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# BIOAVAILABILITY OF MOLYBDENUM IN SOIL AND FODDER CROPS: TOXICITY ANALYSIS AND HEALTH RISK ASSESSMENT

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**Abstract**

Despite the rarity of molybdenum (Mo) metal in nature, significant quantities of Mo in industrial discharges have the potential to represent a serious threat when municipal sewage sludge is applied to agricultural land. Surprisingly little information is available about Mo uptake from sewage sludge-treated soils, especially in terms of field trials. This research looked at the bio accumulative pattern of molybdenum in plants grown at three different sites [Kahoot (32.3837° N, 72.7082° E), Wazidi (32.3917° N, 72.7260° E), and Jhawarian (32.3563° N, 72.6210° E)] of Sargodha district receiving ground water, canal water and sewage water. Concentration of molybdenum was evaluated in soil and water samples. Additionally, the level of molybdenum was also analyzed in hoof samples of animals raised on forage grown on sludge treated soils. Maximum level of molybdenum (43.263 ± 0.3692 mg/L) was observed in water samples collected from SW-III receiving sewage water. Highest level of molybdenum accumulated in *Avena sativa (*20.903 mg/kg ± 0.222) grown at site treated with sewage water. The metal detection in the soil samples among all three sites ranged from 15.96 to 35.59 mg/kg whereas same varied from 17.32 to 43.99 mg/L in water samples. Maximum molybdenum accumulation was observed in hair (3.588 mg/kg ± 0.1252) and hoof (4.5429 mg/kg ± 0.0933) samples collected from buffalos raised on forage grown on sewage waste water. Moreover, the soil in which these crops were grown was also evaluated to get pollution load index which ranged from 0.39 to 0.889. Further indices included BCF ranging from 0.30-0.64, EF: 1.25-2.65 and DIM: 0.04-0.009. Concentration of Mo in plants and soil treated with sewage water and ground water exceeded the international permissible limits.

**Key words:** Sewage water, Molybdenum, Pollution load index, Enrichment factor, Bio concentration factor.

**Abbreviations:** FW: Fresh weight; BCF: Bio concentration factor; DIM: Daily intake of metals; DW: Dry weight; EF: Enrichment factor; LA: Leaf area; LDMC: Leaf dry matter content; LT: Leaf thickness; SLA: Specific leaf area; LTD: Leaf tissue density; LMA: Leaf mass per area; PLI: Pollution load index.

## Introduction

The pollution of groundwater and soil by leachate that seeps out of landfills can have far-reaching repercussions for human health as urbanization and industrialization lead to increased amounts of solid waste. In order to compensate for the lack of available water, urban areas in underdeveloped countries frequently apply raw sewage to the agricultural lands. These kinds of approaches are appealing to low-income farmers for a variety of reasons, including increased agricultural productivity and lowered overall production costs. Utilizing wastewater for agricultural purposes can potentially result in financial gains; nevertheless, this approach is fraught with significant risks to human health and the natural environment (Hanjra & Qureshi, 2010; Murtaza *et al.*, 2010; Raja *et al.*, 2015; Khanum *et al.*, 2017).

Pakistan's metropolitan and peri-urban areas, which lack access to clean or treated water sources, have relied heavily on wastewater. The bulk of Pakistan's industrial sectors discharge untreated wastewater, which contains heavy metals in varying amounts. Unrestricted input of heavy metals is undesirable since it might be challenging to get rid of them after their accumulation in soil. This might lead to heavy metals being absorbed by crops and building up in plant tissues, which could be hazardous to the health of people or animals which consume them. It could also cause heavy metals to be transported from soils to groundwater or surface water (Murtaza *et al.*, 2010).

Irrigation wastewater accumulates heavy metals in agricultural soils, putting residents at risk of contamination. Heavy metal contamination of food is the first sign of degraded food safety and quality (Marshall, 2004; Radwan & Salama, 2006; Khan *et al.*, 2008). It depletes essential nutrients, lowering immunological defenses and psychosocial abilities (Kachenko & Singh, 2006). Heavy metals' toxic properties persist even at concentrations below regulatory limits, compounding these risks.

Wastewater is a byproduct of manufacturing, farming, and other industrial activities. This wastewater can contain significantly higher levels of molybdenum and other heavy metals than standard levels (Asati *et al.*, 2016). The presence of molybdenum in waste water at high concentrations presents a challenge to the environment because molybdenum can seep into the ground and contaminate the water table. At lower concentrations, molybdenum is essential for the development of plants; however, at higher concentrations, it is toxic to plant life. The presence of excessive molybdenum in plant soil poses the greatest threat to forage and silage crops. Ruminant animals that consume plant tissues with high molybdenum content are more likely to suffer from diseases like molybdenosis, a disorder that induces copper deficiencies, and characterized by a low feed intake, diarrhea, and weight loss. Although the long-term effects of drinking water containing high concentrations of molybdenum on human health have not been studied, some scientists suspect a link to several diseases.

The purpose of this study was to determine the amount of molybdenum that was present in soil that had been treated with sewage water and to make a comparison with soil that had been treated with canal water and ground water. The molybdenum bioaccumulation in ruminants and forages grown on these soils were also investigated.

## Material and Methods

**Samples collection:** The variety of existing topsoil, fodder/forages and hair/hooves of mammals were collected from three sites of district Sargodha (Table 1). Three sites in district Sargodha were selected for sampling: Wazidi (GW-I; 32.3917° N, 72.7260° E), Jhawarian (CW-II; 32.3563° N, 72.6210° E) and Kahoot (SW-III; 32.3837° N, 72.7082° E). Sampling was done from October, 2020 to March, 2021.

**Soil:** A total of three triplicates of each site's soil samples, each weighing approximately 600 grams, were taken for analysis. In order to obtain samples of the topsoil, spatulas were used to scrape the surface (0–10 cm). The places where fodder crops were grown and irrigated with sewage, groundwater, and canal water served as the locations for the collection of soil samples. In order to prevent the samples from becoming contaminated, they were preserved by being placed inside a clean polyethylene bag that was also labelled. Before proceeding with the topsoil, soil samples were first weighed in an array to determine the relative humidity of the samples, and then topsoil samples were dehydrated by sunlight for three days before being further dehydrated in an oven to remove any remaining humidity. Approximately 2.0 grams of each sample was utilized following the milling process that was performed on that sample by means of a grinder. After filtering and analyzing the entire sample, it was saved for further evaluation.

**Fodder:** Five different extensively growing fodder species (Oat, Corn/Maize, Mustard, Berseem, Lucerne or alfalfa) used as animal feed (Table 2 states their scientific names) were sampled from three different sites. Each species was collected in duplicate from each site.

**Water:** Water samples were collected for each site depending on the type of irrigation. Half liter of water from each site was collected in separate bottles. Sewage water was collected from drain of particular concerning areas. From every site, three samples were collected and conserved inside labeled, prewashed, poly ethylene bottles. Collection of canal water for irrigation (500 ml) was done inside spotless, labeled plastic bottles.

**Ruminants:** Buffalos were selected for assessment of molybdenum in animal's body. Lactating buffaloes at average age of 7-8 years and average weight 550 kg were selected for the collection of hair and hoof samples.

**Sample analysis:** Prior to wet acid digestion every sample was weighed by means of weight balance.

**Digestion:** In order to digest whole natural stuff within samples of fodder and soil wet digestion was carried out. Chiefly it is done to acquire: i) comprehensive dissolution of biological environment, ii) to avoid contamination and iii) to obtain entire solution of matrix (Jones, 1984).

**Soil digestion:** Method of Sabienë *et al*., (2004) along with slight modification was used. 1-gram top-soil was placed into digestion cavity beside Mehlich-1 (M1) (0.05 mol/L hydrochloric acid + 0.0125 mol/L suplhuric acid); ratio 1:10 solution relative amount and traumatized for 6-8 minutes onto a reciprocate mixer/shaker at 110 oscillations per min and left for whole night approximately for 17 hours. Whole absorption of poisonous minerals inside topsoil was examined subsequently after digestion.

**Water digestion:** Only some drop of $H_2SO_4$ were put in beaker containing water measuring 5.0 ml and boiled it in anticipation of the emergence of smoke. Afterwards, 2.0 ml of $H_2O_2$ hydrogen peroxide was added and process was repeated until water turned out to be clear. The samples were filtered through filter paper and saved in containers.

**Forage digestion:** The forage samples were cleaned through dilute HCl and air dehydrated and preserved inside paper bags. Then the samples were stove dried and digested by means of wet-digestion technique. 0.5 gram of every sample was digested through (2 ml) sulphuric acid and (4 ml) hydrogen peroxide. The samples were heated inside digestion cavity to 40 minutes and subsequently cooled (Allen *et al.*, 1986). Cooled samples were mixed with 2 ml of hydrogen peroxide. Then fusion was heated once more in anticipation of emergence of colorless solution.

**Hooves and hair digestion method:** Wang & Tao (1998), suggested a process in which 20–30 mg of hair or hoof trimmings are weighed and deposited into a test tube. This method was followed for hooves and hair processing. After addition 1 mL of strong $HNO_3$, the test tube was put on a heating plate and heated to 100°C. Each tube had a glass marble put on top of it, and the digestion took 1 hour. The solution was transferred into a measured polypropylene tube and diluted to 10 mL with distilled water after cooling to room temperature.

**Dilution and filtration:** Every sample was then diluted by means of recently primed distilled water amounting to 50 ml solution after digestion. Each sample was then put aside inside plastic bottle.

**Statistical study:** Comparative analysis of soil, water, fodder and animal samples was carried using SPSS Software (Steel *et al.*, 1980). Table 3 points out the standards for analytical assessment.

**Pollution load index (PLI):** PLI was determined by Liu *et al*., (2005).

$$PLI = \frac{\text{Metal concentration (mg per kg) within soil samples}}{\text{Suggested values of metal within soil}}$$

**Bio-concentration factor (BCF):** BCF was evaluated by Cui *et al*., (2004).

$$\text{BCF soil-forage} = \frac{\text{Concentrations of metals in forage}}{\text{Concentrations of metals in soil}}$$

**Enrichment factor (EF):** EF was determined by Buat-Menard & Chesselet (1979).

$$EF = \frac{\text{(Concentration of metal in fodder/conc. of metal in soil)}}{\text{(Concentration of metal fodder/conc. of metal in soil) standard}}$$

**Daily Intake of metals (DIM):** DIM was estimated according to Khan *et al*., (2009).

$$DIM = C_{\text{metal}} * D_{\text{food intake}} / B_{\text{average weight}}$$

Table 4 includes the standard values for daily intake of metals.

**Health risk index (HRI):** HRI is used for finding health risk coupled by means of eating of contaminated forages (Stephens *et al.*, 2001).

$$R_f D = \text{Oral reference dose}$$

Table 5 explains the standard values for oral dose reference of the said metal.

$$HRI = DIM / R_f D$$

HRI above 1 indicates high health risk through dietary intake of metals to consumers (Anon., 2010).

## Results and Discussion

**Molybdenum in water samples:** Molybdenum levels varied significantly in water samples collected from three different sites (Tables 6, 7). The highest level of molybdenum was found in water collected from SW-III (43.99 mg/L), while the lowest level (17.321 mg/L) was found in water collected from GW-I. Sewage wastewater had a minimum concentration of 42.84 mg/L and a maximum concentration of 43.99 mg/L. Fig. 1 depicts the variations in molybdenum concentrations found in water samples collected from all the locations that were studied. In the present study, the maximum concentration of molybdenum was present in SW-III (43.99 mg/L). Greathouse & Osborne (1980) also reported molybdenum in waste water, but at lower levels than our study.

**Table 1. Site description.**

| Site | Irrigation type | Name |
|---|---|---|
| Site-1 | Groundwater (GW-I) | Wazidi |
| Site-2 | Canal water (CW-II) | Jhawarian |
| Site-3 | Sewage wastewater (SW-III) | Kahoot |

**Table 2. List of fodder species with scientific names.**

| Serial no. | Common name | Botanical name |
|---|---|---|
| 1. | Oat | *Avena sativa* |
| 2. | Corn/Maize | *Zea mays* |
| 3. | Mustard | *Brassica campestris* |
| 4. | Berseem | *Trifolium alexandrinum* |
| 5. | Lucerne or alfalfa | *Medicago sativa* |

**Table 3. Analytical characteristics.**

| Standards | Heavy metal | Soil | Plant | Reference |
|---|---|---|---|---|
| Permissible limits(mg/kg) | Molybdenum | 3 | 0.05 | Anon., (1996) |
| Standard concentration (mg/kg) | Molybdenum | 5 | 3 | (Chiroma *et al*., 2014); Dutch Standards (2000) |

**Table 4. Daily intake of metal.**

| Animal | Daily food intake | Average weight | Conversion factor |
|---|---|---|---|
| Buffalos | 12.5 mg/kg (Briggs and Aytenfisu, 1980) | 550 kg (Briggs and Aytenfisu, 1980) | 0.085 (Jan *et al*., 2010) |

**Table 5. Oral reference dose (mg/kg) for heavy metals.**

| Metals (ORfD) Oral reference dosage (mg/kg) | | |
|---|---|---|
| **Metals** | **$R_f D$ (mg/kg/day)** | **Reference** |
| Mo | 0.009 | (Anon., 2010) |

**Table 6. A comparison of mean molybdenum concentrations (mg/L) in water samples.**

| Site | Site GW-I | Site CW-II | Site SW-III |
|---|---|---|---|
| Mean concentration | 17.966 ± 0.4491 | 39.270 ± 0.3300 | 43.263 ± 0.3692 |
| Mean square | 554.85*** | | |

ns = Non-significant *, **, ***= Significant at 0.05, 0.001 and 0.01 level

**Table 7. Analysis data variance of for Molybdenum in soil sewage waste water, canal water and Ground water.**

| Variables | Degree of freedom | Mean square |
|---|---|---|
| Site | 2 | 1106.700*** |
| Plant | 4 | 4.325*** |
| Site * Plant | 8 | 5.221*** |
| Error | 30 | .416 |

ns = Non-significant *, **, ***= Significant at 0.05, 0.001 and 0.01, levels, respectively

**Molybdenum in soil samples:** Plant and site interactive effects showed significant contributions towards molybdenum accumulation (Table 8). Molybdenum concentrations in the soil of GW-I were highest in *B. campestris* grown soil (18.72 mg/kg), and lowest in *T. alexandrinum* plantations (15.96 mg/kg). The order of concentration at GW-I was found as *B. campestris* > *Z. mays* > *M. sativa* > *A. sativa* > *T. alexandrinum*. The mean concentration of molybdenum at CW-II showed an upper peak concentration in *Z. mays* soil (25.19 mg/kg) and lower peak values in *T. alexandrinum* soil (21.123 mg/kg), while the order of metal accumulation was *Z. mays* > *A. sativa* >

*M. sativa* > *B. campestris* > *T. alexandrinum*. Analysis of variance at SW-III showed molybdenum concentration's upper peak in *A. sativa* (35.59 mg/kg) and lowest in *M. sativa* grown soil (33.82 mg/kg) (Table 8; Fig. 2). Current values were higher than those recommended by Dutch Standards in 2000 (29 mg/kg).

**Molybdenum in fodder samples:** The bioaccumulation of molybdenum in samples of forage from the sites studied showed a significant interaction between the site and the forage (Tables 9, 10). Maximum levels of molybdenum metal were found in *A. sativa* (9.413 mg/kg), 15.156 mg/kg), and 20.90 mg/kg) at sites I, II, and III, respectively, while minimum levels were observed in *T. alexandrinum* (4.853 mg/kg, 10.56 mg/kg, and 16.30 mg/kg, respectively). The sequence of metal concentration was observed as *A. sativa* > *Z. mays* > *B. campestris* > *M. sativa* > *T. alexandrinum* for all three sites (Table 10). Fig. 3 explains the molybdenum level in fodder crops studied in this research. High amount of certain metalloids (Cu, Ni, Se, Mo, As, Fe and Zn) was observed in common crop of this region (Khushab city), *Allium staivum*, showing that the sites irrigated with sewage water have ubiquitous spread of metals in soils making their way into the food chains (Khan *et al.*, 2017). Contrary to this, normal levels of metals (Cu, Co, Zn, Fe and Mn) were found in wild plants growing at Soon Valley, Khushab, adjoining district of Sargodha, which indicated that the heavy metals are brought by the irrigational sewage water and that the natural waters of this region are not contaminated (Khan *et al.*, 2021).

The values of the current study were higher than the values (3 mg/kg) suggested by Chiroma *et al*., (2014). The National Research Council (Anon., 1984) says that the most molybdenum that can be in cattle feed is 10 mg Mo/kg, but the current levels are higher than what is safe.

**Table 8. Molybdenum concentration (mg/kg) in soil samples (Mean± S.E).**

| Fodder crops soil | Site GW-I | Site CW-II | Site SW-III |
|---|---|---|---|
| *Trifolium alexandrinum* | 15.966 ± 0.143 | 21.123 ± 0.243 | 33.830 ± 0.069 |
| *Medicago sativa* | 17.140 ± 0.145 | 22.393 ± 0.136 | 33.823 ± 1.234 |
| *Brassica campestris* | 18.720 ± 0.066 | 21.527 ± 0.434 | 34.640 ± 0.077 |
| *Zea mays* | 17.520 ± 0.141 | 25.190 ± 0.128 | 32.666 ± 0.230 |
| *Avena sativa* | 16.970 ± 0.138 | 23.636 ± 0.109 | 35.590 ± 0.335 |

**Table 9. Concentration of Molybdenum (mg/kg) in collected forages samples (Mean +S.E).**

| Fodder crops | Site GW-I | Site CW-II | Site SW-III |
|---|---|---|---|
| *Trifolium alexandrinum* | 4.853 ± 0.287 | 10.560 ± 0.219 | 16.306 ± 0.222 |
| *Medicago sativa* | 5.930 ± 0.254 | 11.710 ± 0.219 | 17.456 ± 0.222 |
| *Brassica campestris* | 7.113 ± 0.222 | 12.860 ± 0.219 | 18.603 ± 0.222 |
| *Zea mays* | 8.263 ± 0.222 | 14.010 ± 0.219 | 19.753 ± 0.222 |
| *Avena sativa* | 9.413 ± 0.222 | 15.156 ± 0.222 | 20.903 ± 0.222 |

**Table 10. Analysis of variance for Molybdenum in forages irrigated with Ground, canal and sewage wastewater.**

| Variables | Degree of freedom | Mean square |
|---|---|---|
| Site | 2 | 495.075*** |
| Plant | 4 | 29.648*** |
| Site * Plant | 8 | 0.001 |
| Error | 30 | 0.157 |

ns = Non-significant *, **, ***= Significant at 0.05, 0.001 and 0.01 level

**Table 11. Analysis of mean concentration of Molybdenum in animal (buffalo) hair.**

| Sites | Site GW-I | Site CW-II | Site SW-III |
|---|---|---|---|
| Mean concentration mg/kg | 0.7327 ± 0.0799 | 2.5859 ± 0.1023 | 3.588 ± 0.1252 |
| Mean square | 10.496*** | | |

ns = Non-significant *, **, ***= Significant at 0.05, 0.001 and 0.01 level

**Table 12. Analysis of mean concentration of Molybdenum in animal (buffalo) hooves.**

| Sites | Site GW-I | Site CW-II | Site SW-III |
|---|---|---|---|
| Mean concentrations mg/kg | 0.5864 ± 0.0837 | 1.6075 ± 0.0862 | 4.5429 ± 0.0933 |
| Mean square | 21.094*** | | |

ns = Non-significant *, **, ***= Significant at 0.05, 0.001 and 0.01 level

**Table 13. Correlation between soil, fodder, hair and hooves collected from different sites.**

| Sites | Soil-fodder | Fodder-hairs | Fodder-hooves |
|---|---|---|---|
| Site GW-I | 0.379*** | 0.910*** | 0.810*** |
| Site CW-II | 0.733*** | 0.990*** | 0.709*** |
| Site SW-III | 0.261*** | 0.940*** | 0.509*** |

ns = Non-significant *, **, ***= Significant at 0.05, 0.001 and 0.01, levels, respectively

**Molybdenum in hair samples of buffalos:** The most molybdenum was found in the hair of animals at SW-III (3.588 mg/kg), and the least was found at GW-I (0.7327 mg/kg) (Table 11). Fig. 4 shows how the amount of molybdenum in hair samples. High amount of another highly toxic heavy metal, lead (Pb), was found in different organs of buffaloes and cattle in Sargodha indicating contamination in the farm soils (Liu *et al.*, 2020).

**Molybdenum in hoof samples of buffalos:** The molybdenum concentration in an animal's hooves was highest at site 3 (4.5429 mg/kg), while the lowest value was observed at site 1 (0.5864 mg/kg). The concentration variation was as follows: SW-III > CW-II > GW-I (Table 12). Fig. 5 depicts the fluctuations of molybdenum values in hooves from all studied samples. Haywood *et al*., (2004) observed 5.0 mg of Mo/kg in hoof samples, which is higher than current values.

## Correlation

All correlations between soil-fodder, fodder-hair, and fodder-hooves at sites GW-I, CW-II and SW-III were significant (Table 13).

**Pollution load index:** The maximum PLI for soil was observed in *A. sativa* (0.889) for SW-III, and the minimum concentration was found in *T. alexandrinum* (0.399). The following sequence was observed at Site 1: *B. campestris*> *Z. mays*> *M. sativa*> *A. sativa*> *T. alexandrinum*. While the order of PLI at CW-II was found as *Z. mays*>*A. sativa*>*M. sativa* >*B. campestris*>*T. alexandrinum*, whereas at site SW-III, PLI was *A. sativa*> *B. campestris*>*T. alexandrinum* >*M. sativa* >*Z. mays* (Table 14). Fig. 6 explains the fluctuations of molybdenum PLI values in all studied samples. Current values were below permissible limits. Current values of PLI were inferior to concentrations determined by Ahmad *et al.,* (2016).

**Bio-concentration factor:** The peak value of the bio-concentration factor was observed in *A. sativa* at GW-I (0.641 mg/kg) at CW-II, and the lowest value was present in *T. alexandrinum* (0.303) at CW-II. The sequence of BCF for GW-I was found as *A. sativa* > *Z. mays* > *B. campestris* > *M. sativa* > *T. alexandrinum*. The order of BCF was *A. sativa* > *B. campestris* > *Z. mays* > *M. sativa* > *T. alexandrinum* on CW-II, and *Z. mays* > *A. sativa* > *B. campestris* > *M. sativa* > *T. alexandrinum* at SW-III (Table 15). Fig. 7 depicts the fluctuations in molybdenum BCF values for all studied samples. Current values were below permissible limits. The present amount of metal is inferior to the value determined by Ahmad *et al*., (2016).

**Enrichment factor:** The enrichment factor for molybdenum metal was found to be maximum at the site irrigated with canal water (CW-II), which was observed in *A. sativa* (2.656) and *T. alexandrinum* (1.259) at GW-I. The sequence of enrichment factors at GW-I remained as *A. sativa* > *Z. mays* > *B. campestris* > *M. sativa* > *T. alexandrinum*. At the second site, the sequence of EF was: *A. sativa* > *B. campestris* > *Z. mays* > *M. sativa* > *T. alexandrinum*, whereas at the third site, the sequence was *Z. mays* > *A. sativa* > *B. campestris* > *M. sativa* > *T. alexandrinum* (Table 16). Fig. 8 explains the fluctuations of molybdenum EF values in all studied samples. Current values were higher than permissible limits but lower than values determined by Ahmad *et al*., (2016).

**Table 14. Pollution load index (PLI) for molybdenum metal.**

| Fodder crops | Site GW-I | Site CW-II | Site SW-III |
|---|---|---|---|
| *Trifolium alexandrinum* | 0.399 | 0.528 | 0.846 |
| *Medicago sativa* | 0.4285 | 0.559 | 0.845 |
| *Brassica campestris* | 0.468 | 0.538 | 0.866 |
| *Zea mays* | 0.438 | 0.629 | 0.816 |
| *Avena sativa* | 0.424 | 0.590 | 0.889 |

**Table 15. Analysis of bio-concentration factor (BCF) for molybdenum metal.**

| Fodder crops | Site GW-I | Site CW-II | Site SW-III |
|---|---|---|---|
| *Trifolium alexandrinum* | 0.303 | 0.499 | 0.482 |
| *Medicago sativa* | 0.345 | 0.522 | 0.516 |
| *Brassica campestris* | 0.379 | 0.597 | 0.537 |
| *Zea mays* | 0.471 | 0.556 | 0.604 |
| *Avena sativa* | 0.554 | 0.641 | 0.587 |

**Table 16. Analysis of enrichment factor (EF) in animals due to bioavailability of molybdenum.**

| Fodder crops | Site GW-I | Site CW-II | Site SW-III |
|---|---|---|---|
| *Trifolium alexandrinum* | 1.259 | 2.071 | 1.997 |
| *Medicago sativa* | 1.433 | 2.167 | 2.138 |
| *Brassica campestris* | 1.574 | 2.475 | 2.225 |
| *Zea mays* | 1.954 | 2.304 | 2.505 |
| *Avena sativa* | 2.298 | 2.657 | 2.433 |

**Table 17. Analysis for daily intake (DIM) of molybdenum metal.**

| Fodder crops | Site GW-I | Site CW-II | Site SW-III |
|---|---|---|---|
| *Trifolium alexandrinum* | 0.0093 | 0.0204 | 0.0315 |
| *Medicago sativa* | 0.0114 | 0.023 | 0.0337 |
| *Brassica campestris* | 0.0137 | 0.0248 | 0.0359 |
| *Zea mays* | 0.0159 | 0.0270 | 0.0381 |
| *Avena sativa* | 0.0181 | 0.029 | 0.0403 |

**Table 18. Analysis for health risk index (HRI) in animals due to molybdenum.**

| Fodder crops | Site GW-I | Site CW-II | Site SW-III |
|---|---|---|---|
| *Trifolium alexandrinum* | 1.042 | 2.267 | 3.5002 |
| *Medicago sativa* | 1.273 | 2.513 | 3.747 |
| *Brassica campestris* | 1.527 | 2.760 | 3.993 |
| *Zea mays* | 1.774 | 3.007 | 4.24 |
| *Avena sativa* | 2.020 | 3.253 | 4.487 |

**Daily intake of metal:** DIM for metal molybdenum showed an upper peak concentration in *A. sativa* (0.0403) at site 3, and lower peak values were found in *T. alexandrinum* at site 1 (0.0093). The sequence for DIM for all sites was followed as *A. sativa* > *Z. mays* > *B. campestris* > *M. sativa* > *T. alexandrinum* (Table 17). Fig. 9 explains the fluctuations of molybdenum DIM values in all studied samples. Present values were lower than the 25 mg/kg suggested by the Anon., (2000). Current values were below permissible limits and also lower than values determined by Ahmad *et al*., (2016).

**Health risk index:** The highest HRI value was found in *A. sativa* (4.486) at SW-III, while the lowest was found in *T. alexandrinum* (1.041) at GW-I. The sequence for HRI for all sites was followed as *A. sativa* > *Z. mays* > *B. campestris* > *M. sativa* > *T. alexandrinum* (Table 18). Fig. 10 explains the fluctuations of molybdenum HRI values in all studied samples. The present amount exceeds permissible limits. Current values were lower than those observed by Ahmad *et al*., (2016).

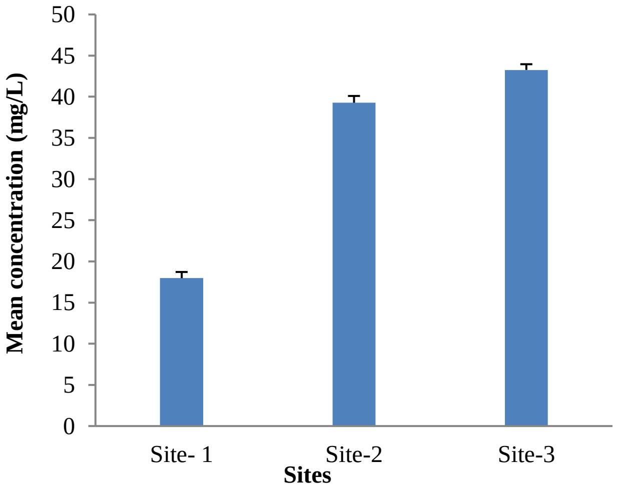


Fig. 1. Fluctuations in concentration of molybdenum in water samples.

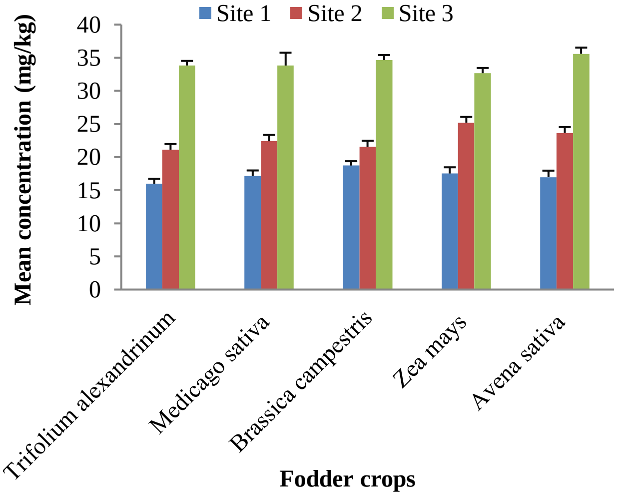


Fig. 2. Fluctuation in level of molybdenum in soil sewage waste water, canal water and ground water.

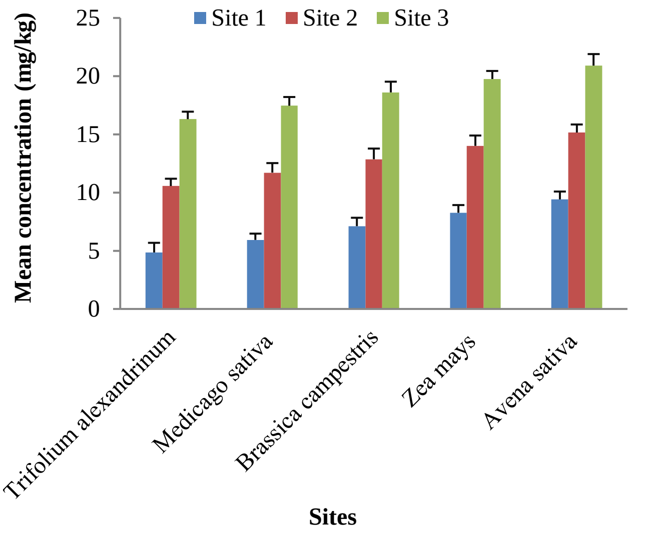


Fig. 3. Fluctuation in level of molybdenum in fodder sewage waste water, canal water and ground water.

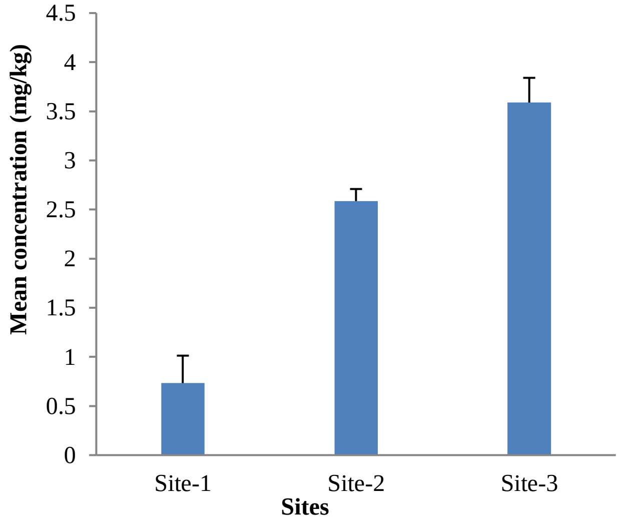


Fig. 4. Fluctuation in concentration of molybdenum in animal (buffalo) hair.

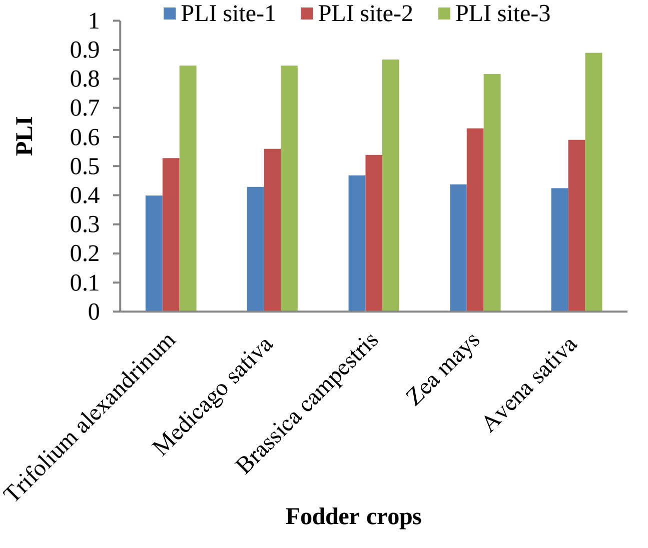


Fig. 6. Fluctuations in PLI level for molybdenum metal.

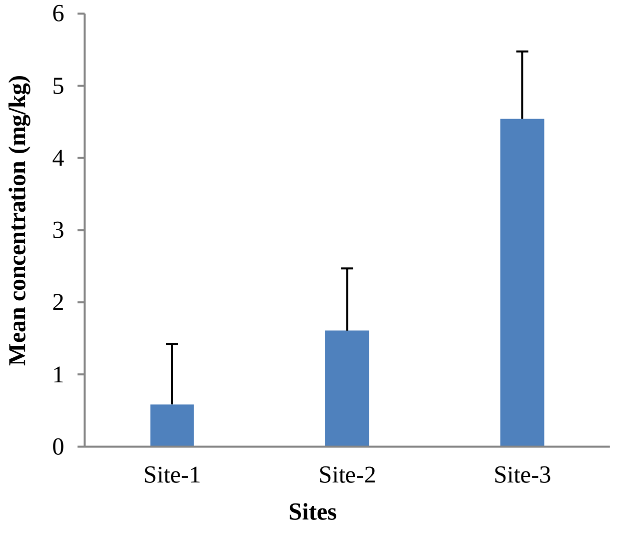


Fig. 5. Fluctuation of molybdenum concentration in animal (buffalo) hooves.

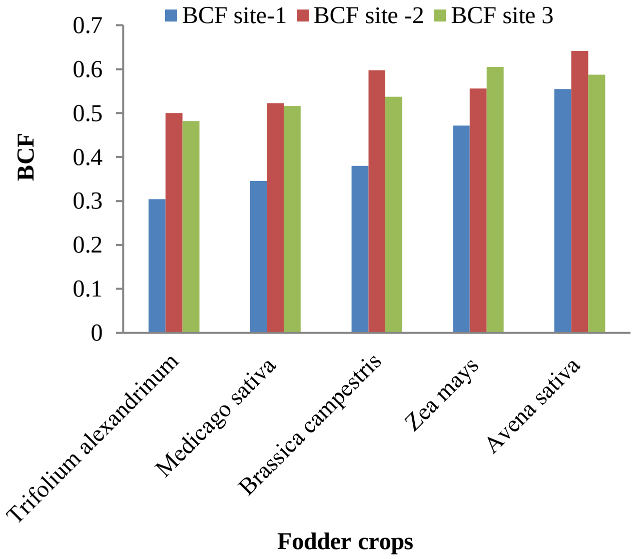


Fig. 7. Fluctuations in levels of molybdenum for BCF.

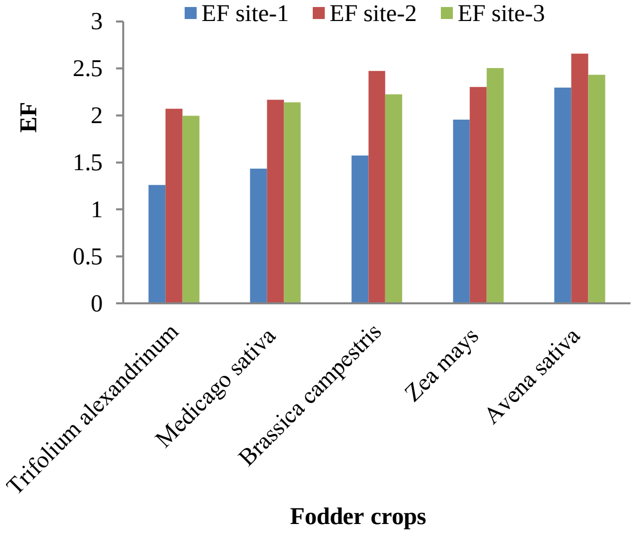


Fig. 8. Fluctuations in EF levels of molybdenum.

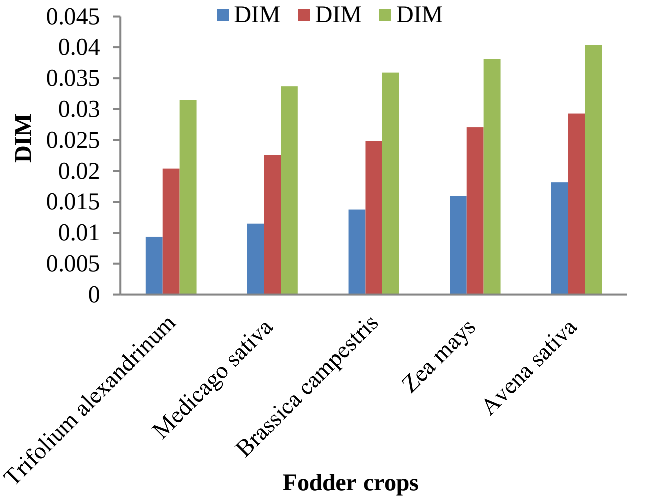


Fig. 9. Fluctuation in level of molybdenum in DIM index.

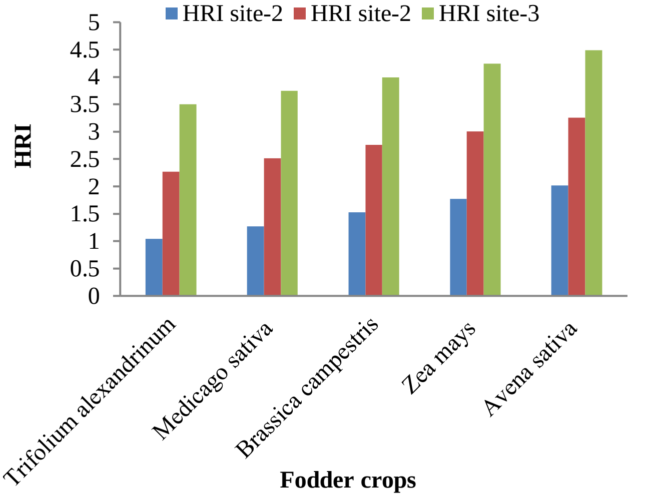


Fig. 10. Fluctuation in level of molybdenum in HRI.

## Conclusion and Recommendation

Heavy metal concentrations in soils and plants have been steadily rising due to years of wastewater irrigation of agricultural lands. This region needs effective measures to cure the toxic metal contamination. The Health Risk Index (HRI > 1) indicated that molybdenum contamination in most vegetables can pose a risk to human health.

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### References

Ahmad, K., A. Ashfaq, Z.I. Khan, M. Ashraf, N.A. Akram, S. Yasmin, A.I. Batool, M. Sher, H.A. Shad and A. Khan. 2016. Health risk assessment of heavy metals and metalloids via dietary intake of a potential vegetable (*Coriandrum sativum* L.) grown in contaminated water irrigated agricultural sites of Sargodha, Pakistan. Human and ecological risk assessment: *An Int. J.*, 22(3): 597-610.

Allen, S., H. Grimshaw and A. Rowland. 1986. Chemical analysis: In: (Eds.): Moore, Pd. and Sb. Chapman. Methods In Plant Ecology. Blackwell Scientific Publication, Oxford, *London*: 285-344.

Anonymous. 1984. NRC, National Research Council. Nutrient requirements of beef cattle -6th Revised Ed. Nutrient Requirements of Domestic Animals, National Academy of Science, Washington DC.

Anonymous. 2000. WHO, World Health Organization. Environmental health criteria. World Health Organization Geneva, Switzerland.

Anonymous. 2010. Guidance for Federal Land Management in the Chesapeake Bay Watershed.

Asati, A., M. Pichhode and K. Nikhil. 2016. Effect of heavy metals on plants: An overview. *Int. J. App. Innov. Engin. & Manag.*, 5(3): 56-66.

Briggs, K.G. and A. Aytenfisu. 1980. Relationships between morphological characters above the flag leaf node and grain yield in spring wheats 1. *Crop Sci.*, 20(3): 350-354.

Buat-Menard, P. and R. Chesselet. 1979. Variable influence of the atmospheric flux on the trace metal chemistry of oceanic suspended matter. *Earth Planet. Sci. Lett.*, 42(3): 399-411.

Chiroma, T., R. Ebewele and F. Hymore. 2014. Comparative assessment of heavy metal levels in soil, vegetables and urban grey waste water used for irrigation in yola and kano. *Int. Refereed J. Eng. Sci*., 3(2): 01-09.

Cui, Y.J., Y.G. Zhu, R.H. Zhai, D.Y. Chen, Y.Z. Huang, Y. Qiu and J.Z. Liang. 2004. Transfer of metals from soil to vegetables in an area near a smelter in nanning, China. *Environ. Int*., 30(6): 785-791.

Dutch Standards. 2000. Circular on Target Values and Intervention Values for Soil Remediation. Ministrie van Volkshuisvesting, Ruimtelijke Ordening en Milieubeheer, pp. 51.

Greathouse, D. and R. Osborne. 1980. Preliminary report on nationwide study of drinking water and cardiovascular diseases. *J. Environ. Pathol. and Toxicol*., 4(2-3): 65-76.

Hanjra, M.A. and M.E. Qureshi. 2010. Global water crisis and future food security in an era of climate change. *Food Policy*, 35(5): 365-377.

Haywood, L.K., G.J. Alexander, M.J. Byrne and E. Cukrowska. 2004. Xenopus laevis embryos and tadpoles as models for testing for pollution by zinc, copper, lead and cadmium. *Afri. Zool.*, 39(2): 163-174.

Jan, F.A., M. Ishaq, S. Khan, I. Ihsanullah, I. Ahmad and M. Shakirullah. 2010. A comparative study of human health risks via consumption of food crops grown on wastewater irrigated soil (Peshawar) and relatively clean water irrigated soil (Lower Dir). *J. Hazard. Mat.*, 179(1-3): 612-621.

Jones, R. 1984. Heavy metals in the sediments of llangorse lake, wales, since celtic-roman times: With 5 figures in the text. Internationale Vereinigung Für Theoretische Und Angewandte Limnologie: *Verhandlungen*, 22(3): 1377-1382.

Kachenko, A.G. and B. Singh. 2006. Heavy metals contamination in vegetables grown in urban and metal smelter contaminated sites in Australia. *Water, Air, Soil Poll.*, 169: 101-123.

Khan, S., Q. Cao, Y. Zheng, Y. Huang and Y. Zhu. 2008. Health risks of heavy metals in contaminated soils and food crops irrigated with wastewater in Beijing, China. *Environ. Poll.*, 152(3): 686-692.

Khan, S., R. Farooq, S. Shahbaz, M.A. Khan and M. Sadique. 2009. Health risk assessment of heavy metals for population via consumption of vegetables. *World Appl. Sci. J*., 6(12): 1602-1606.

Khan, Z.I., I. Ugulu, A. Zafar, N. Mehmood, H. Bashir, K. Ahmad and M. Sana. 2021. Biomonitoring of heavy metals accumulation in wild plants growing at Soon Valley, Khushab, Pakistan. *Pak. J. Bot.*, 53(1): 247-252.

Khan, Z.I., K. Ahmad, N. A. Akram, N. Mehmood and S. Yasmeen. 2017. Heavy metal contamination in water, soil and a potential vegetable garlic (*Allium sativum* L.) in Punjab, Pakistan. *Pak. J. Bot.*, 49(2): 547-552.

Khanum, K., M. Baqar, A. Qadir, M. Mumtaz, A. Tahir, N. Jamil and A. Mahmood. 2017. Heavy metal toxicity and human health risk surveillances of wastewater irrigated vegetables in Lahore District, Pakistan. *Carpathian J. Earth Environ. Sci.*, 12(2): 403-412.

Liu, W.H., J.Z. Zhao, Z.Y. Ouyang, L. Söderlund and G.H. Liu. 2005. Impacts of sewage irrigation on heavy metal distribution and contamination in Beijing, China. *Environ. Int.*, 31(6): 805-812.

Marshall, F. 2004. Enhancing food chain integrity: Quality assurance mechanism for air pollution impacts on fruits and vegetables systems. Crop Post Harvest Program on Food Safety. Final Technical Report, Imperial College London, UK, pp. 165.

Murtaza, G., A. Ghafoor, M. Qadir, G. Owens, M. Aziz and M. Zia. 2010. Disposal and use of sewage on agricultural lands in Pakistan: A review. *Pedosphere*, 20(1): 23-34.

Radwan, M.A. and A.K. Salama. 2006. Market basket survey for some heavy metals in Egyptian fruits and vegetables. *Food Chem. Toxicol.*, 44(8): 1273-1278.

Raja, S., H.M.N. Cheema, S. Babar, A.A. Khan, G. Murtaza and U. Aslam. 2015. Socio-economic background of wastewater irrigation and bioaccumulation of heavy metals in crops and vegetables. *Agri. Water Manag.*, 158: 26-34.

Sabienë, N., D.M. Brazauskienë and D. Rimmer. 2004. Determination of heavy metal mobile forms by different extraction methods. *Ekologija*, 1(1): 36-41.

Steel, R., J. Torrie and D. Dickey. 1980. Principles and procedures of statistics. McGraw-Hill, New York. Principles and Procedures of Statistics. 2nd Ed. McGraw-Hill, New York.

Stephens, S., B. Alloway, J. Carter and A. Parker. 2001. Towards the characterisation of heavy metals in dredged canal sediments and an appreciation of ‘Availability’: Two examples from the Uk. *Environ. Poll.*, 113(3): 395-401.

Wang, X. and S. Tao. 1998. Spatial structures and relations of heavy metal content in wastewater irrigated agricultural soil of Beijing's Eastern Farming Regions. *Bull. Environ. Contamin. and Toxicol.*, 61: 261-268.


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