Heliyon

Received:
18 July 2018
Revised:
28 August 2018
Accepted:
10 September 2018

Cite as: Gashaw Dessalew, Abebe Beyene, Amsalu Nebiyu, Tessema Astatkie. Effect of brewery spent diatomite sludge on trace metal availability in soil and uptake by wheat crop, and trace metal risk on human health through the consumption of wheat grain.
Heliyon 4 (2018) e00783.
doi: 10.1016/j.heliyon.2018.e00783

Effect of brewery spent diatomite sludge on trace metal availability in soil and uptake by wheat crop, and trace metal risk on human health through the consumption of wheat grain

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Abstract

Soil plays a crucial role in food safety as it determines the composition of food at the beginning of the food chain. However, the quality of soil resources in terms of their potential impact on human health caused by harmful elements is poorly understood in Ethiopia due to lack of reliable and appropriate experimental data. In this study, a field experiment was conducted to evaluate trace metal accumulation in soils amended with brewery spent diatomite sludge (BSDS) in comparison to control, recommended inorganic fertilizers (RIF) and integrated BSDS & RIF (BSDS:RIF). Trace metals uptake by wheat crop and the degree of soil contamination, and human health risk were investigated. BSDS application significantly increased the concentration of trace metals (Cu, Zn, Pb and Ni) in soil compared to that in the control, the RIF, and BSDS:RIF applications. It also significantly increased the concentration of Cu, Zn, Pb and Ni in the wheat grain compared to that in the control and the RIF, but the increase was not significantly different from that in BSDS:RIF amendment. All trace metal

https://doi.org/10.1016/j.heliyon.2018.e00783

concentrations in soil and wheat grain (except Pb in wheat grain) were below the maximum permissible limits in some European countries of agricultural soils, and the maximum permissible limit of FAO/WHO. The degree of soil contamination from each of the treatments was below the maximum acceptable degree of contamination. The Health Risk Index (HRI) was <1 for all metals in all treatments. Thus, we conclude that consumption of wheat grain grown on soils amended with BSDS and BSDS:RIF has no human risks and has low likelihood of human exposure to trace metals.

Keyword: Agriculture

1. Introduction

The safe disposal of sewage sludge is one of the major environmental concerns worldwide. Land filling and land application of the sewage sludge are reported as commonly used disposal techniques (Singh and Agrawal, 2008). Land application represents an economically desirable outlet for the producers of wastes by reducing its volumes and its disposal costs and a potentially cheap source of organic matter and plant nutrients for farmers (Iwegbue et al., 2006; Nouri et al., 2008).

Moreover, sewage sludge application is used as a soil conditioner for improving the physical and chemical properties of soils in farmland, forests and home gardens (Wong and Su, 1997; Debosz et al., 2002; Wang et al., 2003; Dolgen et al., 2007). The macronutrients in the sewage sludge serve as a good source of plant nutrients and the organic constituents provide beneficial soil conditioning properties (Singh and Agrawal, 2008). Some waste materials may also contain non essential elements, persistent organic compounds and microorganisms that may be harmful to plants (Mullin and Mitchell, 1994; Chukwuji et al., 2005). For instance, the presence of toxic trace metals in the sludge is a risk that is often cited against the use of sewage sludge for nutrient supplementation of soils (Nicholson et al., 2003; Ayari et al., 2008). High and excessive accumulation of trace metals in soil may result in plants to absorb trace metals above the permitted levels and enter the food chain affecting human health (Arora et al., 2008). Therefore, effective management of sewage sludge must overcome such undesirable effects in crop production systems (Skjelhaugen, 1999). Thus, proper management of sludge utilization should consider many aspects including its trace metal content, impact on crop nutrient requirement and growth, and biological and physico-chemical properties of soils. These aspects are essential to determine the optimum rate, time and method of sludge application (Banerjee et al., 1997; Arora et al., 2008).

In Ethiopia, brewery industries generate large quantities of solid wastes and solid diatomite sludge, and one of these breweries is Borland Graphics Interface (BGI)

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Brewery Limited Company, located in Kombolcha town in northeast Ethiopia. Some farmers in this area have already started using brewery diatomite waste sludge (BSDS) for soil amendment in crop production. Dessalew et al. (2017) studied the impact of BSDS on soil physico-chemical properties and yield and quality of the major cereals (wheat and teff) grown in northeast Ethiopia. However, its impact on uptake of trace metals by wheat and the associated human health risk indices and environmental impacts are poorly understood. Hence, this study aims to evaluate the effects of application of BSDS on yield of wheat grain, trace metals in the soil, trace metals in the wheat grain, and human health risk index due to trace metals.

2. Materials and methods

2.1. Description of the study area

A field experiment was conducted on agricultural lands located on the east side of Kombolcha town in north eastern Ethiopia. Geographically, it is located at 11°04′45.62″ N latitude and 39° 43′30.29″E longitude with an altitude of 1800 m.a.s.l. The area is characterized by bimodal rainfall pattern with mean annual precipitation of 1038.1 mm. The annual maximum and minimum temperatures are 34.0 °C and 4.2 °C, respectively (NMA, 2015).

2.2. Treatments and experimental design

A two-year (2014 and 2015) field experiment was conducted during the main cropping season (from June to November). The test crop was wheat (*Triticum aestivum* L.), which is the most commonly grown cereal in the study area. Within each year, four treatments consisting of (i) control (no fertilizer or brewery diatomite waste sludge [BSDS]), (ii) 100% BSDS at 0.5 t ha^{-1}, (iii) 100% recommended inorganic fertilizer (RIF) consisting of 64 kg N and 46 kg P ha^{-1}, and (iv) equal proportion of BSDS & RIF (BSDS:RIF) were randomized within each of the three blocks of land resulting in a Randomized Blocks Design. Urea (46% N) and Di-Ammonium Phosphate (DAP; 18% N and 46% P_2O_5) were used as sources of N and P, respectively for RIF and BSDS:RIF treatments. The BSDS treatments were broadcasted and thoroughly incorporated into the top 15 cm of the soil 20 days before sowing. Wheat was grown on 4 × 5 m plots at a raw spacing of 20 cm apart. Seeds were drilled in 20 rows, each 5 m long, at a seeding rate of 60 kg ha^{-1}. Plots were kept 1 m apart and blocks at 2 m apart. The full dose of P (46 kg P) and half the dose of N (32 kg) were applied during sowing; the remaining dose of N (32 kg) was applied during heading initiation. Hand weeding was done three times during the growing period. The crop was harvested at grain maturity and grain yield (kg ha^{-1}) was determined.

https://doi.org/10.1016/j.heliyon.2018.e00783

2.3. Soil and BSDS sampling and analyses

Before sowing and applying treatments, three composite soil samples were collected from the experimental site at a depth of 20 cm using a soil auger. Similarly, soil samples from each plot were collected after crop harvest to determine changes in trace metals concentration of the soil because of BSDS, RIF and BSDS:RIF applications. One kg BSDS sample was also collected from the dumping sites of BGI factory. The soil and BSDS samples were air dried, grinded and passed through a sieve of 2 mm mesh and then stored separately for further physico-chemical analyses. The pH of samples was measured in suspension of 1:2.5 soils to potassium chloride with the help of pH meter (Chopra and Kanwar, 1976). Soil and BSDS organic carbon and total N contents were determined by Walkley and Black's rapid titration method (Allison, 1973) and Gerhardt automatic analyzer (Model KB 8S, Germany), respectively. Available P was determined by $NaHCO_3$ extraction method (Olsen and Sommers, 1982).

2.4. Trace metal analysis in wheat grain and soil samples

About 200 g composite wheat grain samples were collected from each experimental unit during harvesting in 2015 and oven-dried at 70 °C for 72 h, and then grounded with an agate motor to fine powder for trace metal analysis. Oven dried wheat grain and soil samples (1 g) were digested after adding 15 ml tri-acidic mixture solution of HNO_3, H_2SO_4 and $HClO_4$ at 5:1:1 ratio at 80 °C till a transparent solution was obtained (Allen et al., 1986). After cooling, the digested sample was filtered using Whatman no. 42 filter paper and the filtrate was finally maintained to 50 ml with distilled water. Concentrations of Zn, Cu, Pb and Ni in the filtrate of digested samples were estimated by using an atomic absorption spectrophotometer (Model 2380, Perkin Elmer, Inc., Norwalk, CT, USA).

2.5. Bioaccumulation factor

The bioaccumulation factor (BAF), the ratio of the concentration of the element in the wheat grain to that in the corresponding soil, was calculated for wheat grain samples to quantify the bioaccumulation effect of wheat grain on the uptake of trace metals from the soils (Liu et al., 2005). BAF was calculated using Equation (1).

$$BAF = \frac{Cr}{Cs} \tag{1}$$

where Cr and Cs are trace metal concentrations in grains and soils, respectively.

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2.6. Enrichment factor

The Enrichment factor (EF) was calculated to evaluate the degree of soil contamination and trace metal accumulation in soil and in the plant grown on contaminated site with respect to soil and plants growing on uncontaminated soil (Kisku et al., 2000) as follows:

$$EF = \frac{\text{Concentration of metals in soil at CS}}{\text{Concentration of metals in soil at NCS}} \text{ Or } \frac{\text{concentration of metal in grain of CS}}{\text{concentration of metals in grain of NCS}} \tag{2}$$

where CS = contaminated site, NCS = Non-contaminated site.

2.7. Daily intake of trace metals and health risk index

The daily intake of metals (DIM) was calculated using the following equation:

$$DIM = \frac{C_{metal} \times D_{food\ intake}}{B_{average\ weight}} \tag{3}$$

where $C_{metal,}$ $D_{food\ intake,}$ and $B_{average\ weight}$ are trace metal concentrations in plants (mg kg^{-1}), daily intake of wheat grain by human, and average body weight, respectively (Khan et al., 2008). The average daily wheat grain intake used was 0.088 kg $person^{-1}$ day^{-1} for wheat (FAO, 2014: Minot et al., 2015), while the average body weight used was 60.7 kg (Walpole et al., 2012). Health risk index (HRI) for intake of trace metals through the consumption of contaminated wheat grain was calculated as the ratio of daily intake of metals (DIM) to oral reference dose (ORfD) (Cui et al., 2004).

$$HRI = \frac{DIM}{ORfD} \tag{4}$$

Oral reference doses were 0.04 and 0.3 mg kg^{-1} day^{-1} for Cu and Zn (USEPA, 2002) and 0.004 and 0.02 mg kg^{-1} day^{-1} for Pb and Ni (USEPA, 1997), respectively. An index more than 1 is considered to be unsafe for human health (USEPA, 2002).

2.8. Statistical analysis

Four treatments (Brewery Spent Diatomite Sludge [BSDS], Recommended Inorganic Fertilizers [RIF], integrated BSDS & RIF with equal proportion [BSDS: RIF], and none of these [Control]) applied to the soil when growing wheat in 2014 and 2015 were compared in terms of trace metals (Cu, Zn, Pb, and Ni) in the soil and in the wheat grain measured in 2015 using ANOVA of a Randomized Blocks Design with 3 blocks. However, since wheat grain yields were measured both in 2014 and 2015, the ANOVA for wheat grain yield was completed using a Randomized Blocks Design with 6 blocks (combination of the 2 years and the 3

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blocks in the field within each year). The analysis was completed using the GLM Procedure of SAS (SAS, 2014), and the validity of model assumptions (normal distribution and constant variance of the error terms) was verified by examining the residuals as described in Montgomery (2017). While all assumptions were met for the 8 response variables, Pb in the grain required a cubic-root transformation. However, the means shown in Table 3 are back-transformed to the original scale. Since the effect of treatment was significant (p-value < 0.05) on all 9 response variables, multiple means comparison was conducted using Fisher's LSD at the 5% level of significance and letter groupings were generated.

3. Results and discussion

3.1. Physico-chemical characteristics of soil and BSDS

As indicated in Table 1, the soil pH is nearly neutral whereas BSDS is slightly basic. Nitrogen, phosphorous, potassium, and organic carbon contents in BSDS were higher than the contents in the soil. The concentrations of heavy metals (Zn, Cu

Table 1. Physico-chemical characteristics of brewery spent diatomite sludge (BSDS) and Soil.

Chemical attributes	Brewery sludge	MPL of heavy metals in sludge	Experimental soil	MPL of heavy metals in soil
Sand (%)	-	-	12.79	-
Silt (%)	-	-	26.05	-
Clay (%)	-	-	60.16	-
Texture class	-	-	Clay	-
EC (mS cm^{-1})	-	-	0.26	-
CEC (meq 100 g $soil^{-1}$)	-	-	61.30	-
pH	8.79	-	6.63	-
Organic carbon (g kg^{-1})	309	-	0.84	-
N (g kg^{-1})	22	-	0.11	-
P (g kg^{-1})	415.91	-	24.61	-
K (g kg^{-1})	1664.98	-	371.33	-
Zn (ppm)	33.19	3000[a]	43	300[b]
Cu (ppm)	25.19	1500[a]	82	100[b]
Pb (ppm)	1.16	1000[a]	0.5	100[b]
Ni (ppm)	31.12	75[c]	81	100[b]

[a] Maximum Permissive Limit (MPL) of heavy metals in sludge (USEPA, 1997).

[b] Maximum permissible agricultural soil concentration in of heavy metals some European countries (Kabata-Pendias and Pendias, 2001).

[c] Maximum Permissive Limit (MPL) of heavy metals in sludge (USEPA, 1993).

https://doi.org/10.1016/j.heliyon.2018.e00783

and Ni) in BSDS were lower than the concentrations in the soil. However, Pb concentration in BSDS (1.16 ppm) was higher than that in the soil (0.5 ppm). Importantly, in reference to the standards shown in Table 1, the studied heavy metals concentrations in both BSDS and soil were lower than the permissible limits of United States Environment Protection Agency (USEPA) (USEPA, 1993, 1997), and the maximum permissible agricultural soil concentration in some European countries (Kabata-Pendias and Pendias, 2001).

3.2. Grain yield

The addition of BSDS resulted in a significantly higher wheat grain yield (2401 kg ha^{-1}) than that obtained from the control (1151 kg ha^{-1}) but was significantly lower than those resulted from the use of RIF (4000 kg ha^{-1}) and BSDS:RIF (4545 kg ha^{-1}). Compared to the control, about two-fold increase in wheat grain yield was observed due to the addition of BSDS; however, this positive effect of BSDS on yield of wheat grain was far below that observed for RIF and BSDS:RIF. The results from the use of BSDS to improve yields of cereal is in agreement with the results of Luque et al. (1990), who reported a significant correlation between the doses of BSDS and yields of corn, sorghum and groundnut. Iliescu et al. (2009) have also observed that the application of BSDS on wheat and tomato seedlings increased vegetative mass and yields. Yield of mung bean has been reported to increase in a soil amended with increasing concentrations of sewage sludge (Singh and Agrawal, 2010). Our study is the first experimental evidence that shows the use of BSDS alone, and BSDS:RIF improves wheat gain yield (giving a yield of 2401 kg ha^{-1} and 4545 kg ha^{-1}, respectively). Thus, the use of BSDS as an organic fertilizer could contribute to improve productivity. As the cost of inorganic fertilizers rise, BSDS may provide a more affordable option integrated with commercial fertilizer or solely.

3.3. Trace metal content in soil

Trace metal contents of the control soil (without treatment), soil amended with BSDS, RIF and BSDS:RIF are shown in Table 2. The application of BSDS on the soil had an effect on trace metal (Cu, Zn, Pb and Ni) content as compared to the soil treated with RIF, BSDS:RIF, and control. Compared to the control, amending the soil with RIF or BSDS:RIF does not significantly affect Cu and Zn content in the soil. However, amending the soil with RIF or BSDS:RIF significantly increases Pb and Ni content in the soil (Table 2). The lowest trace metals content was recorded in control soil. The work of Kumar and Chopra (2012) also showed that amendment of the soil by paper mill sludge increased the concentration of trace metals (Zn, Cu and Pb) in the soil. The trace metals concentration in our experimental soil was below the maximum permissible agricultural soil concentration in some European countries (Kabata-Pendias and Pendias, 2001).

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Table 2. Mean trace metals (Cu, Zn, Pb, and Ni) in the soil (mg kg^{-1}) obtained from the four treatments.

Treatment	Cu in the soil	Zn in the soil	Pb in the soil	Ni in the soil
BSDS	57.7 a[a]	88.3 a	0.096 a	97.0 a
RIF	45.3 b	85.3 ab	0.073 c	85.3 c
BSDS:RIF	46.3 b	83.7 b	0.086 b	89.7 b
Control	43.3 b	83.0 b	0.52 d	81.0 d
MPL	100	300	100	100

MPL = Maximum Permissible Limit in some European countries (Kabata-Pendias and Pendias, 2001).
[a] Within each trace metal, means sharing the same letter are not significantly different at the 5% level of significance.

3.4. Trace metal concentration in grain

Table 3 shows that the concentrations of Cu, Zn and Ni in wheat grain was the highest for plots amended with BSDS as compared to that in the plots amended with RIF, BSDS:RIF and control. Wheat grain Pb concentration did not significantly differ between BSDS and control. Also, there was no significant difference between the concentrations of Cu and Zn in grain of RIF and BSDS:RIF amended soils (Table 3). The control treatment had the lowest concentration of Cu, Zn, Pb and Ni (Table 3). Among the trace metals, grain Zn concentration was found to have elevated values (as high as 3.63 mg kg^{-1}). Singh and Agrawal (2010) also reported that Cu, Pb and Zn contents in the edible part of mung bean increased significantly with sludge application as compared to the control. Kumar and Chopra (2012) have also found that there was accumulation of Zn, Cu and Pb in French beans when paper mill sludge was applied. In the present study, it was interesting to observe that, except Pb in BSDS:RIF treatment, wheat grain Cu, Zn and Ni concentrations were below the maximum permissible limits of FAO/WHO (2001). However, the high concentration of Pb (0.546 mg kg^{-1}), which is higher than the FAO/WHO maximum permissible limit could be due to the high content of Pb in

Table 3. Mean trace metals (Cu, Zn, Pb, and Ni) in wheat grain (mg kg^{-1}) obtained from the four treatments.

Treatment	Cu in the grain	Zn in the grain	Pb in the grain	Ni in the grain
BSDS	2.85 a[a]	3.63 a	0.147 b	0.549 a
RIF	2.12 b	2.53 b	0.096 b	0.450 b
BSDS:RIF	2.22 b	2.67 b	0.546 a	0.550 a
Control	0.60 c	0.93 c	0.059 b	0.239 c
MPL	73.3	99.4	0.3	67.0

MPL = Maximum Permissible Limits, FAO/WHO, 2001.
[a] Within each trace metal, means sharing the same letter are not significantly different at the 5% level of significance.

https://doi.org/10.1016/j.heliyon.2018.e00783

BSDS from this specific brewery, and the porosity nature of BSDS that could increase bio-availability of Pb in the soil.

3.5. Bioaccumulation factors

In our study, the calculated values of bioaccumulation factor (BAF) allowed the evaluation of the ability of wheat plant to collect trace metals present in the soil, and the determination of the uptake rate of metals from the soil solution containing BSDS, RIF and BSDS:RIF to the aerial parts of wheat (Fig. 1). Moreover, the presence and toxicity of the metals will largely depend on the functions they perform in the metabolic processes of the organism and the susceptibility of the plants to bioaccumulation (Bose and Bhattacharyya, 2008).

In the present study, we observed the effect of BSDS, RIF, and BSDS:RIF on the rate of bioaccumulation. The lowest rate of bioaccumulation for all the analyzed metals was found on the plots without amendment (control), while the highest BAF was for the BSDS treated plots (Fig. 1). Among all the analyzed metals, Pb was the highest and Ni was the lowest BAF in all four treatments (Fig. 1). The trend in the BAF values in the study sites was in the ranking order of BSDS > RIF > BSDS:RIF > Control. The potential sorption of metals on soil particles and organic matter could be responsible for a limited transfer to the plants. In addition, some soil characteristics, such as pH, CEC and moisture, can play important roles in the movement of metals from soils to plants (McBride, 2003; Tiwari et al., 2011). BAF of metals decreases with increasing pH of soil (Wuana and Okieimen, 2011; Wołejko et al., 2013).

Plants with different BAF values are categorized as excluder, accumulator and hyper accumulator when BAF values are <1.0, 1.0−10.0 and >10.0, respectively

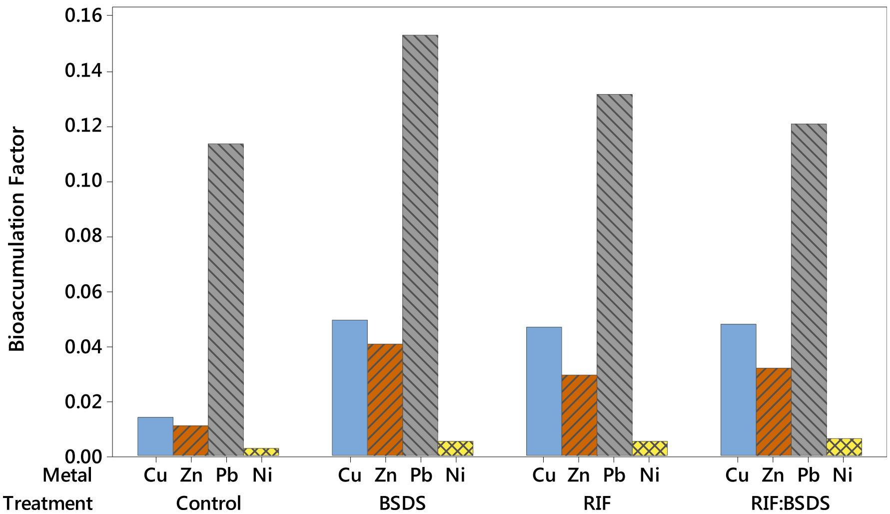

Fig. 1. Bioaccumulation factor of the trace metals in the different treatments.

https://doi.org/10.1016/j.heliyon.2018.e00783

(Ma et al., 2001). When BAF <1, it indicates that the plant only absorbs the trace metal but does not accumulate. When BAF >1, it indicates that the plant accumulates the trace metals. BAF values of all analyzed metals (Cu, Zn, Pb and Ni) in the present study were <1 in the wheat grain of all treatments, which indicates that plants have only absorbed but did not accumulate the trace metals.

3.6. Enrichment factor

The enrichment factors (EF)s of BSDS, RIF and BSDS:RIF amended soils for the trace metals are shown in Fig. 2. In BSDS treated soil, the EFs of trace metals were found to be in the order of Pb > Cu > Ni > Zn while EFs of trace metals in both RIF and BSDS:RIF treated soil were in the order of Pb > Ni > Cu > Zn, respectively. The EF values greater than 1 indicate higher availability and distribution of metals in the contaminated soil, subsequently increasing the metal accumulation in plants grown on the soil (Kisku et al., 2000; Gupta et al., 2008). Among the metals studied, the highest enrichment was recorded for Pb for all treatments (Fig. 2), indicating that Pb has relatively high availability and distribution compared to the other metals in the present study.

3.7. Daily intake of metals and human health risk assessment

Human risk assessment from the pathway of food chain is of prime importance in countries like Ethiopia, where proper usage of sludge for agriculture is still poorly understood. There are several exposure pathways that depend mainly on contaminated sources of air, water, soil, and food (Caussy et al., 2003), but the routes of exposure via food chain is one of the key pathways of trace metals exposure to human (Muchuweti et al., 2006). Cereal crops are staple food for most Ethiopians. In the

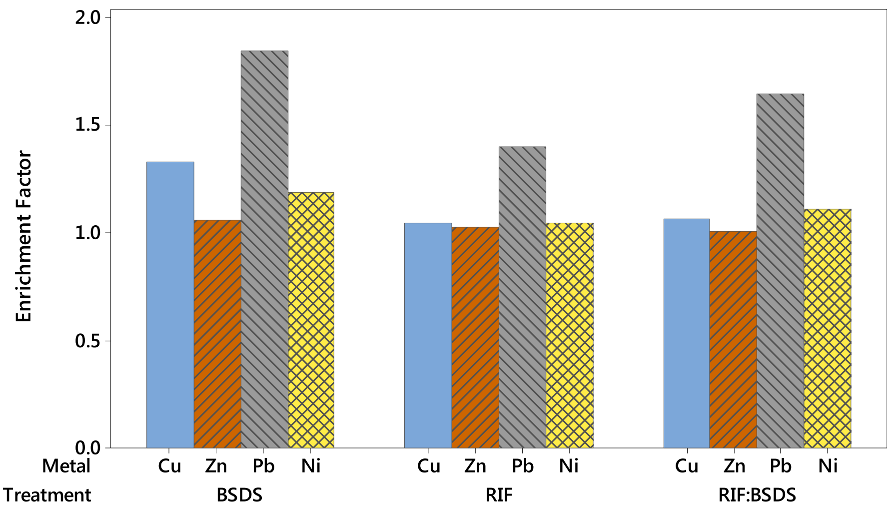

Fig. 2. Enrichment factor of the trace metals in soil in the different treatment.

https://doi.org/10.1016/j.heliyon.2018.e00783

Table 4. Daily intakes of trace metals (DIM) (mg kg^{-1} $person^{-1}$ day^{-1}) and health risk index (HRI) of Cu, Zn, Pb, and Ni trace metals in wheat grown using the control (untreated) and treated with brewery spent diatomite sludge (BSDS), recommended inorganic fertilizer (RIF) and BSDS:RIF soils.

Treatment	Index	Cu	Zn	Pb	Ni
Control	DIM	8.67E-04	13.05E-04	0.86E-04	3.67E-04
BSDS	DIM	41.32E-04	52.63E-04	2.13E-04	7.95E-04
RIF	DIM	30.75E-04	36.75E-04	1.39E-04	6.52E-04
BSDS:RIF	DIM	32.14E-04	38.66E-04	1.51E-04	8.28E-04
Control	HRI	217.46E-04	43.49E-04	213.84E-04	173.25E-04
BSDS	HRI	1032.95E-04	175.42E-04	532.79E-04	397.23E-04
RIF	HRI	768.73E-04	122.50E-04	347.94E-04	326.19E-04
BSDS:RIF	HRI	803.53E-04	128.88E-04	376.94E-04	413.91E-04

present study area, wheat grain is either consumed directly by the local farmers or sold to consumers as a means of income generation for the farmers. Wheat grain is consumed daily by the local population in a form of bread, porridge, roasted grain, boiled grain, pasta, local traditional beer, and other confectionary products. Oral reference dose (ORfD) is the daily exposure of individuals to toxins or pollutants that does not pose substantial hazardous in their lifetime. The ORfDs for trace metals like Cu, Zn, Pb and Ni are 0.04, 0.3, 0.004 and 0.02 mg kg^{-1} day^{-1}, respectively (USEPA, 1997, 2002). In our study the daily intake of trace metals (DIM) and the health risk index (HRI) of these trace metals in the different treatments are shown in Table 4. The HRI of all the studied trace metals was below 1, suggesting that there is no human health risk due to the consumption of wheat grain grown on BSDS, RIF and BSDS:RIF amended soil. This revelation is very important to understand the health risk to human population of Ethiopia from trace metals consumption in wheat grain.

4. Conclusions

Grain yield of wheat increased with the application of BSDS, RIF and BSDS:RIF. Soil and wheat grain also showed greater accumulation of trace metals such as Cu, Zn, Pb and Ni with BSDS amendment compared with the control, RIF and BSDS:RIF applications. Notable findings of the present study were that the concentrations of trace metals both in the soil and wheat grain did not exceed the maximum permissible limit in agricultural soil concentration of some European countries and the permissible limits of FAO/WHO, respectively. Although wheat plants grown on BSDS amended soils either alone or integrated with RIF showed a higher metal uptake compared to the control and RIF, none of the trace metals had HRI >1. Among the trace metals, Zn was less mobile and Pb was highly mobile. Although enrichment

https://doi.org/10.1016/j.heliyon.2018.e00783

factor increased with the BSDS application reflecting the important load/transfer of heavy metals in the soil/plant system, none of the four treatments exceeded the maximum contamination level.

Declarations

Author contribution statement

Gashaw Dessalew: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Abebe Beyene, Amsalu Nebiyu: Conceived and designed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Tess Astatkie: Conceived and designed the experiments; Analyzed and interpreted the data; Wrote the paper.

Funding statement

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Competing interest statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

Acknowledgements

The authors wish to thank Jimma University, Samara University and BGI Ethiopia for their financial and logistic support. We also thank the staff of Bless Agri Food Laboratory Services and the Geological Survey of Ethiopia, Laboratory Center for providing support to conduct the laboratory analysis. Finally, the authors are grateful to the farmers at our study site for their assistance during the field experiments.

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