

# Heavy metals accumulation in forages and bufalo hair on fooded pasture in South Sumatra, Indonesia

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# Heavy metals accumulation in forages and buffalo hair on flooded pasture in South Sumatra, Indonesia

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## Abstract

Lowlands hold important potential for sustainable agriculture in South Sumatra. High acidity of soil and water and practice of domestic waste disposal in the area could be associated with a higher level of micro minerals in forages and grazing animals. This study aimed to evaluate the concentration of copper (Cu), manganese (Mn), zinc (Zn), iron (Fe), lead (Pb), and cadmium (Cd) in the water, forage, and buffalo hair, to compare the concentration of the metals in the forage on residential area and grazing area and to evaluate relationships between age and concentrations of the heavy metals in the buffaloes' hair. The concentrations of the minerals were Fe > Mn > Zn > Pb > Cu > Cd in the water, Fe > Mn > Zn > Pb > Cu > Cd in the forage and Fe > Mn > Cu > Zn > Pb > Cd in the buffalo hair. Concentrations of Pb in water and forages exceeded the permissible limits for drinking water and feeds of livestock. The levels of the heavy metals in forages on the residential and grazing areas were not different and no significant correlation between ages of the animals and concentrations of the metals in the hair. In the forages, Cu–Mn and Fe–Pb while in the hair Cu, Zn and Pb strongly correlated. The principal component analysis revealed that the levels of metals concentration in the forages and buffalo hair associated with two main factors of natural resources.

**Keywords** Acid water and soil · Heavy metals accumulation · Lowlands farming

## Abbreviations

ANOVA	Analysis of variance
Cd	Cadmium
Cu	Copper
FAO	Food and Agriculture Organization
Fe	Iron
HNO <sub>3</sub>	Nitric acid
Mn	Manganese
<i>n</i>	Number
<i>P</i>	Probability
Pb	Lead
PC	Principal component
Zn	Zinc

## Introduction

Lowlands are estimated to be 25% of the total land area in South Sumatra province thus plays an important role in sustainable food security. The landscape is mainly characterized by the high acidity of soil and water and periodic inundation during the rainy season (World Bank 2018). Studies on crops show that low production was related to the low availability of macro-minerals and the presence of excess or toxicity of iron (Fe) and manganese (Mn) (Sahrawat 2004; Noor 2007). The lower pH and higher concentration of the micro minerals in the water result from pyrite oxidation when the acidic soil is drained (Dent 1986; Manders et al. 2002).

Swamp buffalo farming is an important aspect of lowlands especially on deep freshwater swamp areas where crop cultivation is limited by high water level. The low pH of soil and water was related to a deficiency of macro-minerals and excess of micro minerals in the pasture for grazing buffalo (Ali et al. 2013, 2019). Trace and toxic metals may accumulate in the forage and grazing buffalo. However, studies on this aspect especially on flooded pasture are limited. Previous studies showed that availability and plant uptake of copper (Cu), lead (Pb), zinc (Zn), and cadmium (Cd) increased as pH declined (Bang and Hesterberg 2004; Mühlbachová et al. 2005; Zeng et al. 2011).

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Human activities around where facilities for domestic sewage and rubbish processing are not available may contribute to the released of the metals into the environment. A previous study in the flooded pasture showed that concentrations of Cu, Fe, and Mn in forages exceed upper limits for grazing ruminants (Ali et al. 2019) which could be attributed to the ability of several aquatic plants to accumulate the heavy metals from polluted water (Núñez et al. 2011; Veschasit et al. 2012; Wahab et al. 2014).

Concentrations of heavy metals in the hair may serve as a good indicator of heavy metals accumulation in the animal. A study of Rashed and Soltan (2005) with Fe, Mn, Pb and Cd in the hair of goat, sheep, and camel reported relationships between concentrations of the metals in hair and the concentration in forage and soil, while Fe and Mn in the hair showed a strong relation. Cow's hair from a polluted area had a higher level of Cd and Pb than those from an unpolluted area and the Cd level correlated with Cd level in the blood (Patra et al. 2007). A positive correlation between Pb concentrations in cow hair and milk was reported (Gabryszuk et al. 2010). Thus, the objectives of the present study were to evaluate the concentration of Cu, Mn, Zn, Fe, Pb, and Cd in the water, forage, and buffalo hair, to compare the concentration of the metals in the forage on the residential and pasture areas, and to evaluate relationships between age and concentrations of the heavy metals in the buffaloes' hair.

The sampling of water, forage, and buffalo hair was carried out from August to October 2019. Chemical and data analysis was completed in 2020. The locations of the sampling are shown in Fig. 1.

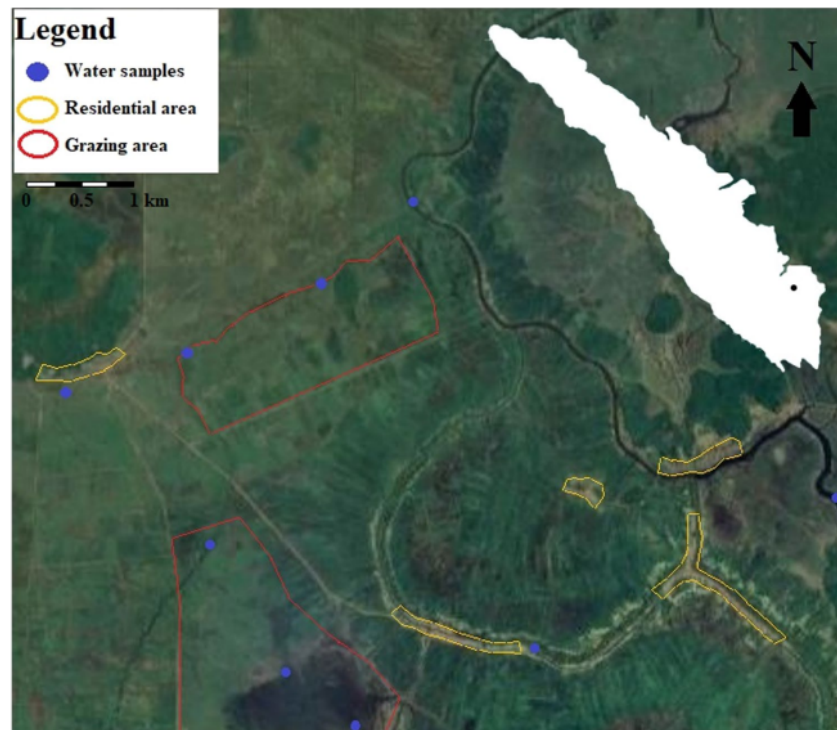
## 5 Materials and methods

### Study area

The study area is a freshwater swamp part of the Batang Hari river watershed and administrated to Ogan Komering Ilir district. The soil is acid fluvipl soil which periodically waterlogged during the rainy season with low availability of calcium, phosphorus, and magnesium and high solubility of Fe and Zn. The study areas are seasonally inundated from three to eight months. The rainy season normally extends from October to March and the dry season occurs from April to September with an annual rainfall 2100–3264 mm.

The housing area of residences is located on the left and right side of the village road. Facilitation for the domestic wastewater processing is not available while domestic waste mostly placed on the abandoned areas or on the roadside at the end of the village. The residential area located in the shallow area of swamp thus has less period of flooding than the grazing area. Grazing area is communal grazing land located in deeper swamp areas

**Fig. 1** Map of Sumatra and satellite image of study location (background image from Google Maps (online), Google, DigitalGlobe, 2020)



and inundated eight to ten months per year where natural species of forages grow without the application of artificial fertilizers. Both housing and grazing areas are reachable by grazing buffaloes.

The landscape of the grazing area exhibits typical aquatic grassland with a scattered population of *Mimosa pigra* and *Maleleuca sp.* The undergrowth is dominated by *Oryza rufipogon*, *Eleocharis dulcis*, *Ischaemum rugosum*, and *Urochloa mutica*. Farming communities of the study area practice seasonal paddy farming in the shallow and middle of the swamp when the water level is decreasing in June to September resulting in shrinkage of grazing area in the dry season.

### Water sampling

Water samples were collected on nine sampling sites (Fig. 1). Water pH was measured on the sites (HI98130, Hanna Instrument). The water samples were filtered with Whatman paper (90 mm Ø) to obtain a 100 ml volume.

### Collection of forage samples

Native plant species consumed by grazing buffalo were sampled included *Mimosa pigra*, *Sesbania exasperata*, *Neptunia oleracea*, *Aeschynomene sensitiva*, *Urochloa mutica*, *Leersia hexandra*, *Hymenoclypeus amplexicaulis*, *Ischaemum rugosum*, *Oryza rufipogon*, *Actinoscirpus grossus*, *Scleria gaertneri*, *Eleocharis dulcis*, *Ludwigia peploides*, *Echinochloa crasipies* and *Ipomea aquatica*. In each plot (100 × 100 m), the aerial part of the vegetation was collected. Samples of stems and leaves of herbaceous plants in the pre-flowering stage and younger twigs of the shrubs were cut by a sharp cutter and placed in the zip plastic bags then pooled per species per plot. After pooling, 78 and 27 samples were obtained on the grazing and residential areas, respectively. The samples (200 g) were washed with tap water before being washed by distilled water then chopped to 5 cm particle size. The samples were transferred to paper bags and then oven-dried at 50 °C for 72 h and milled to pass a 1 mm screen.

### Hair sampling

Buffalo hair samples were collected from male ( $n=17$ ) and female ( $n=82$ ) buffaloes aged 6 to 72 months old raised in the study area. The hair (10 g) was always collected from the same part of the withers and neck of the animals using stainless scissors. Ethyl alcohol was used for cleaning the scissors. The hair samples were washed with tap water and cleaned from foreign materials before being washed in distilled water. After that, the samples were rinsed with acetone for 5 min and then oven-dried at 50 °C for 72 h. In addition, an interview with farmers was conducted to ensure that the animals were raised in this area and to collect the data of the animal's age.

### Chemical analysis of samples

An amount of 1 ml of HNO<sub>3</sub> (65%) was added to the water samples and then heated at 90 °C for 2 h. After cooling, the samples were filtered using 0.2-µm filters for analyses. An amount of the sample (5 and 2 g of forage and hair sample, respectively) was added to 10 ml of concentrated HNO<sub>3</sub> (65%). The mixture was moved to an autoclave for 66 min at 132 °C for digestion. The concentration of the metals was determined by a Shimadzu AA 680 flame atomic absorption. All analyses were performed in triplicate. For each heavy metal, calibration standards were prepared from the stock solution. Concerning the higher concentrations of Fe and Mn in the hair samples, potential contaminations from soil and water to hair samples were accounted for by repetitions of measurement to five samples.

### Data analysis

For statistical analyses, a value of half the detection limit was assigned when the concentration was less than the detection limits. The normal distribution of residual data was checked by the Kolmogorov–Smirnov test. Before the analyses were log-transformed. The Analysis of variance (ANOVA) was used to test the significance of differences in metal accumulation of the forage between the two areas. The average values of the data are presented as geometric means and correlations were calculated by Pearson correlation analysis. Principal component (PC) analysis based on factor analysis was applied for source identification with varimax rotation for factor loading. Statistical analyses were carried out with R 3.6.1.

## Results and discussion

### Heavy metals concentrations in water and forage

The means and ranges of the heavy metals concentrations in water and forage are presented in Table 1. The means and ranges of Mn and Pb in the water and forage exceeded the permissible values, while the means of Cu, Zn, Fe, and Cd were lower than the standard. The high concentration of toxic Pb in water and forage needs serious attention to the health and production of grazing animals. The sampling was conducted in the dry season when the concentrations in the water were always higher than in rainy seasons. However, the lower concentration in the rainy season could not be interpreted as a less toxic effect for animals since the water and forages are the main sources of intake. The Pb concentrations ranged from 3.5 to 23.3 mg/kg in the *I. Aquatica* that exceeded the permissible limit of Food and Agriculture Organization (FAO 2015) in leafy vegetable (0.3 mg/kg). This also presents a potential health problem for villagers since the vegetable is commonly collected and sold in local markets.



**Table 1** Concentration of Cu, Mn, Zn, Fe, Pb and Cd in water (mg/L) and forages (mg/kg) and their permissible limits

	Water		Forages		Permissible limits	
	GM	Range	GM	Range	Water <sup>a</sup>	Forages <sup>b</sup>
Cu	0.02	0.019–0.023	0.53	0.209–0.950	1	35
Mn	0.10	0.094–0.098	216.54	36.044–415.917	0.05 <sup>c</sup>	150
Zn	0.08	0.080–0.086	18.10	7.817–38.534	25	100
Fe	0.42	0.211–0.236	77.29	27.755–171.367	2	750
Pb	0.06	0.043–0.077	9.42	0.609–27.831	0.05	5
Cd	0.0043	0.0036–0.0051	0.0031	ND–0.0147	0.05	1

GM geometric mean, ND not detected

<sup>a</sup>For Livestock, United States Environmental Protection Agency (Bagley et al. 1997)

<sup>b</sup>European Union legislation (Hejna et al. 2018)

<sup>c</sup>Food and Agriculture Organization (2002)

**Table 2** Concentration of Cu, Mn, Zn, Fe, Pb (mg/kg) and Cd (µg/kg) in forages on grazing and residential area

	Grazing, n = 78		Residence, n = 27		P
	GM	Range	GM	Range	
Cu	0.54	0.209–0.951	0.52	0.233–0.951	0.469
Mn	224.23	58.325–399.626	205.92	36.044–415.918	0.390
Zn	18.80	9.372–38.534	17.13	7.817–38.056	0.159
Fe	79.63	27.755–171.368	74.04	29.292–151.044	0.463
Pb	9.78	0.609–27.832	8.93	3.489–19.986	0.425
Cd	4.24	0.003–14.776	1.93	0.003–13.325	0.105

GM geometric mean, n number of samples analyzed, P probability

Higher Pb concentrations of the metals were found in the floating plants compared to the rooted plant ( $P=0.03$ ) revealed the bioaccumulation of the metals in the floating plants of the previous study such as *E. crasipies* (Núñez et al. 2011), *N. oleracea*, and *I. aquatica* (Veschasit et al. 2012; Wah<sup>7</sup> et al. 2014). The order of element concentrations was  $Fe > Mn > Zn > Pb > Cu > Cd$  for the water and  $Mn > Fe > Zn > Pb > Cu > Cd$  for the forages. The pH value measured in the water ranged from 3.5 to 5.1 (data not shown). The highest concentration of Fe and Mn in the forages was also reported in the previous studies (Rashed and Soltan 2005; Ali et al. 2019) and was considered as a toxic level for crops (Sahrawat 2004) and water biota (Manders et al. 2002) and related to the higher solubility in the acid water (Bang and Hesterberg 2004; Mühlbachová et al. 2005).

Table 2 presents non-significant differences in the concentration of the elements of forages on grazing and residential area. The household activities, mainly the uncontrolled rubbish disposal, did not result in a higher concentration of the metals in the forages. The Pearson correlation analysis revealed significant ( $P < 0.05$ ) positive correlations among Cu, Mn, Zn, Fe, and Pb concentration of forages (Table 3). The Mn concentrations correlated with the concentrations of Cu, Zn, Fe, while the concentrations of Pb and Zn correlated with the concentrations of Fe

**Table 3** Pearson's correlation among heavy metals in the forages

	Cu	Mn	Zn	Fe	Pb	Cd
Cu	1					
Mn	0.81**	1				
Zn	0.63**	0.78**	1			
Fe	0.43*	0.78**	0.70**	1		
Pb	0.55*	0.82**	0.74**	0.90**	1	
Cd	0.36*	0.36*	0.30*	0.22	0.28*	1

\*\*Correlation is significant at 0.01 level

\*Correlation is significant at 0.05 level

and Pb. In the PC analysis (Table 4), two principal components were obtained and the first two components accounted for 76.5% of variances of data. The greater contribution to the variation in the first component was Cu and Mn, whereas Fe and Pb in the second component.

The result of ANOVA does not reflect a non-significant effect of the antropogenic activities to the concentration of metals. The effect could not differ in the different locations though the intens of human activities are different between the two areas. However, the acid water and soil, the period of flooding, and the higher concentration of the metals in surrounding water need to be accounted for. The PC analysis grouped the source of variation. However, since non-significant influence of the locally human activities, natural processes such as acidity and flooding may more dominate the source of the variation of the heavy metals concentration in the forages.

### Heavy metals concentrations in buffaloes hair

The concentrations of Cu, Mn, Zn, Fe, Pb, and Cd measured in the hair of the buffaloes sampled in this study are summarized in Table 5. The order of metal concentrations was  $Fe > Mn > Cu > Zn > Pb > Cd$ . The concentrations of Cu, Mn, Fe, and Pb were out of the normal range, while Zn concentrations

**Table 4** Factor loading for selected heavy metals in forages

	Factor 1	Factor 2
Cu	<b>0.88<sup>a</sup></b>	0.26
Mn	<b>0.77</b>	0.57
Zn	0.65	0.51
Fe	0.32	<b>0.94</b>
Pb	0.46	<b>0.73</b>
Cd	0.53	0.34
% of variance	39.90	36.60
Cumulative %	39.90	76.50

<sup>a</sup>Values of dominant elements in each factor are indicated bold

**Table 5** Concentration of Cu, Mn, Zn, Fe, Pb (mg/kg) and Cd ( $\mu\text{g}/\text{kg}$ ) in buffalo hair and values cited in the literature

	Hair		Literature values
	GM	Range	
Cu	18.37	1.521–71.252	6.7–32.0 <sup>a</sup>
Mn	122.55	24.264–594.541	0.5–1.32 <sup>a</sup>
Zn	10.84	2.498–32.852	100–150 <sup>a</sup>
Fe	1320.77	61.938–14,737.46	59–200 <sup>a</sup>
Pb	1.15	0.000–36.459	0.0003–0.033 <sup>b</sup>
Cd	ND	ND	0.004–2.700 <sup>b</sup>

In cow hair <sup>a</sup>Puls (1994), <sup>b</sup>Gabryszuk et al. (2010) and Miroshnikov et al. (2019), ND non detected

were in the normal range of cow hair (Puls 1994). The concentrations of Cu were above the acceptable range in 24.8% animals and below the lower acceptable range in 4.8% animals and Fe concentrations were above the acceptable range in 88.0% animals. Comparing to the previous studies in hair of cows, Pb concentrations in 93.0% animals were above the maximum value (0.03  $\mu\text{g}/\text{kg}$ ) in the previous studies (Gabryszuk et al. 2010; Miroshnikov et al. 2019) but lower than those in cows hair in polluted (11.7 mg/kg) and unpolluted area (2.9 mg/kg) in the study of Patra et al. (2007). Compare to the study of Rashed

and Soltan (2005) <sup>10</sup> concentrations of Fe and Mn were also the highest among concentrations of the metals in hair of sheep, goat and camels. Their values (45–996 g/kg and 2.7–55 g/kg for Fe and Mn, respectively) were lower, while a range of the Cd concentrations (0.10–29 g/kg) was higher than the range in the present study.

The concentrations of Cu correlated positively with the concentrations of Zn ( $P < 0.01$ ) and Fe ( $P < 0.05$ ) and negatively with the concentrations of Pb ( $P < 0.01$ ). The Pb concentrations also correlated negatively with the concentrations of Zn and positively with Mn ( $P < 0.01$ ). However, Ages of animals did not relate to the concentrations of the metals (Table 6). In the PC analysis (Table 7), two components were obtained that account for 50.60% of samples variation where Cu and Zn in the first components and Pb in the second component as the highest loading. The highest values of Cu, Zn, and Pb confirm the significant <sup>4</sup>relations of the elements, whereas Rashed and Soltan (2005) reported a strong correlation between Fe and Mn in the hair of sheep, goat, and camels.

Lead <sup>4</sup>nonessential mineral widely distributed in the environment that persist in the environment for a long time and could be detected in most living organisms (Mahaffey 1977). The higher Pb in the buffalo hair might attributed to the higher Pb concentration in the water and forages though this could not be evidenced in this study. The non-significant correlations between the ages and the concentrations of the metals in the hair could relate to the hair molting of the animals (Combs 1987) and the concentrations of the metals in the water and forages.

## Conclusion

<sup>3</sup>The study appraised the concentrations of Cu, Mn, Zn, Fe, Pb, and Cd in the water, forage, and buffalo hair in the flooded pasture where the acidity and flooding are the main characteristics of the land. The levels of the heavy metals in the forages on residential and grazing area were not different and no significant correlation between ages of the animals and the concentrations of the metals. In the forages, Cu–Mn and Fe–Pb while in the

**Table 6** Pearson's correlation among selected heavy metals in buffalo hair

	Cu	Mn	Zn	Fe	Pb	Cd	age
Cu	1						
Mn	0.01	1					
Zn	0.92**	0.00	1				
Fe	0.18	–0.16	0.10	1			
Pb	–0.22*	0.37*	–0.27*	–0.02	1		
Cd	0.00	0.00	0.00	0.00	0.00	1	
age	0.00	0.12	0.07	0.12	0.00	0.00	1

\*\*Correlation is significant at 0.01 level

\*Correlation is significant at 0.05 level



**Table 7** Factor loading for selected heavy metals in Buffalo hair

	Factor 1	Factor 2
Cu	<b>0.99<sup>a</sup></b>	
Mn		0.38
Zn	<b>0.91</b>	-0.15
Fe	0.18	
Pb	-0.13	<b>0.99</b>
Cd	0.13	
% of variance	31.30	19.30
Cumulative %	31.30	50.60

<sup>a</sup> Values of dominant elements in each factor are indicated bold

hair Cu, Zn and Pb were correlated. The PC analysis revealed that the levels of metals concentration in the forages and buffalo hair associated with two main factors of natural resources. The higher concentrations of Pb might indicate a potential accumulation of the metal in other tissues of buffalo. **Further studies are required to evaluate the concentration of the minerals in the liver, kidney, muscle, and milk of the grazing animal to reduce the risk of toxicity to humans.**

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## Declarations

**Conflict of interest** The authors declare that there is no conflict of interest.

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