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 artikecle 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) <em@editorialmanager.com> 17 November 2020 pukul 19.18
Balas Ke: "International Journal of Environmental Science and Technology (JEST)" <karthick.sankar@springernature.com>
Kepada: asep indra munawar ali <asep_ali@fp.unsri.ac.id>

Dear Dr. ali,

Your submission entitled "Heavy metals accumulation in forages and buffalo hair on flooded pasture in South Sumatra, Indonesia" has been received by journal International Journal of Environmental Science and Technology.

This submission is related to: .

You will be able to check on the progress of your paper by logging on to Editorial Manager as an author. The URL is https://www.editorialmanager.com/jest/.

Thank you for submitting your work to this journal.

Kind regards,

International Journal of Environmental Science and Technology

Our flexible approach during the COVID-19 pandemic

If you need more time at any stage of the peer-review process, please do let us know. While our systems will continue to remind you of the original timelines, we aim to be as flexible as possible during the current pandemic.

This letter contains confidential information, is for your own use, and should not be forwarded to third parties.

Recipients of this email are registered users within the Editorial Manager database for this journal. We will keep your information on file to use in the process of submitting, evaluating and publishing a manuscript. For more information on how we use your personal details please see our privacy policy at https://www.springernature.com/production-privacy-policy. If you no longer wish to receive messages from this journal or you have questions regarding database management, please contact the Publication Office at the link below.

In compliance with data protection regulations, you may request that we remove your personal registration details at any time. (Use the following URL: https://www.editorialmanager.com/jest/login.asp?a=r). Please contact the publication office if you have any questions.

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:	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.
Suggested Reviewers:	

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

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

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

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

Acknowledgements

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

Abstract

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.

Keywords: Acid water, flooded pasture, heavy metals

Introduction

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

Swamp buffalo farming is an important aspect of lowlands especially on deep freshwater swamp areas where crop cultivation is limited by high water level. The low pH of soil and water was related to a deficiency of macro minerals and excess of micro minerals in the pasture for grazing buffalo (Ali et al. 2013; 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 in the forage on the residential and pasture areas and to evaluate relationships between age and concentrations of the heavy metals in the buffaloes' hair.

Materials and methods

Study area

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

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

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

Figure 1 near here

Water sampling

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

Collection of forage samples

Native plant species consumed by grazing buffalo were sampled included *Mimosa pigra*, *Sesbania exasperata*, *Neptunia oleracea*, *Aeschynomene sensitiva*, *Urochloa mutica*, *Leersia hexandra*, *Hymenachne amplexicaulis*, *Ischaemum rugosum*, *Oryza rufipogon*, *Actinoscirpus grossus*, *Scleria gaertneri*, *Eleocharis dulcis*, *Ludwigia peploides*, *Echinocloa crasipies* and *Ipomea aquatica* (n=105). In each plot (100 × 100 m), the aerial part of the vegetation was collected. Samples of stems and leaves of herbaceous plants in the pre-flowering stage and younger twigs of the shrubs were cut by a sharp cutter and placed in the zip plastic bags then pooled per species per plot. Samples (200 g) were washed with tap water before being washed by distilled water then chopped to 5 cm of particle size. The samples were transferred to paper bags and then oven-dried at 50 ^{0}C for 72 hours and milled to pass a 1 mm screen.

Hair sampling

Buffalo hair samples were collected from male (n=17) and female (n=82) buffaloes aged 6 to 72 months old raised in the study area. The hair (10 g) was always collected from the same part of the withers and neck of the animals using stainless scissors. Ethyl alcohol was used for cleaning the scissors. The hair samples were washed with tap water and cleaned from foreign materials before being washed in distilled water. After that, the samples were rinsed with acetone for 5 minutes and then oven-dried at 50 ^{0}C for 72 hours. In addition, an interview with farmers was conducted to ensure that the animals were raised in this area and to collect the data of the animal's age.

Chemical analysis of samples

An amount of 1 ml of HNO_3 (65%) was added to the water samples and then heated at 90 ^{0}C for 2 hours. After cooling, the samples were filtered using 0.2-µm filters for analyses. An amount of the sample (5 and 2 g of forage and hair sample, respectively) was added to 10 mL of concentrated HNO_3 (65%). The mixture was moved to an autoclave for 66 min at 132 ^{0}C for digestion. The concentration of the metals was determined by a Shimadzu AA 680 flame atomic absorption. All analyses were performed in triplicate. For each heavy metal, calibration standards were prepared from the stock solution. Concerning the higher concentrations of Fe and Mn in the hair samples, potential contamination from soil and water to hair samples was accounted for by a repetition of measurement to five samples.

Data analysis

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

Results and discussion

Heavy metals concentrations in water and forage

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

Table 1 near here

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

Table 2 near here

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

Table 3 & Table 4 near here

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

Heavy metals concentrations in buffaloes hair

The concentrations of Cu, Mn, Zn, Fe, Pb, and Cd measured in hair of the buffaloes sampled in this study are summarized in Table 5. The order of metal concentrations was Fe > Mn > Cu > Zn > Pb > Cd. Concentrations of Cu, Mn, Fe, and Pb were out of the normal range while Zn concentrations were in normal range of cow hair (Puls 1994). The concentratoions of Cu were above acceptable range in 24.8% animals and below the lower acceptable range in 4.8% animals and Fe concentrations were above acceptable range in 88.0% animals. Comparing to the previous studies in hair of cows, Pb concentrations in 93.0% animals were above the maximum value (0.03 µg/kg) in the previous studies (Gabryszuk et al. 2010; Miroshnikov et al. 2019) but lower than those in cows hair in polluted (11.7 mg/kg) and unpolluted area(2.9 mg/kg) in the study of Patra et al. (2007). Compare to the study of Rashed and Soltan (2005) the concentrations of Fe and Mn were also the highest among concentrations of the metals in hair of sheep, goat and camels. Their values (45 – 996 g/kg and 2.7 – 55 g/kg for Fe and Mn, respectively) were lower while Cd range (0.10 – 29 g/kg) was higher than those in the present study.

Table 5 near here

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

Table 6 and 7 near here

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

Conclusions

The present study 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. The levels of the heavy metals in forages on residential and grazing area were not different and no significant correlation between ages of the animals and concentrations of the metals. In the forages, Cu-Mn and Fe-Pb while in the hair Cu, Zn and Pb were correlated. Principal component analysis revealed that the levels of metals concentration in the forages and buffalo hair associated with two main factors of natural resources.

References

Ali AIM, Sandi S, Muhakka et al. (2013) The Grazing of Pampangan buffaloes at non tidal swamp in South Sumatra of Indonesia. International Conference on Asia Agriculture and Animal. Moscow. Elsevier, pp: 87-92.

Ali AIM, Sandi S, Riswandi et al. (2019) Seasonal influence on mineral concentration of forages on flooded pastures in South Sumatra, Indonesia. Trop Grasslands-Forrajes Trop 7:527-532. DOI http://dx.doi.org/10.17138/TGFT(7)527-532.

Bagley C, Kotuby-Amacher J,Farrell-Poe K (1997) Analysis of water quality for livestock. Utah State University. https://digitalcommons.usu.edu/extension_histall/106/. 9 October 2020

Bang JS,Hesterberg D (2004) Dissolution of trace element contaminants from two coastal plain soils as affected by pH. J Environ Qual 33:891-901. DOI https://doi.org/10.2134/jeq2004.0891.

Combs DK (1987) Hair analysis as an indicator of mineral status of livestock. J Anim Sci 65:1753-1758. DOI https://doi.org/10.2527/jas1987.6561753x.

Dent (1986) Acid Sulphate Soils. A Baseline for research and development. Publication 39. Wageningen, Netherland: ILRI.

FAO (2002) Water quality for livestock and poultry. Food and Agriculture Organization. http://www.fao.org/3/T0234E/T0234E07.htm.

Gabryszuk M, Sloniewski K, Metera E et al. (2010) Content of mineral elements in milk and hair of cows from organic farms. J Elemen 15:259-267.

Hejna M, Gottardo D, Baldi A et al. (2018) Review: Nutritional ecology of heavy metals. Animal 12:2156–2170. DOI https://doi.org/10.1017/S175173111700355X.

Mahaffey K (1977) Quantities of lead producing health effects in humans: sources and bioavailability. Environ Health Perspect 19:285-295. DOI https://doi.org/10.1289/ehp.7719285.

Manders JA, Smith CD, Watling KM et al. (2002) An Investigation of Acid Sulfate Soils in the Logan-Coomera Area. Volume 1 Report on Acid Sulfate Soil Mapping. Department of Natural Resources and Mines, Indooroopilly, Queensland, Australia.

Miroshnikov S, Zavyalov O, Frolov A et al. (2019) The content of toxic elements in hair of dairy cows as an indicator of productivity and elemental status of animals. Environ Sci Pollut Res 26:18554-18564. DOI https://doi.org/10.1007/s11356-019-05163-5.

Mühlbachová G, Šimon T,Pechová M (2005) The availability of Cd, Pb and Zn and their relationships with soil pH and microbial biomass in soils amended by natural clinoptilolite. Plant Soil Environ 51:26-33. DOI https://doi.org/10.17221/3552-PSE.

Noor M (2007) Fresh water swamp: ecology and development. Jakarta. Raja Grafindo: (in Indonesian).

Núñez SER, Negrete JLM, Rios JEA et al. (2011) Hg, Cu, Pb, Cd, and Zn accumulation in macrophytes growing in tropical wetlands. Water, Air, Soil Pollut 216:361-373. DOI http://dx.doi.org/10.1007%2Fs11270-010-0538-2.

Patra RC, Swarup D, Naresh R et al. (2007) Tail hair as an indicator of environmental exposure of cows to lead and cadmium in different industrial areas. Ecotoxicol Environ Saf 66. DOI https://doi.org/10.1016/j.ecoenv.2006.01.005.

Puls R (1994) Mineral levels in animal health. 2nd Edn., Clearbrook, Canada: Sherpa International.

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/s10661-005-6288-8.

Sahrawat K (2004) Iron toxicity in wetland rice and the role of other nutrients. J Plant Nutr 27:1471-1504. DOI https://doi.org/10.1081/PLN-200025869.

Veschasit O, Meksumpun S,Meksumpun C (2012) Heavy metals contamination in water and aquatic plants in the Tha Chin River, Thailand. Agric Nat Resour 46:931-943.

Wahab ASA, Ismail SNS, Praveena SM et al. (2014) Heavy metals uptake of water mimosa (*Neptunia oleracea*) and its safety for human consumption. Iranian J Public Health 43:103-111.

World Bank (2018) Lowland Spatial Analyses (Water Management for Climate Change Mitigation and Adaptive Management Development). Jakarta. World Bank.

Zeng F, Ali S, Zhang H et al. (2011) The influence of pH and organic matter content in paddy soil on heavy metal availability and their uptake by rice plants. Environ Pollut 159:84-91. DOI https://doi.org/10.1016/j.envpol.2010.09.019.

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)

[b]European Union legislation (Hejna et al. 2018)

[c] Food and Agriculture Organization (2002)

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

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

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

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

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

**Correlation is significant at 0.01 level

*Correlation is significant at 0.05 level

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

[a]Values of dominant elements in each factor are indicated bold

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

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

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

**Correlation is significant at 0.01 level
*Correlation is significant at 0.05 level

Table 7 Factor loading for selected heavy metals in 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

[a]Values of dominant elements in each factor are indicated bold

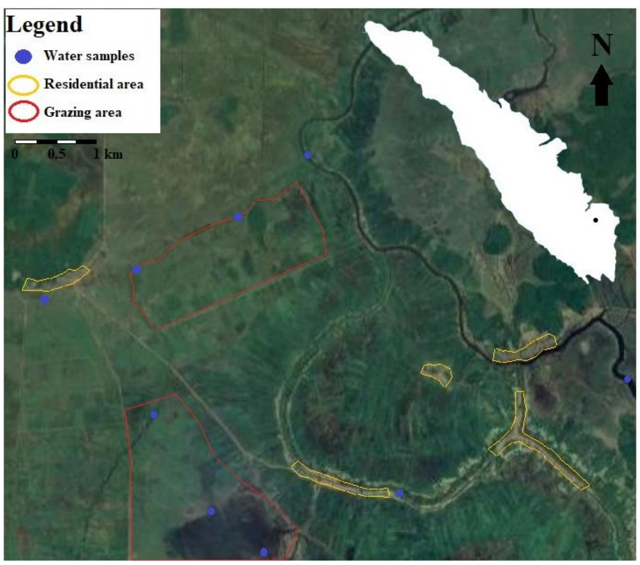

Fig. 1 Map of Sumatra and satellite image of study location

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

Status of Our manuscript (JEST-D-20-02975)

Asep Indra Munawar Ali fp <asep_ali@fp.unsri.ac.id> 17 Januari 2021 pukul 09.36
Kepada: karthick.sankar@springernature.com

Dear Dr. Sankar,
Could you please inform me about the current status of our manuscript? either the manuscript is still at editors or peer reviewers?.
We submitted the manuscript (JEST-D-20-02975, Heavy metals accumulation in forages and buffalo hair on flooded pasture in South Sumatra, Indonesia) on November 17th, 2020.

Thank you
Asep Ali

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

JEST-D-20-02975

Karthick Sankar <karthick.sankar@springernature.com> 18 Januari 2021 pukul 21.42
Kepada: International Journal of Environmental Science and Technology <ijest.publication@gmail.com>
Cc: Asep Indra Munawar Ali fp <asep_ali@fp.unsri.ac.id>

Dear Mirkia,

Please find the below email from author of the manuscript JEST-D-20-02975.

Hope you will be taking care of the same.

Thank you very much.

Best regards,

Karthick

Karthick Sankar (Mr.)

JEO Assistant

Journals Editorial Office (JEO)

Springer Nature

T +91 44 42197752

F +91 44 42197763

Karthick.Sankar@springernature.com

www.springernature.com

From: Asep Indra Munawar Ali fp <asep_ali@fp.unsri.ac.id>
Sent: Sunday, January 17, 2021 8:06 AM
To: Karthick Sankar <karthick.sankar@springernature.com>
Subject: Status of Our manuscript (JEST-D-20-02975)

[External - Use Caution]

Dear Dr. Sankar,
Could you please inform me about the current status of our manuscript? either the manuscript is still at editors or peer reviewers?.

We submitted the manuscript (JEST-D-20-02975, Heavy metals accumulation in forages and buffalo hair on flooded pasture in South Sumatra, Indonesia) on November 17th, 2020.

Thank you

Asep Ali

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:	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.

Abstract

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.

Keywords: Acid water, flooded pasture, heavy metals

Introduction

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

Swamp buffalo farming is an important aspect of lowlands especially on deep freshwater swamp areas where crop cultivation is limited by high water level. The low pH of soil and water was related to a deficiency of macro minerals and excess of micro minerals in the pasture for grazing buffalo (Ali et al. 2013; 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 in the forage on the residential and pasture areas and to evaluate relationships between age and concentrations of the heavy metals in the buffaloes' hair.

Materials and methods

Study area

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

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

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

Figure 1 near here

Water sampling

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

Collection of forage samples

Native plant species consumed by grazing buffalo were sampled included *Mimosa pigra*, *Sesbania exasperata*, *Neptunia oleracea*, *Aeschynomene sensitiva*, *Urochloa mutica*, *Leersia hexandra*, *Hymenachne amplexicaulis*, *Ischaemum rugosum*, *Oryza rufipogon*, *Actinoscirpus grossus*, *Scleria gaertneri*, *Eleocharis dulcis*, *Ludwigia peploides*, *Echinocloa crasipies* and *Ipomea aquatica* (n=105). In each plot (100 × 100 m), the aerial part of the vegetation was collected. Samples of stems and leaves of herbaceous plants in the pre-flowering stage and younger twigs of the shrubs were cut by a sharp cutter and placed in the zip plastic bags then pooled per species per plot. Samples (200 g) were washed with tap water before being washed by distilled water then chopped to 5 cm of particle size. The samples were transferred to paper bags and then oven-dried at 50 ^{0}C for 72 hours and milled to pass a 1 mm screen.

Hair sampling

Buffalo hair samples were collected from male (n=17) and female (n=82) buffaloes aged 6 to 72 months old raised in the study area. The hair (10 g) was always collected from the same part of the withers and neck of the animals using stainless scissors. Ethyl alcohol was used for cleaning the scissors. The hair samples were washed with tap water and cleaned from foreign materials before being washed in distilled water. After that, the samples were rinsed with acetone for 5 minutes and then oven-dried at 50 ^{0}C for 72 hours. In addition, an interview with farmers was conducted to ensure that the animals were raised in this area and to collect the data of the animal's age.

Chemical analysis of samples

An amount of 1 ml of HNO_3 (65%) was added to the water samples and then heated at 90 ^{0}C for 2 hours. After cooling, the samples were filtered using 0.2-μm filters for analyses. An amount of the sample (5 and 2 g of forage and hair sample, respectively) was added to 10 mL of concentrated HNO_3 (65%). The mixture was moved to an autoclave for 66 min at 132 ^{0}C for digestion. The concentration of the metals was determined by a Shimadzu AA 680 flame atomic absorption. All analyses were performed in triplicate. For each heavy metal, calibration standards were prepared from the stock solution. Concerning the higher concentrations of Fe and Mn in the hair samples, potential contamination from soil and water to hair samples was accounted for by a repetation of measurement to five samples.

Data analysis

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

Results and discussion

Heavy metals concentrations in water and forage

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

Table 1 near here

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

Table 2 near here

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

Table 3 & Table 4 near here

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

Heavy metals concentrations in buffaloes hair

The concentrations of Cu, Mn, Zn, Fe, Pb, and Cd measured in hair of the buffaloes sampled in this study are summarized in Table 5. The order of metal concentrations was Fe > Mn > Cu > Zn > Pb > Cd. Concentrations of Cu, Mn, Fe, and Pb were out of the normal range while Zn concentrations were in normal range of cow hair (Puls 1994). The concentratoions of Cu were above acceptable range in 24.8% animals and below the lower acceptable range in 4.8% animals and Fe concentrations were above acceptable range in 88.0% animals. Comparing to the previous studies in hair of cows, Pb concentrations in 93.0% animals were above the maximum value (0.03 µg/kg) in the previous studies (Gabryszuk et al. 2010; Miroshnikov et al. 2019) but lower than those in cows hair in polluted (11.7 mg/kg) and unpolluted area(2.9 mg/kg) in the study of Patra et al. (2007). Compare to the study of Rashed and Soltan (2005) the concentrations of Fe and Mn were also the highest among concentrations of the metals in hair of sheep, goat and camels. Their values (45 – 996 g/kg and 2.7 – 55 g/kg for Fe and Mn, respectively) were lower while Cd range (0.10 – 29 g/kg) was higher than those in the present study.

Table 5 near here

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

Table 6 and 7 near here

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

Conclusions

The present study 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. The levels of the heavy metals in forages on residential and grazing area were not different and no significant correlation between ages of the animals and concentrations of the metals. In the forages, Cu-Mn and Fe-Pb while in the hair Cu, Zn and Pb were correlated. Principal component analysis revealed that the levels of metals concentration in the forages and buffalo hair associated with two main factors of natural resources.

References

Ali AIM, Sandi S, Muhakka et al. (2013) The Grazing of Pampangan buffaloes at non tidal swamp in South Sumatra of Indonesia. International Conference on Asia Agriculture and Animal. Moscow. Elsevier, pp: 87-92.

Ali AIM, Sandi S, Riswandi et al. (2019) Seasonal influence on mineral concentration of forages on flooded pastures in South Sumatra, Indonesia. Trop Grasslands-Forrajes Trop 7:527-532. DOI http://dx.doi.org/10.17138/TGFT(7)527-532.

Bagley C, Kotuby-Amacher J,Farrell-Poe K (1997) Analysis of water quality for livestock. Utah State University. https://digitalcommons.usu.edu/extension_histall/106/. 9 October 2020

Bang JS,Hesterberg D (2004) Dissolution of trace element contaminants from two coastal plain soils as affected by pH. J Environ Qual 33:891-901. DOI https://doi.org/10.2134/jeq2004.0891.

Combs DK (1987) Hair analysis as an indicator of mineral status of livestock. J Anim Sci 65:1753-1758. DOI https://doi.org/10.2527/jas1987.6561753x.

Dent (1986) Acid Sulphate Soils. A Baseline for research and development. Publication 39. Wageningen, Netherland: ILRI.

FAO (2002) Water quality for livestock and poultry. Food and Agriculture Organization. http://www.fao.org/3/T0234E/T0234E07.htm.

Gabryszuk M, Sloniewski K, Metera E et al. (2010) Content of mineral elements in milk and hair of cows from organic farms. J Elemen 15:259-267.

Hejna M, Gottardo D, Baldi A et al. (2018) Review: Nutritional ecology of heavy metals. Animal 12:2156–2170. DOI https://doi.org/10.1017/S175173111700355X.

Mahaffey K (1977) Quantities of lead producing health effects in humans: sources and bioavailability. Environ Health Perspect 19:285-295. DOI https://doi.org/10.1289/ehp.7719285.

Manders JA, Smith CD, Watling KM et al. (2002) An Investigation of Acid Sulfate Soils in the Logan-Coomera Area. Volume 1 Report on Acid Sulfate Soil Mapping. Department of Natural Resources and Mines, Indooroopilly, Queensland, Australia.

Miroshnikov S, Zavyalov O, Frolov A et al. (2019) The content of toxic elements in hair of dairy cows as an indicator of productivity and elemental status of animals. Environ Sci Pollut Res 26:18554-18564. DOI https://doi.org/10.1007/s11356-019-05163-5.

Mühlbachová G, Šimon T,Pechová M (2005) The availability of Cd, Pb and Zn and their relationships with soil pH and microbial biomass in soils amended by natural clinoptilolite. Plant Soil Environ 51:26-33. DOI https://doi.org/10.17221/3552-PSE.

Noor M (2007) Fresh water swamp: ecology and development. Jakarta. Raja Grafindo: (in Indonesian).

Núñez SER, Negrete JLM, Rios JEA et al. (2011) Hg, Cu, Pb, Cd, and Zn accumulation in macrophytes growing in tropical wetlands. Water, Air, Soil Pollut 216:361-373. DOI http://dx.doi.org/10.1007%2Fs11270-010-0538-2.

Patra RC, Swarup D, Naresh R et al. (2007) Tail hair as an indicator of environmental exposure of cows to lead and cadmium in different industrial areas. Ecotoxicol Environ Saf 66. DOI https://doi.org/10.1016/j.ecoenv.2006.01.005.

Puls R (1994) Mineral levels in animal health. 2nd Edn., Clearbrook, Canada: Sherpa International.

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/s10661-005-6288-8.

Sahrawat K (2004) Iron toxicity in wetland rice and the role of other nutrients. J Plant Nutr 27:1471-1504. DOI https://doi.org/10.1081/PLN-200025869.

Veschasit O, Meksumpun S,Meksumpun C (2012) Heavy metals contamination in water and aquatic plants in the Tha Chin River, Thailand. Agric Nat Resour 46:931-943.

Wahab ASA, Ismail SNS, Praveena SM et al. (2014) Heavy metals uptake of water mimosa (*Neptunia oleracea*) and its safety for human consumption. Iranian J Public Health 43:103-111.

World Bank (2018) Lowland Spatial Analyses (Water Management for Climate Change Mitigation and Adaptive Management Development). Jakarta. World Bank.

Zeng F, Ali S, Zhang H et al. (2011) The influence of pH and organic matter content in paddy soil on heavy metal availability and their uptake by rice plants. Environ Pollut 159:84-91. DOI https://doi.org/10.1016/j.envpol.2010.09.019.

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)

[b]European Union legislation (Hejna et al. 2018)

[c] Food and Agriculture Organization (2002)

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

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

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

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

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

**Correlation is significant at 0.01 level

*Correlation is significant at 0.05 level

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

[a]Values of dominant elements in each factor are indicated bold

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

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

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

**Correlation is significant at 0.01 level
*Correlation is significant at 0.05 level

Table 7 Factor loading for selected heavy metals in 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

[a]Values of dominant elements in each factor are indicated bold

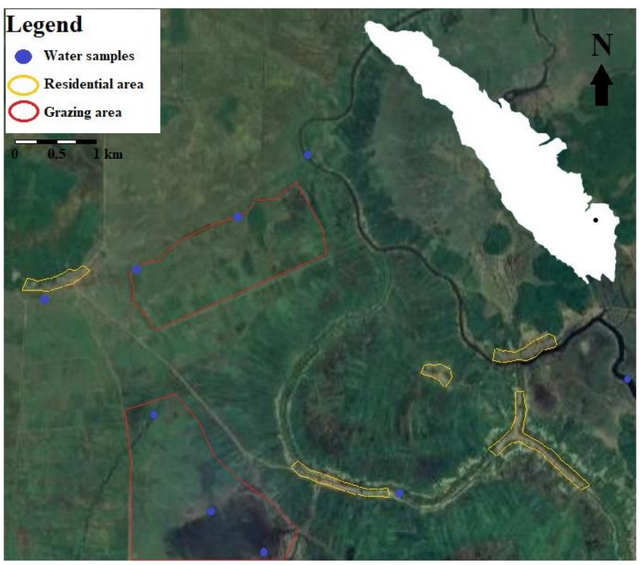

Fig. 1 Map of Sumatra and satellite image of study location

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 betters 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) <em@editorialmanager.com>
Balas Ke: "International Journal of Environmental Science and Technology (JEST)" <karthick.sankar@springernature.com>
Kepada: asep indra munawar ali <asep_ali@fp.unsri.ac.id>

11 April 2021 pukul 12.06

Ref.: Ms. No. JEST-D-20-02975R1
Heavy metals accumulation in forages and buffalo hair on flooded pasture in South Sumatra, Indonesia

Dear Dr. ali,

International Journal of Environmental Science and Technology has received your revised submission.

You may check the status of your manuscript by logging onto Editorial Manager at https://www.editorialmanager.com/jest/.

Kind regards,

Editorial Office
International Journal of Environmental Science and Technology

Our flexible approach during the COVID-19 pandemic

If you need more time at any stage of the peer-review process, please do let us know. While our systems will continue to remind you of the original timelines, we aim to be as flexible as possible during the current pandemic.

This letter contains confidential information, is for your own use, and should not be forwarded to third parties.

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-20-02975R1
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:

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.

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 the paragraph and Table 2.	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/s10661-237 005-6288-8.		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			
Overall 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	1 & 1	1. At first mention the metals are written in full 2. Abbreviations have been checked through all sections of the manuscript	
Abstract 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 betters 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		The journal guideline suggest the tittle without colon	
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		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	

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

Heavy metals on flooded pasture

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

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

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

Acknowledgements

The authors are thankful for financial support through a competitive grant SP DIPA-023.17.2.677515 provided by Universitas Sriwijaya.

Abstract

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

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

List of symbols

ANOVA	Analysis of variance
Cd	Cadmium
Cu	Copper
FAO	Food and Agriculture Organization
Fe	Iron
HNO_3	Nitric acid

Mn	Manganese
n	Number
P	Probability
Pb	Lead
PC	Principal Component
Zn	Zinc

Introduction

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

Swamp buffalo farming is an important aspect of lowlands especially on deep freshwater swamp areas where crop cultivation is limited by high water level. The low pH of soil and water was related to a deficiency of macro minerals and excess of micro minerals in the pasture for grazing buffalo (Ali et al. 2013; 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 copper (Cu), lead (Pb), zinc (Zn), and cadmium (Cd) increased as pH declined (Bang and Hesterberg 2004; Mühlbachová et al. 2005; Zeng et al. 2011). Human activities around where facilities for domestic sewage and rubbish processing are not available may contribute to the released of the metals into the environment. A previous study in the flooded pasture showed that concentrations of Cu, Fe, and

Mn in forages exceed upper limits for grazing ruminants (Ali et al. 2019) which could be attributed to the ability of several aquatic plants to accumulate the heavy metals from polluted water (Núñez et al. 2011; Veschasit et al. 2012; Wahab et al. 2014).

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

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

Materials and methods

Study area

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

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

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

Figure 1 near here

Water sampling

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

Collection of forage samples

Native plant species consumed by grazing buffalo were sampled included *Mimosa pigra*, *Sesbania exasperata*, *Neptunia oleracea*, *Aeschynomene sensitiva*, *Urochloa mutica*, *Leersia hexandra*, *Hymenachne amplexicaulis*, *Ischaemum rugosum*, *Oryza rufipogon*, *Actinoscirpus* *grossus, Scleria gaertneri, Eleocharis dulcis, Ludwigia peploides, Echinocloa crasipies* and *Ipomea aquatica*. In each plot (100 × 100 m), the aerial part of the vegetation was collected. Samples of stems and leaves of herbaceous plants in the pre-flowering stage and younger twigs of the shrubs were cut by a sharp cutter and placed in the zip plastic bags then pooled per species per plot. After pooling, 78 and 27 samples were obtained on the grazing and residential areas, respectively. The samples (200 g) were washed with tap water before being washed by distilled water then chopped to 5 cm of particle size. The samples were transferred to paper bags and then oven-dried at 50 ^{0}C for 72 hours and milled to pass a 1 mm screen.

Hair sampling

Buffalo hair samples were collected from male (n=17) and female (n=82) buffaloes aged 6 to 72 months old raised in the study area. The hair (10 g) was always collected from the same part of the withers and neck of the animals using stainless scissors. Ethyl alcohol was used for cleaning the scissors. The hair samples were washed with tap water and cleaned from foreign materials before being washed in distilled water. After that, the samples were rinsed with acetone for 5 minutes and then oven-dried at 50 ^{0}C for 72 hours. In addition, an interview with farmers was conducted to ensure that the animals were raised in this area and to collect the data of the animal's age.

Chemical analysis of samples

An amount of 1 ml of HNO_3 (65%) was added to the water samples and then heated at 90 ^{0}C for 2 hours. After cooling, the samples were filtered using 0.2-µm filters for analyses. An amount of the sample (5 and 2 g of forage and hair sample, respectively) was added to 10 mL of concentrated HNO_3 (65%). The mixture was moved to an autoclave for 66 min at 132 ^{0}C for digestion. The concentration of the metals was determined by a Shimadzu AA 680 flame atomic absorption. All analyses were performed in triplicate. For each heavy metal, calibration standards were prepared from the stock solution. Concerning the higher concentrations of Fe and Mn in the hair samples, potential contaminations from soil and water to hair samples were accounted for by repetitions of measurement to five samples.

Data analysis

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

Results and discussion

Heavy metals concentrations in water and forage

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

Table 1 near here

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

Table 2 near here

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

Table 3 & Table 4 near here

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

Heavy metals concentrations in buffaloes hair

The concentrations of Cu, Mn, Zn, Fe, Pb, and Cd measured in the hair of the buffaloes sampled in this study are summarized in Table 5. The order of metal concentrations was Fe > Mn > Cu > Zn > Pb > Cd. The concentrations of Cu, Mn, Fe, and Pb were out of the normal range while Zn concentrations were in the normal range of cow hair (Puls 1994). The concentratoions of Cu were above the acceptable range in 24.8% animals and below the lower acceptable range in 4.8% animals and Fe concentrations were above the acceptable range in 88.0% animals. Comparing to the previous studies in hair of cows, Pb concentrations in 93.0% animals were above the maximum value (0.03 µg/kg) in the previous studies (Gabryszuk et al. 2010; Miroshnikov et al. 2019) but lower than those in cows hair in polluted (11.7 mg/kg) and unpolluted area (2.9 mg/kg) in the study of Patra et al. (2007). Compare to the study of Rashed and Soltan (2005) the concentrations of Fe and Mn were also the highest among concentrations of the metals in hair of sheep, goat and camels. Their values (45 – 996 g/kg and 2.7 – 55 g/kg for Fe and Mn, respectively) were lower while range of the Cd concentrations (0.10 – 29 g/kg) was higher than the range in the present study.

Table 5 near here

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

Table 6 and 7 near here

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

Conclusions

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

Conflict of interest

The authors declare that there is no conflict of interest.

References

Ali AIM, Sandi S, Muhakka et al. (2013) The Grazing of Pampangan buffaloes at non tidal swamp in South Sumatra of Indonesia. International Conference on Asia Agriculture and Animal. Moscow. Elsevier, pp: 87-92

Ali AIM, Sandi S, Riswandi et al. (2019) Seasonal influence on mineral concentration of forages on flooded pastures in South Sumatra, Indonesia. Tropical Grasslands-Forrajes Tropicales 7:527-532. http://dx.doi.org/10.17138/TGFT(7)527-532

Bagley C, Kotuby-Amacher J, Farrell-Poe K (1997) Analysis of water quality for livestock. Utah State University. https://digitalcommons.usu.edu/extension_histall/106/. Accessed 9 October 2020

Bang JS, Hesterberg D (2004) Dissolution of trace element contaminants from two coastal plain soils as affected by pH. Journal of Environmental quality 33:891-901. https://doi.org/10.2134/jeq2004.0891

Combs DK (1987) Hair analysis as an indicator of mineral status of livestock. J Anim Sci 65:1753-1758. https://doi.org/10.2527/jas1987.6561753x

Dent (1986) Acid Sulphate Soils. A Baseline for research and development. Publication 39. Wageningen, Netherland: ILRI

FAO (2002) Water quality for livestock and poultry. Food and Agriculture Organization. http://www.fao.org/3/T0234E/T0234E07.htm. Accessed 3 October 2020

FAO (2015) General standard for contaminants and toxins in food and feed. Food and Agriculture Organization. http://www.fao.org/download/standards/CXS_193e_2015.pdf. Accessed 2 April 2021

Gabryszuk M, Sloniewski K, Metera E et al. (2010) Content of mineral elements in milk and hair of cows from organic farms. Journal of Elementology 15:259-267.

Hejna M, Gottardo D, Baldi A et al. (2018) Review: Nutritional ecology of heavy metals. Animal 12:2156–2170. https://doi.org/10.1017/S175173111700355X

Mahaffey K (1977) Quantities of lead producing health effects in humans: sources and bioavailability. Environ Health Perspect 19:285-295. https://doi.org/10.1289/ehp.7719285

Manders JA, Smith CD, Watling KM et al. (2002) An Investigation of Acid Sulfate Soils in the Logan-Coomera Area. Volume 1 Report on Acid Sulfate Soil Mapping. Department of Natural Resources and Mines, Indooroopilly, Queensland, Australia

Miroshnikov S, Zavyalov O, Frolov A et al. (2019) The content of toxic elements in hair of dairy cows as an indicator of productivity and elemental status of animals. Environmental Science and Pollution Research 26:18554-18564. https://doi.org/10.1007/s11356-019-05163-5

Mühlbachová G, Šimon T, Pechová M (2005) The availability of Cd, Pb and Zn and their relationships with soil pH and microbial biomass in soils amended by natural clinoptilolite. Plant Soil and Environment 51:26-33. https://doi.org/10.17221/3552-PSE

Noor M (2007) Fresh water swamp: ecology and development. Jakarta. Raja Grafindo: (in Indonesian)

Núñez SER, Negrete JLM, Rios JEA et al. (2011) Hg, Cu, Pb, Cd, and Zn accumulation in macrophytes growing in tropical wetlands. Water, Air, Soil Pollut 216:361-373. http://dx.doi.org/10.1007%2Fs11270-010-0538-2

Patra RC, Swarup D, Naresh R et al. (2007) Tail hair as an indicator of environmental exposure of cows to lead and cadmium in different industrial areas. Ecotoxicology and environmental safety 66. https://doi.org/10.1016/j.ecoenv.2006.01.005

Puls R (1994) Mineral levels in animal health. 2nd Edn., Clearbrook, Canada: Sherpa International

Rashed MN, Soltan ME (2005) Animal hair as biological indicator for heavy metal pollution in urban and rural areas. Environ Monit Assess 110:41-53. https://doi.org/10.1007/s10661-005-6288-8

Sahrawat K (2004) Iron toxicity in wetland rice and the role of other nutrients. J Plant Nutr 27:1471-1504. https://doi.org/10.1081/PLN-200025869

Veschasit O, Meksumpun S, Meksumpun C (2012) Heavy metals contamination in water and aquatic plants in the Tha Chin River, Thailand. Agriculture and Natural Resources 46:931-943.

Wahab ASA, Ismail SNS, Praveena SM et al. (2014) Heavy metals uptake of water mimosa (*Neptunia oleracea*) and its safety for human consumption. Iranian Journal of Public Health 43:103-111.

World Bank (2018) Lowland Spatial Analyses (Water Management for Climate Change Mitigation and Adaptive Management Development). Jakarta: World Bank

Zeng F, Ali S, Zhang H et al. (2011) The influence of pH and organic matter content in paddy soil on heavy metal availability and their uptake by rice plants. Environ Pollut 159:84-91. https://doi.org/10.1016/j.envpol.2010.09.019

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)

[b]European Union legislation (Hejna et al. 2018)

[c] Food and Agriculture Organization (2002)

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

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

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

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

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

**Correlation is significant at 0.01 level

*Correlation is significant at 0.05 level

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

[a]Values of dominant elements in each factor are indicated bold

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

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

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

**Correlation is significant at 0.01 level
*Correlation is significant at 0.05 level

Table 7 Factor loading for selected heavy metals in 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

[a]Values of dominant elements in each factor are indicated bold

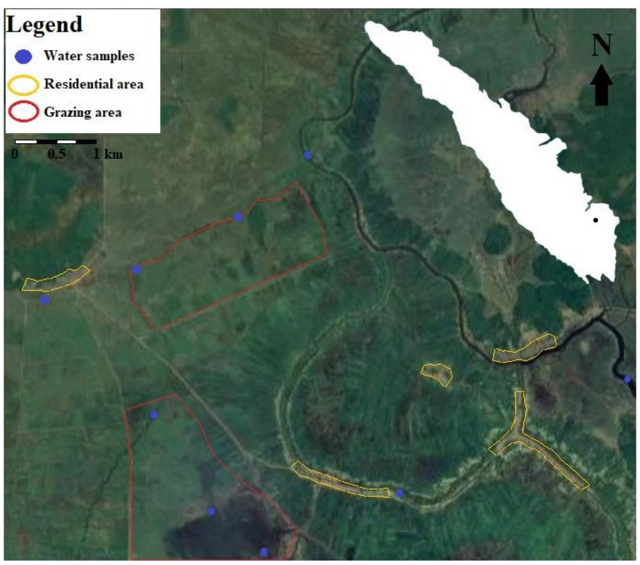

Fig. 1 Map of Sumatra and satellite image of study location (background image from Google Maps (online), Google, DigitalGlobe, 2020)

Table

1

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)

[b]European Union legislation (Hejna et al. 2018)

[c] Food and Agriculture Organization (2002)

2

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

3

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

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

**Correlation is significant at 0.01 level

*Correlation is significant at 0.05 level

4

5

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

[a]Values of dominant elements in each factor are indicated bold

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

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

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

**Correlation is significant at 0.01 level
*Correlation is significant at 0.05 level

Table 7 Factor loading for selected heavy metals in 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

[a]Values of dominant elements in each factor are indicated bold

Figure


Legend
Water samples
Residential area
Grazing area
0 0.5 1 km
N

Unsri Google Apps

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

Decision on your manuscript - JEST-D-20-02975R1 - [EMID:b8541cb7a84df1e9]

M. Abbaspour <em@editorialmanager.com> 28 Mei 2021 pukul 21.03
Balas Ke: "M. Abbaspour" <editor@ijest.org>
Kepada: asep indra munawar ali <asep_ali@fp.unsri.ac.id>

Ref.:Ms.No.JEST-D-20-02975R1
Heavy metals accumulation in forages and buffalo hair on flooded pasture in South Sumatra, Indonesia
International Journal of Environmental Science and Technology

Dear Dr. ali,

I am pleased to tell you that your work has now been accepted for publication in International Journal of Environmental Science and Technology.

Comments from the Editor and Reviewers can be found below.

Thank you for submitting your work to this journal

With kind regards
M. Abbaspour
Editor-in-Chief
International Journal of Environmental Science and Technology

Comments from the Editors and Reviewers:

Please note that this journal is a Transformative Journal (TJ). Authors may publish their research through the traditional subscription access route or make their paper open access through payment of an article-processing charge (APC). Find out more about Transformative Journals

Our flexible approach during the COVID-19 pandemic

If you need more time at any stage of the peer-review process, please do let us know. While our systems will continue to remind you of the original timelines, we aim to be as flexible as possible during the current pandemic.

Unsri Google Apps

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

Proofs for your article in International Journal of Environmental Science and Technology (3424)

spr_corrections@springer.com <spr_corrections@springer.com> 3 Juni 2021 pukul 13.01
Kepada: asep_ali@fp.unsri.ac.id

SPRINGER NATURE

Article Title : Heavy metals accumulation in forages and buffalo hair on flooded pasture in South Sumatra, Indonesia
DOI : 10.1007/s13762-021-03424-w
JEST-D-20-02975R1

Dear Author,

We are pleased to inform you that your paper is nearing publication. You can help us facilitate quick and accurate publication by using our e.Proofing system. The system will show you an HTML version of the article that you can correct online. In addition, you can view/download a PDF version for your reference.

As you are reviewing the proofs, please keep in mind the following:

- This is the only set of proofs you will see prior to publication.
- Only errors introduced during production process or that directly compromise the scientific integrity of the paper may be corrected.
- Any changes that contradict journal style will not be made.
- Any changes to scientific content (including figures) will require editorial review and approval.

Please check the author/editor names very carefully to ensure correct spelling, correct sequence of given and family names and that the given and family names have been correctly designated (NB the family name is highlighted in blue).

Please submit your corrections within 2 working days and make sure you fill out your response to any AUTHOR QUERIES raised during typesetting. Without your response to these queries, we will not be able to continue with the processing of your article for Online Publication.

Your article proofs are available at:

https://eproofing.springer.com/ePj/index/PhfdMBL2PS1E-Yx6kmswbq_1H31L3Wxg9Hp1pD0YMQjPP7aphY9R6F-VxspaF0-Cb1yVxEyuSAr5uW5plji9SNHqrs8d6Lyg8u7rUALV62sP9DjvFg3mLPL3Z9u4RPYA

The URL is valid only until your paper is published online. It is for proof purposes only and may not be used by third parties.

Should you encounter difficulties with the proofs, please contact me.

We welcome your comments and suggestions. Your feedback helps us to improve the system.

Thank you very much.

Sincerely yours,

Springer Nature Correction Team

No. 6&7, 5th Street, Radhakrishnan Salai,
Mylapore, Chennai, Tamilnadu
India, Pincode 600 004
e-mail: spr_corrections@springer.com
Fax: