HEAVY METAL CONTAMINATION IN THE SOIL AND FODDER GRASS OF MUSI RIVER REGION, HYDERABAD.

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Received: April 09, 2018 Accepted: May 14, 2018

ABSTRACT *Pollution is a worldwide problem. Heavy metals are well established environmental pollutants of a great concern due to their serious implications in plant, animal and human health. Higher levels of heavy metals in soil, water and animals have been reported in different parts of India. Present study investigates heavy metal contamination of soil and its uptake by fodder grass (Brachiaria mutica) collected from field sites covering areas across the Musi River, Hyderabad. The soil and plant samples were collected from the field and the control sites during the summer season for two consecutive years and analysed for heavy metal content by atomic absorption spectroscopy. The mean concentrations of total metal in study site soil were 24.14±4.14, 60.35±9.33, 8.47±0.60, 106.32±24.09 and 135.09±27.65 mg Kg^{-1} for Cr, Cu, Ni, Pb and Zn respectively. Except Pb, all the other studied metals were within permissible limit for WHO/FAO soil standards. Grass samples had an average metal content of 0.046±0.002, 8.193±0.86, 1.026±0.130, 15.653±0.671 and 61.327±1.283 mg Kg^{-1} for Cr, Cu, Ni, Pb and Zn respectively. The content of Pb in grass samples exceeded the critical toxicity limit for animal feed, indicating a potential risk of food chain accumulation and contamination by this heavy metal.*

Keywords: *Pollution, Heavy Metal, Atomic Absorption Spectroscopy, Food chain*

Introduction:

Heavy metals are chemical elements and their metalloids supposed to be at least five times denser than water and have an atomic number greater than 20. Studies on heavy metal contamination are a highly researched area in the scientific world (Tchounwou, Yedjou, Patlolla, & Sutton, 2012). Chromium, Copper, Cadmium, Lead, Nickel and Zinc are some of the most common analysed metals in soil due to their possible entry into food chain especially by the way of the forage plants grown on these soils(Yan et al., 2012; Balli, Shittu, & Inuwa, 2013; Moreki, Woods, & Nthoiwa, 2013). Some of these metals like Copper(Cu), Zinc(Zn), Manganese (Mn), Nickel(Ni) and Cobalt(Co) are micronutrients necessary for plant growth, while others like Lead (Pb) have no known biological functions (Tangahu et al, 2011).

Multiple factors such as tire wear, brake wear, road abrasion, galvanised fuel tank parts, lubricant oil, exhaust gas and worn out engines made of metal alloys other than traffic volume and urbanization are some of the reasons attributed for the mobility and elevated levels of heavy metals like Pb, Cd, Cu, Zn and Cr in atmosphere (Yan et al., 2012; Venkateshwarlua, Tejavath Nageswra Rao, Dasharamb, 2014).

Heavy metal gets accumulated in animals consuming contaminated fodder which is supposed to have a significant impact on livestock health through the long term toxic effects via metabolic interference. Human beings are the likely recipients of its toxic effects being the potential livestock consumers (Gall, Boyd, & Rajakaruna, 2015). Metals like Cu, Ni, Cr and Zn are known to cause deleterious health effects in human where as Pb is classified as human carcinogens (known or probable) by the U.S. EPA, and the International Agency for Research on Cancer(Singh, Gautam, Mishra, & Gupta, 2011; Tchounwou et al, 2012).

Hyderabad has a hot semi-arid climate which limits the quality and quantity of forage and grazing activity during the dry periods dependent mostly on Musi river waters (Van Rooijen, Biggs, Smout, & Drechsel, 2010). The Musi river water quality flowing through the city of Hyderabad is reported to be deteriorating with continuous inflows of waste water from industries and urban generated sewage water released into it. When used as a dependent water source for irrigation, a reduction in yield of major crops like paddy along with human health implications in the form of stomach pain, body pains, anaemia and skin allergies has been reported (Buechler & Mekala, 2002; Cheepi,2012). The soil and vegetables grown in peri urban and urban areas irrigated with Musi River water have been observed to contain heavy metals (Mekala, Davidson, Samad, & Boland, 2007; Sekhar Chary, Kamala, Shanker & Frank, 2005; Karamtothu, Devi, & Naik, 2015).

The present study was conducted to know the extent of heavy metal (Cr, Cu, Ni, Pb and Zn) contamination of soil and its uptake by fodder grass (*Brachiaria mutica*) which is one of the highly cultivated pasture plants around the Musi river region. With high transport connectivity and a feasible market around the study sites, long term pastoral activity cannot be completely ruled out. This plant has been widely researched upon for its higher tolerance to excessive concentrations of Pb highlighting the potential hazard to the free grazing animals and cattle fed with such contaminated fodder grass (Khan et al, 2018). Thus knowing the contamination levels in soil and fodder grass for the heavy metals (Cr, Cu, Ni, Pb and Zn) at the study sites will provide base information for its safe use.

Material and Methodology

Sampling Sites

The GPS coordinates for the sampling areas is between 17°22'05.1"N 78°27'33.5"E and 17°22'46.2"N 78°29'33.0"E. The total length along the river stream covered was approximately 4.06 Km. Six sampling sites were included in the study ,of which four had natural pastures where cattle, goats and sheep are grazed freely and forage harvesting for subsistence have been observed in the recent past. The six sites have been indicated in the Fig.1.

Control samples were collected from site behind R.I.T.S campus, Chevella (17°18'40.1"N 78°06'33.5"E) which was free of obvious sources of pollution and where normal irrigation was practiced with treated water.

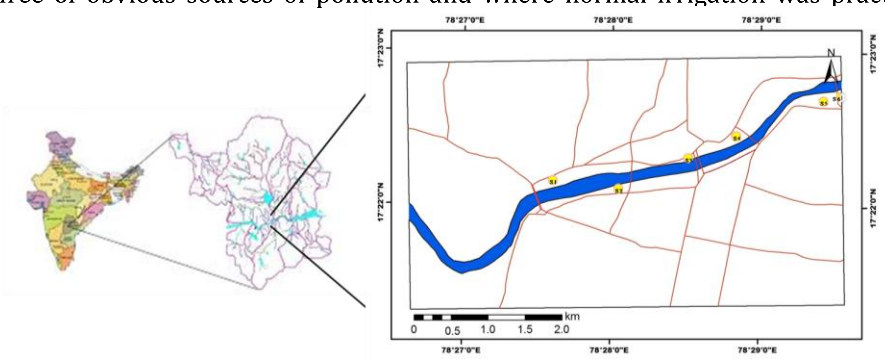

Figure 1- Location map of study area

Sample Collection-

Sampling was done in summer seasons of 2016 and 2017. The surface soil samples (15–20 cm depth) were collected from the study area with stainless steel hand trowel; 1 kg of soil was collected for each sample. Plant and soil samples were obtained with around 10-15 spots taken for one composite sample across each study site area. The soil subsamples were made by cone and quart method.

Preparation of sample for analysis-

Soil:

After removal of large stones the soil samples were spread on glass plates, air dried, mechanically ground to fine powder and sieved through 100 mesh (150 µm). It was then oven dried at 100±1° C for 2 hrs and stored in clean polythene bags for further analysis. Hydrogen Ion concentration (pH) was analyzed using on pH meter (M/s. EUTECH Instruments PC650), using standard procedures (IS: 2720, 1973).

For acid digestion of samples 1 gm of sieved material was treated with HNO_3, H_2O_2 and HCl in a stepwise procedure recommended by Environmental Protection Agency (EPA, Method 3050B). After digestion the samples were cooled, filtered into pre-cleaned plastic bottles using Whatmann filter paper 40, made up to 50 ml using Milli Q water and stored until analysed for metal content.

Plant Material:

Test plants were thoroughly cleaned and washed under a running tap water followed by distilled water to remove elements like dust, dirt and possible parasite or their eggs adsorbed to it. Samples were cut into small pieces; air dried for 2 days and kept in hot air oven at 60°C ± 5°C to obtain a constant weight. Dried samples were grounded to powder, passed through a 1mm steel mesh, and then mixed to homogenize and stored in polythene bags.

Dried sample material was digested by a modified method describe by Rodríguez-Seijo, Lago-Vila, Andrade, and Vega (2016). For digestion 6 ml of concentrated nitric acid and 2 ml of hydrogen peroxide was added to 0.2 g of sieved plant material. The mixture was heated at 60ºC for 2-3 hours, until fumes stop and resulting solution is clear. The digested samples were cooled, filtered using Whatman filter paper no. 40 into pre-cleaned plastic bottles, made upto 50 ml using Milli Q water and stored until analysed for metal content.

The concentrations of the elements in both plant and soil samples was determined using Atomic Absorption Spectrophotometer (Make: GBC-932AA) with the analyses being done in triplicate. The results were expressed in mg Kg^{-1} dry weight.

Quality Assurance (QA) and Quality Control (QC):

Reference standards from M/s. Inorganic Ventures, USA, Analytical reagent (AR) grade chemicals and Milli Q water were used throughout the study. Nitric acid samples were digested without the addition of any plant material, which acts as the reagent blank for plant samples, was used to check the contamination possibility from the glassware or the acid. QC is carried out at every 10 – 15 samples intervals.

Data analysis

The mean levels, range and standard deviation of the metals under study in soil and fodder grasses are compared with the control samples and WHO/FAO permissible limits in irrigation soil and assessed for its safe use comparing with critical levels for toxicity to plants and animals. The summary for the obtained results are presented in table 1 and 2 and the distributions of each metal across the five sampling stations are presented in figures 2-6.

Data analysis was done to know the association between metals in soil and plants in all the present study samples using Karl Pearson's correlation (significance tested at α = 0.05 and 0.01) and the result are summarized in table 3 and 4. The analysis of variance ANOVA (anoVa) was used for comparison sources of variation for heavy metal (Pb, Cu, Cr, Ni and Zn) data in soil with sampling year and source (number of bridges nearest to site) as two factors. Probabilities less than 0.05 ($p<0.05$) were considered as statistically significant. All statistical analyses were done by SPSS software 17.0 for windows.

Results

Descriptive Statistics –Soil

Analysis of soil type indicated it as brownish sandy soil with black clay subsoil. pH of the studied sites was in the range of 7.9 to 8.3. Table 1 shows descriptive analysis of data on soil for metal content in mg Kg^{-1}.

Table 1- Analysis of data on soil (mg Kg^{-1})

Metal	Present study			WHO/FAO Permissible Value for irrigation soil *
	Control Site Mean ± S.D	Field Site Range	Field Site Mean ± S.D	
Cr	9.56±0.16	16.97-28.34	24.14±4.14	100
Cu	17.21±0.71	42-68.89	60.35±9.33	100
Ni	3.16±0.25	7.64-9.46	8.47±0.60	50
Pb	22.55±1.20	67.52-129.4	106.32±24.09	100
Zn	29.91±0.31	78.38-153.41	135.09±27.65	300

*Chiroma, Ebewele, & Hymore, 2014

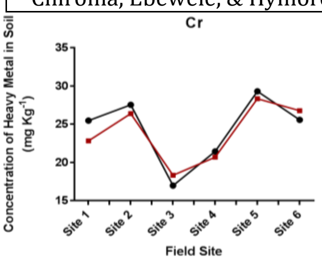

Figure 2-Concentration of Chromium in soil.

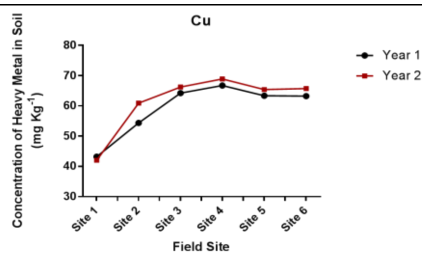

Figure 3-Concentration of Copper in soil.

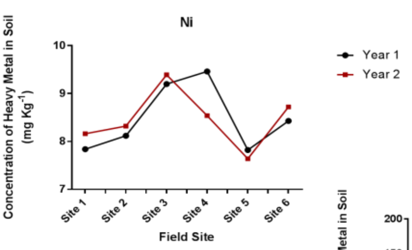

Figure 4-Concentration of Nickel in soil.

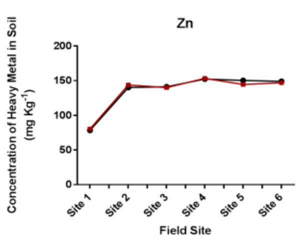

Figure 6-Concentration of Zinc in soil.

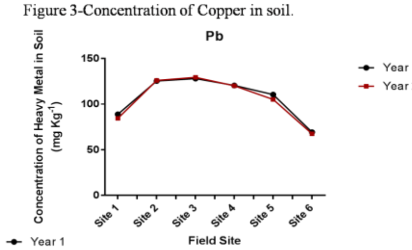

Figure 5-Concentration of Lead in soil.

Figure 2-6: Site wise and Year wise distribution of Heavy Metals (Cr, Cu, Ni, Pb and Zn) in

Descriptive Statistics – Fodder Plant

The concentrations of total Cr, Cu, Ni and Zn were also higher in the vegetation samples of study area compared to the control. The summary of obtained results of heavy metals in plants for control and study sites is presented in table 2.

Table 2-Analysis of data on metal in fodder plant (mg Kg^{-1})

Metal	Present Study		
	Control Fodder Mean ± S.D	Musi Fodder Range	Musi Fodder Mean ± S.D
Cr	0.03±0.005	0.027- 0.073	0.046±0.017
Cu	4.75±0.127	4.67-14.00	8.193±2.854
Ni	0.36±0.006	0.015 -2.34	1.027±0.715
Pb	5.65±0.339	9.51-23.96	15.653±5.660
Zn	16.95±0.919	51.02-83	61.326±14.014

Correlation Studies in Soil-

Table 3- Correlation coefficients between metals in soil

	Cr	Cu	Ni	Pb	Zn
Cr	1				
Cu	-0.183	1			
Ni	-.880*	0.517	1		
Pb	-0.400	0.315	0.340	1	
Zn	0.023	.956**	0.370	0.339	1

* Correlation is significant at the 0.05 level (2-tailed).
** Correlation is significant at the 0.01 level (2-tailed).

Results for correlation analysis of metal in soil, presented in table 3, gave an inverse correlation of Chromium with Cu, Pb, Ni and a weak positive correlation with Zinc. Nickel with respect to other metals (Pb, Cu and Zn) had a moderate positive correlation, similar results were observed for metal lead with respect to Cu, Ni, Zn. The correlation coefficient between Copper-Zinc was found to be significant at α 0.01.

Correlation Studies of Metal in Soil and Fodder Plant-

Table 4- Correlation coefficients between metals in soil and grass

	Cr(Soil)	Cr(Grass)	Cu(Soil)	Cu(Grass)	Ni(Soil)	Ni(Grass)	Pb(Soil)	Pb(Grass)	Zn(Soil)	Zn(Grass)
Cr(Soil)	1									
Cr(Grass)	-0.455	1								
Cu(Soil)	-0.542	-0.152	1							
Cu(Grass)	-0.853	0.067	0.355	1						
Ni(Soil)	-.986*	0.368	0.477	0.926	1					
Ni(Grass)	-0.842	0.451	0.043	0.892	0.897	1				
Pb(Soil)	-0.687	0.502	-0.216	0.772	0.754	.966*	1			
Pb(Grass)	-0.769	0.846	-0.055	0.574	0.751	0.858	0.865	1		
Zn(Soil)	0.101	-0.87	0.62	0.109	-0.067	-0.352	-0.521	-0.708	1	
Zn(Grass)	0.369	0.113	-.963*	-0.098	-0.272	0.18	0.421	0.166	-0.575	1

* Correlation is significant at the 0.05 level (2-tailed).

Correlation analysis result for data on metal concentrations in soil and grass plant (table 4), indicated a positive correlation for Ni (soil)-Ni (grass), Cu(soil)-Cu(grass), Pb(soil)- Pb(grass) and a negative correlation for Cr (soil)-Cr (grass), Zn(soil)-Zn(grass). The correlation between Cu (soil) - Zn (grass) was -0.963 and Pb (soil) - Ni (grass) was 0.966, which were statistically significant at $p < 0.05$.

ANOVA analysis-

On performing two factor ANOVA analysis (Bridges and Year as two factors) metal Copper showed significant differences ($p<0.01$) between sampling points (a) for soil and grass samples and also significant differences between the sampling period (B) of grass samples and also significant interaction ($p<0.01$) between sampling points and sampling periods (axB) for soil samples was observed.

DISCUSSION

Heavy metal content in soil:

pH is one of the most important effective parameters on biosorption processes. When compared to control sites similar range of pH was observed indicating sewage contaminated waters had no significant affect on soil pH at the sites under study. Alkaline pH of the soil upto pH 8.8 makes the metals like Cu, Pb and Ni slightly mobile enriching them in soil which in the long run creates related problems (Parth, Murthy, & Saxena, 2011).

The concentrations of heavy metals in soil (mg Kg^{-1}) as indicated in fig 2-6 ranged between 16.97 – 28.34, 42-68.89, 7.64-9.46, 67.52-129.4, 78.38- 153.41 for Cr, Cu, Ni, Pb and Zn respectively among the study sites. The results indicated in table 1 and fig 2-6 emphasizes that there was a non uniform trend observed amongst the sites but the elements concentrations were within the WHO/FAO permissible values for irrigation soils except for metal lead. At the sites (S2 to S5), the irrigation was seen to be carried out using pumps for pulling water from the Musi River. The soil metal concentrations were higher at these sites compared to site S1 and S6 where irrigation is not a practise. The metal content in soil, at all the studied sites was higher than control sites where normal irrigation was practised. The metal load is therefore elevated due to the use of Musi river waters which is popularly known as sewage drain more for its deteriorating status. The trend of the metals in soil was as follows: Pb > Cu > Zn > Cr > Ni.

Dust fall containing upto 20% toxic heavy metals like Cu, Cr, Pb and Zn is considered a non point source, polluting nearby soil and water bodies through the storm water runoff and drains (Prestes, Anjos, Sodré, & Grassi, 2006; Trujillo-González, Torres-Mora, Keesstra, Brevik, & Jiménez-Ballesta, 2016). Road dust in the areas near to the present study sites have been reported by several researcher to be elevated on metals like Cu and Pb which is attributed to the irregular and high density of vehicular activity here.(Venkateshwarlua et al, 2014; Mathur, Balaram, Satyanarayanan, & Sawant, 2016). Urban runoff and storm water drains which are bound to be metal rich are elevating the metal load in Musi river and its surrounding and is rationalized as a reason for higher metal content in the soils irrigated with Musi waters compared to the control sites.

Heavy metal content in plant:

The content of heavy metals in plants is dependent both on plant species and climatic conditions (Tangahu et al, 2011). The results for heavy metal content in study plant (*Brachiara mutica*) were compared with the normal and critical ranges of heavy metals in plants to assess its toxicity levels.

The normal plant concentrations are 4-12 mg Kg^{-1} for Cu, 0.01-1 mg Kg^{-1} for Cr and 0.13-1.1 mg Kg^{-1} for Ni. For plant to experience heavy metal toxicity, the critical levels are 20-30, 10-18 and 2-50 mg Kg^{-1} for Cu, Cr and Ni respectively (Onder, Dursun, Gezgin, & Demirbas, 2007; Sadeghi, S. A. T., Sadeghi, M. H., & Dashtizadeh, 2014). The average content of copper, chromium and nickel in the field and control plants was observed to be in the safe and normal metal concentration ranges.

Heavy metal lead is found to get easily absorbed and accumulated in different plant parts although it is not an essential element. In the present study, the average concentration of lead in fodder plant from field sites was 15.653±5.660 mg Kg^{-1}.This value is much higher than normal range of 5-10 mg Pb per Kg dry matter, but within the plant toxicity level range of 25 mg Kg^{-1} (Sekhar et al., 2005; Moreki et al., 2013)

The feed from grassland, are assumed to have 50–100 mg Zinc metal per Kg dry matter and the concentrations greater than 100 are found toxic for plant health. (Onder, et al., 2007; Laţo, A., Radulov, Berbecea, Laţo, K., & Crista, 2012). The concentration of Zn in the fodder from present study sites ranged between 51-83 mg Zn per Kg of dry matter, which was within the expected and safe range of feed from grasslands.

Correlation and ANOVA Studies for metal content in Soil and Plant:

In correlation matrix for metal content in soil, a significant positive correlation was observed between Cu (soil) –Zn (soil) at α 0.01 indicating a common source or activity leading to its elevated levels. Result for correlation analysis on metal concentrations in soil and grass plant (table 4), indicated metals like Cu, Ni and Pb are positively correlated and a direct proportionality between the metals in soil and uptake by the plant is observed. Further the correlation coefficient values of Cu (soil) - Zn (grass) and Pb (soil) - Ni (grass) suggest that the study plant is balancing metals Ni and Zn in the required ranges needed for plant.

Optimal levels of Ni and lower values of Cr are found beneficial for animal development and are suggested for animal diets (Ahmad et al., 2009; Balli et al., 2013). The correlation between Cr (grass)-Ni (grass) was negative and was found to be significant at $p< 0.05$, thus a favour in the balance of nutrient in animal feed was observed.

A significant ($p<0.05$) value in F test for copper metal was observed both for year and source of sampling, indicating number of bridges close to the sampling point and copper concentration in the soils were in a direct relationship. However for the other metals a significant F-test result was not obtained indicating that the same source does not exists for all metals under study. The low degree of positive correlation (0.35) between Cu (grass) and Cu (Soil) is indicative of the fact that this micronutrient for plants will be taken up as per the need of plant even if the metal is elevated in soil.

Potential Health Risk Due To Heavy Metal Contaminated Soil Exposure and Fodder Intake:

The analysis of data on metal content in study plant (*B.mutica*) indicated that except of metal Lead this plant is free of Cr, Cu, Ni and Zn metal contamination. Also it was found to be in safe range from exerting toxicity effects in the plant due to any of the metals presently studied.

Safety of *B.mutica* as animal feed-

The lower limits of critical concentration of heavy metal through animal feed is supposed to be 50 mg Cr, 50 mg Ni and 300 mg Zn, all in mg Kg^{-1}dry matter (Sekhar et al , 2006). The average concentration of these metals in the studied fodder plant was found to be within the minimum critical value and hence safe for animal consumption with respect to Cr, Ni and Zn metal.

Copper deficient pastures through several scientific studies are related to conditions of defective keratinisation of sheep wool and reduced fertility in cattle. A concentration of 7-14 mg Kg^{-1} of Cu is recommended through the pasture feed and single feed source having content between 15-30 mg $Kg^{-1}$is found toxic for animal health (Underwood, 2012). The average content of copper in the present fodder plant was 8.193±2.854 mg Kg^{-1}which was well within the recommended range for animal feed and the observed values of Cu content in plant was much below the levels needed to exert toxicity in animal.

The recommended levels of heavy metal lead in cattle feed ration is 1-8 mg Kg^{-1} and values above these are found to be toxic(Gall et al., 2015). The average concentration of Pb in fodder grass from the studied sites (15.652±0.671 mg kg^{-1}) was two-fold higher than recommended standard in animal feed. Studies have shown higher content of Pb in milk of cattle fed with fodder grass (*Panicum maximum*) cultivated on lead polluted soils of Musi river region (Sekhar et al., 2005) As the fodder grass under present study (*Brachiara mutica*) is closely speciated to *Panicum maximum* it also has a the potential of bioaccumulation and biomagnification in the animal grazing on them or being fed with them.

Thus the fodder from the study area can be considered as high risk category for lead metal and a toxicological risk to livestock and its dependent consumers.

Risk to human health:

Soil from sites S1 and S6 had an average lead metal concentration of 86.71±3.203 and 68.42±1.272 mg Kg^{-1}in respectively. These sites are in very close vicinity to human habitation. According to World Health Organization (WHO, 2006) guidelines the maximum tolerable soil concentrations of lead as a toxic chemicals based on human health protection is 84 mg Kg^{-1}. Therefore sites(S1 and S6) are to be addressed for human safety measures due to direct risk of exposure to metal lead through soil.

As human beings are the potential consumers of the livestock and their products, an indirect risk to human health is through the soil and fodder from the four studied sites (S2 to S4), which are contaminated with metal lead and are likely to enter the food chain.

CONCLUSION

Site location (lands close to roadways) and environmental factors (urban runoff, storm water drains, air borne pollutants and irrigation using sewage mixed waters) is intensifying the content of heavy metal Lead in soil and *Brachiara mutica* grass plant from Musi river region. The studied area has both direct and indirect risk of heavy metal lead exposure to humans, which have implications to human health and quality of life. The present study concludes that Lead metal is a harmful pollutant and human exposure to such sources should be minimized. A remediation method for soil is proposed to be sought at the study area for risk mitigation.

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