnO can produce intoxication in any of the biotic forms if consume in high proportion via inhalational route as well neural mutilations. A few times the neural mutilations get irreversible [36].

For several industrialized and trade units 'set up and to regulate the emanation of metalloids, protocols have been made in numerous states which are highly significant for the routine monitoring and risk assessment and regulation of the environment. Regular survey and monitoring programs of heavy metal contents in foodstuffs have been carried out for decades in most developed countries [37-40]. Precautions are needed to decrease the contagion level at the point of H_2O source, improve operations following harvesting, develop healthier management in newly harvests cultivation marketing coordination to progress dietetic criteria, improve hygiene standards for the metropolis foodstuff bazaars and intensify awareness in customers and policers on the insalubrity of metallurgic alloys of contagion in the food consumption [41].

This investigation emphasis on bio-surveillance pollution of metallurgic ores Pb^{2+}, Fe_2, Cu^+, MnO, and Cd^{2+} in fruit testers such as grape-berries assembled from the chosen non-rural regions of Spani Road and Qambrani Road of Quetta. Further, this study was done to detect the heavy metal content in the fruit of grapes in order to assure a significant improvement in food safety. ml of 1% HNO_3 was added to the sample. The solution was filtered with Whatman no. 42 filter paper and < 0.45 lm Millipore filter paper. It was then transferred quantitatively to a 25 ml volumetric flask by adding distilled water.

2. EXPERIMENTAL

Samplings of newly ripened grapes were conducted from the two primary sites of Quetta city namely Spani Road and Qambrani Road. Sampling was done on the basis of grapes irrigated by dirty water at Spani Road and irrigated by clean water at Qambrani Road.

2.1 Sampling Formulation

The assembled grape samples were meticulously cleaned up with $d.H_20$. These samplings were then slashed into tiny fragments and desiccated in the oven at 340° F for 48 hours. The desiccated samples were then comminuted into a pulverulent structure and stockpiled in a synthetic polyethylene sack prepared for the digestive process. A.A.S (Atomic-Absorption-Spectroscopic) conventional resolutions for $Zn^{2+,}$ Fe+, Cu^{2+}, MnO, Pb^{2+} and Cd^{2+} were procured from Fisher-Scientific-Company, U.S.A. Operational conventional resolutions were organized by means of making dilutions of the mother liquors. Entire liquors were equipped with the dual distillation of deionized H_2O acquired by filtrations of $d.H_2O$ via applying "Milli-Q-purifier system (Millipore, Direct-Q5, France)" instantaneously prior to it's application. Specimens were undergoing mineralization by applying 65%HNO_3, Merck-Suprapure, and Perchloric acid ($HClO_4$). Objects were retained in a hygienic place to circumvent contagion. Samplings' absorptivity and fabrication for analytic solution for A.A.S earlier to quantization of methodical by Atomic Absorption Spectrometry, it's generally indispensable to abolish the carbon-based medium and get the constituent into distinct systematic liquor. For this reason, the fruit sample was first digested with chemicals where the organic matrix of fruit was destroyed and left the element into a clear solution. In this investigation 'Wet Digestion' method was used. 90ml fruit squashes sample was taken in a 250 ml conical flask and 10 ml digestion mixture (HNO_3 and $HClO_4$). Assimilation occurred in 1.5 hrs at 248°F. When cool, the mixture was diluted to a 30 ml volume with 2 N HNO3 dilution mixtures.

One gram of sample was placed in a 250 ml digestion tube and 10 ml of concentrated HNO3 and HClO4 were added [42]. The specimens were made undergo heating for 0.75 hrs at 194°F and subsequently, the thermal reading was augmented up to 150°C afterward that the specimens were made to undergo boiling for a minimum of eight hrs while waiting for obtaining unambiguous liquor. Intensive absorptive fusion was included in the samplings (5ml was inserted thrice) and assimilation arose 'til the dimensions abridged to nearly 1ml. The inner tubular walls were cleaned with a petite $d.H_2O$ and the tube was twirled during the course of incorporation and absorption to retain the tubular walls sanitized and avert the declining of the specimens. Later chilling, 5ml of 1%HNO_3 was included in the samplings. The liquor was strained and purified with Whatman#42 filtering paper and<0.45lm Millipore filter paper. Later on relocation was done quantifiably to a 25ml volumetrical flagon by addition of $d.H_2O$.

2.2 Statistical Evaluation

2.3.1 Computations of orally consumed metallurgies through grape samplings

The orally consumed metallic ores via grape specimens were computed in accordance with Jolly *et al.*, [43]. Daily intake of metals (DIM)=diurnal fruit intake x average fruit metallic proportion (mg/day-1,fresh mass). The prerequisite quantity of fruit in diurnal nutrition needed to be 300g/individual [44].

2.3 Computations of health menace index of metallurgical contagion of grape specimens

By the consumption of metallurgies polluted grapes, the health menaces through specimens was considered by applying a Hazard-Quotient [45]. H.Q is the proportion betwixt disclosure and the reference oral dosage (Rf.D). If the proportion is lesser than 1, there is no apparent menace. An approximation of the prospective menace of metallurgy to hominoid health (H.Q) from the ingestion of grape specimen is evaluated by the subsequent equation:

H.Q=(Div) x (cmetal)/RfD,

However (Div) is the daily intake of fruits (kg.day-1), (cmetal) is the proportion of metallic ores in the fruit, *e.g.*grape (mg.kg-1), Rf.D is the oral reference dosage for the metallic ores (mg.kg-1 of b.w/day-1). Even though the H.Q-based menace evaluation procedure doesn't deliver a quantifiable assessment for the prospective of a disclosed populace undergoing a converse healthiness impact, it certainly offers a sign of health menace point because of disclosure to contaminants [43].

3. RESULTS AND DISCUSSION

Fruits are important sources of nutrients and offer advantages over dietary supplements, because of their low cost and wide availability. Fruits are strongly recommended to include in daily diet for the reduction of various forms of tumors, cancer, cardiac pathogenicity, stroke and many other persistent infections. This analysis is designed to trace the intensities of metalloids in fruits of grapes that are selected from the Spini Road and Qambrani Road, Quetta. The findings demonstrate that the freshly ripened fruits found with the increased quotient of metallurgies yonder the lawfully allowable restrictions. It needs to be observed here that these standards are less austere International-Standards. The iron level permitted for food is 15 mg/kg according to the standards of WHO. WHO has established a provisional tolerable weekly intake for the lead of 0.025 mg/kg of body weight. The permissible limit of Cd is 0.01 mg/g, according to Food Additive Organization/World Health Organisation 1999. Fe and Cd concentration was found higher than other metals. In all the samples of grapes, the concentration of Fe is the highest while Cd is the lowest. Concentration of heavy metals Cd^{2+},Fe^{+},Cu^{2+},Zn^{2+},MnO, Pb^{2+} found in sample of fruits of grapes follows in Table 1-2 and Figure-2&3. In all the samples of fruits, the concentration of Feb was the highest while Cd is the lowest. The resultant consequences specify the direction of the profusion of metallurgies in the assembled fresh specimens of fruits as follows: $Cd^{+}<Fe^{+}<Cu^{+}<Zn^{+}<MnO<Pb^{+}$ Furthermore the S.D standards also specify advanced inconsistency in applications assortments of the metallurgic alloys.

Grapes samplings were evaluated on the proportion of Cd^{+},Fe^{+},Cu^{+},Zn^{+},MnO,Pb^{+} mention to clean filthy H_2O irrigation in the Qta.city.

Table-1. The concentration of heavy metals ug/g in grapes irrigated by dirty water at Spani Road, Quetta.

S.NO.	Name of Site	Fe	Zn	Cu	Mn	Pb	Cd
1	Spani Road	263.3	0.46	6.9	0.2395	0.102	0.06

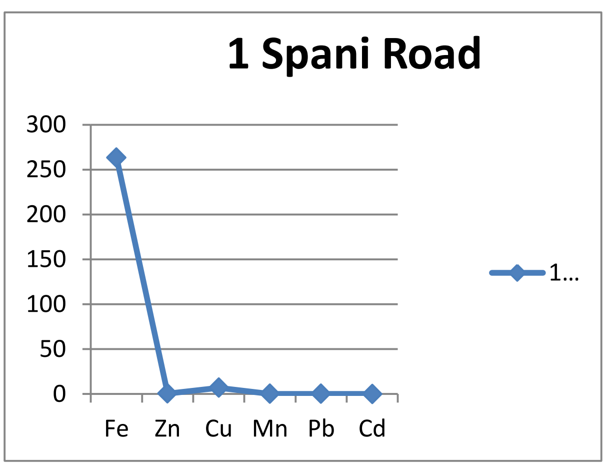

Figure-2. The concentration of heavy metals ug/g in grapes irrigated by dirty water at Spani Road, Quetta.

Estimated day-to-day consumptions of metallic alloys thru humanoids from grapes may be calculated from tables 1-2 and Figure-2&3. Consumption figures statistically were computed by applying the mean figures of metallurgies in grapes samplings and in view of that all individuals (supposing70.kg of b.w) intakes nearly 300g of fruits/day [44]. Several fruits are ingested inconsistently by diverse sections of the populace at altered periods all over the year; and thus, the overhead approximation is well-thought-out accurate for the average deviation consumption of metalloids from fruits. The consumptions of chosen metalloids aren't excessive and within the permissible restrictions suggested by several organizations [46-48]

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Table-2. The concentration of heavy metals ug/g in grapes irrigated by clean water at Qambrani Road, Quetta.

S.NO.	Name of Site	Fe	Zn	Cu	Mn	Pb	Cd
1	Qumbrani Road	165.5	0.41	3.6	0.0156	0.092	nd

The Hazard-Quotient (H.Q) figures for Fe^+, Zn^+ and Cu^+ were computed correspondingly. The sequence of H-Q for the components trailed the declining direction $Cu^+ > Fe^+ > Zn^+$. The H.Q figures for entire alloys were less than 1, which can be considered benign.

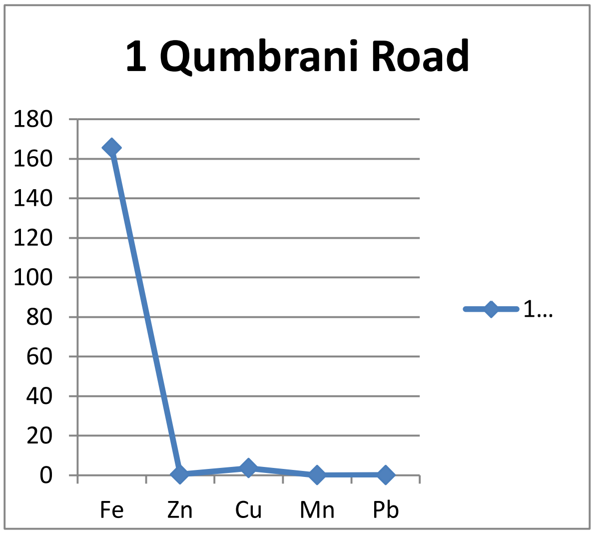

Figure-3. The concentration of heavy metals ug/g in grapes irrigated by clean water at Qambrani Road, Quetta.

The aforementioned analytical study discloses the elevated disclosure of newly ripened fruit merchandise to the metalloids. Marshall *et al.*[49] demonstrated through his investigational findings that the harvests are a lot of times cultivated in filthy and despoiled environs in farming capacities and are focused to more effluence from automobiles and production units in the course of package forming, delivering and advertising in markets. An additional aspect is considered a realistic approach that maximum pastures and woodlands currently are profoundly dampened by applying mechanical thrusts; and even though this has facilitated increments in agronomic production. Economically H_2O performs a dispensable role in evaluating the excellence of aqua (H_2O) being consumed. The trouble ascends when that irrigational water emanates from sewer drainage and commercial sectors' refusal into seas, streams, tributaries, or dirtied underground water. In such a situation when the fruit samplings are assembled from the designated Bangalore's marketplaces, we trust that a group of influences comprising the application of contaminated H_2O, depraved practical and conventional applications management after the harvesting of the fruit merchandises with indifference to the protocols for safe diet, and the corporeal marketplace surroundings in these localities enclosed by a substantial metropolitan effluence testimony may have worsened the effluence intensities of these fruit testers. As discussed earlier by Marshall *et al.*[49] proper protocols and management tools are requisite for the reduction in effluence at aqua resources; advance management and organizational operations after harvesting yields; develop healthier synchronization in fresh harvests conventional marketing scheme to progress protocols for better nutrition.

4. CONCLUSION

For several organic and functional actions, these metallic constituents are indispensable for sustaining health all over life. The problem arises when the irrigation water comes from sewage and industrial fed lakes, rivers, or contaminated groundwater. Some deleterious heavy metals elements are such as Cd^+, Fe^+, Cu^+, Zn^+, MnO, Pb^+ are transmitted into fruits and another farm produces. Some of them are transited into highly toxic compounds. By giving consideration to the prospective harmfulness, determined features and amassed actions besides the intake of vegetables and fruits, it is essential to examine and evaluate the foodstuffs to guarantee that the intensities of these effluents in metalloids come across the approved global requirements. The aforementioned analysis discloses the excessive disclosure of freshly ripened fruit merchandise to the metallurgies and alloys in direction Fe>Cu>Zn>Mn>Pb> Cd, which causes a problem to the urban environment and ultimately to human food chain safety. In the instance of the ripened fruit samplings of grapes assembles from the designated Spani Road and Qambrani Road. Utilization of dirtied and filthy water, depraved managemental operations after the harvesting period of the fruit yields with disdain with the safety protocols for better nutrition may possibly have ascended contagion intensities of these fruit samplings. The statistical record will assist to deliver the eminence of metallurgies in fruits and correspondingly to guarantee safe nutrition and to guard the user of fruits and fruit juices that might affect our health. It is concluded that fruits consumed by urban consumers contain heavy metals more than their permissible levels.

5. RECOMMENDATIONS

- For the protection of humans and animals from the lethal impacts of metallurgies and alloys, "Joint-Expert-Committee-of-Food-Additives-and-Contaminants, FAO/WHO (J.E.C.F.A)" has fixed corresponding standards for lethal effects, designated "Provisional-Maximum-Tolerable-Weekly-Intakes (P.T.W.I)" and "Provisional-Maximum-Tolerable-Daily-Intakes (P.M.T.D.I). P.M.T.D.I is the termination point for pollutants having no accumulative features and correspondingly, P.T.W.I is the termination point for foodstuff toxins such as metallurgies and alloys having accumulative features, applied by WHO.
- Irrigational resources need to be carefully chosen grape sites are suggested for food intake.

- To develop hygienic circumstances and standards for the food merchandisers and marketplaces, and intensify awareness in customers and policers on the threats of metallurgic effluence in the consumption of food products.
- The Govt. of Pakistan should stress the development of education for the eradication of illiteracy and ignorance of the Balochistan backward areas for the purpose of the development of prosperous Pakistan.

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