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# ARSENIC AND CADMIUM RISK ASSESSMENT IN A DOMESTIC WASTEWATER IRRIGATED AREA USING SAMPLES OF WATER, SOIL AND FORAGES AS INDICATORS

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## ABSTRACT

The recent research was performed to investigate the toxicity of As and Cd in suburban area of Sargodha, Punjab, Pakistan. Water, soil and forage samples were collected for this purpose in summer and winter season and analysis was done via wet digestion to determine the concentration of selected heavy metals. The mean concentration of As and Cd in water was found above the permissible maximum limit. Mean concentration of Cd in soil and forages was lower than the allowed limit whereas As which was higher than the PML in forages. So, continuous use of domestic wastewater for irrigation purpose should be avoided in order to secure the area from any hazard in near future.

**Keywords:** Toxicity, cadmium, arsenic, wastewater, irrigation.

## INTRODUCTION

Pakistan covers 34.4 square kilometer area as agricultural land. An arable part of land is referred as agricultural land which is utilized for the establishment of pastures and crops production (Nath et al., 2009; Nagajyothi et al., 2010). A substance either natural or synthetic present in water, soil, air that damages the human beings and leads to the impairment of environment is known as pollutant (EPA 2008). Varying degrees of pollution sensitivity are represented by biotic communities, animals, plants, microbes and bacteria. Owing to this fact, these could be utilized as indicators to approach and evaluate contamination of environment (Ali et al., 2011). The most applicative means for environmental administrators, water quality managers and decision makers are pollution indices which have merge the impacts of every single parameter (Rajkumar et al., 2015).

Fodder occupies a key position in livestock feed because it is a good provider of minerals for the ruminants (Ahmad et al., 2008). Sheep, goats, cows and buffaloes are simply assorted as ruminants since they assimilate the plant-based diet by gradually dissolving it in rumen which is the initial stomach of animals (Zukowska and Biziuk, 2008). Despite the enough supply of forages to animals, growth and reproductive difficulties are induced in them owing to minerals insufficiency in soil and forages (Tiffany et al., 2000). It has been traced that the existence of heavy metals in forages leads to imbalance in nutrient attainability, various diseases and physiological problems (Khan et al., 2007). Moreover, only anthropogenic sources are not responsible for the presence of heavy metals in water and soil, these metals are also artificially supplemented into the commercial feeds which are filled with As, Cu and Zn as essential elements to stimulate the optimal growth and to impart anti-microbial characteristics (Sager 2007; Moral et al., 2008). Soil as a pivotal part of ecologic systems and plays a significant role in the existence of human beings contributing to the quality of human life either directly or indirectly (Yu et al., 2012). Domestic wastewaters as a chief part of municipal wastewater emerge from food preparation, kitchens, restroom usage, laundry and baths (Ma et al., 2019). In Pakistan, an area of approximately 32,500 hectare is recently irrigated with waste water (Saleem, 2005). Gastrointestinal cancer is caused by excess amount of soil and plant heavy metals (Zhang and Liu 2002). Cd causes nephrotoxicity particularly to the nearest tubular cells. It is also known to induce bone demineralization either directly via bone damage or indirectly via renal dysfunction (Bernard 2008). In human beings, As leads to conjunctivitis, skin cancer and brain damage while in plants it leads to deactivation of different enzymes, oxidative stress, inhibit root elongation, damaged cell membrane and inhibit root elongation (Finnegan and Chen, 2012). The main objective of study is to evaluate the As and Cd risk owing to the application of domestic wastewater in investigated area.

## MATERIALS AND METHODS

### *Study Area*

The present investigation was conducted at three sites of Sahiwal, District Sargodha, Pakistan. Samples were collected during summer and winter seasons in 2018-2019. One of the selected sites was irrigated with tube well water, 2nd with canal water and 3rd with sewage water. Population of this area is increasing day by day. To meet the demands of the Public, sewage water is used by farmers which improve their pasture quality and enhance crop production. Owing to presence of various toxic metals in sewage water there's an excessive chance of contamination in the environment of the area. This is the main reason that stimulated the selection of that site for current study.

### *Sample Collection*

#### *i. Water Sampling*

Total 24 water samples, 12 from each season (summer and winter) and 4 from each sampling site (tube well site, canal site and sewage site) were gathered. From the reservoirs of water, the samples were taken in plastic bottles which were properly cleaned with double distilled water. A sieve of 75µm was used to filter water in order to remove any impurity such as stone or roots. A final sample of 5 ml was labelled and saved at room temperature for further analysis of water.

#### *ii. Soil Sampling*

Three sites (Tube well water irrigated site, Canal water irrigated site and Sewage water irrigated site) of suburban area of Sargodha were selected for the purpose of sampling. Both in summer and winter season, total 120 soil samples were collected from the rhizosphere of forages. 20 soil samples were collected from each site in each season. Each sample along with 4 replicates was taken from almost 15-30 cm depth with the help of shovel. To remove the moisture content from soil, the samples were air dried and then subjected to oven drying at about 70-75$^0$ C for 3 days. The oven dried soil samples were pulverized and a fine powder was made by the use of a grinder and further hard particles were removed by passing the powder through a sieve of 2 mm and 1g of each soil sample was sealed in paper envelop for analysis.

### *iii. Forage Sampling*

During summer and winter season, forage samples were randomly gathered from three sites of study area. Total 120 forage samples were taken, 60 in each season and 20 from each site (tube well site, canal site and sewage site) along with 4 replicates of each forage plant in polythene bags. All samples were well washed with pure water to remove any impurity attached with forages. After air drying, the forage samples were oven dried for 3 days at nearly 70-75$^o$ C. Crushing of weighed forage samples in a grinder and screening through a mesh of 1mm was done and 1g of each forage sample was stored for the purpose of analysis.

## *Samples Preparation*

### *i. Water Samples Preparation*

5 ml of water sample was put in a flask, digested with 3 ml $H_2SO_4$ and 5ml of $H_2O_2$ and placed in chamber of digestion for approximately 30 minutes. When evaporation of fumes was ceased, removal of water sample from the chamber of digestion was done and heated again by adding 2ml of $H_2O_2$ until the disappearance of solution color. Sample was removed from digestion chamber and after cooling its final volume was made 50ml with double distilled water. After that solution was filtered and preserved in plastic bottles. The same preparation method was repeated for all water samples.

### *ii. Soil Samples Preparation*

After removal of soil samples from incubator, “Wet digestion Method” was used for the digestion of soil (Vukadinovic and Bertic, 1988). 1 g of soil taken in a flask was placed in digestion chamber and digested by using 4 ml of $H_2SO_4$ and 8ml of $H_2O_2$ for almost 30 minutes. The soil sample was removed from digestion chamber when evaporation ceased. 2 ml of $H_2O_2$ was added and heated again in digestion chamber. Same procedure was carried on till the sample became colourless. Whatmann No.42 filter paper was used for the filtration of digested material. Double distilled water was added to make the final volume of sample up to 50 ml and stored in plastic bottles which were tagged. All soil samples were digested by the same method.

### *iii. Forage Samples Preparation*

1 g of forage sample which was taken from forage samples after oven drying was put in a flask, 2 ml of $H_2SO_4$ and 4ml of $H_2O_2$ was used for sample digestion and then placed in chamber of digestion for at least 30 minutes. When evaporation from the flask ceased to seem, sample was removed from the chamber of digestion and again heated in it by adding 2 ml of $H_2O_2$. Until the disappearance of sample colour, the same process was continued. After removal of digested sample from chamber of digestion, double distilled water was added to dilute the sample up to 50ml, passed through Whatmann No. 42 filter paper for filtration and saved in tagged bottles of plastic. Same procedure was utilized for the digestion of all oven dried forage samples.

## *Analysis of Samples*

To evaluate the concentrations of cadmium (Cd) in samples, analysis was done using AASP (Atomic Absorption Spectrophotometer). For assessment of arsenic (As), A Perkin - Elmer Model 460 atomic absorption spectrophotometer equipped with an MHS-10 Mercury/Hydride System was used. A Perkin-Elmer arsenic electrode less discharge lamp was used as a light source. Measurements were made with deuterium background correction. The filtrate (10 ml) was taken in 50 ml volumetric flask and 5 ml conc. HCl and 1 ml mixed reagent [5% KI (w/v) + 5% ascorbic acid (w/v)] added to it. The flasks were kept as such for 45min, to ensure complete reaction and volume was made up to 50 ml. The total Arsenic content in solution was determined by using atomic absorption spectrophotometer (AAS), Perkin Elmer Analyst 200 coupled with Flow Injection Analysis System (FIAS 400) where the carrier solution was 10% HCl (v/v) (Dubey et al., 2018).

### *Statistical Analysis*

Through the use of special program for social sciences (SPSS) software version No.20 variance analysis and correlations were determined. Two-way ANOVA was applied on the data of water, soil, and forage to find mean significance. By the application of two-way ANOVA, variance for heavy metals concentration in soil, water and forages was observed. At 0.001, 0.01 and 0.05 probability level the mean significance difference was tested as given by Steel et al., (2006).

## RESULTS AND DISCUSSION

### *Variance Analysis of Metals in Water*

According to ANOVA results the Season, Site, Season*Site had significant ($P< 0.05$) effect on the concentration of As while Season*Site, showed significant ($P< 0.05$) effect on the concentration of Cd, and non-significant effect on Cd concentration was exhibited by Season in water (Table 1).

**Table 1: Analysis of Variance (Mean squares) for As and Cd in water samples.**

| Sources | As | Cd |
|---|---|---|
| Season | 14.645*** | .244$^{ns}$ |
| Site | .362*** | .037** |
| Season*Site | .328*** | .059* |

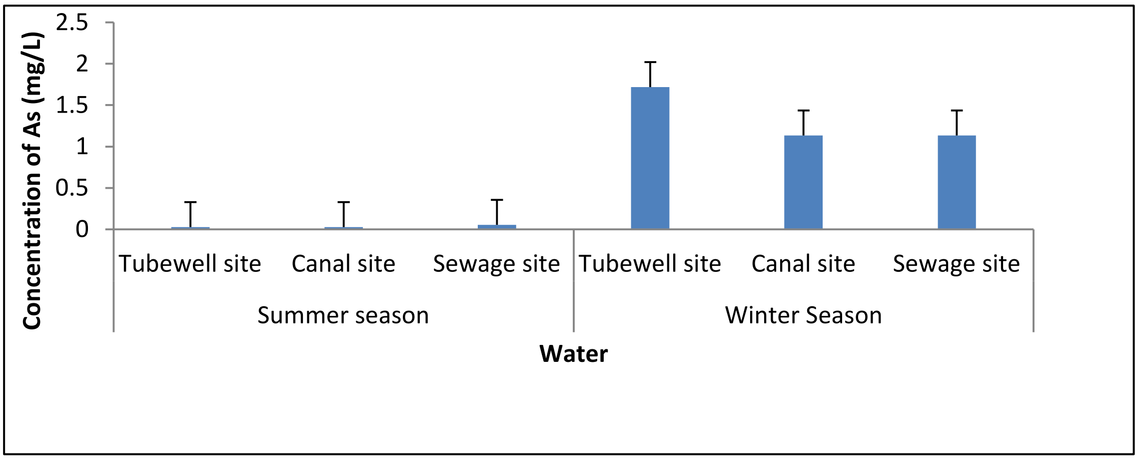


**Figure 1: As Arsenic Fluctuations in Water samples of summer and winter season.**

### *Mean Concentration of Metals in Water*

The range of mean values of As in water was 0.0517 to 0.0247 mg/kg in summer and during winter season variation exhibited by As values was 1.9361 to 1.1336 mg/kg. Sewage water of both seasons showed highest concentration of As. While tube well water of summer season and canal water of winter showed least As values (Fig. 1).

In summer season results maximum mean concentration of Cd was found in sewage water i.e. 0.1645 mg/kg whereas minimum Cd concentration was observed in tube well water i.e.0.0240 mg/L. During winter season higher Cd content i.e. 0.4206 mg/L was exhibited by tube well water while lower concentration was found in canal water i.e.0.1624 mg/L.

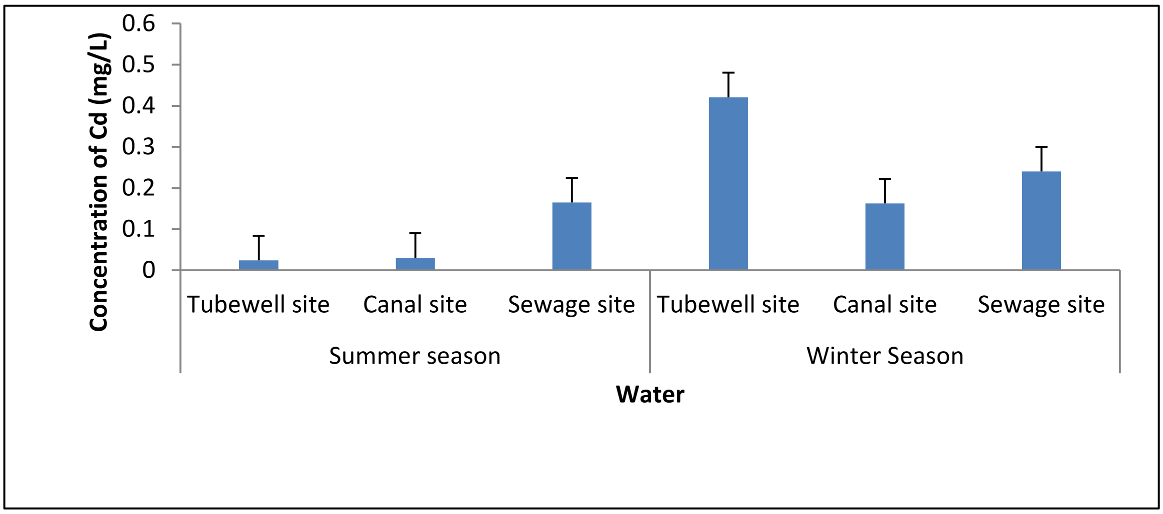


**Figure 2: Cd Fluctuations in Water samples of summer and winter season.**

Cd mean concentration was highest in winter as compared to summer season concentration (Fig.1).

**Table 2: Mean Concentrations of metals in water of sampling sites.**

| Season | Sites | As | Cd |
|---|---|---|---|
| Summer | Tube well Site | 0.0247 | 0.0240 |
| | Canal Site | 0.02608 | 0.02992 |
| | Sewage Site | 0.0517 | 0.1645 |
| Winter | Tube well Site | 1.71962 | 0.4206 |
| | Canal Site | 1.1336 | 0.1624 |
| | Sewage Site | 1.9361 | 0.24025 |

### *Variance Analysis of Metals in Soil*

The ANOVA results exhibited the significant (P< 0.05) effect of Season, Site, Soil, Season*Site, Season*Soil. While non-significant effect was shown by Site*Soil and Season*Site*Soil on the concentration of As in soil (Table 3). According to ANOVA results only Season showed significant (P< 0.05) effect on the concentration of Cd in soil whereas non-significant effect was exhibited by Site, Soil, Season*Site, Season*Soil, Site*Soil and Season*Site*Soil (Table 3).

**Table 3: Analysis of Variance (Mean Squares) for As and Cd in Soil samples.**

| Sources | As | Cd |
|---|---|---|
| Season | 141.138*** | .067* |
| Site | 7.527*** | $.007^{ns}$ |
| Soil | .459*** | $.029^{ns}$ |
| Season*Site | 7.464*** | $.012^{ns}$ |
| Season*soil | .404*** | $.012^{ns}$ |
| Site*Soil | $.001^{ns}$ | $.008^{ns}$ |
| Season*Site*Soil | $.001^{ns}$ | $.022^{ns}$ |

### *Mean Concentrations of Metals in Soil*

During summer, soil taken along the *S. vulgare* plant of canal water irrigated site showed higher mean value of As i.e. (0.0914 mg/kg) and the lowest mean value (0.0499 mg/kg) was observed in *Z. mays* mays of tube well site. During winter maximum (3.4819 mg/kg) mean concentration of As was found in forage plants treated with sewage water and minimum mean concentration of As was observed in tube well water irrigated forage i.e. A. *A. sativa* having 1.0560 mg/kg value (Fig. 3).

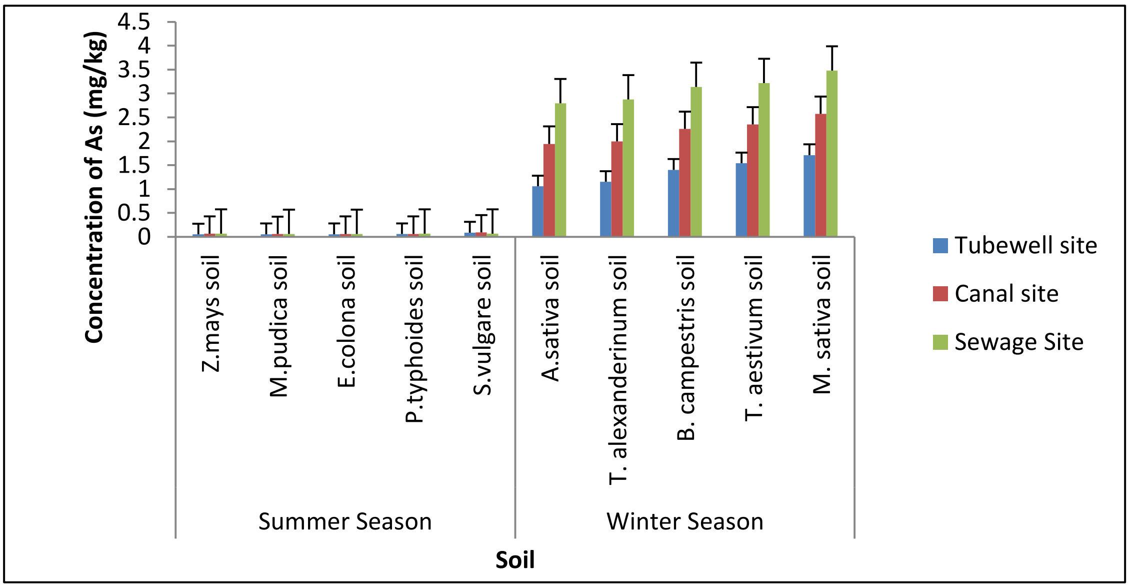


**Figure 3: As Fluctuations in Soil samples of summer and winter season.**

Results indicated that in summer, soil found around the *S. vulgare* which was irrigated with tube well water showed highest Cd concentration. Whereas soil found along the *M. pudica* plant exhibited the lowest concentration of Cd. Mean value of Cd ranged from 0.2641 to 0.0647 mg/kg during the summer season. Whereas in winter season higher Cd contamination was observed in soil taken along with *M. sativa* and less concentration of Cd was shown by soil collected along the forage (*A. sativa*) which was treated with tube well water. Cd mean concentration differed from 0.3243 to 0.0807 mg/kg during winter. Winter season showed more Cd contamination than summer (Fig. 3).

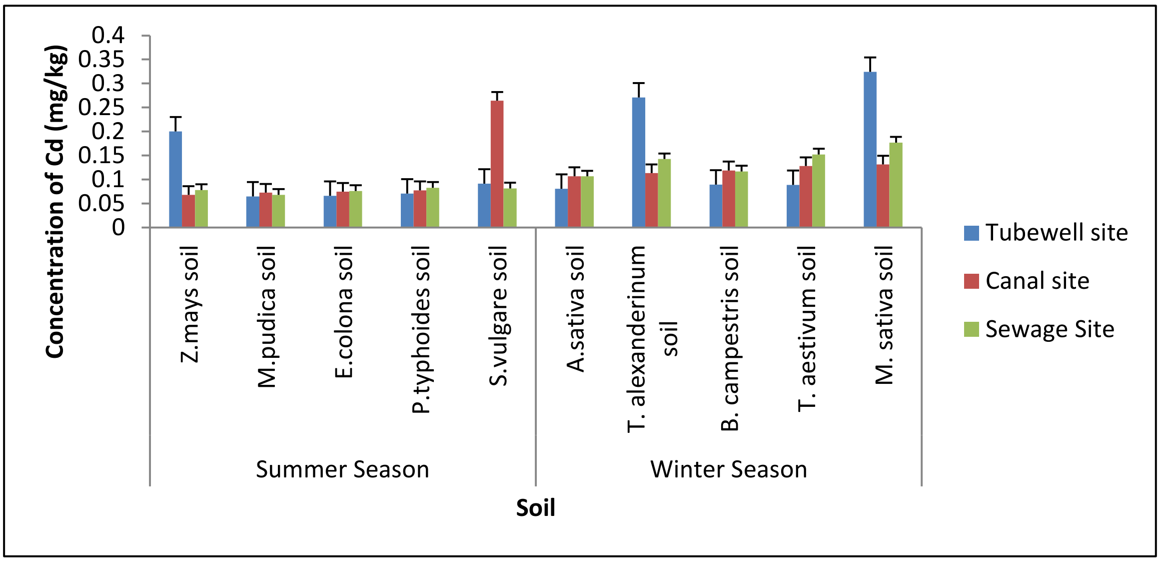


**Figure 4: Cd Fluctuations in Soil samples of summer and winter season.**

**Table 4: Maximum permissible limit of metals in soil.**

| Metal | Permissible Limit | Source |
|---|---|---|
| **As** | 20 mg/kg | O'Neill (1995) |
| **Cd** | 3-6 mg/kg | EU, (2006) and WHO/FAO, (2007) |

## *Variance Analysis of Metals in Forage*

The ANOVA results showed significant ($P<0.05$) effect of Season, Site, Forages, Season*Site, Season*Forages, Site*Forages and Season*Site*Forages on the concentration of As in forages (Table 5). It was showed by the ANOVA results that Season and Site had significant ($P< 0.05$) effect on the Cd concentration in forages while non-significant effect of Forages, Season*Site, Season*Forages, Site*Forages and Season*Site*Forages was exhibited on the concentration of Cd in forages (Table 5).

**Table 5: Analysis of Variance (Mean squares) for As and Cd in forage samples.**

| Sources | As | Cd |
|---|---|---|
| Season | 109.511*** | 0.083*** |
| Site | 12.448*** | .080*** |
| Forages | 0.536*** | $.016^{ns}$ |
| Season*Site | 12.004*** | $.033^{ns}$ |
| Season*Forages | 0.514*** | $.005^{ns}$ |
| Site*Forages | 0.044*** | $.018^{ns}$ |
| Season*Site*Forages | 0.063*** | $.005^{ns}$ |

## *Mean Concentrations of Metals in Forage*

The mean concentration of As in forages of summer season ranged from 0.13078 to 0.02782 mg/kg. Among forage species of summer season , higher (0.13078 mg/kg) concentration of As was observed in *Z. mays* irrigated with sewage water and lower (0.02782 mg/kg) As contamination was shown by *Z. mays* treated with tube well water. As values of winter season were ranged from 3.49598 to 0.74640 mg/kg. Maximum contamination level of As was found in sewage water irrigated forage i.e. *M. sativa* (3.49598 mg/kg). While minimum value i.e. 0.74640 mg/kg was observed in *A. sativa* of tube well water site (Fig. 5). During summer season, the mean value of Cd showed variation from 0.1869 to 0.03485 mg/kg. Highest mean concentration was 0.1869 mg/kg in E. colona irrigated with sewage water and *Z. mays* treated with tube well water showed least mean value of Cd i.e. 0.03485 mg/kg. During winter season, the contamination level of forages differed from (0.3249 to 0.0535 mg/kg). In *B. campestris* maximum (0.3249 mg/kg) Cd value was found, recorded at sewage water irrigated site. While *T. alexanderinum* exhibited minimum (0.0535 mg/kg) concentration of Cd which was recorded at tube well water treated site (Fig. 5).

**Table 6: Maximum permissible limit of metals in forage.**

| Metal | Permissible Limit | Source |
|---|---|---|
| **As** | 3.0mg/kg | O'Neill (1995) |
| **Cd** | 0.5 mg/kg | CERSPC(2009) |

## *Correlation*

Significant and positive correlation was found in soil and forage of both seasons for As (Table 7). For Cd, non-significant and positive correlation was found when Soil-Forage of summer seasons was correlated. While soil-forage of winter season showed negative and non-significant correlation (Table 7).

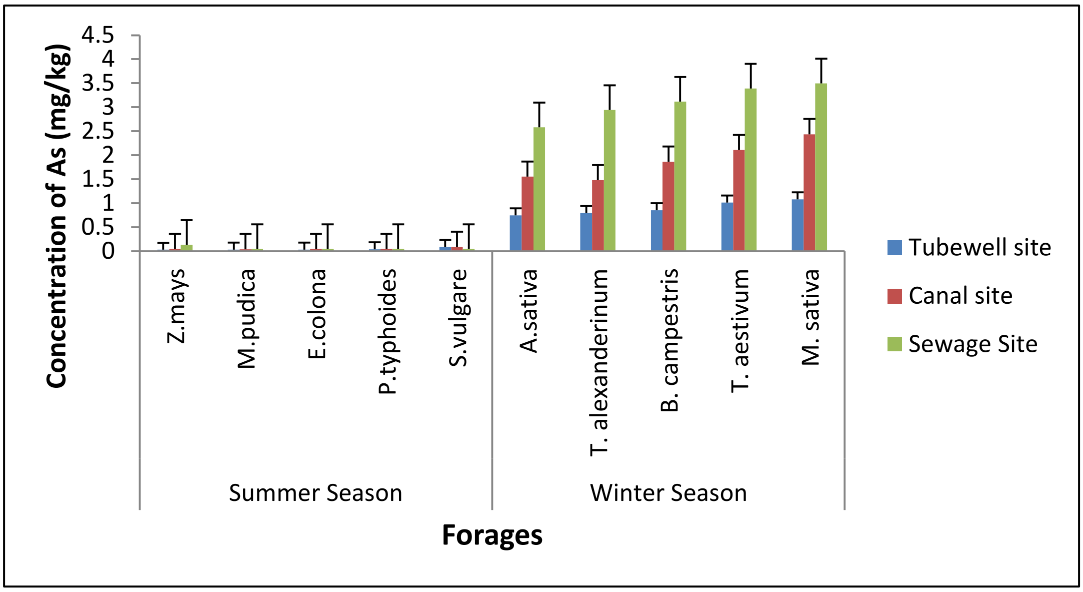


**Figure 5: As Fluctuations in forage samples of summer and winter season.**

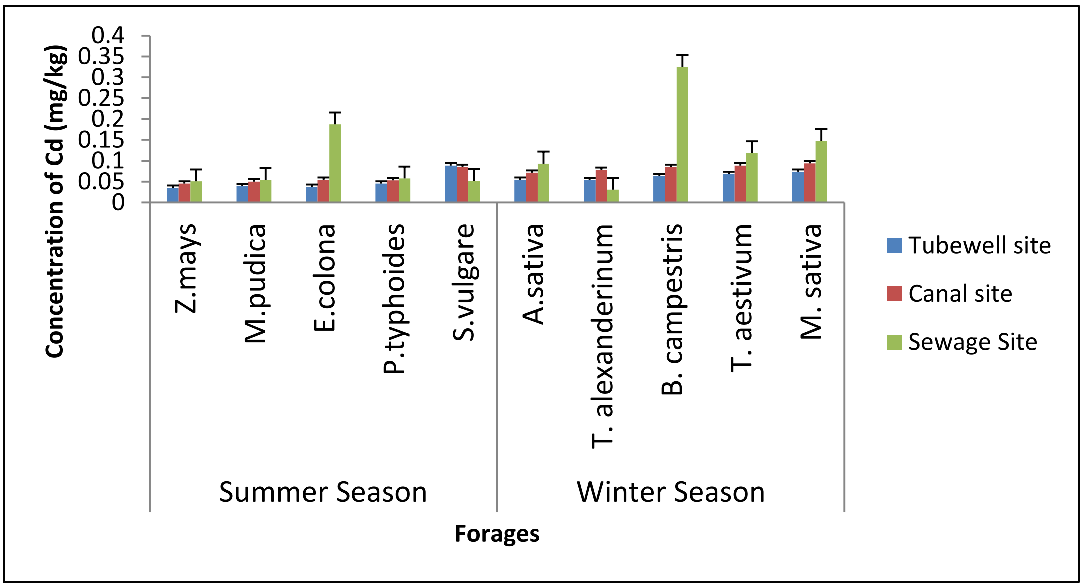


**Figure 6: Cd Fluctuations in Forage samples of summer and winter season**

**Table 7: Correlation coefficient between concentrations of As and Cd in Soil-Forage.**

| Sampling Season | Correlation coefficient | As | Cd |
|---|---|---|---|
| Summer | Soil-Forage | 0.070 | 0.602* |
| Winter | Soil-Forage | -0.086 | 0.989** |

## DISCUSSION

For enhanced growth of crops in the study area, fertilizers containing arsenic were used, which ultimately lead to the contamination of that region. During agriculture practices the use of agrochemicals might contribute to some concentration of As in water (Kumar et al., 2007). Soil irrigated with sewage water showed high level of As but this value was found less than the PML i.e. 20mg/kg established by O'Neill (1995). Ahmad et al., (2014) observed higher As value in soil as compared to that found in recent study. The availability of heavy metals was more in soil treated under contaminated water as compared to uncontaminated water treated soil (Mahmood and Malik 2014). As contamination might be attributed to the use of phosphatic fertilizers (Hartley et al., 2013). Soils contaminated with As have hazardous effects on forages and livestock feeding on these forages. During present study, the arsenic concentration was higher in forages than the allowed limit of As i.e. 3.0 mg/kg as reported by O'Neill (1995). Higher concentration of As might be due to the sewage water irrigation. The mean concentration of As found in forage samples by Ahmad et al., (2014) was 3.25mg/kg which is lower than observed in the current study. According to previous studies, the low amount of As has beneficial action. Cardiac diseases, kidney problems, osteoporosis and central nervous system disorders are caused by excessive levels of As (Duruibe et al., 2007). Natural geological source or particular food additives give rise to As in food material (EFSA 2005). The correlation value of As between soil-forage was found higher in current study than reported by Ahmad et al., (2014) and Pérez et al., (2014). Weak connection between soil- plant shows non-significant and positive correlation (Ekmekyapar et al., 2012). Strong connection between soil and plant shows non-significant and negative correlation. Positive correlation means alteration in one variable is closely correlated with alteration in second variable. The Cd value in water found in present study was higher than the recommended limit of water 0.005mg/L established by USEPA (2000) whereas the current value was lower as compared to that reported by Iqbal et al., (2016). In study area, the use of sewage water with numerous hazardous substances might be the cause of high level of Cd in water. Because of easy transfer in food chain, Cd is labelled as a serious environmental contaminant. Man and animals show unwanted physiological and biochemical changes as a result of exposure to Cd (Ogundiran et al., 2012). Human sources such as combustion of fossil fuel and agrochemicals might be the source of Cd in water (Kumar et al., 2007). The Cd range observed in soil of present study was far lower than 3-6mg/kg which is the safe limit according to European Union (EU) (2006) and WHO/FAO (2007). The range of Cd concentration reported by Siddique et al., (2019) was higher than found in our research. The use of fertilizers suggests the level of Cd in agricultural soil and leaching might be the cause of low concentration of cadmium. Least Cd value displays low anthropogenic influence in the research area. Cadmium concentration in plants was 3.0mg/kg according to Ciecko et al., (2001) which was higher than the present finding. In current findings, the mean concentration of Cd in forages was lower than the decisive level (0.5mg/kg) established by CERSPC (2009). Cd concentration in forages was less than that (0.85mg/kg) reported by

Sathyamoorthyl et al., (2016). As Cd occurrence in soil, water and air depends upon industries and anthropogenic activities and study area has no such influences so forages were free from Cd contamination. Cd concentration being higher than regular level poses a considerable danger for livestock (Aksoy et al., 1999). Human beings suffer from nervous system, liver and cardiovascular disorders by the Cd accumulation in body (Tataruch and Kierdorf, 2003). The Cd correlation coefficient found in the recent study between forage-blood was non-significant and positive while Siddique et al., (2019) reported significant and positive correlation in forage-blood samples and negative correlation in soil-forage was observed. Khan et al., (2008) observed positive and significant correlation between soil and forage. Edaphic factors might be responsible for non-significant correlation between soil-forage-blood. The positive correlation for cadmium was due to the metals effective translocation from soil (Amlan et al., 2012).

## CONCLUSION

Domestic waste water exhibited health risk to livestock as well as human beings. Heavy metal content in soil and forages was lower than the permissible limit because study area has no industries and least influence of anthropogenic activities but, continuous use of domestic waste water in study area for the irrigation of agricultural fields might be harmful for animals and humans in the near future. Overuse of fertilizers to enhance the crop production and illegal dumping of domestic waste contaminate the tube well and canal water utilized by animals for drinking is a source of heavy metals assemblage in animal blood and ultimately to human beings via food chain. Government should make legislations to secure the public health.

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