Effects of brewery sludge on soil chemical properties, trace metal availability in soil and uptake by wheat crop, and bioaccumulation factor

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ABSTRACT

Brewery sludge is the solid residue obtained from agro-industrial processing. It is possible to utilize the waste products in an environment friendly and economical way to replace mineral fertilizer due to its sufficient macronutrients and organic carbon content. However, its use is limited due to heavy metal concentration that may contaminate crops and then the food chain. The objective of this study was to assess the suitability of brewery sludge for using to grow bread wheat (Triticum aestivum L.) by determining the effect of brewery sludge (7 levels: 0, 3, 6, 9, 12 and 15 t ha⁻¹, and 1 recommended rate of NPS only) on soil chemical properties, bioaccumulation factor, and heavy metal absorption in the soil and in the bread wheat grain using a Randomized Blocks Design field experiment conducted at two sites during the 2018 cropping season. Amendment of brewery sludge at a rate of 15 t ha⁻¹ led to substantial variations in soil chemical properties except for Mg²⁺ content at both study sites. Concentrations of the studied heavy metals (except Zn in the soil) increased with increasing brewery sludge application rate in the soil and in the wheat grain. However, heavy metal uptake by wheat grain and heavy metal concentration in the soil were below the allowed limits. The bioaccumulation factor in the wheat grain was < 1.0 for the studied heavy metals. The findings of the study suggest that brewery sludge at a rate of 15 t ha⁻¹ could be recommended due to its high nourishing effect for soil and for promoting nutritional quality of wheat crop and is safe for human consumption. However, since sludge application may lead to increase in the amount of trace metals in the soil-plant system, a long-term study is recommended.

1. Introduction

The recent expansion of brewery factories in Ethiopia may lead to a substantial ecological pollution (Alayu and Yirgu, 2017). The brewery industry discharges a large amount of waste products including spent grain, sludge, hot trub, and yeast cells that contain a large amount of contaminants (Kanagachandran and Jayaratne, 2006) and threatens the surrounding areas (Rajagopal et al., 2013). Therefore, appropriate removal of the waste of brewery products is an important environmental concern worldwide. Currently, landfilling and land application are considered as the best disposal mechanisms of waste brewery sludge in Ethiopia (Alayu and Yirgu, 2017).

According to Nouri et al. (2008), land application is considered as an alternative strategy for brewery producers to minimize dumping expenses and to avoid land space restrictions, and it serves as an economical source of organic fertilizer that provides macro and micronutrients for crop producers. Furthermore, brewery sludge application is used for improving the physiochemical properties of agricultural soils (Alayu et al., 2018). Additionally, brewery sludge serves as a good supply of plant macronutrients and organic ingredients that improve several aspects of soil quality (Singh and Agrawal, 2008). Conversely, sludge intended for agricultural use may encompass heavy metals, non-biodegradable properties, tenacious organic pollutants, accumulative behaviors, and microorganisms that have detrimental effects to the plant (Chukwuji et al., 2005; Wang and Chen, 2009) and can cause serious health risks via transmission into the food chain if their level exceeds the recommended (Arthurson, 2008; Arora et al., 2008).

In Ethiopia, bread wheat (Triticum aestivum L.) is one of the major crops and an important constituent of the national diet and provides income to growers to reduce poverty and increase the socio-economic growth of the country (Mulatu and Dechassa, 2015). Furthermore, it supplies carbohydrates, proteins, and micronutrients for human growth.

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Bioaccumulation factor is an important method that measures the accumulation and uptake of trace metals by wheat crops from the surrounding soil. It also assesses the efficiency of wheat in heavy metal accumulation and translocation. Therefore, the transfer and accumulation of heavy metals in soil-plant frameworks is an essential research topic. When the accumulation exceeds the allowable limit, it exerts toxic effects and may harm immune, nervous, and reproductive systems (Al-Othman et al., 2012; Aladesanmi et al., 2019). As a result, it is imperative to assess the concentration of trace metals in crops to guarantee safe food consumption and minimize public health and environmental risks.

In Ethiopia, beer companies produce large amounts of waste products (spent grain, yeast cells, and brewery sludge) and one of these breweries is Raya Beer Limited Company (BGI groups), located in Maychew town in Southern Tigray Region. Some farmers in this vicinity have started using waste sludge for crop production and animal feeding. However, no scientific research has been carried out to study the effect of sludge on wheat heavy metal uptake, bioaccumulation factor, and environmental hazards. Therefore, the overall objectives of this study were (1) to examine the concentration of heavy metal in the soil as well as in the wheat grain, and the bioaccumulation factor of the test crop, and (2) to determine the effect of brewery sludge on soil chemical properties.

2. Materials and methods

2.1. Description of the study area

A field experiment was conducted at Ofa district in Southern Tigray Region, Ethiopia during the main cropping season of 2018. Geographically, it is located at 12°30’28”N latitude and 39°51’50”E longitude, and has an altitude of 2468 masl. The two experimental sites (S1 and S2) have a unimodal rainfall pattern with a mean annual precipitation is 986 mm, have a mean annual temperature of 15.3 °C with a mean maximum and minimum temperatures of 5.4 °C and 24.7 °C, respectively (Kidane et al., 2018). According to the classification of the IUSS Working Group (IUSS Working Group WRB, 2015), the soils of Ofa district are dominated by vertisols, which are characterized by very deep, black soil, well structured, well drained, and clayey with no clear pattern along soil depth. The physiochemical properties of the soil at the two experimental sites are shown in Table 1.

2.2. Treatments and experimental design

Field experiments were conducted at 2 sites during the main cropping season (June–November) of 2018. Since bread wheat (King bird variety) is widely grown in the study area, it was used as the test crop for the experiment. The treatments were based on previous unpublished and published reports on cereal crops, initial heavy metal concentration of the soil, the nature of brewery sludge (nutrient content), the raw materials used, and the processing systems. Accordingly, at each site, the experiment, laid out as Randomized Blocks Design (with 3 blocks) has 7 treatments: 1 control (no application), 5 rates of brewery sludge (3, 6, 9, 12 and 15 t ha⁻¹), and 1 recommended rate of NPSBZn (17.8N-35.7 P-7.75S-0.1B-2.22Zn) + Urea (46 %N), from now on referred as NPS. That is, there were 21 plots at each site, and the size of each plot was 2 m × 3 m (6 m²) and the distance between the plots and the blocks were 0.5 m and 1 m, respectively. Treatments were randomized within each block, and all the NPS and half of the N fertilizers were applied in rows and were incorporated into the soil at the time of sowing. The remaining N (31.9 kg) fertilizer was side-dressed at the tillering stage of wheat. Wheat seeds were sown by drilling in 3 m long rows in each plot at a spacing of 20 cm rows at a seeding rate of 150 kg ha⁻¹. Weeds were removed by hand at the early tillering, the maximum tillering, and the booting stages of growth.

2.3. Determination of soil physiochemical properties

At each site, composite surface (0–30 cm) soil samples were collected from 5 randomly selected spots of the experimental field prior to seeding. Soil samples were also collected at the same spots after harvest to determine variations in the concentration of heavy metals and physiochemical properties of the soil due to the application of waste brewery sludge and mineral fertilizer. The soil samples were mixed, air dried, ground to pass through a 2 mm sieve, and analyzed for soil texture, pH, total N, organic carbon, available P, CEC, exchangeable cations (Ca²⁺ and Mg²⁺), and the extractable heavy metals (Pb, Zn, Cu, Mn, Cd and Cr). Soil particle distribution of the samples were determined using the Hydrometer method described in Bouyoucos (1951). The pH of the Soil was measured potentiometrically in the supernatant suspension of a soil-water mixture of 1:2.5 using a pH meter as outlined in Sahlemedhin and Taye (2000).

Total nitrogen in the soil was determined by the micro-Kjeldahl method as described in Jackson (1958). Organic carbon was determined according to the wet digestion method described in Walkley and Black (1934). Available P was determined following the method of Olsen et al. (1954). CEC of the soil was determined from NH₄OAc saturated samples and measured through distillation using the micro-Kjeldahl procedure as described by Brenner and Mulvaney (1982), Exchangeable Ca²⁺ and Mg²⁺ were measured using Atomic Absorption Spectrophotometry. Sodium and Potassium were measured by using flame photometry (Toth and Prince, 1949). The extractable heavy metal elements (Pb, Zn, Mn, Cu, Cd and Cr) were digested in HNO₃/HCl and measured by using atomic absorption spectrophotometry (AAS). All
determinations were done in triplicates, and the accuracy of the procedure was verified by using a spike sample as described in Lindsay and Norvell (1978). The concentration of brewery sludge heavy metals (Cu, Zn, Mn, Cr, Pb, and Cd) before application (Table 2) were extracted using the DTPA extraction method (Lindsay and Norvell, 1978).

2.4. Determination of heavy metals in wheat grain

Wheat grain samples were obtained from each experimental plot at the time of harvest and oven dried for 3 days by setting the temperature at 70 °C, and then ground in an agate motor thoroughly for heavy metal determination. Then the wheat grain and soil samples (1 g) were treated by 15 ml of HNO3, H2SO4 and HClO4 at a ratio of 5:1:1 at 80 °C and digested until a transparent solution was gained as described in Allen et al. (1986). Following cooling, Whatman no. 42 filter paper was used to filter the digested sample, and the filtrate was preserved to 50 ml with distilled water. Finally, the concentrations of Pb, Zn, Mn, Cu, Cd, and Cr in the filtrate of digested samples were determined using atomic absorption spectrophotometer (AAS).

2.5. Bioaccumulation factor (BAF)

The bioaccumulation factors for the analyzed trace metals were determined as proportions of the concentration of a given metal in wheat grain to its subsequent concentration in the soil. These values indicate the plant’s capacity to accumulate heavy metals to its soil substrate. Accordingly, as indicated in Dessalew et al. (2018), BAF was calculated using the following formula.

\[
BAF = \frac{C_g}{C_s},
\]

where \(C_g\) is heavy metal concentration in the grain, and \(C_s\) is heavy metal concentration in the soil.

2.6. Statistical analysis

The effect of brewery sludge (7 levels) on the studied response variables (soil chemical properties, bioaccumulation factor, and heavy metal absorption in the soil and in the wheat grain) was determined at each of the two sites using a Randomized Blocks Design with 3 blocks. For each response variable, the normal distribution and homogenous variance model assumptions on the error terms were verified by examining the residuals as described in Montgomery (2020). Independence assumption is valid because the 6 treatments were randomized within each block. Whenever a treatment effect was significant, multiple means comparison was done using the least significance difference (Fisher’s LSD) method at 5% level of significance to generate letter groupings. The analysis was done using the General Linear Model (GLM) procedure of SAS (SAS, 2014).

3. Results and discussion

3.1. Short term effect on soil physiochemical properties

The analysis of variance revealed that the effect of brewery sludge is significant on all response variables other than exchangeable Mg\(^{2+}\) ions. The multiple means comparison results of the physical and biochemical properties obtained from the plots treated with the control, the 5 levels of brewery sludge, and the NPS are shown in Table 3. The sludge-amended plots showed significant differences in soil pH, electrical conductivity (EC), organic carbon (OC), total nitrogen (TN), available phosphorus (AvP), exchangeable (EX) cations (Ca\(^{2+}\), K\(^{+}\), and Na\(^+\)) and cation exchange capacity (CEC) as compared to the NPS and the control plots; however, there was no significant difference in terms of Mg\(^{2+}\) ions.

The highest reduction in pH (resulting in pH of 6.5) was recorded from the application of 12 t ha\(^{-1}\) at Site 2, which was statistically at par with 15 t ha\(^{-1}\) indicating further increment of brewery sludge from 12 t ha\(^{-1}\) to 15 t ha\(^{-1}\) did not reduce potential of hydrogen in the soil. All 5 rates of brewery sludge at Site 1 did not result in a significantly different pH. However, NPS treatment at both sites increased pH value as compared to the background soil (Table 1 and Table 3). The soil pH of both sites decreased with the application of brewery sludge, which is in agreement with the results reported by Alayu and Leta (2020). Similarly, Obbard et al. (1993) did their experiment with two clusters of soils with identical textures, but one with low pH (4.8–5.8) and the other one with a higher pH (5.0–7.0), and both soils decreased their pH, which is attributed to sludge’s nitrification of the bio residue. The reduction in soil pH might be from the decomposition (aerobic conditions) of organic matter rich compound, thus it produces organic (humic acid) and inorganic acid from mineralization organic matter of brewery sludge treated soils. Furthermore, a small dilution effect might decrease soil pH when the soil is treated with high rates of brewery sludge.

Documented results also indicate that organic nitrogen mineralization of brewery sludge produces protons through nitrification (Oxidation of NH\(_4\) or NH\(_3\)) and mineralization process that releases soluble electrolytes (NO\(_3\) and SO\(_4\)) in the soil solution, that results in pH decrease in the soil (Yilmaz and Temizgül, 2014; Eid et al. 2017, 2018).

Regarding to electrical conductivity, the highest (0.5 mS\(^{-1}\)) was recorded in the 12 t ha\(^{-1}\) treatment at Site 1 (Table 3); however, this result was not significantly different from those obtained at the 9 and 15 t ha\(^{-1}\) application rates. The lowest (0.1 mS\(^{-1}\)) electrical conductivity was obtained from the control treatment at Site 2 (Table 3). Soil salinity, across the entire treatments was directly related to increasing dose of amendment of brewery sludge. This finding maybe explained by the high permeation of salinity due to the introduction of brewery sludge. Like in this study, Alayu and Leta (2020) reported that a higher amount of brewery sludge increases soil salinity, without causing serious stress on the development of maize plant. Latare et al. (2014) reported EC ranging from 0.15 to 0.37 and 0.3 to 0.45 mS\(^{-1}\) in post-harvest rice soils and

### Table 2. Heavy metal concentration of the soil at the two sites and brewery sludge.

| Heavy metal | Concentration (mg/kg) | Site 1 | Site 2 | Standard (mg/kg) | CCME\(^{1}\) Limit | USEPA\(^{2}\) Limit |
|-------------|-----------------------|--------|--------|------------------|---------------------|------------------|
|             | Brewery sludge        |        |        |                  |                     |                  |
| Zn          | 26.5                  | 79     | 65     | 700              | 7500                |          |
| Cd          | 1.2                   | 0.3    | 0.3    | 3                | 85                  |          |
| Ni          | 28.5                  | 91     | 79     | 62               | 420                 |          |
| Pb          | 0.6                   | 0.4    | 0.7    | 150              | 840                 |          |
| Mn          | 0.5                   | 1907   | 1983   | -                | -                   |          |
| Cu          | 16.2                  | 49     | 46     | 400              | 4300                |          |
| Cr          | 0.5                   | 68     | 61     | 210              | 3000                |          |

\(^{1}\) Canadian Council of Ministers of the Environment (CCME) (2005), no limits in use.

\(^{2}\) United States Environmental Protection Authority (USEPA) (1994), ceiling limits for all sludge applied to land.
wheat soils, respectively. The soil salinity found in the current study was not at a level that could restrict the rate of plant development because the EC values meet the mediocre salinity cutoff of most plants, which is between 3 and 4 mS m⁻¹ (Abdullah et al., 2015).

Cation exchange capacity (CEC) is widely used by agronomists and soil scientists to quantify the surface charge in soil. The highest (55.2 cmol (+) kg⁻¹) CEC was recorded from the plot that received the highest application rate (15 t ha⁻¹) at Site 2 (Table 3); however, this result was not significantly different from that obtained from the 12 t ha⁻¹ rate. The results showed that amendment of brewery sludge at both sites consistently increased CEC of the soil as the application rate increases; and the lowest (31.7 cmol (+) kg⁻¹) CEC was recorded in the mineral fertilizer (NPS) treatment at Site 1 (Table 3). A plausible explanation for such improvement of soil CEC might be the impact of organic matter on chemical properties of the soil that altered its colloidal complex. It represents the rise in negative surface charges (CEC) and consequently the rise in cation retention (Abreu et al., 2005).

Exchangeable Ca²⁺ (EX.Ca²⁺) was highly affected by the application rate of brewery sludge. Although the exact values of EX.Ca²⁺ at the two sites were a bit difference, the significance of the treatment differences and the increasing response with increasing application rate were consistent at both sites (Table 3). The lowest mean EX.Ca²⁺ was recorded from the control treatment at both sites (Table 3). Sodium content varied with background of the soil and the brewery sludge application rate. Increasing the application rate of brewery sludge increased sodium content at both sites. As a result, the highest (434.6 mg kg⁻¹) sodium content was obtained from the control treatment at both sites (Table 3). These results suggest that applying 

| Treatment | Site | pH | EC (mS m⁻¹) | OC (%) | TN (%) | AvP (mg kg⁻¹) |
|-----------|-----|----|-----------|-------|-------|-------------|
| 0 t ha⁻¹  | S1  | 7.6 | 0.2        | 1.3   | 0.1   | 99.5        |
| 3 t ha⁻¹  | S1  | 6.5 | 0.4        | 14.7  | 2.3   | 712.8       |
| 6 t ha⁻¹  | S1  | 6.5 | 0.4        | 15.7  | 2.6   | 719.2       |
| 9 t ha⁻¹  | S1  | 6.5 | 0.4        | 16.0  | 2.7   | 755.3       |
| 12 t ha⁻¹ | S1  | 6.5 | 0.5        | 17.0  | 2.8   | 779.2       |
| 15 t ha⁻¹ | S1  | 6.6 | 0.5        | 17.6  | 3.1   | 794.5       |
| NPS       |     | 8.0 | 0.3        | 12.0  | 1.8   | 103.4       |
| 0 t ha⁻¹  | S2  | 7.4 | 0.1        | 0.7   | 0.4   | 85.4        |
| 3 t ha⁻¹  | S2  | 6.9 | 0.3        | 14.0  | 2.5   | 682.3       |
| 6 t ha⁻¹  | S2  | 6.7 | 0.4        | 14.5  | 2.8   | 683.6       |
| 9 t ha⁻¹  | S2  | 6.7 | 0.4        | 15.4  | 3.0   | 701.4       |
| 12 t ha⁻¹ | S2  | 6.5 | 0.4        | 16.5  | 3.6   | 731.8       |
| 15 t ha⁻¹ | S2  | 6.5 | 0.4        | 17.0  | 3.7   | 734.8       |
| NPS       |     | 7.5 | 0.2        | 0.6   | 2.0   | 95.4        |

EX.Ca²⁺ (cmol (+) kg⁻¹) CEC (cmol (+) kg⁻¹) EX.K⁺ (mg kg⁻¹) EX.Na⁺ (mg kg⁻¹) EX.Mg²⁺ (cmol (+) kg⁻¹)

Within each column and within each site, means sharing the same letter are not significantly different at the 5% level of significance.
brewery sludge is an effective way of improving soil exchangeable K in the soil. One of the important benefits of incorporating waste brewery sludge in agrarian soils was reported by Singh and Agrawal (2008) to be increased accessibility of macronutrients and micronutrients to the crop. In our experiment, soil NPK content increased with each brewery sludge addition, attaining its largest value with the highest rate (15 t ha$^{-1}$).

Mohammad and Athamneh (2004) also reported that soils amended with sludge tended to have a high phosphorus and organic matter content, and a neutral pH. Similarly, Alcantara et al. (2009) obtained significant increments of P nutrient in the soil up to 40 cm soil layer, and the soil P content improved from 1 mg kg$^{-1}$ (the initial value) to 114 mg kg$^{-1}$ after sludge was incorporated. Moreover, soil treated with brewery sludge at a rate of 0.96 t ha$^{-1}$ increased N, P, and K content by 1.35, 2 and 23-fold, respectively compared to the control treatment (Alayu and Leta, 2020). This confirms that brewery sludge can be used to improve soil quality and source of macronutrients to enhance crop growth and development as suggested previously (Yilmaz and Temizgül, 2014; Zhao et al., 2013).

Brewery sludge has numerous functions in elevating soil with macro-nutrient (N, P, K), micronutrient, and improving soil physicochemical properties (Bouriouj et al., 2015), which makes it an inexpensive and a productive form of brewery sludge disposal. However, an elevated level of contaminants, particularly of pathogenic microorganisms and heavy metals, could create danger in soil (Nogueiro et al., 2013).

Brewery sludge application on the soil had a highly significant effect on organic carbon (OC) content compared to that of NPS and control. The OC content increased with increasing application rate, and the highest (17.6 and 17.0) OC were obtained from the application of 15 t ha$^{-1}$ at Site 1 and Site 2, respectively, which are 13.7- and 27.3-times improvement of the soil compared to the NPS fertilizer. The lowest (0.6) OC was obtained from NPS amended plots at Site 2 (Table 3). The findings of the current study are in line with previous reports. For example, Bai et al. (2017) observed that a combined application of sewage sludge and green manuring significantly elevated organic carbon content by 97.5–122.2%, 167.9–205.7%, 320.2–345.2%, and 352.8–427.8% at the rate of 30, 75, 150, and 300 t ha$^{-1}$ in 2013 and 2014 seasons, respectively as compared to the control plots. Moreover, Alcantara et al. (2009) reported that increasing the concentration of sewage sludge increased the amount of OC in the soil at both 0–20 and 20–40 cm depths, which was possibly directly affecting soil cation exchange capacity (CEC) at both depths. The OC results in our study were expected because the initial OC content at the study sites were very low compared to that in the applied brewery sludge (Table 1), which corroborates the findings of Singh and Agrawal (2010).

3.2. Trace metals in the soil

Multiple means comparison results of the trace metal analysis are shown in Table 4. The concentrations of the metals are in the order of Mn $>$ Zn $>$ Ni $>$ Cr $>$ Cu $>$ Cd $>$ Pb and Mn $>$ Ni $>$ Zn $>$ Cr $>$ Cu $>$ Cd $>$ Pb at Site 1 and Site 2, respectively. Furthermore, the results showed that the concentration of heavy metals increased with increasing brewery sludge application rate. Compared to the initial content in the soil, the application of brewery sludge at 15 t ha$^{-1}$ rate increased the concentration of the heavy metals by 1.47, 2.25, 4.33, 1.08, 1.2, 1.00 and 1.22 folds, and by 1.48, 1.86, 4.33, 1.11, 1.23, 1.00 and 1.21 folds for Zn, Pb, Cd, Cr, Ni, Mn, and Cu, at Site 1 and Site 2, respectively. Thus, the application of brewery sludge has a higher impact on the concentrations of Cd and Pb than on the other trace metals at both sites. However, the analyzed heavy metals concentration in the studied sites showed that the concentrations of trace metals are within the limit values for agricultural soils as per the standard of some European countries.

Brewery sludge can exhibit high or low heavy metal content depending on its source (Viola et al., 2014). In addition, the phytotoxicity of brewery sludge derived trace metals consists of several factors viz., quantity and type of trace metals, soil and plant related characteristics, degree of trace metals concentrations in brewery sludge, and environmental factors (Qian et al., 1996). The current study revealed a concomitant increase in the contents of trace metals in soils incorporated with brewery sludge. This might be attributed to the application of metal rich brewery sludge to the soil. The study by Singh and Agrawal (2009) also showed increased availability of heavy metals with reducing pH.

The work of Dessalew et al. (2018) showed that amending the soil by brewery spent diatomite sludge significantly increases the concentration of wheat grain trace metals, such as Cu, Zn and Ni by 1.33, 1.06 and 1.2 folds compared to that in the control plots, respectively. Moreover, Jamali et al. (2009) reported that soil treated with sludge improved both bio-available and total forms of Cd, Ni, Cr, and Pb in the soil. In our study, in all treatment, the heavy metals were below the legally acceptable levels of USEPA (1997). The findings of this study that revealed acceptably low levels of heavy metals in the brewery sludge treated soils is exciting because it encourages reutilizing these waste materials as good and economical sources of fertilizer for agricultural production in an environmentally sustainable way with negligible chance of potential exposure to heavy metals.

### Table 4. Mean accumulation of trace metals in the soil (mg/kg) after harvest.

| Treatment | Site | Zn (mg/kg) | Pb (mg/kg) | Cd (mg/kg) | Cr (mg/kg) | Ni (mg/kg) | Mn (mg/kg) | Cu (mg/kg) |
|-----------|-----|-----------|------------|------------|------------|------------|------------|------------|
| 0 t ha$^{-1}$ | S1  | 85.0$^a$ | 0.5$^d$ | 0.2$^d$ | 65.0$^f$ | 90.6$^d$ | 1988.5$^f$ | 49.7$^c$ |
| 3 t ha$^{-1}$ | S1  | 112.2$^d$ | 0.7$^c$ | 1.2$^b$ | 70.5$^g$ | 106.9$^d$ | 1910.3$^d$ | 57.1$^c$ |
| 6 t ha$^{-1}$ | S1  | 113.9$^e$ | 0.7$^c$ | 1.3$^a$ | 72.0$^c$ | 108.1$^h$ | 1911.3$^a$ | 58.0$^c$ |
| 9 t ha$^{-1}$ | S1  | 114.5$^b$ | 0.8$^d$ | 1.4$^c$ | 72.5$^{0.0}$ | 108.7$^{0.0}$ | 1912.7$^a$ | 57.9$^f$ |
| 12 t ha$^{-1}$ | S1  | 116.2$^a$ | 0.9$^c$ | 1.5$^c$ | 73.4$^{0.0}$ | 109.4$^{0.0}$ | 1913.4$^c$ | 59.4$^b$ |
| 15 t ha$^{-1}$ | S1  | 116.2$^a$ | 0.9$^c$ | 1.5$^c$ | 73.8$^{0.0}$ | 109.5$^{0.0}$ | 1913.7$^d$ | 59.6$^c$ |
| NPS | S1  | 86.8$^d$ | 0.5$^c$ | 0.4$^d$ | 67.2$^a$ | 90.3$^d$ | 1907.7$^d$ | 49.2$^a$ |
| 0 t ha$^{-1}$ | S2  | 70.3$^c$ | 0.7$^d$ | 0.3$^e$ | 58.3$^c$ | 78.8$^c$ | 1965.8$^e$ | 46.5$^f$ |
| 3 t ha$^{-1}$ | S2  | 93.1$^a$ | 0.9$^d$ | 1.2$^c$ | 63.4$^a$ | 93.0$^c$ | 1967.6$^a$ | 53.4$^f$ |
| 6 t ha$^{-1}$ | S2  | 94.3$^a$ | 1.0$^d$ | 1.3$^c$ | 64.8$^{0.0}$ | 94.0$^{0.0}$ | 1968.7$^c$ | 54.3$^f$ |
| 9 t ha$^{-1}$ | S2  | 95.0$^a$ | 1.1$^d$ | 1.3$^c$ | 65.2$^{0.0}$ | 94.6$^{0.0}$ | 1970.0$^c$ | 54.1$^f$ |
| 12 t ha$^{-1}$ | S2  | 96.4$^a$ | 1.2$^d$ | 1.3$^c$ | 67.5$^{0.0}$ | 97.4$^{0.0}$ | 1990.0$^c$ | 55.5$^f$ |
| 15 t ha$^{-1}$ | S2  | 96.5$^a$ | 1.3$^d$ | 1.3$^c$ | 67.7$^{0.0}$ | 97.4$^{0.0}$ | 1990.2$^c$ | 55.8$^f$ |
| NPS | S2  | 72.0$^d$ | 0.5$^c$ | 0.4$^d$ | 61.8$^a$ | 80.4$^d$ | 1984.0$^d$ | 46.0$^f$ |

Within each column and within each site, means sharing the same letter are not significantly different at the 5% level of significance.
the wheat grain at both sites. However, the accumulation of Zn increased at Site 1, but decreased at Site 2 with increasing application rate of brewery sludge (Table 5). The concentration of heavy metals was higher in wheat grains at various sludge rates as compared to those in the control and the NPS treatments. The highest concentration was recorded for Mn followed by Cu, Zn, Ni, Cr, Pb and Cd (Table 5). These results suggest that incorporation of brewery sludge in soil at a rate of 15 t ha⁻¹ is a good alternative for wheat grain due to its good soil nourishment benefit of yeast waste sludge. The highest concentration of Zn at Site 2 was obtained from the control treatment, whereas the concentration of the rest of the heavy metals were recorded from waste brewery sludge at an application rate of 15 t ha⁻¹. This result indicated that larger amount of Mn, Cu, and Ni were maintained in wheat grain. The trace metal concentration in wheat grain increased as follows: Mn < Cu < Ni < Zn < Cr < Pb < Cd (Table 5). Hence, amendment of soil with waste brewery sludge at both sites showed that this waste material can be utilized as a good source of fertilizer for agricultural use in an eco-friendly manner and to enrich the soil with micronutrients without heavy metal toxicity in the food chain.

Amendment of brewery sludge elevated heavy metal concentration in wheat grain. Similarly, Singh and Agrawal (2010a) reported that Ni, Pb, Mn, Cu, Cd, Cr, and Zn contents increased significantly with the application of sludge as compared to the unamended plots in mung bean seeds. Moreover, Mazen et al. (2010) reported that the application of sewage sludge (at a rate of 75%) elevated heavy metals’ concentration of wheat grain. Thus, Cd and Cu increased by 3.0 and 1.53 folds, while Pb and Zn increased by 1.2 folds over the control.

In our study, wheat grain exhibited continuous increase in the concentration of the studied metals as brewery sludge rate increased. Low soil pH facilitates metals ions uptake by plants (Tiwari et al., 2011) and hence, raising it with lime is the best strategy to minimize uptake. Likewise, previous studies documented that sludge increases the concentration of heavy metals in different crops. Accordingly, the concentration of Ni and Pb in broccoli crops at an application rate of 74 t ha⁻¹ (Antionious, 2009), Cu content in soybean crop at an application rate of 124 t ha⁻¹ (Sridhar et al., 2011), the content of Zn and Cu in wheat grain at an application rate of 9 t ha⁻¹ (Singh and Agrawal, 2007), and palak plants at 20% (w/w) sludge rate (Li et al., 2012). Additionally, higher Zn concentration in the soil may create competitive absorption with Cd that tend to reduce Cd uptake by crops (Oliver et al., 1994). The increment of organic carbon after brewery sludge application may also decrease Cd uptake by plants (Chaudri et al., 2007). The present study showed that the concentration of all heavy metals in wheat grain were below the maximum permissible limits according to Weigert (1991), and Kabata-Pendias and Pendias (1984).

3.4. Bioaccumulation factors

The bioaccumulation factor (BAF) values that allow the assessment of wheat plants’ ability to accumulate trace metals from the soil and their uptake rate of the metals to the aerial parts of the wheat from the soil treated with control, brewery sludge, and NPS fertilizer are shown in Table 6. Among all the analyzed metals, Cu had the highest BAF and Cd had the lowest BAF in all treatments. The trend in the BAF values at Site 1 was in the following order of Cu > Zn > Pb > Ni > Mn > Cr. From this study area, the BAF of Cu, Zn, Pb, Ni, Mn, and Cd in wheat grain fell in 0.48–0.61, 0.19–0.28, 0.18–0.27, 0.12–0.16, 0.07–0.08, and 0.03–0.09 ranges, respectively (Table 6). The existence and toxicity of the trace metals depend mainly on their functions in the metabolic processes of the organism and the vulnerability of the plants to bioaccumulation (Bose and Bhattacharyya, 2008).

Plants are categorized as excluder if their BAF values are <1.0, as accumulator if their BAF values are between 1.0 and 10, and as hyper accumulator if their BAF values are >10 (Dessalew et al., 2018). When BAF <1.0, it means that the plant absorbs the trace metal but does not accumulate it. When BAF >1.0, it means that the plant accumulates the trace metal. As shown in Table 6, the BAF values of all trace metals (Zn, Cd, Cu, Cr, Mn, and Pb) were <1.0 in all treatments, which indicates that the plants in all treatments absorbed but did not accumulate the trace metals.

The bioaccumulation factor values at Site 2 were in the order of Cu > Pb > Zn > Cd > Ni > Mn > Cr. The highest BAF values for Zn and Cd were obtained from the control and the lowest BAF values were obtained from the 15 t ha⁻¹ and 3 t ha⁻¹ brewery sludge application rate (Figure 1a and b), respectively; while the highest Pb was obtained from the plots treated with NPS mineral fertilizer (Figure 1a), and for the other heavy metals (Cu, Mn, Cr, and Ni) the highest concentration was obtained from the 15 t ha⁻¹ brewery sludge application rate (Figure 1b, c and d).

High level of heavy metal in plant body was reported to significantly affect the rate of transpiration and photosynthetic capacity, which reduces the activity of light harvesting complex (Singh and Agrawal, 2010b), and to increase reactive oxygen species (ROS) in plants (Halliwell and Gutteridge, 2015). The availability of ROS possesses strong oxidizing properties that degrade enzymes, proteins and nucleic acids and affect cellular and structural function by breaking bonds (Panda et al., 2016).

### Table 5. Concentration of heavy metals in wheat grain (mg/kg).

| Treatment | Site | Zn (mg/kg) | Pb (mg/kg) | Cd (mg/kg) | Cr (mg/kg) | Ni (mg/kg) | Mn (mg/kg) | Cu (mg/kg) |
|-----------|-----|------------|------------|------------|------------|------------|------------|------------|
| 0 t ha⁻¹  | S1  | 17.5       | 0.1        | 0.02       | 0.5        | 10.0       | 119.9      | 25.1       |
| 3 t ha⁻¹  | S1  | 20.6       | 0.1        | 0.08       | 0.6        | 13.0       | 125.1      | 27.0       |
| 6 t ha⁻¹  | S1  | 23.3       | 0.1        | 0.03       | 0.7        | 13.5       | 125.0      | 28.5       |
| 9 t ha⁻¹  | S1  | 24.1       | 0.1        | 0.05       | 0.7        | 14.1       | 129.2      | 29.0       |
| 12 t ha⁻¹ | S1  | 26.7       | 0.2        | 0.09       | 1.0        | 15.7       | 136.2      | 34.6       |
| 15 t ha⁻¹ | S1  | 32.0       | 0.2        | 1.1        | 17.0       | 138.0      | 36.2       |
| NPS       |     | 19.5       | 0.1        | 0.02       | 0.5        | 11.3       | 118.8      | 25.8       |
| 0 t ha⁻¹  | S2  | 14.0       | 0.1        | 0.08       | 0.5        | 9.0        | 134.3      | 22.3       |
| 3 t ha⁻¹  | S2  | 11.5       | 0.2        | 0.08       | 0.5        | 11.0       | 140.1      | 24.0       |
| 6 t ha⁻¹  | S2  | 11.6       | 0.2        | 0.03       | 0.6        | 11.3       | 142.3      | 25.4       |
| 9 t ha⁻¹  | S2  | 9.6        | 0.2        | 0.13       | 0.7        | 12.0       | 142.3      | 26.3       |
| 12 t ha⁻¹ | S2  | 7.7        | 0.2        | 0.15       | 0.9        | 13.3       | 149.8      | 30.5       |
| 15 t ha⁻¹ | S2  | 7.3        | 0.2        | 0.18       | 1.0        | 14.4       | 151.8      | 31.9       |
| NPS       |     | 13.4       | 0.2        | 0.07       | 0.4        | 9.6        | 130.2      | 23.1       |
| MPL (mg/kg)| 99.40 | 0.30 | 0.20 | 2.30 | 67.90 | 500 | 73.30 |

Within each column and within each site, means sharing the same letter are not significantly different at the 5% level of significance; MPL (Maximum Permissible Limits) (Weigert, 1991; Kabata-Pendias and Pendias, 1984).
Bread wheat BAF significantly increased with an increase in the brewery sludge rate except for Zn, Cd and Pb. This suggests that the uptake of heavy metals from the soil by wheat crop might be linked to their availability under various applications of brewery sludge.

Furthermore, the results showed that all the studied heavy metals are not accumulated consistently in wheat grain, and even the absorption of the metals does not depend on the concentration of the brewery sludge. Several factors including, the chemical form of the heavy metals, pH of the soil, soil organic matter content, plant species, irrigation water, and climatic conditions govern the transfer and accumulation of significant heavy metals in a soil-plant system. Soils differ in their physiochemical properties, which makes the transfer and accumulation of heavy metals in the soil-plant system complex. The accumulation of heavy metals is affected by several factors including organic acid exudation that modifies the surface of Rhizosphere, which controls the transfer and accumulation of trace metals responsible for metal accumulation in plant parts. For example, flavonoid exudation together with protein increases the resistance of protein nitrogen to microbial degradation and will have an impact on soil pH, as a result it influences the activity of trace metals in the soil. Bioavailability of heavy metals might be swayed by clay particles and soil colloids. For a given soil pH, Zhou and Li (1996) reported that the capacity of the soil for Zn adsorption, which in turn limits the transfer of Zn to crops can be increased by increasing the percentage of particles by < 0.002 mm. Soil microbes are other factors that affect heavy metal absorption depending on whether the area is close to the root hair or not. Bacteria and fungi interacting with plant roots increase heavy metal concentrations by changing the activity of the metals, viz the concentration of As, Hg and Se is minimized by some soil bacteria, while heavy metals like Fe and As can oxide (Cetin et al., 2011; Zhang and Xia, 2000).

The availability of heavy metals is highly affected by the plant’s absorption mechanism, especially through passive uptake via root cell wall (McLaughlin et al., 2011). Soil nitrogen plays a significant role in transferring heavy metals from soil to plant. Many studies (e.g., Zhou et al., 2014; Gazzato et al., 2012; Perilli et al., 2010) confirmed the vital role of soil nitrogen in affecting the bioavailability and access of metals by modifying soil pH. Heavy metal uptake and effective mobilization are affected by soil pH. Among heavy metals, Mn and Cd are very sensitive to soil acidity when pH ranges between 5.5 and 6.0 while Zn, Ni and Cu are less sensitive when the pH ranges between 5.0 and 5.5, and Pb is not mobilized until the pH < 4.5 (Blake and Goulding, 2002). Furthermore, cations like NH₄⁺ can shift soil chemical equilibrium that affects the exchangeable forms of heavy metals, main elements, and hydrogen ion (H⁺), implying that changes in the soil pH and concentration of free bases (NH₃) further increase the solubility of heavy metals from the soil colloids (Lorenz et al., 1994). More specifically, the low bioaccumulation factor of Cd metal in a plot treated with brewery sludge might be explained by a couple reasons: (1) the addition of co-cations (Ca²⁺) in

![Figure 1. Bioaccumulation factor (BAF) of the trace metals in wheat grain at Site 2.](image-url)
brewery sludge, which competitively prevents uptake of Cd by wheat crop, and (2) the dissolved organic carbon, perhaps diminishing the availability of Cd in soil solution to plants through complexation of free Cd$^{2+}$ (McLaughlin et al., 2007). Our results are also in line with those of Wuana and Okieimen (2011) and Wolejko et al. (2013), who reported an increment in bioaccumulation factor of heavy metals with decreasing soil pH. In summary, the bioaccumulation factors of wheat crop at both experimental sites were <1.0 for all types of heavy metals, which suggest that the wheat only absorbed but not accumulate the heavy metals. Therefore, the grain produced by using brewery sludge is safe for human consumption.

4. Conclusion

To sum up, the amendment of agriculture soil with brewery sludge can advance soil nourishment and nutritional quality of wheat grain. Based on the findings of this study, the amendment of soil with brewery sludge at an application rate of 15 t ha$^{-1}$ is recommended for the production of wheat crop. Although the results at both study sites showed that the concentration of heavy metals in wheat grain increased with increasing application rate of brewery sludge, the concentrations of all heavy metals measured within the consumable tissues of wheat grain were within the acceptable range and remained below the phytotoxic levels. Therefore, the use of brewery sludge does not cause environmental risks or concerns, hence, the problem of dumping brewery sludge in the vicinity can be solved by utilizing it for crop production. But regular monitoring of heavy metal concentrations in the soil and in the growing crops is needed to avoid food chain contamination and risks to human health. Also, further long-term studies across diverse environments and different test crops would be necessary to widen the recommendation.

Declarations

Author contribution statement

Wakijra Tesfahun: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.
Ambachew Zerfu, Mesera Shumye, Gezai Abera, Asmeret Kidanea, Tessema Astatkie: Conceived and designed the experiments; Analyzed and interpreted the data; Wrote the paper.

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The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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