# Hematological screening of heavy metals among patients of asthma using medicinal herbs in Karachi, Pakistan

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**Abstract**: Prevalence and concentration of some toxic (Pb, Cd, and As) and essential metals (Zn, Cu, Cr, Ni, and Co) were determined in the blood samples collected from asthmatic patients in Karachi, Pakistan using atomic absorption spectrophotometer. All selected patients were habitual of taking crude drugs and home remedy as self medication to treat and prevent asthma. We detected large proportion of the patients with detectable levels of heavy metals. The percentage prevalence of Pb, Cr, Ni, and Co, were found 100% while that of As and Cd were 55% and 60% respectively. Atomic absorption spectroscopic data showed the presence of metals in the analyzed samples in the range of 0.00-37.08 µg/dl for As, 8.1-41.4µg/dl for Pb, 0.0-9.6µg/l for Cd, 2.0-55.6µg/l for Cr, 20.0-85.9µg/l for Ni, and 0.15-3.6 µg/l for Co. The study will be helpful in creating awareness regarding the use of quality herbal medicines in asthma. It is suggested that along with avoidance of other risk factors, reliance on the use of quality traditional medicines free from heavy metal contamination is very essential especially incase of bronco asthmatic conditions.

**Keywords**: As, Cd, hematological heavy metal screening, essential metals, Pb, respiratory diseases, trace elements.

## INTRODUCTION

Heavy metal toxicity is a major environment related health hazard to the populace and a risk factor for both acute and chronic respiratory illnesses, asthma, and allergies. According to WHO some 235 million people are currently suffering from asthmatic affections (WHO, 2011a). Chronic lung diseases are included in the four main non-communicable diseases (NCDs) that kill three in five people worldwide. Of the 57 million global deaths in 2008, 36 million (63%), were due to NCDs, particularly in developing nations (WHO, 2011b).

Respirable particulate matter ($PM_{2.5}$ and $PM_{10}$) in the ambient air, are often enriched in potentially toxic trace elements (Sternbeck *et al.,* 2002; Xia and Gao, 2011). Credible scientific data proved that if the concentrations of heavy metals like Cd, Pb, Ni, As, Mn, Hg, Co, and Cr present in ambient particulate matter (PM) exceed critical thresholds, they may pose potential toxic effects on ecosystem and have a strong impact on human health (WHO, 2007; Janko *et al.,* 2000). The amount of metal ingested are transmitted to several organs through circulatory system, and is particularly significant to the overall heavy metal load in the human body especially in lungs (Descotes, 1992).

Toxic manifestations of metals range from minor upper respiratory irritation, flue, cough, rhinitis, nasal allergy, to asthmatic attacks, chronic bronchitis, congestive chest diseases, high incidence of chronic obstructive pulmonary diseases (COPD), and even lung cancer (Verougstraete *et al.,* 2004; Fengyuan *et al.,* 2008; Kampa and Castanas, 2008). Even very low concentrations of heavy metals are sufficient to adversely affect the respiratory tract (Gillialand *et al.*, 1999; Carter*et al.,* 1998; and Nemery, 1990).

At cellular level metals produce toxic effects on respiratory organs by direct affecting the alveolar macrophage, epithelial, and endothelial cells that are known to play significant role in the immune system of air ways (Carter*et al.,* 1998). The harmonious interplay and targeted regulation of the immune system are either stimulated or inhibited by heavy metals leading to chain reactions and pathological responses (Descotes, 1992). For example Cd inhibit the synthesis and expression of inflammatory cytokines like IL-1β, IL-4, IL-6, TNF-α, IFN-γ, and ICAM-1leading to the prevalence asthma. On the other hand tissue damage caused by toxic metals results in decreased pulmonary growth and lung function (Marth *et al.,* 2001). Abnormal distributions of these trace minerals (Cu, Zn, Se) may aggravate oxidative damage and inflammation, increased CD4/CD8 lymphocyte ratios and disturbed and decreased lung function in asthma (Guo *et al.,* 2011).

Prevalence of asthma is very common in Karachi city. Regarding their treatment medicinal plants and herbal formulations are usually the choice as a first line of therapy for the prevention and treatment along with conventional drugs. Heavy metals contaminated crude medicinal plants and herbal formulations may act as potential gradual source of heavy metal exposure to such patients (Hina *et al.,* 2011 and 2014). Such contaminated herbal drugs may increase the concentration of metals in blood of asthmatic patients and may aggravate the condition upon long-term therapeutic duration. The

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objective of the study was to evaluate the prevalence of toxic and essential metals in the blood samples collected from asthmatic patients frequently using sub standard herbal medicines.

## MATERIAL AND METHOD

### *Sample collection*

Blood samples of twenty randomly selected adult human subjects from both genders were collected from different zones of Karachi Pakistan, in order to screen out the hematological heavy metal profile of patients suffering from asthma. All selected patients utilize medicinal plants and home remedy (mostly *P. nigrum, P. longum, Z. officinalis, M. nigra, V.odorata, C. cyminum, C. sativum, T. ammi,* and *O. bracteatum etc*) as self-medication. 5 ml of blood was collected from each patient using sterilized disposable syringes (5-cc) and refrigerated immediately after addition of EDTA in each sample to avoid blood clotting and any kind of degradation. Table- 01 contained the information about patient I.D, gender, and smoking habit of all twenty selected patients.

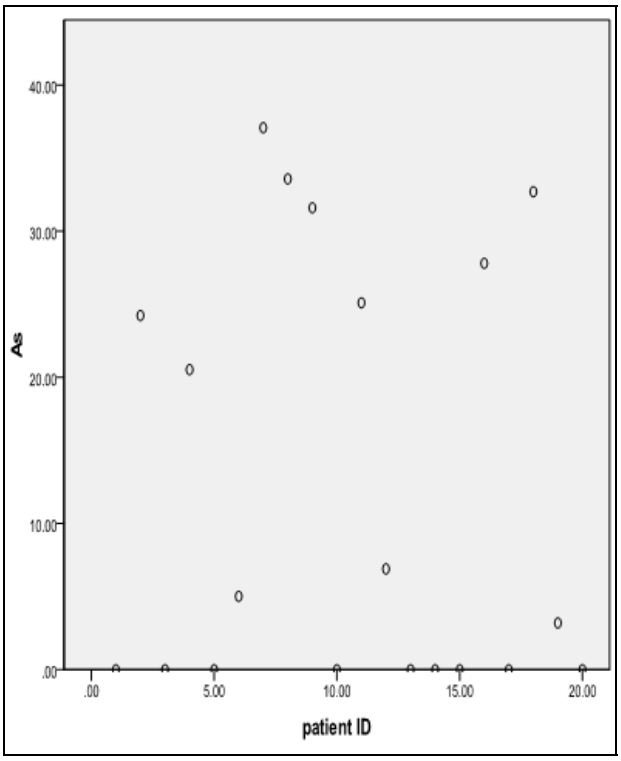


**Fig. 1**: Concentration of AS (µg/dl) among patients of asthma using herbal medicines.

### *Sample preparation*

To carry out wet digestion of blood samples procedure of Narjis *et al.,* 2000 was followed with some modifications. Combination of Nitric acid ($HNO_3$) and Hydrochloric acid (HCL) were prepared in the ration of 20:1.1ml blood was mixed with 3 ml of this acid mixture and diluted to 10 ml by adding deionized double distilled water. The solutions were subjected to shaking on a vibrator for 10 minutes. After being shaken, each sample was heated on a water bath at 60º C. 2 ml Hydrogen Peroxide ($H_2O_2$) was added slowly as oxidizing agent to fasten the process of digestion. Upon complete digestion, solutions were filtered out using Whatman filter paper No.42. Appropriate dilutions were made accordingly for the analysis of metals using deionized double distilled water.

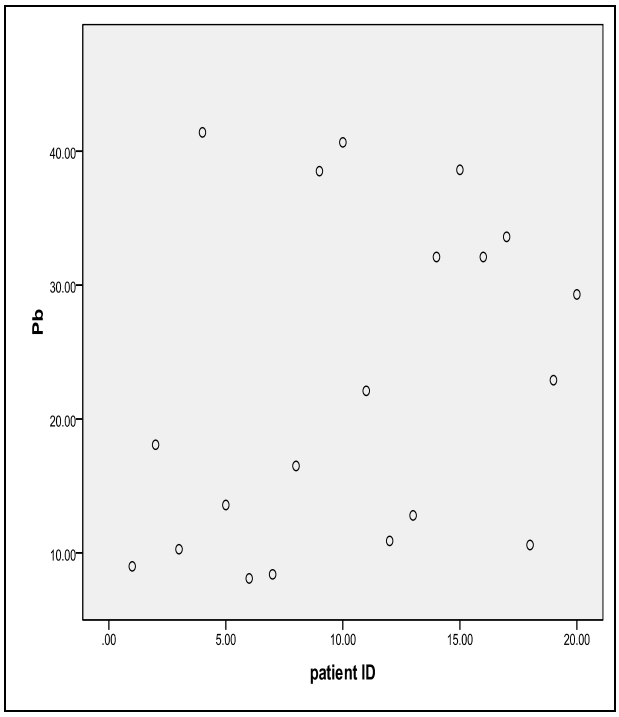


**Fig. 2**: Concentration of Pb (µg/dl) among patients of Asthma using Herbal Medicines

### *Analytical technique*

*Instrumentation*

Atomic absorption spectroscopy of all blood samples were performed using AAnalyte 700 Perkin Elmer atomic absorption spectrophotometer. All instrumental parameters were set according to the standard working conditions summarized in table 02.

### *Preparation of standard solution of metals*

Calibration curves for As, Pb, Cd, Cr, Ni, and Co were obtained by diluting certified standard stock solution (1000ppm ±5) of these metals in at least three different concentration range. Fresh standard solutions were prepared at the working day to avoid any kind of degradation. Analytical grade volumetric flasks (Pyrex) were used during analysis. All glass wears, apparatus, and disposable tips of micropipettes were washed with double distilled water and rinsed with the solution to be dispensed prior to use.

## STATISTICAL ANALYSIS

The concentration of As, Pb, Cd, Cr, Ni, and Co in blood samples of all twenty patients were subjected to statistical

evaluation using the software "Statistical package for Social Sciences (SPSS) V 17". Statistical treatment includes the calculations of descriptive statistics (overall mean, range statistics, minimum and maximum values) of metals found in the blood samples collected from all twenty patients (table 3).

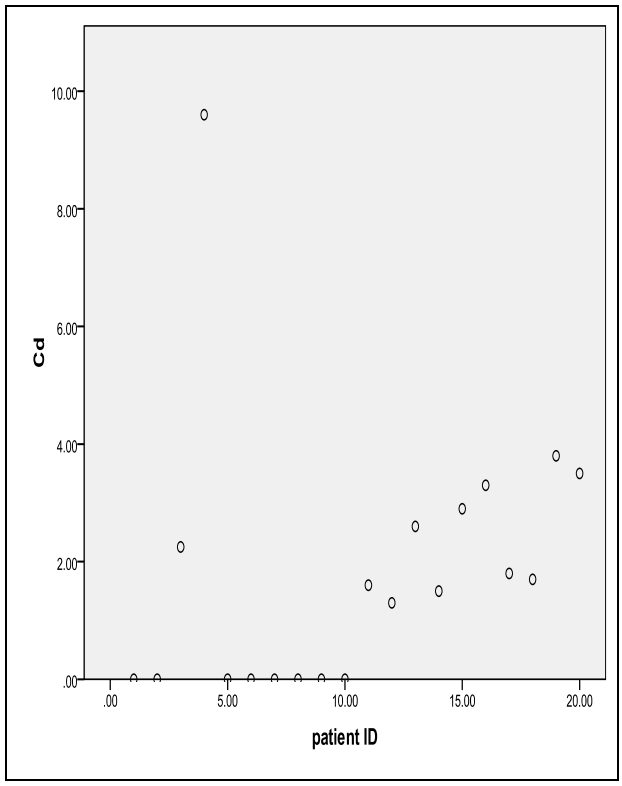


**Fig. 3**: Concentration of Cd (µg/l) among patients of asthma using herbal medicines.

## RESULTS

Concentrations of Pb (µg/dl), Cd (µg/l), As (µg/dl), Cr (µg/l), Co (µg/l), and Ni (µg/l) were calculated using the atomic absorption spectroscopic data that was obtained from the analysis of blood samples of the twenty patients of asthma and results are mentioned in figs. 01-06. All parameters regarding Descriptive statistics (minimum and maximum concentrations, range, mean and standard error) were also calculated using "Statistical Package for Social Sciences (SPSS V17)" and given in table 03. Complete heavy metal profiles of all twenty patients (01-20) are represented in fig. 7.

Table 4 showed an account of percentage prevalence of metals among the patients of asthma, different laboratory reference ranges, and the percentages of patients crossing those allowable limits.

## DISCUSSION

Asthma is one of the major respiratory tract diseases that have been found common among the local population of Karachi Pakistan. Use of medicinal plants in crude form and herbal formulations are generally the first line of therapy for the treatment and prophylaxis by the patients. People often use to buy herbal drugs themselves from shops locally known as "pansaar stores" in such conditions, especially in the pre and post winter seasons when prevalence of asthma and allergies are common. Due to environmental pollution and lack of pharmacovigilance these herbs are contaminated with environmental pollutants especially heavy metals.

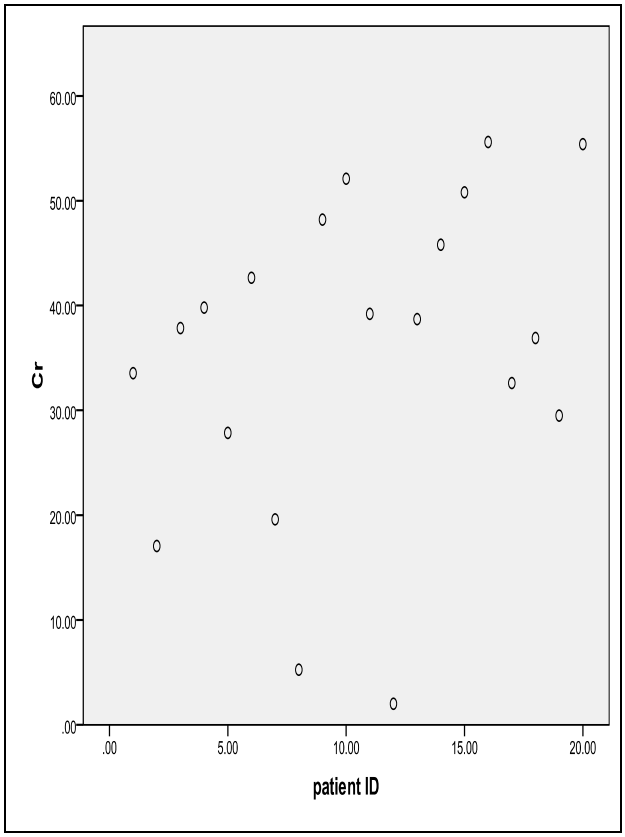


**Fig. 4**: Concentration of Cr (µg/l) among patients of asthma using herbal medicines.

As shown in the results of this study, we detected large proportion of the patients of asthma with detectable levels of heavy metals taking TM in such conditions (figs 01-07 and table 03). The percentage prevalence of Pb, Cr, Ni, Co, were found 100% while that of As and Cd were 55% and 60% respectively. Various reference laboratory ranges have been described to limit the concentration of heavy metals in the blood of human being that were used to compare the detected level of heavy metals in all analyzed blood samples. Concentrations of all heavy metals in most of the tested samples were found towards higher side crossing the one or more laboratory reference ranges (table 4). These findings suggest that the role of heavy metals in prognosis of asthma can never be ignored.

Detection of such higher levels of heavy metals in the blood samples of local population of Karachi city can be

associated with the higher level of environmental pollution in this cosmopolitan city. Increasing level of environmental pollution in Karachi is resulting in gradual release of heavy metals in the atmosphere, hence badly affects both humanbeing (especially respiratory system) as well as other component of ecosystem (fauna and flora) of the respective zone. Rehman and Ali in 1993 also reported increasing level of respiratory tract diseases in areas of highly pollution containing heavy metals as major pollutant in Karachi city (Rehman and Ali, 1993). This environmental pollution also act as major contributing factor responsible for heavy metal contamination of the medicinal plants and related products, that has been turned out as a serious health issue now a days. Metal contaminated traditional medicines may act as potential source of metal accumulation in the body. Moreover increasing amounts of heavy metals particularly Pb, Cd, As, Ni and Cr may lead to severe metabolic changes in the long run habitual use of herbal medicines. Therefore only quality herbal concoctions free from heavy metal contamination should be administered. Otherwise these drugs may also act as potential source of metal contamination especially when prescribed for prolonged therapeutic durations.

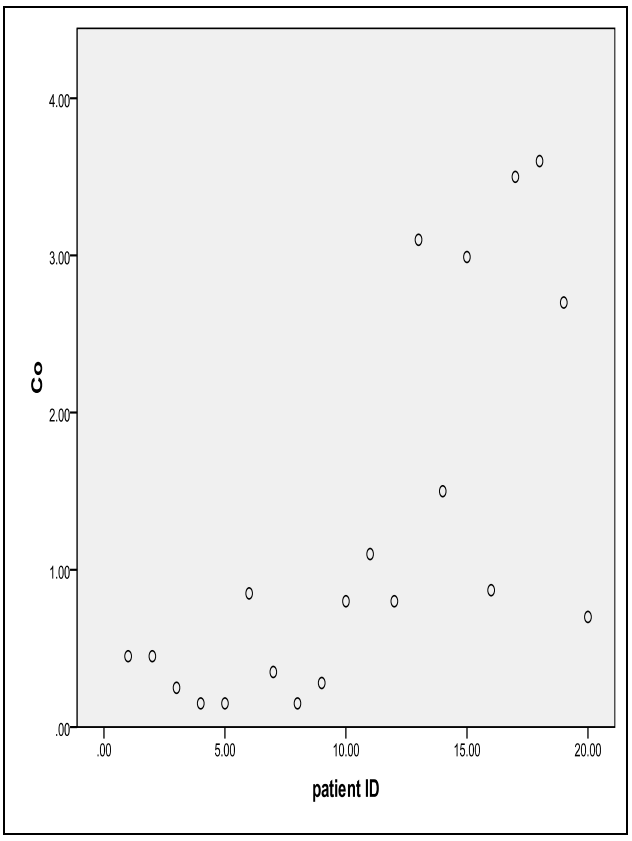


**Fig. 5**: Concentration of Co (µg/l) among patients of asthma using herbal medicines.

In the light of above facts, it is recommended that along with avoidance of other risk factors, reliance on the use of traditional medicines with care especially in case of bronco asthmatic patients should be made prerequisite for the sake of general public health.

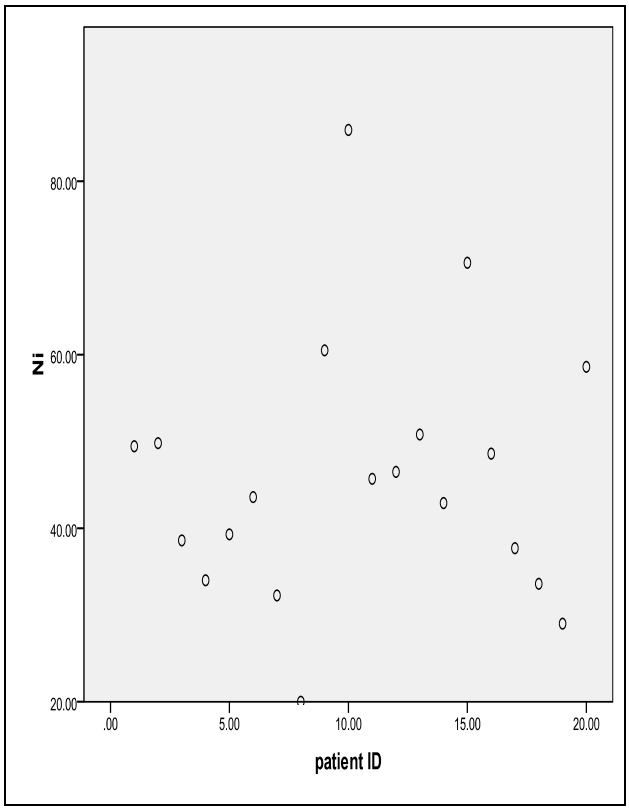


**Fig. 6**: Concentration of Ni (µg/l) among patients of Asthma using Herbal Medicines

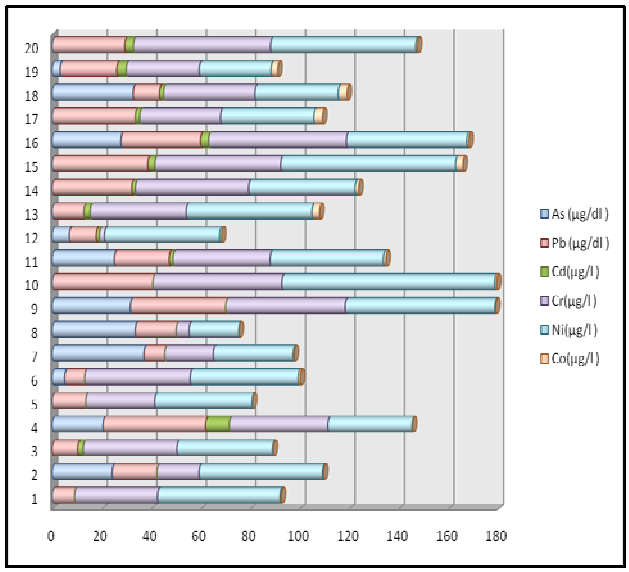


**Fig. 7**: Hematological heavy metals profile of patients of asthma (01-20) taking herbal medicines.

## CONCLUSION

Hematological screening of heavy metal contents among users of traditional medicines in asthmatic patients ex-

**Table 1**: Information about selected patients of respiratory diseases using herbal medicines

| Patient' I.D | Gender | Smoking Habit | Type of Respiratory Disease | Use of Herbal Medicines | Patient' I.D | Gender | Smoking Habit | Type of Respiratory Disease | Use of Herbal Medicines |
|---|---|---|---|---|---|---|---|---|---|
| 01 | Female | Non smoker | Asthma | +ve | 11 | Male | Smoker | Asthma | +ve |
| 02 | Female | Non smoker | Asthma | +ve | 12 | Male | Smoker | Asthma | +ve |
| 03 | Female | Non smoker | Asthma | +ve | 13 | Male | Non smoker | Asthma | +ve |
| 04 | Male | Non smoker | Asthma | +ve | 14 | Male | Non smoker | Asthma | +ve |
| 05 | Male | Smoker | Asthma | +ve | 15 | Female | Non smoker | Asthma | +ve |
| 06 | Male | Smoker | Asthma | +ve | 16 | Male | smoker | Asthma | +ve |
| 07 | Female | Non smoker | Asthma | +ve | 17 | Male | Smoker | Asthma | +ve |
| 08 | Female | Non smoker | Asthma | +ve | 18 | Male | Smoker | Asthma | +ve |
| 09 | Male | Smoker | Asthma | +ve | 19 | Male | Smoker | Asthma | +ve |
| 10 | Male | Smoker | Asthma | +ve | 20 | Male | Smoker | Asthma | +ve |

**Table 2**: Standard operating parameters of AAnalyte 700 Perkin Elmer Spectrophotometer for hematological heavy metal analysis

| S. No. | Heavy Metals | Application on AAS | Weave Length (nm) | Slit Width (nm) | Type of Lamps |
|---|---|---|---|---|---|
| 01 | As | *MHS-AAS | 193 | 0.7 | EDL |
| 02 | Pb | **GF-AAS | 283.3 | 0.7 | EDL |
| 03 | Cd | GF-AAS | 228.8 | 0.7 | EDL |
| 04 | Cr | GF-AAS | 357.9 | 0.7 | EDL |
| 05 | Ni | GF-AAS | 232 | 0.2 | EDL |
| 06 | Co | GF-AAS | 240.7 | 0.2 | EDL |

*Mercury Hydride system **Graphite Furnace

**Table 3**: Descriptive statistics of heavy metals among patients of asthma using herbal medicines

| Heavy Metals | N | Range | Minimum | Maximum | Mean | |
|---|---|---|---|---|---|---|
| | Statistic | Statistic | Statistic | Statistic | Statistic | Std. Error |
| As (µg/dl) | 20 | 37.08 | .00 | 37.08 | 12.3870 | 3.24285 |
| Pb (µg/dl) | 20 | 33.30 | 8.10 | 41.40 | 22.4745 | 2.71401 |
| Cd (µg/l) | 20 | 9.60 | .00 | 9.60 | 1.7925 | .50872 |
| Cr (µg/l) | 20 | 53.60 | 2.00 | 55.60 | 35.5200 | 3.40871 |
| Ni (µg/l) | 20 | 65.90 | 20.00 | 85.90 | 45.8695 | 3.34747 |
| Co (µg/l) | 20 | 3.45 | .15 | 3.60 | 1.2370 | .27092 |

**Table 4**: Percentage of heavy metals among patients of asthma compared with different laboratory reference ranges

| S. No. | Heavy Metals | Percentage of pre-valence of heavy metals | Laboratory reference range | Percentage of patients of RTIs crossing the allowable limits |
|---|---|---|---|---|
| 01 | As | 55 % | 2-23 µg/dl (Iverson *et al.*, 2007) | 35-55 % |
| 02 | Pb | 100% | 20 µg/dl (Iverson *et al*., 2007-Kenneth D Mclatchey, 2002) | 50 % |
| 03 | Cd | 60 % | 0.3-1.2µg/l (Iverson *et al.*, 2007)<br>0.5-1µg/l Kenneth Mclatchey, 2002) | 60 %<br>60 % |
| 04 | Co | 100% | 0.05-2.7µg/l (Cataloni and Leon, 2011) | 100-20% |
| 05 | Ni | 100% | 3-7µg/l (Kenneth D Mclatchey, 2002) | 100% |
| 06 | Cr | 100% | 0.7-28 µg/l (Iverson *et al.*, 2007) | 100-75 % |

hibited the significant presence of toxic (As, Pb, Cd) and essential heavy metals (Cr, Ni, and Co) in almost all tested samples causing alarming situation. It suggests that might be the role of these heavy metals in asthma. Furthermore the drugs of natural origin that is usually taken as self medications by a large portion of local population must be pure and free from all contaminations especially heavy metals for the sake of public health. Standardization protocol of herbal raw material according to WHO guidelines in this regard is also very important to be adopted.

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