

BUKTI KORESPONDENSI
ARTIKEL JURNAL INTERNASIONAL BEREPUTASI

Judul artikel : Heavy metals accumulation in forages and buffalo hair on flooded pasture in South Sumatra, Indonesia

Jurnal : International Journal of Environmental Science and Technology 19, 4137–4142 2022

Penulis : A.I.M. Ali, S. Sandi, Riswandi

No	Perihal	Tanggal
1	Bukti konfirmasi submit artikel dan artikel yang disubmit	17/11/2020
2	Mempertanyakan status artikel	17/01/2021
3	Respon editor/review	18/01/2021
4	Bukti konfirmasi submit artikel dan artikel yang disubmit	11/04/2021
5	Bukti konfirmasi artikel accepted	28/05/2021
6	Bukti konfirmasi artikel proof for publishing	03/06/2021

[Terpublish](#)



Asep Indra Munawar Ali fp <asep_ali@fp.unsri.ac.id>

JEST-D-20-02975 - Submission Confirmation

International Journal of Environmental Science and Technology (JEST)

17 November 2020 pukul

<em@editorialmanager.com>

19.18

Balas Ke: "International Journal of Environmental Science and Technology (JEST)"

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International Journal of Environmental Science and Technology

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

--Manuscript Draft--

Manuscript Number:	JEST-D-20-02975	
Full Title:	Heavy metals accumulation in forages and buffalo hair on flooded pasture in South Sumatra, Indonesia	
Short Title:	heavy metals accumulation in forages and buffalo hair	
Article Type:	Original Paper	
Keywords:	Acid water; flooded pasture; Heavy metals	
Corresponding Author:	asep indra munawar ali, Ph.D Universitas Sriwijaya Fakultas Pertanian Ogan Ilir, Sumatera Selatan INDONESIA	
Corresponding Author Secondary Information:		
Corresponding Author's Institution:	Universitas Sriwijaya Fakultas Pertanian	
Corresponding Author's Secondary Institution:		
First Author:	asep indra munawar ali, Ph.D	
First Author Secondary Information:		
Order of Authors:	asep indra munawar ali, Ph.D	
	Sofia Sandi	
	Riswandi Riswandi, Dr.	
Order of Authors Secondary Information:		
Funding Information:	universitas sriwijaya (SP DIPA-023.17.2.677515)	Mr. asep indra munawar ali
Abstract:	<p>This study was conducted to evaluate the concentration of Cu, Fe, Zn, Mn, Pb, and 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 result demonstrated that concentrations of Cu, Mn, Zn, Fe, Pb 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 were 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.</p>	
Suggested Reviewers:		

1 **Heavy metals accumulation in forages and buffalo hair on flooded pasture in South**
2 **Sumatra, Indonesia**

3 **A.I.M. Ali*, S. Sandi, Riswandi**

4 Department of Animal Science, Faculty of Agriculture, Universitas Sriwijaya, South Sumatra,
5 Indonesia.

6 * Email: asep_ali@fp.unsri.ac.id, ORCID: 0000-0001-6330-8748

7 **Acknowledgements**

8 The authors are thankful for financial support through a competitive grant No SP DIPA-
9 023.17.2.677515 provided by Universitas Sriwijaya.

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1 **Abstract**

2 This study was conducted to evaluate the concentration of Cu, Fe, Zn, Mn, Pb, and Cd in the water,
3 forage, and buffalo hair, to compare the concentration of the metals in the forage on residential
4 area and grazing area and to evaluate relationships between age and concentrations of the heavy
5 metals in the buffaloes' hair. The result demonstrated that concentrations of Cu, Mn, Zn, Fe, Pb
6 were Fe > Mn > Zn > Pb > Cu > Cd in the water, Fe > Mn > Zn > Pb > Cu > Cd in the forage and
7 Fe > Mn > Cu > Zn > Pb > Cd in the buffalo hair. Concentrations of Pb in water and forages
8 exceeded the permissible limits for drinking water and feeds of livestock. The levels of the heavy
9 metals in forages on the residential and grazing areas were not different and no significant
10 correlation between ages of the animals and concentrations of the metals in the hair. In the forages,
11 Cu-Mn and Fe-Pb while in the hair Cu, Zn and Pb were strongly correlated. The principal
12 component analysis revealed that the levels of metals concentration in the forages and buffalo hair
13 associated with two main factors of natural resources.

14 **Keywords:** Acid water, flooded pasture, heavy metals

16 **Introduction**

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

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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; Ali et al. 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 Cu, Pb, Zn, and Cd increased as pH declined (Bang and Hesterberg 2004; Mühlbachová et al. 2005; Zeng et al. 2011). 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, Co, Ni, 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's hair and milk was reported (Gabryszuk et al. 2010). Thus, the objectives of the present study are to evaluate the concentration of Cu, Fe, Zn, Mn, Pb, and Cd in the water, forage, and buffalo hair, to compare the concentration of the metals

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46 in the forage on the residential and pasture areas and to evaluate relationships between age and
47 concentrations of the heavy metals in the buffaloes' hair.

48 **Materials and methods**

49 **Study area**

50 The study area is a freshwater swamp part of the Batang Hari river watershed and
51 administrated to Ogan Komering Ilir district. The soil is acid fluvisol soil which periodically
52 waterlogged during the rainy season with low availability of Ca, P, and Mg and high solubility of
53 Fe and Zn. The study areas are seasonally inundated from three to eight months. The rainy season
54 normally extends from October to March and the dry season occurs from April to September with
55 an annual rainfall 2,100 to 3,264 mm.

56 The housing area of residences is located on the left and right side of the village road.
57 Facilitation for domestic wastewater processing is not available while domestic garbage mostly
58 placed on the abandoned areas or on the roadside at the end of the village. The residential area
59 located in the shallow area of swamp thus has less period of flooding than the grazing area. Grazing
60 area is communal grazing land located in deeper swamp areas and inundated eight to ten months
61 per year where natural species of forages grow without application of artificial fertilizers. Both
62 housing and grazing areas are reachable by grazing buffaloes.

63 The landscape of the grazing area exhibits typical aquatic grassland with a scattered
64 population of *Mimosa pigra* and *Maleleuca sp.* The undergrowth is dominated by *Oryza rufipogon*,
65 *Eleocharis dulcis*, *Ischaemum rugosum*, and *Urochloa mutica*. Farming communities of the study
66 area practice seasonal paddy farming in the shallow and middle of the swamp when the water level
67 is decreasing in June to September resulting in shrinkage of grazing area in the dry season. The
68 sampling was carried out from August to October 2019.

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69 *Figure 1 near here*

70 **Water sampling**

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

74 **Collection of forage samples**

75 Native plant species consumed by grazing buffalo were sampled included *Mimosa pigra*,
76 *Sesbania exasperata*, *Neptunia oleracea*, *Aeschynomene sensitiva*, *Urochloa mutica*, *Leersia*
77 *hexandra*, *Hymenachne amplexicaulis*, *Ischaemum rugosum*, *Oryza rufipogon*, *Actinoscirpus*
78 *grossus*, *Scleria gaertneri*, *Eleocharis dulcis*, *Ludwigia peploides*, *Echinochloa crasipies* and
79 *Ipomea aquatica* (n=105). In each plot (100 × 100 m), the aerial part of the vegetation was
80 collected. Samples of stems and leaves of herbaceous plants in the pre-flowering stage and younger
81 twigs of the shrubs were cut by a sharp cutter and placed in the zip plastic bags then pooled per
82 species per plot. Samples (200 g) were washed with tap water before being washed by distilled
83 water then chopped to 5 cm of particle size. The samples were transferred to paper bags and then
84 oven-dried at 50 °C for 72 hours and milled to pass a 1 mm screen.

85 **Hair sampling**

86 Buffalo hair samples were collected from male (n=17) and female (n=82) buffaloes aged 6
87 to 72 months old raised in the study area. The hair (10 g) was always collected from the same part
88 of the withers and neck of the animals using stainless scissors. Ethyl alcohol was used for cleaning
89 the scissors. The hair samples were washed with tap water and cleaned from foreign materials
90 before being washed in distilled water. After that, the samples were rinsed with acetone for 5
91 minutes and then oven-dried at 50 °C for 72 hours. In addition, an interview with farmers was

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4 92 conducted to ensure that the animals were raised in this area and to collect the data of the animal's
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6 93 age.

9 94 **Chemical analysis of samples**

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11 95 An amount of 1 ml of HNO₃ (65%) was added to the water samples and then heated at 90
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14 96 °C for 2 hours. After cooling, the samples were filtered using 0.2-µm filters for analyses. An
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16 97 amount of the sample (5 and 2 g of forage and hair sample, respectively) was added to 10 mL of
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19 98 concentrated HNO₃ (65%). The mixture was moved to an autoclave for 66 min at 132 °C for
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21 99 digestion. The concentration of the metals was determined by a Shimadzu AA 680 flame atomic
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24 100 absorption. All analyses were performed in triplicate. For each heavy metal, calibration standards
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26 101 were prepared from the stock solution. Concerning the higher concentrations of Fe and Mn in the
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29 102 hair samples, potential contamination from soil and water to hair samples was accounted for by a
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31 103 repetition of measurement to five samples.

32 33 104 **Data analysis**

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36 105 For statistical analyses, a value of half the detection limit was assigned when the
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38 106 concentration was less than detection limits. The normal distribution of residual data was checked
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41 107 by the Kolmogorov–Smirnov test. Before the analyses data were log-transformed. ANOVA was
42
43 108 used to test the significance of differences in metal accumulation of the forage between the two
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46 109 areas. The average values of the data are presented as geometric means and correlations were
47
48 110 calculated by Pearson correlation analysis. Principal component (PC) analysis based on factor
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51 111 analysis was applied for source identification with varimax rotation for factor loading. Statistical
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53 112 analyses were carried out with R 3.6.1.

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4 **113 Results and discussion**

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7 **114 Heavy metals concentrations in water and forage**

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9 115 The means and ranges of the heavy metals concentrations in water and forage are presented
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11 116 in Table 1. The means and ranges of Mn and Pb in the water and forage exceeded the permissible
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14 117 values while the means of Cu, Zn, Fe, and Cd were lower than the standard. The high concentration
15
16 118 of toxic Pb in water and forage needs serious attention to the health and production of grazing
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19 119 animals. The sampling was conducted in the dry season when the concentrations in the water were
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21 120 always higher than in rainy seasons. However, the lower concentration in the rainy season could
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24 121 not be interpreted as a less toxic effect for animals since water and forage are the main sources of
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26 122 intake. Pb concentrations ranged from 3.5 to 23.3 mg/kg in the *I. Aquatica* that exceeded the
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29 123 permissible limit of WHO (2 mg/kg) for human consumption. This also presents a potential health
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31 124 problem for villagers since the vegetable is commonly collected and sold in local markets.

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34 125 [Table 1 near here](#)

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36 126 Higher Pb concentrations of the metals were found in the floating plants compared to the rooted
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38 127 plant ($P = 0.03$) revealed the bioaccumulation of the metals in the floating plants of the previous
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41 128 study such as *E. Crasipies* (Núñez et al. 2011), *N. oleracea*, and *I. aquatica* (Veschasit et al. 2012;
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43 129 Wahab et al. 2014). The order of element concentrations was $Fe > Mn > Zn > Pb > Cu > Cd$ for
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45
46 130 the water and $Mn > Fe > Zn > Pb > Cu > Cd$ for the forages. The pH value measured in the water
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48 131 ranged from 3.5 – 5.1 (data not shown). The highest concentration of Fe and Mn in the forages
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51 132 was also reported in the previous studies (Rashed and Soltan 2005; Ali et al. 2019) and was
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53 133 considered as a toxic level for crops (Sahrawat 2004) and water biota (Manders et al. 2002) and
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55 134 related to the higher solubility in the acid water (Bang and Hesterberg 2004; Mühlbachová et al.
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58 135 2005).

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136 [Table 2 near here](#)

137 Table 2 presents non-significant differences in the concentration of the elements of forages on
138 grazing and residential area. The household activities, mainly the uncontrolled rubbish discharged,
139 did not result in a higher concentration of the metals in the forages. Pearson correlation analysis
140 revealed significant ($P < 0.05$) positive correlations among Cu, Mn, Zn, Fe, and Pb concentration
141 of forages (Table 3). Mn correlated with Cu, Zn, Fe, and Pb, Zn correlated with Fe and Pb while
142 Fe correlated with Pb. In factor analysis (Table 4), two principal components were obtained and
143 the first two components accounted for 76.5% of variances of data. The greater contribution to the
144 variation in the first component was Cu and Mn whereas Fe and Pb in the second component.

145 [Table 3 & Table 4 near here](#)

146 The result of ANOVA does not reflect a non significant effect of antropogenic activities to the
147 concentration of metals. The effect could not differ in the different locations though the intens of
148 human activities is differ between two areas. However, the acid water and soil, period of flooding
149 and the higher concentration of the metals in the sorounding water need to be accounted. The PC
150 analysis grouped the source of variation. However, since non significat influence of the locally
151 human activities, natural process such as acidity and flooding may more dominate the source of
152 the variation of the heavy metals concentration in the forages.

153 **Heavy metals concentrations in buffaloes hair**

154 The concentrations of Cu, Mn, Zn, Fe, Pb, and Cd measured in hair of the buffaloes
155 sampled in this study are summarized in Table 5. The order of metal concentrations was $Fe > Mn$
156 $> Cu > Zn > Pb > Cd$. Concentrations of Cu, Mn, Fe, and Pb were out of the normal range while
157 Zn concentrations were in normal range of cow hair (Puls 1994). The concentratoions of Cu were
158 above acceptable range in 24.8% animals and below the lower acceptable range in 4.8% animals

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4 159 and Fe concentrations were above acceptable range in 88.0% animals. Comparing to the previous
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7 160 studies in hair of cows, Pb concentrations in 93.0% animals were above the maximum value (0.03
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9 161 $\mu\text{g}/\text{kg}$) in the previous studies (Gabryszuk et al. 2010; Miroshnikov et al. 2019) but lower than
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11 162 those in cows hair in polluted (11.7 mg/kg) and unpolluted area(2.9 mg/kg) in the study of Patra
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14 163 et al. (2007). Compare to the study of Rashed and Soltan (2005) the concentrations of Fe and Mn
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16 164 were also the highest among concentrations of the metals in hair of sheep, goat and camels. Their
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19 165 values (45 – 996 g/kg and 2.7 – 55 g/kg for Fe and Mn, respectively) were lower while Cd range
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21 166 (0.10 – 29 g/kg) was higher than those in the present study.

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24 167 [Table 5 near here](#)

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26 168 Concentrations of Cu correlated positively with Zn ($P < 0.01$) and Fe ($P < 0.05$) and
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29 169 negatively with Pb ($P < 0.01$). Concentrations of Pb concentrations also correlated negatively with
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31 170 Zn and positively with Mn ($P < 0.01$). However, Ages of animals did not relate to the
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33 171 concentrations of the metals (Table 6). In the PC analysis (Table 7), two components were obtained
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36 172 that account for 50.60% of samples variation where Cu and Zn in the first components and Pb in
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38 173 the second component as the highest loading. The highest values of Cu, Zn, and Pb confirm the
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41 174 significant correlations of the elements. Rashed and Soltan (2005) reported a strong correlation
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43 175 between Fe and Mn in the hair of sheep, goat and camels.

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46 176 [Table 6 and 7 near here](#)

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48 177 Lead is nonessential mineral widely distributed in the environment that persist in the
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50 178 environment for a long time and could be detected in most living organisms (Mahaffey 1977). The
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53 179 higher Pb in the buffalo hair might attributed to the higher Pb concentration in the water and
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55 180 forages though this could not be evidenced in this study. The non significant correlations between
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4 181 ages and the concentrations of the metals in hair could be relate to the hair moulting of the animals
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6 182 (Combs 1987) and the concentrations of the metals in the water and forages.

9 183 **Conclusions**

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11 184 The present study demonstrated that concentrations of Cu, Mn, Zn, Fe, Pb were Fe > Mn
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14 185 > Zn > Pb > Cu > Cd in the water, Fe > Mn > Zn > Pb > Cu > Cd in the forage and Fe > Mn > Cu
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16 186 > Zn > Pb > Cd in the buffalo hair. The levels of the heavy metals in forages on residential and
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19 187 grazing area were not different and no significant correlation between ages of the animals and
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21 188 concentrations of the metals. In the forages, Cu-Mn and Fe-Pb while in the hair Cu, Zn and Pb
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24 189 were correlated. Principal component analysis revealed that the levels of metals concentration in
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26 190 the forages and buffalo hair associated with two main factors of natural resources.

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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 ^a	Forages ^b
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 ^c	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

^a for Livestock, United States Environmental Protection Agency (Bagley et al. 1997)^bEuropean Union legislation (Hejna et al. 2018)^c Food and Agriculture Organization (2002)

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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

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

	<i>Cu</i>	<i>Mn</i>	<i>Zn</i>	<i>Fe</i>	<i>Pb</i>	<i>Cd</i>
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

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Table 4 Factor loading for selected heavy metals in forages

	Factor 1	Factor 2
Cu	0.88^a	0.26
Mn	0.77	0.57
Zn	0.65	0.51
Fe	0.32	0.94
Pb	0.46	0.73
Cd	0.53	0.34
% of Variance	39.90	36.60
Cumulative %	39.90	76.50

^aValues of dominant elements in each factor are indicated bold

257

Table 5 Concentration of Cu, Mn, Zn, Fe, Pb (mg/kg) and Cd (µg/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*
Mn	122.55	24.264-594.541	0.5 - 1.32*
Zn	10.84	2.498-32.852	100 – 150*
Fe	1320.77	61.938-14737.46	59 – 200*
Pb	1.15	0.000-36.459	0.0003-0.033 [#]
Cd	ND	ND	0.004-2.700 [#]

in cow hair * Puls (1994), [#] (Gabryszuk et al. 2010; Miroshnikov et al. 2019), ND: non detected

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

	<i>Cu</i>	<i>Mn</i>	<i>Zn</i>	<i>Fe</i>	<i>Pb</i>	<i>Cd</i>	<i>age</i>
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

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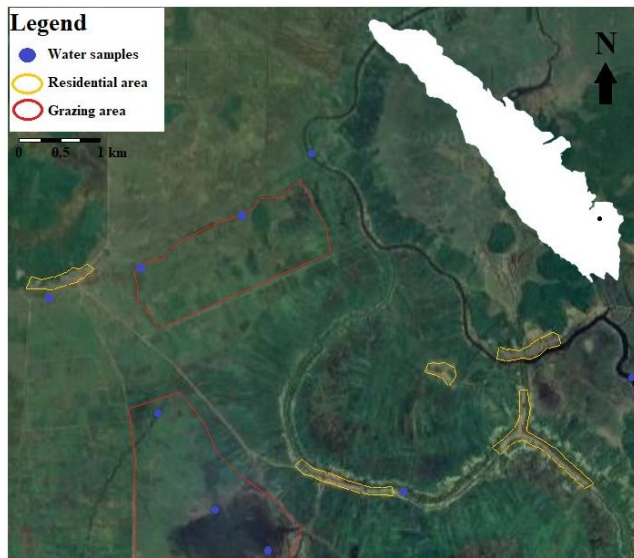
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Table 7 Factor loading for selected heavy metals in buffalos hair

	Factor 1	Factor 2
Cu	0.99^a	
Mn		0.38
Zn	0.91	-0.15
Fe	0.18	
Pb	-0.13	0.99
Cd	0.13	
% of Variance	31.30	19.30
Cumulative %	31.30	50.60

^aValues of dominant elements in each factor are indicated bold

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Fig. 1 Map of Sumatra and satellite image of study location

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Asep Indra Munawar Ali fp <asep_ali@fp.unsri.ac.id>

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Karthick Sankar (Mr.)

JEO Assistant

Journals Editorial Office (JEO)

Springer Nature

T +91 44 42197752

F +91 44 42197763

Karthick.Sankar@springernature.com

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International Journal of Environmental Science and Technology
Heavy metals accumulation in forages and buffalo hair on flooded pasture in South Sumatra, Indonesia
 --Manuscript Draft--

Manuscript Number:	JEST-D-20-02975
Full Title:	Heavy metals accumulation in forages and buffalo hair on flooded pasture in South Sumatra, Indonesia
Short Title:	heavy metals accumulation in forages and buffalo hair
Article Type:	Original Paper
Keywords:	Acid water; flooded pasture; Heavy metals
Abstract:	<p>This study was conducted to evaluate the concentration of Cu, Fe, Zn, Mn, Pb, and 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 result demonstrated that concentrations of Cu, Mn, Zn, Fe, Pb 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 were 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.</p>

1 **Abstract**

2 This study was conducted to evaluate the concentration of Cu, Fe, Zn, Mn, Pb, and Cd in the water,
3 forage, and buffalo hair, to compare the concentration of the metals in the forage on residential
4 area and grazing area and to evaluate relationships between age and concentrations of the heavy
5 metals in the buffaloes' hair. The result demonstrated that concentrations of Cu, Mn, Zn, Fe, Pb
6 were Fe > Mn > Zn > Pb > Cu > Cd in the water, Fe > Mn > Zn > Pb > Cu > Cd in the forage and
7 Fe > Mn > Cu > Zn > Pb > Cd in the buffalo hair. Concentrations of Pb in water and forages
8 exceeded the permissible limits for drinking water and feeds of livestock. The levels of the heavy
9 metals in forages on the residential and grazing areas were not different and no significant
10 correlation between ages of the animals and concentrations of the metals in the hair. In the forages,
11 Cu-Mn and Fe-Pb while in the hair Cu, Zn and Pb were strongly correlated. The principal
12 component analysis revealed that the levels of metals concentration in the forages and buffalo hair
13 associated with two main factors of natural resources.

14 **Keywords:** Acid water, flooded pasture, heavy metals

16 **Introduction**

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

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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; Ali et al. 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 Cu, Pb, Zn, and Cd increased as pH declined (Bang and Hesterberg 2004; Mühlbachová et al. 2005; Zeng et al. 2011). 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, Co, Ni, 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's hair and milk was reported (Gabryszuk et al. 2010). Thus, the objectives of the present study are to evaluate the concentration of Cu, Fe, Zn, Mn, Pb, and Cd in the water, forage, and buffalo hair, to compare the concentration of the metals

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46 in the forage on the residential and pasture areas and to evaluate relationships between age and
47 concentrations of the heavy metals in the buffaloes' hair.

48 **Materials and methods**

49 **Study area**

50 The study area is a freshwater swamp part of the Batang Hari river watershed and
51 administrated to Ogan Komering Ilir district. The soil is acid fluvisol soil which periodically
52 waterlogged during the rainy season with low availability of Ca, P, and Mg and high solubility of
53 Fe and Zn. The study areas are seasonally inundated from three to eight months. The rainy season
54 normally extends from October to March and the dry season occurs from April to September with
55 an annual rainfall 2,100 to 3,264 mm.

56 The housing area of residences is located on the left and right side of the village road.
57 Facilitation for domestic wastewater processing is not available while domestic garbage mostly
58 placed on the abandoned areas or on the roadside at the end of the village. The residential area
59 located in the shallow area of swamp thus has less period of flooding than the grazing area. Grazing
60 area is communal grazing land located in deeper swamp areas and inundated eight to ten months
61 per year where natural species of forages grow without application of artificial fertilizers. Both
62 housing and grazing areas are reachable by grazing buffaloes.

63 The landscape of the grazing area exhibits typical aquatic grassland with a scattered
64 population of *Mimosa pigra* and *Maleleuca sp.* The undergrowth is dominated by *Oryza rufipogon*,
65 *Eleocharis dulcis*, *Ischaemum rugosum*, and *Urochloa mutica*. Farming communities of the study
66 area practice seasonal paddy farming in the shallow and middle of the swamp when the water level
67 is decreasing in June to September resulting in shrinkage of grazing area in the dry season. The
68 sampling was carried out from August to October 2019.

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69 *Figure 1 near here*

70 **Water sampling**

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

74 **Collection of forage samples**

75 Native plant species consumed by grazing buffalo were sampled included *Mimosa pigra*,
76 *Sesbania exasperata*, *Neptunia oleracea*, *Aeschynomene sensitiva*, *Urochloa mutica*, *Leersia*
77 *hexandra*, *Hymenachne amplexicaulis*, *Ischaemum rugosum*, *Oryza rufipogon*, *Actinoscirpus*
78 *grossus*, *Scleria gaertneri*, *Eleocharis dulcis*, *Ludwigia peploides*, *Echinochloa crasipies* and
79 *Ipomea aquatica* (n=105). In each plot (100 × 100 m), the aerial part of the vegetation was
80 collected. Samples of stems and leaves of herbaceous plants in the pre-flowering stage and younger
81 twigs of the shrubs were cut by a sharp cutter and placed in the zip plastic bags then pooled per
82 species per plot. Samples (200 g) were washed with tap water before being washed by distilled
83 water then chopped to 5 cm of particle size. The samples were transferred to paper bags and then
84 oven-dried at 50 °C for 72 hours and milled to pass a 1 mm screen.

85 **Hair sampling**

86 Buffalo hair samples were collected from male (n=17) and female (n=82) buffaloes aged 6
87 to 72 months old raised in the study area. The hair (10 g) was always collected from the same part
88 of the withers and neck of the animals using stainless scissors. Ethyl alcohol was used for cleaning
89 the scissors. The hair samples were washed with tap water and cleaned from foreign materials
90 before being washed in distilled water. After that, the samples were rinsed with acetone for 5
91 minutes and then oven-dried at 50 °C for 72 hours. In addition, an interview with farmers was

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4 92 conducted to ensure that the animals were raised in this area and to collect the data of the animal's
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6 93 age.

9 94 **Chemical analysis of samples**

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11 95 An amount of 1 ml of HNO₃ (65%) was added to the water samples and then heated at 90
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14 96 °C for 2 hours. After cooling, the samples were filtered using 0.2-µm filters for analyses. An
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16 97 amount of the sample (5 and 2 g of forage and hair sample, respectively) was added to 10 mL of
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18 98 concentrated HNO₃ (65%). The mixture was moved to an autoclave for 66 min at 132 °C for
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21 99 digestion. The concentration of the metals was determined by a Shimadzu AA 680 flame atomic
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23 100 absorption. All analyses were performed in triplicate. For each heavy metal, calibration standards
24
25 101 were prepared from the stock solution. Concerning the higher concentrations of Fe and Mn in the
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27 102 hair samples, potential contamination from soil and water to hair samples was accounted for by a
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29 103 repetition of measurement to five samples.

33 104 **Data analysis**

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36 105 For statistical analyses, a value of half the detection limit was assigned when the
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38 106 concentration was less than detection limits. The normal distribution of residual data was checked
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41 107 by the Kolmogorov–Smirnov test. Before the analyses data were log-transformed. ANOVA was
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43 108 used to test the significance of differences in metal accumulation of the forage between the two
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45 109 areas. The average values of the data are presented as geometric means and correlations were
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48 110 calculated by Pearson correlation analysis. Principal component (PC) analysis based on factor
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50 111 analysis was applied for source identification with varimax rotation for factor loading. Statistical
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53 112 analyses were carried out with R 3.6.1.

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4 **113 Results and discussion**

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7 **114 Heavy metals concentrations in water and forage**

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9 115 The means and ranges of the heavy metals concentrations in water and forage are presented
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11 116 in Table 1. The means and ranges of Mn and Pb in the water and forage exceeded the permissible
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14 117 values while the means of Cu, Zn, Fe, and Cd were lower than the standard. The high concentration
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16 118 of toxic Pb in water and forage needs serious attention to the health and production of grazing
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19 119 animals. The sampling was conducted in the dry season when the concentrations in the water were
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21 120 always higher than in rainy seasons. However, the lower concentration in the rainy season could
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24 121 not be interpreted as a less toxic effect for animals since water and forage are the main sources of
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26 122 intake. Pb concentrations ranged from 3.5 to 23.3 mg/kg in the *I. Aquatica* that exceeded the
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29 123 permissible limit of WHO (2 mg/kg) for human consumption. This also presents a potential health
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31 124 problem for villagers since the vegetable is commonly collected and sold in local markets.

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34 125 [Table 1 near here](#)

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36 126 Higher Pb concentrations of the metals were found in the floating plants compared to the rooted
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38 127 plant ($P = 0.03$) revealed the bioaccumulation of the metals in the floating plants of the previous
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41 128 study such as *E. Crasipies* (Núñez et al. 2011), *N. oleracea*, and *I. aquatica* (Veschasit et al. 2012;
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43 129 Wahab et al. 2014). The order of element concentrations was $Fe > Mn > Zn > Pb > Cu > Cd$ for
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45
46 130 the water and $Mn > Fe > Zn > Pb > Cu > Cd$ for the forages. The pH value measured in the water
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48 131 ranged from 3.5 – 5.1 (data not shown). The highest concentration of Fe and Mn in the forages
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51 132 was also reported in the previous studies (Rashed and Soltan 2005; Ali et al. 2019) and was
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53 133 considered as a toxic level for crops (Sahrawat 2004) and water biota (Manders et al. 2002) and
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55 134 related to the higher solubility in the acid water (Bang and Hesterberg 2004; Mühlbachová et al.
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58 135 2005).

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136 [Table 2 near here](#)

137 Table 2 presents non-significant differences in the concentration of the elements of forages on
138 grazing and residential area. The household activities, mainly the uncontrolled runoff discharged,
139 did not result in a higher concentration of the metals in the forages. Pearson correlation analysis
140 revealed significant ($P < 0.05$) positive correlations among Cu, Mn, Zn, Fe, and Pb concentration
141 of forages (Table 3). Mn correlated with Cu, Zn, Fe, and Pb, Zn correlated with Fe and Pb while
142 Fe correlated with Pb. In factor analysis (Table 4), two principal components were obtained and
143 the first two components accounted for 76.5% of variances of data. The greater contribution to the
144 variation in the first component was Cu and Mn whereas Fe and Pb in the second component.

[Table 3 & Table 4 near here](#)

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

153 **Heavy metals concentrations in buffaloes hair**

154 The concentrations of Cu, Mn, Zn, Fe, Pb, and Cd measured in hair of the buffaloes
155 sampled in this study are summarized in Table 5. The order of metal concentrations was $Fe > Mn$
156 $> Cu > Zn > Pb > Cd$. Concentrations of Cu, Mn, Fe, and Pb were out of the normal range while
157 Zn concentrations were in normal range of cow hair (Puls 1994). The concentrations of Cu were
158 above acceptable range in 24.8% animals and below the lower acceptable range in 4.8% animals

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4 159 and Fe concentrations were above acceptable range in 88.0% animals. Comparing to the previous
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7 160 studies in hair of cows, Pb concentrations in 93.0% animals were above the maximum value (0.03
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9 161 $\mu\text{g}/\text{kg}$) in the previous studies (Gabryszuk et al. 2010; Miroshnikov et al. 2019) but lower than
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11 162 those in cows hair in polluted (11.7 mg/kg) and unpolluted area(2.9 mg/kg) in the study of Patra
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14 163 et al. (2007). Compare to the study of Rashed and Soltan (2005) the concentrations of Fe and Mn
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16 164 were also the highest among concentrations of the metals in hair of sheep, goat and camels. Their
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19 165 values (45 – 996 g/kg and 2.7 – 55 g/kg for Fe and Mn, respectively) were lower while Cd range
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21 166 (0.10 – 29 g/kg) was higher than those in the present study.

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24 167 [Table 5 near here](#)

25
26 168 Concentrations of Cu correlated positively with Zn ($P < 0.01$) and Fe ($P < 0.05$) and
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28
29 169 negatively with Pb ($P < 0.01$). Concentrations of Pb concentrations also correlated negatively with
30
31 170 Zn and positively with Mn ($P < 0.01$). However, Ages of animals did not relate to the
32
33 171 concentrations of the metals (Table 6). In the PC analysis (Table 7), two components were obtained
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35
36 172 that account for 50.60% of samples variation where Cu and Zn in the first components and Pb in
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38 173 the second component as the highest loading. The highest values of Cu, Zn, and Pb confirm the
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41 174 significant correlations of the elements. Rashed and Soltan (2005) reported a strong correlation
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43 175 between Fe and Mn in the hair of sheep, goat and camels.

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46 176 [Table 6 and 7 near here](#)

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48 177 Lead is nonessential mineral widely distributed in the environment that persist in the
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50 178 environment for a long time and could be detected in most living organisms (Mahaffey 1977). The
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53 179 higher Pb in the buffalo hair might attributed to the higher Pb concentration in the water and
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55 180 forages though this could not be evidenced in this study. The non significant correlations between
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4 181 ages and the concentrations of the metals in hair could be relate to the hair moulting of the animals
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6 182 (Combs 1987) and the concentrations of the metals in the water and forages.

9 183 **Conclusions**

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11 184 The present study demonstrated that concentrations of Cu, Mn, Zn, Fe, Pb were Fe > Mn
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14 185 > Zn > Pb > Cu > Cd in the water, Fe > Mn > Zn > Pb > Cu > Cd in the forage and Fe > Mn > Cu
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16 186 > Zn > Pb > Cd in the buffalo hair. The levels of the heavy metals in forages on residential and
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19 187 grazing area were not different and no significant correlation between ages of the animals and
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21 188 concentrations of the metals. In the forages, Cu-Mn and Fe-Pb while in the hair Cu, Zn and Pb
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24 189 were correlated. Principal component analysis revealed that the levels of metals concentration in
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26 190 the forages and buffalo hair associated with two main factors of natural resources.

29 191 30 192 **References**

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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 ^a	Forages ^b
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 ^c	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

^a for Livestock, United States Environmental Protection Agency (Bagley et al. 1997)^bEuropean Union legislation (Hejna et al. 2018)^c Food and Agriculture Organization (2002)

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Table 2 Concentration of Cu, Mn, Zn, Fe, Pb (mg/kg) and Cd ($\mu\text{g/kg}$) 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

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

	<i>Cu</i>	<i>Mn</i>	<i>Zn</i>	<i>Fe</i>	<i>Pb</i>	<i>Cd</i>
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

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Table 4 Factor loading for selected heavy metals in forages

	Factor 1	Factor 2
Cu	0.88^a	0.26
Mn	0.77	0.57
Zn	0.65	0.51
Fe	0.32	0.94
Pb	0.46	0.73
Cd	0.53	0.34
% of Variance	39.90	36.60
Cumulative %	39.90	76.50

^aValues of dominant elements in each factor are indicated bold

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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*
Mn	122.55	24.264-594.541	0.5 - 1.32*
Zn	10.84	2.498-32.852	100 – 150*
Fe	1320.77	61.938-14737.46	59 – 200*
Pb	1.15	0.000-36.459	0.0003-0.033 [#]
Cd	ND	ND	0.004-2.700 [#]

in cow hair * Puls (1994), [#] (Gabryszuk et al. 2010; Miroshnikov et al. 2019), ND: non detected

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

	<i>Cu</i>	<i>Mn</i>	<i>Zn</i>	<i>Fe</i>	<i>Pb</i>	<i>Cd</i>	<i>age</i>
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

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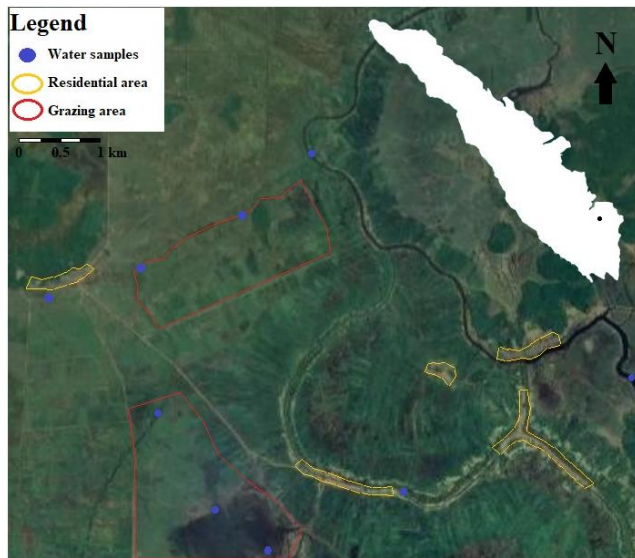
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Table 7 Factor loading for selected heavy metals in buffalos hair

	Factor 1	Factor 2
Cu	0.99^a	
Mn		0.38
Zn	0.91	-0.15
Fe	0.18	
Pb	-0.13	0.99
Cd	0.13	
% of Variance	31.30	19.30
Cumulative %	31.30	50.60

^aValues of dominant elements in each factor are indicated bold

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Fig. 1 Map of Sumatra and satellite image of study location

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The study titled “**Heavy metals accumulation in forages and buffalo hair on flooded pasture in South Sumatra, Indonesia**” has been reviewed. In my opinion, the study is an interesting one and it is beautifully presented. I recommend acceptance and publication after authors have carried out some minor corrections. My worries are as follows:

Abstract

While this section is good and informative, there are some flaws such as:

1. All metals should be written in full at first mention before subsequent abbreviation
2. Abbreviations should not be used to start a new statement

Keywords:

The keywords used are not convincing and only 3 is too small. I suggest authors increase them and choose better words or phrases that best describe the content of the manuscript.

Introduction:

Well written

Materials and Methods

Very well written and highly scientific

Results and Discussion

These sections are well written. Authors should just avoid using abbreviations to start a new sentence or headings as the case may be.

Table and Figures

1. In Table titles, there should be a colon after the number
2. The same should be adopted for figure labelling

References

Okay

General comment

1. The entire manuscript should be thoroughly checked for English Language correction.
2. I strongly suggest that authors include a section showing the list of all abbreviations and their full meanings



Asep Indra Munawar Ali fp <asep_ali@fp.unsri.ac.id>

JEST: Submission Confirmation for JEST-D-20-02975R1 - [EMID:d77139e67f4f6627]

International Journal of Environmental Science and Technology (JEST)

11 April 2021 pukul
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Heavy metals accumulation in forages and buffalo hair on flooded pasture in South Sumatra, Indonesia

--Manuscript Draft--

Manuscript Number:	JEST-D-20-02975R1	
Full Title:	Heavy metals accumulation in forages and buffalo hair on flooded pasture in South Sumatra, Indonesia	
Short Title:	Heavy metals on flooded pasture	
Article Type:	Original Paper	
Keywords:	Acid water and soil; heavy metals accumulation; lowlands farming	
Corresponding Author:	asep indra munawar ali, Ph.D Universitas Sriwijaya Fakultas Pertanian Ogan Ilir, Sumatera Selatan INDONESIA	
Corresponding Author Secondary Information:		
Corresponding Author's Institution:	Universitas Sriwijaya Fakultas Pertanian	
Corresponding Author's Secondary Institution:		
First Author:	asep indra munawar ali, Ph.D	
First Author Secondary Information:		
Order of Authors:	asep indra munawar ali, Ph.D	
	Sofia Sandi	
	Riswandi Riswandi, Dr.	
Order of Authors Secondary Information:		
Funding Information:	universitas sriwijaya (SP DIPA-023.17.2.677515)	Mr. asep indra munawar ali
Abstract:	<p>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.</p>	

Reviewer Comments		Authors Responses	
Comments	Page & line	Comments/Correction	New Page & line
Reviewer 1			
Is the water used for examination in this manuscript is swamp water or any type of water? Please explain it in the manuscript.		The water used is swamp water in the study area. This is explained in Materials and Methods (water sampling)	4 & 88
(n=105). In each plot (100 × 100 m), Need clarification.	5 & 79	(n=105) is the total number of samples of forage in grazing (78) and residential (27) area for the analyses. This has been clarified in <u>the paragraph</u> and <u>Table 2</u> .	5 & 100
Check the doi of the reference: Rashed M,Soltan M (2005) Animal hair as biological indicator for heavy metal pollution in urban and rural areas. Environ Monit Assess 110:41-53. DOI https://doi.org/10.1007/s10661-237005-6288-8 .		The doi of Rashed MN,Soltan ME and others DOI in the reference list have been checked	12 & 267
The conclusions are very similar to the summary of the manuscript that leads to a review.		The conclusion and abstract have been revised	Abstract Conclusions
Certain grammar corrections are indicated throughout the manuscript. It is preferred to take it.			
by the high	1 & 18	“The” has been added	2 & 34
the rainy	1 & 19	“The” has been added	2 & 35
water have resulted or water result	1 & 22	The words have been changed to ...water result....	2 & 38
the application	3 & 61	“The” has been added	4 & 80
were	5 & 102	It has been corrected	6 & 121
repetitions	5 & 103	It has been corrected	6 & 122
the	5 & 106	“The” has been added	6 & 125
rubbish	7 & 138	Rubbish has been changed to rubbish	7 & 158
the Analysis of variance (ANOVA)	7 & 146	The words have been added at first mention	6 & 127
the period	7 & 148	“The” has been added	8 & 169
sorounding	7 & 149	“The” has been deleted	8 & 170
accounted for	7 & 149	“for” has been added	8 & 170
natural processes	7 & 151	The word has been corrected	8 & 172
Reviewer 2			
<u>Overall</u> The study titled "Heavy metals accumulation in forages and buffalo hair on flooded pasture in South Sumatra, Indonesia" has been reviewed. In my opinion, the study is an interesting one and it is beautifully presented. I recommend acceptance and publication after authors have carried out some minor corrections. My worries are as follows:			
<u>Abstract</u> While this section is good and informative, there are some flaws such as: 1. All metals should be written in full at first mention before subsequent abbreviation 2. Abbreviations should not be used to start a new statement	1 & 1	1. At first mention the metals are written in full 2. Abbreviations have been checked through all sections of the manuscript	
<u>Abstract</u> The keywords used are not convincing and only 3 is too small. I suggest authors increase them and		The keywords have been revised	1 & 17

choose better words or phrases that best describe the content of the manuscript.			
<u>Introduction</u> Well written			
<u>Materials and Methods</u> Very well written and highly scientific			
<u>Results and Discussion</u> These sections are well written. Authors should just avoid using abbreviations to start a new sentence or headings as the case may be.			
<u>Table and Figures</u> 1. In Table titles, there should be a colon after the number 2. The same should be adopted for figure labelling		The journal guideline suggest the title without colon	
<u>References</u> Okay			
<u>General comment</u> 1. The entire manuscript should be thoroughly checked for English Language correction. 2. I strongly suggest that authors include a section showing the list of all abbreviations and their full meanings		1. English language has been corrected 2. The section has been included	1 & 18
Editorial comments			
Declaration on conflict of interest should be provided in the manuscript		It has been provided	10 & 217
At this stage, the manuscript "English language" must not bear any error. Otherwise, it will not be processed further. Please highlight all the edited corrections		The edited corrections have been highlighted	
Running title which is the short version of the main title shall be provided and included after the full title.		The running title has been provided	Title page & 3
All abbreviations should be removed from the TITLE, RUNNING TITLE, ABSTRACT and KEYWORDS. In return, they should be placed in the manuscript body and defined completely for the first time; afterward only the abbreviated form can be applied throughout the text .		The abbreviations have been checked. As reviewer recommendation, the list has been added. The use in all sections have been checked	
Abstract shall be written briefly within 150-250 words.		The abstract has been written with 243 words	
Date and location of the research carried out throughout the study must be mentioned at the end of INTRODUCTION section. [IMPORTANT]		Date and location of the research have been added	3 & 64
The whole manuscript shall be structured according to the IJEST style as: Title; Running title; Abstract; Keywords; Introduction; Materials and Methods; Results and Discussion; Conclusion; Acknowledgements and References		The running title has been added in Title page The structure of the manuscript has been checked	1 & 3
Figures/ photos should be originally in JPEG or TIFF format within 200-300 dpi resolution as they are attached separately.		The figure is JPEG/TIFF format with 243 dpi	
Tables should be the same as manuscript in MS-Word 2003 format and also uploaded separately in the same Word format in the system.		The file with all tables has been uploaded	

1 **Abstract**

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7 2 **Lowlands hold important potential for sustainable agriculture in South Sumatra. High acidity of**
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10 3 **soil and water and practice of domestic waste disposal in the area could be associated with a higher**
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12 4 **level of micro minerals in forages and grazing animals.** This study aimed to evaluate the
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14 5 concentration of **copper (Cu), manganese (Mn), zinc (Zn), iron (Fe), lead (Pb), and cadmium (Cd)**
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16 6 in the water, forage, and buffalo hair, to compare the concentration of the metals in the forage on
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18 7 residential area and grazing area and to evaluate relationships between age and concentrations of
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20 8 the heavy metals in the buffaloes' hair. **The concentrations of the minerals** were Fe > Mn > Zn >
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22 9 Pb > Cu > Cd in the water, Fe > Mn > Zn > Pb > Cu > Cd in the forage and Fe > Mn > Cu > Zn >
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24 10 Pb > Cd in the buffalo hair. Concentrations of Pb in water and forages exceeded the permissible
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26 11 limits for drinking water and feeds of livestock. The levels of the heavy metals in forages on the
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28 12 residential and grazing areas were not different and no significant correlation between ages of the
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30 13 animals and concentrations of the metals in the hair. In the forages, Cu-Mn and Fe-Pb while in the
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32 14 hair Cu, Zn and Pb strongly correlated. The principal component analysis revealed that the levels
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34 15 of metals concentration in the forages and buffalo hair associated with two main factors of natural
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36 16 resources.

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39 17 **Keywords:** Acid water **and soil**; heavy metals **accumulation**; **lowlands farming**

40 41 42 43 44 45 46 18 **List of symbols**

47 19	ANOVA	Analysis of variance
48 20	Cd	Cadmium
49 21	Cu	Copper
50 22	FAO	Food and Agriculture Organization
51 23	Fe	Iron
52 24	HNO ₃	Nitric acid

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4	25	Mn	Manganese
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6	26	n	Number
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8	27	P	Probability
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10	28	Pb	Lead
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12	29	PC	Principal Component
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14	30	Zn	Zinc
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18	32	Introduction	
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21	33		Lowlands are estimated to be 25% of the total land area in South Sumatra province thus
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23	34		play an important role in sustainable food security. The landscape is mainly characterized by the
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25	35		high acidity of soil and water and periodic inundation during the rainy season (World Bank 2018).
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27	36		Studies on crops show that low production was related to the low availability of macro minerals
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29	37		and the presence of excess or toxicity of iron (Fe) and manganese (Mn) (Sahrawat 2004; Noor
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31	38		2007). The lower pH and higher concentration of the micro minerals in the water result from pyrite
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33	39		oxidation when the acidic soil is drained (Dent 1986; Manders et al. 2002).
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38	40		Swamp buffalo farming is an important aspect of lowlands especially on deep freshwater
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40	41		swamp areas where crop cultivation is limited by high water level. The low pH of soil and water
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42	42		was related to a deficiency of macro minerals and excess of micro minerals in the pasture for
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44	43		grazing buffalo (Ali et al. 2013; Ali et al. 2019). Trace and toxic metals may accumulate in the
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46	44		forage and grazing buffalo. However, studies on this aspect especially on flooded pasture are
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48	45		limited. Previous studies showed that availability and plant uptake of copper (Cu), lead (Pb), zinc
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50	46		(Zn), and cadmium (Cd) increased as pH declined (Bang and Hesterberg 2004; Mühlbachová et
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52	47		al. 2005; Zeng et al. 2011). Human activities around where facilities for domestic sewage and
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54	48		rubbish processing are not available may contribute to the released of the metals into the
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56	49		environment. A previous study in the flooded pasture showed that concentrations of Cu, Fe, and
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4 50 Mn in forages exceed upper limits for grazing ruminants (Ali et al. 2019) which could be attributed
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6 51 to the ability of several aquatic plants to accumulate the heavy metals from polluted water (Núñez
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9 52 et al. 2011; Veschasit et al. 2012; Wahab et al. 2014).

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11 Concentrations of heavy metals in the hair may serve as a good indicator of heavy metals
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14 54 accumulation in the animal. A study of Rashed and Soltan (2005) with Fe, Mn, Pb and Cd in the
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16 55 hair of goat, sheep, and camel reported relationships between concentrations of the metals in hair
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19 56 and the concentration in forage and soil while Fe and Mn in the hair showed a strong relation.
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21 57 Cow's hair from a polluted area had a higher level of Cd and Pb than those from an unpolluted
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24 58 area and the Cd level correlated with Cd level in the blood (Patra et al. 2007). A positive correlation
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26 59 between Pb concentrations in cow's hair and milk was reported (Gabryszuk et al. 2010). Thus, the
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29 60 objectives of the present study were to evaluate the concentration of Cu, Mn, Zn, Fe, Pb, and Cd
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31 61 in the water, forage, and buffalo hair, to compare the concentration of the metals in the forage on
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34 62 the residential and pasture areas, and to evaluate relationships between age and concentrations of
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36 63 the heavy metals in the buffaloes' hair.

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38 64 The sampling of water, forage, and buffalo hair was carried out from August to October
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40
41 65 2019. Chemical and data analysis was completed in 2020. The locations of the sampling are shown
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43 66 in Fig. 1.

45 67 **Materials and methods**

47 68 **Study area**

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49
50 69 The study area is a freshwater swamp part of the Batang Hari river watershed and
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53 70 administrated to Ogan Komering Ilir district. The soil is acid fluvisol soil which periodically
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55 71 waterlogged during the rainy season with low availability of calcium, phosphorus, and magnesium
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58 72 and high solubility of Fe and Zn. The study areas are seasonally inundated from three to eight
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4 73 months. The rainy season normally extends from October to March and the dry season occurs from
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7 74 April to September with an annual rainfall 2,100 to 3,264 mm.

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9 75 The housing area of residences is located on the left and right side of the village road.
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11 76 Facilitation for the domestic wastewater processing is not available while domestic waste mostly
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14 77 placed on the abandoned areas or on the roadside at the end of the village. The residential area
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16 78 located in the shallow area of swamp thus has less period of flooding than the grazing area. Grazing
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19 79 area is communal grazing land located in deeper swamp areas and inundated eight to ten months
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21 80 per year where natural species of forages grow without the application of artificial fertilizers. Both
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24 81 housing and grazing areas are reachable by grazing buffaloes.

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26 82 The landscape of the grazing area exhibits typical aquatic grassland with a scattered
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29 83 population of *Mimosa pigra* and *Maleleuca sp.* The undergrowth is dominated by *Oryza rufipogon*,
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31 84 *Eleocharis dulcis*, *Ischaemum rugosum*, and *Urochloa mutica*. Farming communities of the study
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34 85 area practice seasonal paddy farming in the shallow and middle of the swamp when the water level
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36 86 is decreasing in June to September resulting in shrinkage of grazing area in the dry season.

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38 87 *Figure 1 near here*

39 40 41 88 **Water sampling**

42
43 89 Water samples were collected on nine sampling sites (Fig. 1). Water pH was measured on
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46 90 the sites (HI98130, Hanna Instrument). The water samples were filtered with Whatman paper (90
47
48 91 mm Ø) to obtain a 100 ml volume.

49 50 51 92 **Collection of forage samples**

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53 93 Native plant species consumed by grazing buffalo were sampled included *Mimosa pigra*,
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55 94 *Sesbania exasperata*, *Neptunia oleracea*, *Aeschynomene sensitiva*, *Urochloa mutica*, *Leersia*
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58 95 *hexandra*, *Hymenachne amplexicaulis*, *Ischaemum rugosum*, *Oryza rufipogon*, *Actinoscirpus*

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96 *grossus*, *Scleria gaertneri*, *Eleocharis dulcis*, *Ludwigia peploides*, *Echinochloa crasipies* and
97 *Ipomea aquatica*. In each plot (100 × 100 m), the aerial part of the vegetation was collected.
98 Samples of stems and leaves of herbaceous plants in the pre-flowering stage and younger twigs of
99 the shrubs were cut by a sharp cutter and placed in the zip plastic bags then pooled per species per
100 plot. After pooling, 78 and 27 samples were obtained on the grazing and residential areas,
101 respectively. The samples (200 g) were washed with tap water before being washed by distilled
102 water then chopped to 5 cm of particle size. The samples were transferred to paper bags and then
103 oven-dried at 50 °C for 72 hours and milled to pass a 1 mm screen.

Hair sampling

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

Chemical analysis of samples

112 An amount of 1 ml of HNO₃ (65%) was added to the water samples and then heated at 90
113 °C for 2 hours. After cooling, the samples were filtered using 0.2-µm filters for analyses. An
114 amount of the sample (5 and 2 g of forage and hair sample, respectively) was added to 10 mL of
115 concentrated HNO₃ (65%). The mixture was moved to an autoclave for 66 min at 132 °C for
116 digestion. The concentration of the metals was determined by a Shimadzu AA 680 flame atomic

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119 absorption. All analyses were performed in triplicate. For each heavy metal, calibration standards
120 were prepared from the stock solution. Concerning the higher concentrations of Fe and Mn in the
121 hair samples, potential contaminations from soil and water to hair samples **were** accounted for by
122 **repetitions** of measurement to five samples.

123 **Data analysis**

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

132 **Results and discussion**

133 **Heavy metals concentrations in water and forage**

134 The means and ranges of the heavy metals concentrations in water and forage are presented
135 in Table 1. The means and ranges of Mn and Pb in the water and forage exceeded the permissible
136 values while the means of Cu, Zn, Fe, and Cd were lower than the standard. The high concentration
137 of toxic Pb in water and forage needs serious attention to the health and production of grazing
138 animals. The sampling was conducted in the dry season when the concentrations in the water were
139 always higher than in rainy seasons. However, the lower concentration in the rainy season could
140 not be interpreted as a less toxic effect for animals since the water and forages are the main sources
141 of intake. **The Pb concentrations** ranged from 3.5 to 23.3 mg/kg in the *I. Aquatica* that exceeded

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142 the permissible limit of Food and Agriculture Organization (FAO, 2015) in leafy vegetable (0.3
143 mg/kg). This also presents a potential health problem for villagers since the vegetable is commonly
144 collected and sold in local markets.

145 [Table 1 near here](#)

146 Higher Pb concentrations of the metals were found in the floating plants compared to the rooted
147 plant (P = 0.03) revealed the bioaccumulation of the metals in the floating plants of the previous
148 study such as *E. Crasipies* (Núñez et al. 2011), *N oleracea*, and *I. aquatica* (Veschasit et al. 2012;
149 Wahab et al. 2014). The order of element concentrations was Fe > Mn > Zn > Pb > Cu > Cd for
150 the water and Mn > Fe > Zn > Pb > Cu > Cd for the forages. The pH value measured in the water
151 ranged from 3.5 – 5.1 (data not shown). The highest concentration of Fe and Mn in the forages
152 was also reported in the previous studies (Rashed and Soltan 2005; Ali et al. 2019) and was
153 considered as a toxic level for crops (Sahrawat 2004) and water biota (Manders et al. 2002) and
154 related to the higher solubility in the acid water (Bang and Hesterberg 2004; Mühlbachová et al.
155 2005).

156 [Table 2 near here](#)

157 Table 2 presents non-significant differences in the concentration of the elements of forages on
158 grazing and residential area. The household activities, mainly the uncontrolled **rubbish disposal**,
159 did not result in a higher concentration of the metals in the forages. The Pearson correlation
160 analysis revealed significant (P < 0.05) positive correlations among Cu, Mn, Zn, Fe, and Pb
161 concentration of forages (Table 3). **The Mn concentrations** correlated with the concentrations of
162 Cu, Zn, Fe, whilst the concentrations of Pb and Zn correlated with the concentrations of Fe and
163 Pb. In the PC analysis (Table 4), two principal components were obtained and the first two

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4 164 components accounted for 76.5% of variances of data. The greater contribution to the variation in
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7 165 the first component were Cu and Mn whereas Fe and Pb in the second component.

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9 166 [Table 3 & Table 4 near here](#)

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11 167 The result of ANOVA does not reflect a non significant effect of the antropogenic activities to the
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14 168 concentration of metals. The effect could not differ in the different locations though the intens of
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16 169 human activities are different between the two areas. However, the acid water and soil, the period
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19 170 of flooding, and the higher concentration of the metals in sorounding water need to be accounted
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21 171 for. The PC analysis grouped the source of variation. However, since non significat influence of
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24 172 the locally human activities, natural processes such as acidity and flooding may more dominate
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26 173 the source of the variation of the heavy metals concentration in the forages.

27 28 29 174 **Heavy metals concentrations in buffaloes hair**

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31 175 The concentrations of Cu, Mn, Zn, Fe, Pb, and Cd measured in the hair of the buffaloes
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34 176 sampled in this study are summarized in Table 5. The order of metal concentrations was Fe > Mn
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36 177 > Cu > Zn > Pb > Cd. The concentrations of Cu, Mn, Fe, and Pb were out of the normal range
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38 178 while Zn concentrations were in the normal range of cow hair (Puls 1994). The concentratoions of
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41 179 Cu were above the acceptable range in 24.8% animals and below the lower acceptable range in
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43 180 4.8% animals and Fe concentrations were above the acceptable range in 88.0% animals.
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46 181 Comparing to the previous studies in hair of cows, Pb concentrations in 93.0% animals were above
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48 182 the maximum value (0.03 µg/kg) in the previous studies (Gabryszuk et al. 2010; Miroshnikov et
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51 183 al. 2019) but lower than those in cows hair in polluted (11.7 mg/kg) and unpolluted area (2.9
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53 184 mg/kg) in the study of Patra et al. (2007). Compare to the study of Rashed and Soltan (2005) the
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56 185 concentrations of Fe and Mn were also the highest among concentrations of the metals in hair of
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58 186 sheep, goat and camels. Their values (45 – 996 g/kg and 2.7 – 55 g/kg for Fe and Mn, respectively)

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4 187 were lower while **range of the Cd concentrations** (0.10 – 29 g/kg) was higher than the range in the
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6 188 present study.

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9 189 **Table 5 near here**

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11 190 **The concentrations of Cu correlated positively with the concentrations of Zn** ($P < 0.01$) and
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14 191 Fe ($P < 0.05$) and negatively with **the concentrations of Pb** ($P < 0.01$). The Pb concentrations also
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16 192 correlated negatively with **the concentrations of Zn** and positively with Mn ($P < 0.01$). However,
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19 193 Ages of animals did not relate to the concentrations of the metals (Table 6). In the PC analysis
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21 194 (Table 7), two components were obtained that account for 50.60% of samples variation where Cu
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24 195 and Zn in the first components and Pb in the second component as the highest loading. The highest
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26 196 values of Cu, Zn, and Pb confirm the significant correlations of the elements whereas Rashed and
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29 197 Soltan (2005) reported a strong correlation between Fe and Mn in the hair of sheep, goat and
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31 198 camels.

32
33 199 **Table 6 and 7 near here**

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36 200 Lead is nonessential mineral widely distributed in the environment that persist in the
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38 201 environment for a long time and could be detected in most living organisms (Mahaffey 1977). The
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41 202 higher Pb in the buffalo hair might attributed to the higher Pb concentration in the water and
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43 203 forages though this could not be evidenced in this study. The non significant correlations between
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46 204 the ages and the concentrations of the metals in the hair could relate to the hair molting of the
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48 205 animals (Combs 1987) and the concentrations of the metals in the water and forages.

50 206 **Conclusions**

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53 207 **The study appraised the concentrations of Cu, Mn, Zn, Fe, Pb, and Cd in the water, forage,**
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55 208 **and buffalo hair in the flooded pasture where the acidity and flooding are the main characteristics**
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58 209 **of the land.** The levels of the heavy metals in **the forages** on residential and grazing area were not
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210 different and no significant correlation between ages of the animals and the concentrations of the
211 metals. In the forages, Cu-Mn and Fe-Pb while in the hair Cu, Zn and Pb were correlated. The PC
212 analysis revealed that the levels of metals concentration in the forages and buffalo hair associated
213 with two main factors of natural resources. The higher concentrations of Pb might indicate a
214 potential accumulation of the metal in other tissues of buffalo. Further studies are required to
215 evaluate the concentration of the minerals in the liver, kidney, muscle, and milk of the grazing
216 animal to reduce the risk of toxicity to humans.

Conflict of interest

The authors declare that there is no conflict of interest.

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284

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 ^a	Forages ^b
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 ^c	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

^a for Livestock, United States Environmental Protection Agency (Bagley et al. 1997)^bEuropean Union legislation (Hejna et al. 2018)^c Food and Agriculture Organization (2002)

285

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

286

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

	<i>Cu</i>	<i>Mn</i>	<i>Zn</i>	<i>Fe</i>	<i>Pb</i>	<i>Cd</i>
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

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Table 4 Factor loading for selected heavy metals in forages

	Factor 1	Factor 2
Cu	0.88^a	0.26
Mn	0.77	0.57
Zn	0.65	0.51
Fe	0.32	0.94
Pb	0.46	0.73
Cd	0.53	0.34
% of Variance	39.90	36.60
Cumulative %	39.90	76.50

^aValues of dominant elements in each factor are indicated bold

290

Table 5 Concentration of Cu, Mn, Zn, Fe, Pb (mg/kg) and Cd (µg/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*
Mn	122.55	24.264-594.541	0.5 - 1.32*
Zn	10.84	2.498-32.852	100 – 150*
Fe	1320.77	61.938-14737.46	59 – 200*
Pb	1.15	0.000-36.459	0.0003-0.033 [#]
Cd	ND	ND	0.004-2.700 [#]

in cow hair * Puls (1994), [#] (Gabryszuk et al. 2010; Miroshnikov et al. 2019), ND: non detected

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292

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

	<i>Cu</i>	<i>Mn</i>	<i>Zn</i>	<i>Fe</i>	<i>Pb</i>	<i>Cd</i>	<i>age</i>
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

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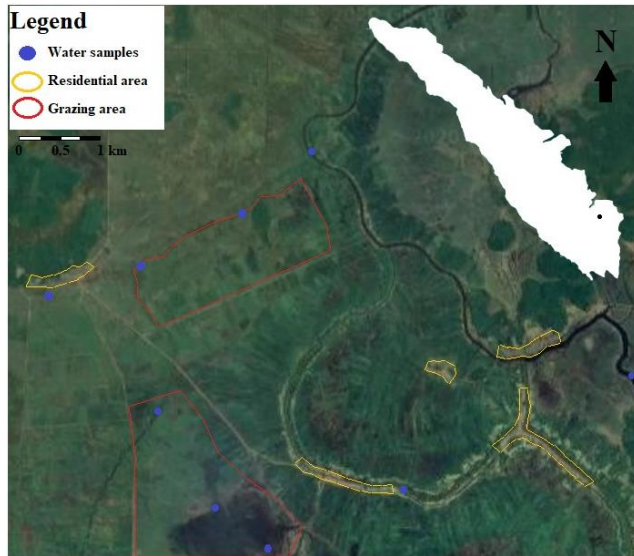
Table 7 Factor loading for selected heavy metals in buffalos hair

	Factor 1	Factor 2
Cu	0.99 ^a	
Mn		0.38
Zn	0.91	-0.15
Fe	0.18	
Pb	-0.13	0.99
Cd	0.13	
% of Variance	31.30	19.30
Cumulative %	31.30	50.60

^aValues of dominant elements in each factor are indicated bold

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

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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

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Asep Indra Munawar Ali fp <asep_ali@fp.unsri.ac.id>

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