

## Assessment of Available Manganese in Milk by Using fodders Grown in Long-Term Wastewater Irrigated Soil

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## ASSESSMENT OF AVAILABLE MANGANESE IN MILK BY USING FODDERS GROWN IN LONG-TERM WASTE WATER IRRIGATED SOIL

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### ABSTRACT

Heavy metals are considered as most important contaminations due to industrialization of countries and an influence on its existence in soil, plant and milk. A study was carried out to check manganese content in soil, forage and milk at three sites of city Jhang, Punjab, Pakistan. All samples (milk, soil, water, fodder plants and ground water) were analyzed for manganese by Atomic Absorption Spectrophotometer. Different health indices were also studied to check Mn flow in food chain. Level of Mn in samples was found within acceptable limits. Manganese level was higher in soil samples collected from Site-III than other sites. Manganese showed higher value (2.595 to 10.402 mg/kg) in soil than other samples. Fodders were found to accumulate manganese from 0.008 to 0.022 mg/kg. Manganese concentration was found to be 0.1482 to 1.241 mg/L, 0.164 to 0.9708 mg/L in water and milk, respectively. BCF and PLI values for manganese were also found to be less than 1. Estimated daily intake (EDI) and THQ of manganese are found within permissible limits in milk of cows feeding on fodders irrigated with wastewater and ground water. So, use of wastewater for irrigation purpose should be properly checked due to possible toxic effects.

**Keywords:** Wastewater, fodders, cow milk, bio-concentration factor, pollution load index.

### INTRODUCTION

Irrigation of land is done by sewage water in several areas of Pakistan due to continuous increase in water shortage. Among cultivated areas of Pakistan, water shortage has been estimated to be 33% in 2025 (Anonymous, 2005). Because of water shortage, irrigation in 32500 hectares land is done with wastewater in Pakistan (Ensink et al., 2004).

Milk's contamination is believed to be one of the most hazardous features from last few years (Malhat et al., 2012). The troubles of milk quality and pollution have enhanced by environmental pollution (Farid and Baloch, 2012). Although, iron and manganese are necessary in plant growth but their accumulation up to toxic

level is harmful in plants (Khan et al., 2018).

Due to global milk contamination, the public health is believed to be at a greater risk because of xenobiotic compounds and environmental pollutants such as toxic heavy metals, mycotoxin and other pollutants through cattle feeds (Seyed and Ebrahim, 2012). Intake of this contaminated milk acts like supplementary source of heavy metal exposure (Ruqia et al., 2015).

Environmental pollution is a most important universal problem facing severe risks by humans and animals (Rajaganapathy et al., 2011). It is believed that, rapid industrialization and the development of modern farming

technology are principal issues for environmental pollution. Chemical contaminants or remains in animal feed are dangerous for the lives of living organisms (Leeman et al., 2007).

As population increases, water shortage has become a limiting factor for economic development of the country (Lu et al., 2015). In cities, environmental pollution has been enhanced due to a large amount of effluents produced from human settlements and industries which are collectively called municipal waste (public waste). The amount of the effluents has been increased due to speedy growth population and increased number of industries in the country (Dogan et al., 2014).

Milk plays a significant role in diet of humans because it contains a variety of valuable minerals (Buldini et al., 2002). The root cause of environmental pollution is technological advancement, excessive road traffic and industrialization. Some pollutants particularly lead and cadmium are commonly present in atmosphere and increase the risks of human and animal health by entering into food chain (Licata et al., 2004).

Cow milk is considered to be priceless human diet in comparison to goat or sheep milk because of its nutritional values as it is an excellent source of fats, proteins and minerals (Sayed & Ebrahim, 2012; Enb et al., 2009). Farm animal's intake metals when they are supplied with condense polluted feeds or pastures. But, metal transfer to milk of cow is extremely changeable (Maas et al., 2011).

Milk and dairy products are significant building blocks of human diet. The presence of important energetic nutrients such as proteins, lactose, vital fatty acids, minerals and vitamins in balanced amount in milk has made it a complete food. Although, milk and dairy products contains some pollutants which increases risk factors especially for health of the people (Licata et al., 2004).

The objective of this study is to assess transfer of manganese from different sources of wastewater to soil, fodders and milk and its possible health risks on human health.

## MATERIALS AND METHODS

### *Study Area*

Jhang is 18<sup>th</sup> largest and central city of Pakistan. It is situated at junction of rivers Chenab and Jehlum which is known as Trimmu Head. It is recognized as agricultural city divided into four Tehsils Jhang, Ahmad Pur Siyal, Shorkot and Athara Hazari. The climate of Jhang city is extreme hot and cold in summer and winter, respectively. In summer season, temperature reaches upto 52 °C. While, minimum temperature varies between 21 and 4.5 °C.



Figure 1: Location map of study area.

### *Introduction to Three Selected Sites*

Wastewater irrigation in various areas of Jhang city is widespread practice due to its easy availability including all effluents of city such as industrial and domestic wastes. Sites were selected on basis of sources of irrigation. Site-I is known as Jhang-chiniot road, Site-II, Jhang-Faisalabad road. Site-III was Jhang-Sargodha road. Site-II and Site-III were irrigated with wastewater but Site-I was irrigated with tube well water and also known as control site.

### *Plant Sampling*

During 2018-2019, each site was further divided into 5 plots. All soil, fodder

samples were collected from every plot of site to make one composite sample. Four replicates of soil and fodders were prepared.

Total 72 samples were collected from three sites. Plant samples were cleaned with distilled water and placed in labeled paper bags. Brassica, millet, barseem, lucern and maize plants were general crops across three sites. Eight different types of fodders were collected from selected three sites which are given in table 1.

**Table 1: List of fodders collected from three sites.**

Sr. No.	Common Name	Scientific Name
1	Maize	<i>Zea mays</i>
2	Millet	<i>Pennisetum laucum</i>
3	Mustard	<i>Brassica campestris</i>
4	Barseem	<i>Trifolium alexandrinum</i>
5	Lucern	<i>Medicago sativa</i>
6	Bathu	<i>Chenopodium album</i>
7	Jai	<i>Avena sativa</i>
8	Methi	<i>Trigonella foenum-graecum</i>

### **Sampling of Soils across Three Sites**

Total 72 composite samples of soil from three sites were collected. 5 soil samples (one from every corner and one from center of fields) from 0-15 cm depth were assembled from all sites. Soil samples were placed into air tight bags.

Soil samples (10 g each) were collected from every plot of site where fodders were grown. Five composite samples were collected and labeled. All samples were air dried and placed in incubator at 70 °C for 05 days.

### **Milk Sampling**

Total 75 samples were randomly collected from cattle farms located in these areas. 25 cows were selected from each site for milk sampling. 20 ml milk was

taken and stored in freezer at -20 °C for next processing.

### **Digestion of Samples**

Plant samples were washed with distilled water. Edible parts of fodders and soil samples were oven-dried at 70°C. Dried samples were ground into a fine powder form with help of grinder and stored in polyethylene packets for acid digestion. For soil and plant digestion, wet digestion method was used. Sample (1g) was digested in a flask with addition of 4.0 ml of H<sub>2</sub>SO<sub>4</sub> and 8.0 ml of H<sub>2</sub>O<sub>2</sub> by placing it in digestion chamber. When fumes congested evaporating, remove samples from digestion chamber after disappearance of evaporating fumes. Each sample was further digested with 2 ml of H<sub>2</sub>O<sub>2</sub> until solution becomes clear. Filter solution and make final volume of 50 ml by adding distilled water. All prepared samples were kept in plastic bottles for further analysis.

### **Milk Digestion**

In a digestion flask, 10 ml of 65% HNO<sub>3</sub> and 3 ml of 30% H<sub>2</sub>O<sub>2</sub> were used to digest 10 ml of milk and heated until solution became clear. Whatman filter paper 40 was also used for filtration and use distilled water to make final volume of 20 ml (Jigam et al., 2011).

### **Instrumentation**

Glassware (conical flasks, measuring flasks, test tubes, burette, pipette, measuring cylinders, hot plate, reagent bottles, Whatman filter paper # 42 and funnels), oven, 20 ml sterilized plastic tubes, labeled paper envelop, 20 ml syringe, grinder and Atomic Absorption Spectrophotometer (AAS) was used to complete this study.

### **Analysis**

Manganese analysis of samples was approved out by using Atomic Absorption Spectrophotometer Perkin-Elmer AAS-5000 (Perkin-Elmer Corp., 1980) after their digestion. Samples were examined for evaluation of manganese content in all samples. The observed mean concentrations were compared with permissible values and were used for further calculations to evaluate possible risks. Mean content of heavy metal were also observed in soils in which different fodders were grown to compute pollution load index (PLI) across all sites.

Target health quotient (THQ) of manganese in groundwater and selected fodders were calculated according to prescribed standard methods to know whether said crop produce at each site poses a carcinogenic health risk to exposed population or not.

### **Statistical Analysis**

Significance level was found out at probability levels of 0.05, 0.001 and 0.01 as suggested by Steel and Torrie, 2006.

### **Bio-Concentration Factor**

Bio-concentration factor (BCF) is determined using formula worked out by Cui et al., 2004.

$$\text{Bio-concentration factor} = (M)^{\text{Plant}} / (M)^{\text{Soil}}$$

Where,

$(M)^{\text{Plant}}$  = Concentration of metal (mg/kg) in plant

$(M)^{\text{Soil}}$  = Concentration of metal (mg/kg) in soil

### **Pollution Load Index (PLI)**

Pollution Load Index is determined by following formula given by Liu et al., 2005.

$$\text{Pollution Load Index} = \frac{(M)^{\text{IS}}}{(M)^{\text{RS}}}$$

Where,

$(M)^{\text{IS}}$  = Concentration of metal (mg/kg) in investigated soil

$(M)^{\text{RS}}$  = Reference value of metal in soil

Reference value of Mn in soil is 46.75 (Singh et al., 2010a).

### **Estimated Daily Intake (EDI)**

Estimated daily intake of metals (EDI) was computed as given by Yu et al., 2015.

$$\text{EDI} = C_{\text{metal}} \times I \times C_{\text{factor}} / B_{\text{average weight}}$$

Where  $C_{\text{metal}}$  is mean concentration of metal in milk (mg/L),  $I$  is daily intake of milk (kg/day) and its value was taken as kg/person/day.  $C_{\text{factor}}$  is conversion factor (0.085).  $B_{\text{average weight}}$  is average body weight (kg) taken as 60 kg.

### **Target Health Quotient (THQ)**

THQ is described as ratio of estimated daily intake of metals in milk to oral reference dose (RfD) and was calculated with suggestion of Yu et al., 2015.

$$\text{THQ} = \text{EDI} / \text{RfD}$$

EDI = Estimated daily intake of metal

RfD = Oral reference dose

If  $\text{THQ} > 1.0$  for single metal indicates that health of consumers is at high risk or it is carcinogenic (USEPA, 2002). Oral reference dose for Mn is 0.014 (USEPA, 2010).

## RESULTS AND DISCUSSION

### Soil

In soil, Mn level was significantly ( $p < 0.05$ ) influenced by supply of various types of water (Table 2). Results of manganese concentration are shown in Table 3. In soil, its concentration ranged between 2.595 to 10.402 mg/kg. Calculated Mn value was highest in S4 soil and lowest in F2 at Site-III and Site-II, respectively. Transfer of manganese from soil to plants is mainly affected by pH of soil. Acidic soil was considered to be good source of Mn in forage crops. Badly

depleted soils have high concentration of Mn in plants. It is also noticed that with increase in Mn accumulation may cause decrease the ability of fodders to absorb cobalt (Vatansever et al., 2016). Results of this study falls within the range (2.5-31.5 mg/kg) reported by Hassan et al., (2013). Khan et al., (2018) (0.119, 1.415 mg/kg) and Ahmad et al., (2018) (0.60, 0.81 mg/kg) reported the lower level of Mn in control and waste water irrigated soils as compared to present study. Our study reported lower value than permissible maximum limits (80 mg/kg) given by USEPA, 2010.

**Table 2: Analysis of variance of Mn in soil, fodder, water and milk.**

Source	Degree of freedom	Mean Square	
		Soil	Fodder
Sites	2	233.586 <sup>***</sup>	33.118 <sup>***</sup>
Soil	7	14.373 <sup>***</sup>	8.371 <sup>***</sup>
Sites * Soil	14	3.868 <sup>***</sup>	2.545 <sup>***</sup>
Error	72	0.040	0.064

  

Water		
Source of Variation	Degree of freedom	Mean Square
Sites	2	1.267 <sup>ns</sup>
Error	9	0.309

  

Milk		
Source of Variation	Degree of freedom	Mean Square
Sites	2	4.125 <sup>***</sup>
Error	72	0.147

\*, \*\*, \*\*\*: Significant at 0.05, 0.01 and 0.001 levels; ns: non-significant

**Table 3: Concentrations of manganese (mg/kg) in soils.**

Sites	S1	S2	S3	S4	S5	S6	S7	S8
Site-I	4.0368	2.5955	3.3888	3.4572	4.3610	4.9745	2.8892	3.0358
Site-II	8.0475	9.0540	4.0675	9.3600	7.0518	8.7300	6.5415	7.0442
Site-III	9.5637	9.9115	6.1200	10.4025	8.2025	9.5690	8.5795	7.9190

**Table 4: Concentration of manganese (mg/kg) in fodders.**

Sites	F1	F2	F3	F4	F5	F6	F7	F8
Site-I	1.027	0.9218	1.0628	1.0578	1.5802	2.088	1.0152	0.2548
Site-II	3.483	4.0395	1.0058	4.3515	2.4162	3.7208	1.717	2.0385
Site-III	2.027	4.0515	1.0268	4.0368	1.772	4.6532	2.9662	2.88

### **Fodders**

Soil and water exhibit highly significant ( $P < 0.05$ ) results in diverse types of fodders. Range 0.008 to 0.022 mg/kg was reported for Mn content in fodders (Tables 4). Maximum value was reported in F6 at Site-III while least at Site-I in F8 during findings of this study. This value was found to be within permissible limit 30 mg/kg suggested by FAO/WHO (2001). Present Mn results were not seemed to cause any toxicity. Khan et al., (2018) found elevated Mn values (76.65, 115.50 mg/kg) in forages than our results.

Manganese (Mn) is a necessary micronutrient for plants. It plays remarkable role in biosynthesis of, carbohydrate and lipid, photosynthesis and oxidative stress protection. Its deficiency affects plant growth and development, chlorosis and necrosis in tissues (European Commission, 2006).

### **Water**

In this study, Mn level demonstrated non-significant ( $P > 0.05$ ) affects in all water sources used for irrigation of fodder by farmers. Range of Mn concentration in water was found to be 0.1482-1.241 mg/L (Table 5).

**Table 5: Concentration of Mn (mg/L) in water.**

Sites	Mn
Site-I	0.1482
Site-II	0.929
Site-III	1.241

Control site showed lower contamination as compared to other sites. But at site-III, water is more polluted due to addition of city effluents. Mn concentration (0.063 mg/L, 0.067 mg/L) reported by Khan et al., (2018) in tap and sewage water was lower than our values. Metal level under this study was higher than permissible limits of 0.2 mg/L demonstrated by Pescod, 1994. Aurangzeb

et al., (2011) found value 22.007 mg/L for Mn in water which was higher than present investigation.

### **Milk**

It was described that appraisal of mineral profile of milk involved manipulation of fodders consumed by cows and soil in which fodder was cultivated. Therefore, soil-plant system as well as dietary analysis showed highly significant ( $P < 0.05$ ) effect on mineral content of milk. While, range of Mn concentration was found to be 0.164 - 0.9708 mg/L recording 0.9708 mg/L as highest value at Site-III (Table 6).

**Table 6: Concentration of Mn (mg/L) in milk.**

Sites	Mn
Site-I	0.1621
Site-II	0.6334
Site-III	0.9708

Salah et al. (2013) reported the same concentration of Mn (0.497 mg/kg) in milk as in this work. Parween et al., (2016) analyzed milk samples and found that Mn content (0.001-0.002 mg/kg) was lower than our values. Noticeable difference was detected in Mn (1.788 mg/l) in cattle milk of industrial areas Kano suggested by Ogabiela et al., (2011) and these examined values.

### **Bio-Concentration Factor**

Bio-concentration factor of Mn for F8 was lower (0.083) at control Site-I than other fodders. Maximum BCF value (0.4649) of Mn was observed by F4 at Site-III. Although, all examined Mn BCF values were found to be less than 1 (Table 7). BCF values for Mn were found to be in accordance with findings of Alrawiq et al., 2014 (0.221-0.490) due to different water irrigation. Mn has an important job to do in plants systems such as photosynthesis, role in nitrate assimilation and chlorophyll synthesis

**Table 7: Bio-concentration factor for manganese.**

Sites	F1	F2	F3	F4	F5	F6	F7	F8
Site-I	0.254409	0.355153	0.313621	0.30597	0.362348	0.419741	0.351378	0.083932
Site-II	0.432805	0.446156	0.247277	0.464904	0.342636	0.426208	0.262478	0.289387
Site-III	0.211947	0.408768	0.167778	0.388061	0.216032	0.486279	0.345731	0.363682

. Mn plays role in synthesis of chlorophyll, due to this reason their presence is necessary for Photosystem II.

**Pollution Load Index**

Pollution factor of Mn for S2 was lower at control site-I than other soils. Maximum PLI value of Mn was showed by S4 at Site-III (irrigated with wastewater). Examined values of PLI were seen to be within range of 0.0555-0.2225 for all types of soils (Table 8).

Ahmad et al., 2014 found higher PLI values (0.305-0.379) in soil at two different sites in comparison to our results. Khan et al. (2018) reported lower PLI for Mn (0.01, 0.02) in soil irrigated with different sources. All the samples in current study showed PLI <1 for all samples. Mn is a necessary element required for growth of plants, stop yellowing of leaves. Sources of Mn are usually present underground in the form of salts of manganese and high Mn concentration in may contaminate soil samples in this way (Al-Othman et al., 2012).

**Table 8: Pollution load index for manganese.**

Sites	S1	S2	S3	S4	S5	S6	S7	S8
Site-I	0.086349	0.055519	0.072488	0.073951	0.093283	0.106406	0.061801	0.064937
Site-II	0.172139	0.193668	0.087005	0.200214	0.150841	0.186738	0.139925	0.150678
Site-III	0.204571	0.212011	0.130909	0.222513	0.175455	0.204684	0.183519	0.16939

**Estimated Daily Intake (EDI)**

The Daily intake value for Mn was ranged between 0.0027-0.016 mg/kg (Table 9) by milk consumption by humans. Khan et al., (2019) observed similar values of daily intake (0.001-0.009 mg/kg) for manganese in relation with present research. Daily intake of Mn (0.05 mg/kg) recorded by Khan et al. (2013) was higher than this observation. Calculated EDI

values were found to be within tolerable limits.

**Table 9: Estimated Daily Intake for Mn.**

Sites	Mn
Site-I	0.016403
Site-II	0.064094
Site-III	0.098236

### Target Health Quotient (THQ)

THQ for manganese through the intake of cow milk varied from 0.193-1.155 (Table 10). Data showed that THQ for Mn was greater than 1 at one site indicating zinc toxicity. Khan et al. (2015) concluded that THQ for Mn was also found to be more than 1 for vegetables.

THQ value (0.1) for Mn investigated by Salah et al., (2013) was found within the range given by present study. Khan et al. (2019) recorded MnTHQ (0.024-0.219) which was in accordance with variation of our study. Muhibet al. (2016) recorded much lower THQ value (0.004) for Mn.

**Table 10: Target Health Quotient for Mn.**

Sites	Mn
Site-I	0.000229642
Site-II	0.000897317
Site-III	0.0013753

### CONCLUSION

It was concluded that use of wastewater gave maximum growth of various crops because it acts like an organic manure and as well as fertilizer. The concentrations of Mn were within safe limit given by FAO/WHO.BCF, PLI & HRI were less than 1. Results indicated that it is safe to use wastewater as source of mineral but it should not be used for long duration due to excessive accumulation of different heavy metals. This will also cause contamination of food chain.

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