

# Heavy Metals Accumulation in Cauliflower (*Brassica Oleracea* L. var. *Botrytis*) Grown in Brewery Sludge Amended Sandy Loam Soil

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## Abstract

An experiment was conducted to study the effects of five levels of brewery sludge (BS) (0, 5, 10, 15 and 20 Mg ha<sup>-1</sup>) and recommended dose of chemical fertilizers (100:60:50 NPK ha<sup>-1</sup>) on heavy metals content of soil and plant parts (leaf and Curd) of cauliflower. Brewery sludge application significantly increased the heavy metals (Zn, Cu, Pb, Cr and Cd) concentration in both soil and plant parts of cauliflower as compared to the unamended soil. The highest heavy metals concentrations in soil and plant parts (curd and leaf) were recorded from 20 Mg ha<sup>-1</sup> BS application. Accumulation of heavy metals in leaf was higher than in curd. The curd yield of cauliflower significantly increased with brewery sludge application as compared to control. Heavy metals in soil were within the USEPA permissible limits. Leaf and curd in all treatments assimilated Zn and Cu below the FAO/WHO maximum permissible value. But Pb and Cd concentrations in leaf and curd exceeded the FAO/WHO maximum permissible value, except Pb concentration in curd from control and 5 Mg ha<sup>-1</sup> BS application. Cr concentration was below the FAO/WHO maximum permissible value in curd; whereas the concentration in leaf surpassed the maximum permissible value except in unamended soil and 5 Mg ha<sup>-1</sup> BS application. Thus, this study highlights the risk involved in consumption of cauliflower receiving brewery sludge and likelihood of human exposure to heavy metals.

## Key words

Brewery Sludge; Heavy Metals; Leaf; Curd

## Introduction

Sludge, one of the principal byproducts produced from various wastewater treatment plants (Odegaard, Paulsrud, and Karlsson, 2002), can be used as fertilizer in agricultural lands for the production of agricultural crops. Application of sewage sludge serves numerous functions including macro and micro nutrients supplement to crops, ameliorating the soil physical properties and augmenting the organic matter content

in soil; all contributing to an increment in crop production (Mantovi, Baldoni, and Toderi, 2005). However, the sewage sludge application may result in heavy metals accumulation in the soil (Chen, Wang, and Wang, 2005; Arora *et al.*, 2008). As a result, plants may absorb heavy metals above the permitted levels and enter the food chain affecting the human beings health (Arora *et al.*, 2008). Effective management of sewage sludge must overcome such undesirable effects in crop production systems (Skjelhaugen, 1999). Therefore, the proper management of sludge utilization must consider many aspects including its heavy metal content, crop type and its nutrient requirement, and biological and physico-chemical properties of soil. These aspects are essential to determine the optimum rate, time and method of sludge application (Banerjee, Burton, and Depoe, 1997; Arora *et al.*, 2008).

In Nawalparasi, Nepal, brewery industries generate large quantities of solid wastes. Farmers residing in this area exploit solid waste (sludge) for agricultural production. Heavy metals concentrations in soil and plant tissue may increase with the use of sludge for vegetable production posing threats to health of consumers. Hence, this study aims to assess the influence of brewery industry sludge application on soil heavy metals content, yield of cauliflower and heavy metals uptake by leaf and curd.

## Materials and Methods

A field experiment was carried out at Divyapuri 5, Nawalparasi, Nepal from October to December 2008 to assess the effects of brewery sludge on heavy metals contents of soils and plant parts of cauliflower (*Brassica oleracea* L. var. *botrytis*). The experiment was laid out in Randomized Complete Block Design with four

replications in sandy loam soil. There were six treatment combinations having five rates of brewery sludge (0, 5, 10, 15, 20 Mg ha<sup>-1</sup>) and the recommended doses of chemical fertilizers (100:60:50 kg NPK ha<sup>-1</sup>). Cauliflower, a popular vegetable in Nepal, was selected for the experiment. The dewatered brewery sludge (BS) produced from beer factory of Chaudhary Udhog; Nepal was applied into the plot seven days before transplanting cauliflower seedlings. Nitrogen was supplied through urea in two split application and phosphorous and potash was supplied through Diammonium Phosphate and Muriate of Potash as basal application, respectively.

Soil samples, prior to experiment and after the crop harvest, were collected from each plot. The BS was sampled from the brewery waste water treatment plant. The soil and BS samples were air dried, grinded and passed through a 1mm sieve to analyze their chemical properties. The pH level was determined by the Beckman electrode pH meter in a suspension of soil and water at 1:2.5 ratio (Cottenie *et al.*, 1982), soil and BS organic matter content by using modified Walkley and Black method (Houba *et al.*, 1989), total soil and BS nitrogen by the Kjeldahl distillation method (Bremner and Mulvaney, 1982), available soil Phosphorous by the NaHCO<sub>3</sub> extraction method (Olsen, Sommers, and Page, 1982) using spectrophotometer, and available soil Potassium by the ammonium extraction method (Pratt, 1965) using flame photometry. Air dried soil and sludge samples were acid digested using HNO<sub>3</sub> and HCl mixture at 3:1 ratio along with 30% H<sub>2</sub>O<sub>2</sub> (Edgell, 1989) and analyzed for total Zn, Cu, Pb, Cr and Cd content. Total sludge K and total sludge P were determined by Atomic absorption Spectrophotometer.

Leaf and curd samples, collected separately from each plot, were oven dried, grinded and passed through 1

mm sieve. Oven dried samples were acid digested with the mixture solution of HNO<sub>3</sub>, H<sub>2</sub>SO<sub>4</sub> and HClO<sub>4</sub> at 5:1:1 ratio (Allen, Grimshaw, and Rowland, 1986). The solution obtained after the filtration of digested sample was analyzed to determine heavy metals (Zn, Cu, Pb, Cr and Cd) by Atomic Absorption Spectrophotometer. Data were subjected to Analysis of Variance using MSTAT-C software and Duncan's Multiple Range Test was performed to separate different treatment means using DMRT at 5% probability level.

## Results

### *Characteristics of Soil and Brewery Sludge*

The selected characteristics of soil and brewery sludge were presented in Table 1. The soil and brewery sludge are slightly acidic in nature. Nitrogen, phosphorous, potash, and heavy metals (Zn, Cu, Cd, Pb and Cr) content in brewery sludge were higher than in the soil. The Zn concentration was highest whereas the Cd concentration was lowest in both soil and brewery sludge. Importantly, both in soil and BS, all of these heavy metals were lower than the permissible limits of United States Environment Protection Agency (USEPA) (USEPA, 1997).

### *Yield of Cauliflower*

The effects of brewery sludge (BS) on curd yield of cauliflower were presented in Table 2. With the increasing application of BS, progressive increase in curd yield was observed. The highest curd yield (12.43 Mg ha<sup>-1</sup>) was obtained from 20 Mg ha<sup>-1</sup> BS rate whereas the lowest curd yield (7.01 Mg ha<sup>-1</sup>) was recorded in control (Table 2). The curd yield obtained from 20 Mg ha<sup>-1</sup> BS application was significantly higher compared to control and 5 Mg ha<sup>-1</sup> BS application.

TABLE 1 SELECTED PROPERTIES OF BREWERY SLUDGE AND EXPERIMENTAL SOIL

Parameters	Brewery sludge	MPL of heavy metals in sludge*	Experimental Soil	MPL of heavy metals in soil*
pH	6.2	–	6.01	–
Organic matter (g/kg)	265.3	–	14.1	–
N (g/kg)	25.3	–	0.9	–
**P (g/kg)	6.7	–	1.15	–
***K (g/kg)	7.3	–	0.34	–
Zn (µg g <sup>-1</sup> )	558.47	3000	38.09	300
Cu (µg g <sup>-1</sup> )	75.23	1500	10.66	100
Pb (µg g <sup>-1</sup> )	33.88	1000	11.91	350
Cr (µg g <sup>-1</sup> )	34.98	1200	9.18	250
Cd (µg g <sup>-1</sup> )	2.25	20	0.73	0.6

\*USEPA, 1997, \*\* Total phosphorous for BS and available phosphorous for soil

\*\*\* Total Potassium for BS and available potassium for soil, MPL = Maximum Permissible Limits

### Heavy Metals Content in Soils

Soil Zn from 20 Mg ha<sup>-1</sup>BS application was significantly greater (44.32 µg g<sup>-1</sup>) as compared to 5 and 10 Mg ha<sup>-1</sup> sludge, and chemical fertilizer application, but similar to 15 Mg ha<sup>-1</sup> application (Table 3). The application of 20 Mg ha<sup>-1</sup> BS increased Cu concentration in soil significantly compared to other treatments. The lowest (10.92 µg g<sup>-1</sup>) Cu concentration was found in control.

Accumulation of lead (Pb) in soil was highest (13.02 µg g<sup>-1</sup>) in 20 Mg ha<sup>-1</sup> BS application. The value was significantly higher compared to other treatments except in 15 Mg ha<sup>-1</sup> BS application. The lowest (11.06 µg g<sup>-1</sup>) Pb concentration was recorded in control (Table 3). The application of 5 and 10 Mg ha<sup>-1</sup> BS, and chemical fertilizer had similar effects.

TABLE 2 EFFECTS OF BREWERY SLUDGE ON BIOLOGICAL AND CURD YIELDS OF CAULIFLOWER AT DIVYAPURI, NAWALPARASI, NEPAL

Treatments	Curd yield (Mg ha <sup>-1</sup> )
BS <sub>0</sub>	7.01±0.42 <sup>c</sup>
BS <sub>5</sub>	9.46±1.09 <sup>bc</sup>
BS <sub>10</sub>	9.81±0.71 <sup>ab</sup>
BS <sub>15</sub>	10.44±1.23 <sup>ab</sup>
BS <sub>20</sub>	12.43±0.94 <sup>a</sup>
CF	11.00±0.95 <sup>ab</sup>
LSD <sub>0.05</sub>	2.46
CV%	16.31

<sup>a</sup>Means followed by different letter(s) in column are significantly different at 0.05 probability level by DMRT

The concentration of Cr in soil obtained from the application of 20 Mg ha<sup>-1</sup> BS was significantly higher than control and chemical fertilizer application. The highest concentration (9.98 µg g<sup>-1</sup>) of Cr was obtained in 20 Mg ha<sup>-1</sup> brewery sludge additions whereas the lowest (8.21 µg g<sup>-1</sup>) concentration was recorded in the treatment with no sludge (Table 3). The cadmium concentration in soil increased with increasing the level of BS application from 0 to 20 Mg ha<sup>-1</sup>, with highest (0.94 µg g<sup>-1</sup>) value in 20 Mg ha<sup>-1</sup> BS application.

This treatment had significantly higher concentration of cadmium than BS<sub>0</sub>, BS<sub>5</sub>, and chemical fertilizer application, but similar concentration to BS<sub>10</sub> and BS<sub>15</sub> applications (Table 3).

### Heavy Metals Concentration in Leaf and Curd of Cauliflower

Addition of BS increased Zn concentration of leaves from 38.76 (µg g<sup>-1</sup>) in control to 60.91 (µg g<sup>-1</sup>) in 20 g ha<sup>-1</sup> BS (Table 4). There were no significant differences in Zn concentration among 5 to 20 Mg ha<sup>-1</sup> BS additions but Zn concentration in leaves was significantly higher with BS addition as compared to chemical fertilizer. Similar pattern of increasing Zn concentration was also observed in curd but the concentration was lower compared to leaves (Table 5). Treatments receiving 15 and 20 Mg ha<sup>-1</sup> BS rates resulted into the highest concentrations (5.36 µg g<sup>-1</sup>) in leaf, which were significantly higher than control and 5 Mg ha<sup>-1</sup> BS rates. Non-significant differences in curd Zn concentration were found among 10 to 20 Mg ha<sup>-1</sup> sludge rates. However, the concentration of zinc in curd was highest in 20 Mg ha<sup>-1</sup>BS application. The highest Cu concentration (5.35µg g<sup>-1</sup>) in curd was obtained in 20 Mg ha<sup>-1</sup> BS rate which was significantly higher compared to control and chemical fertilizer, but it was similar to other BS rates (Table 5).

The curd Pb concentration was highest (0.59 µg g<sup>-1</sup>) in 20 Mg ha<sup>-1</sup> BS rate which was significantly higher than control, 5 and 10 Mg ha<sup>-1</sup> BS, and chemical fertilizer treatments (Table 5). The values obtained in 10 and 15 Mg ha<sup>-1</sup> BS application were significantly higher than control and 5 Mg ha<sup>-1</sup> BS. Chromium concentration in leaf increased with increasing sludge application ranging from 2.15 to 2.55 µg g<sup>-1</sup>, and resulting the highest concentration in 20 Mg ha<sup>-1</sup> BS (Table 4). Similarly, highest chromium concentration in cauliflower curd was obtained in 20 Mg ha<sup>-1</sup> BS application. The Cr concentration in this treatment was

TABLE 3 EFFECTS OF BREWERY SLUDGE ON HEAVY METALS (ZN, CU, PB, CR AND CD) CONTENTS OF SOIL AT DIVYAPURI, NAWALPARASI, NEPAL

Treatments	Zinc (Zn) (µg g <sup>-1</sup> )	Copper (Cu) (µg g <sup>-1</sup> )	Lead (Pb) (µg g <sup>-1</sup> )	Chromium (Cr) (µg g <sup>-1</sup> )	Cadmium (Cd) (µg g <sup>-1</sup> )
BS <sub>0</sub>	37.82±1.73 <sup>c</sup>	10.92±0.86 <sup>b</sup>	11.06±0.43 <sup>c</sup>	8.21±0.32 <sup>b</sup>	0.73±0.03 <sup>d</sup>
BS <sub>5</sub>	38.95±1.39 <sup>bc</sup>	11.48±0.94 <sup>b</sup>	11.28±0.55 <sup>c</sup>	9.08±0.33 <sup>ab</sup>	0.83±0.02 <sup>bc</sup>
BS <sub>10</sub>	40.25±0.98 <sup>bc</sup>	11.89±0.83 <sup>b</sup>	11.95±0.21 <sup>bc</sup>	9.55±0.45 <sup>a</sup>	0.86±0.03 <sup>ab</sup>
BS <sub>15</sub>	42.32±1.10 <sup>ab</sup>	12.33±0.27 <sup>b</sup>	12.53±0.43 <sup>ab</sup>	9.60±0.65 <sup>a</sup>	0.90±0.02 <sup>ab</sup>
BS <sub>20</sub>	44.32±1.65 <sup>a</sup>	15.85±1.23 <sup>a</sup>	13.02±0.40 <sup>a</sup>	9.98±0.61 <sup>a</sup>	0.94±0.03 <sup>a</sup>
CF	36.56±0.30 <sup>c</sup>	11.23±0.46 <sup>b</sup>	11.94±0.37 <sup>bc</sup>	8.56±0.01 <sup>b</sup>	0.75±0.02 <sup>cd</sup>
MPL*	200	50	300	400	3
LSD <sub>0.05</sub>	3.47	2.34	1.00	0.84	0.08
CV%	5.75	12.66	5.58	6.11	6.47

\*USEPA, 1997, MPL=Maximum Permissible Limits

<sup>a</sup>Means followed by different letter(s) in column are significantly different at 0.05 probability level by DMRT

TABLE 4 EFFECTS OF BREWERY SLUDGE APPLICATION ON HEAVY METALS (Zn, Cu, Pb, Cr AND Cd) UPTAKE BY CAULIFLOWER LEAF AT DIVYAPURI, NAWALPARASI, NEPAL

Treatments	Zinc (Zn) ( $\mu\text{g g}^{-1}$ )	Copper (Cu) ( $\mu\text{g g}^{-1}$ )	Lead (Pb) ( $\mu\text{g g}^{-1}$ )	Chromium (Cr) ( $\mu\text{g g}^{-1}$ )	Cadmium (Cd) ( $\mu\text{g g}^{-1}$ )
BS <sub>0</sub>	38.76±5.96 <sup>bc</sup>	6.37±0.76 <sup>c</sup>	3.23±0.29 <sup>c</sup>	2.15±0.08 <sup>c</sup>	1.30±0.26 <sup>bc</sup>
BS <sub>5</sub>	50.76±0.94 <sup>ab</sup>	7.13±0.71 <sup>bc</sup>	3.76±0.18 <sup>bc</sup>	2.25±0.23 <sup>bc</sup>	1.21±0.22 <sup>c</sup>
BS <sub>10</sub>	54.09±2.96 <sup>a</sup>	8.80±0.77 <sup>ab</sup>	4.42±0.21 <sup>ab</sup>	2.33±0.13 <sup>abc</sup>	1.32±0.25 <sup>bc</sup>
BS <sub>15</sub>	56.67±2.39 <sup>a</sup>	8.95±0.09 <sup>ab</sup>	5.36±0.56 <sup>a</sup>	2.45±0.20 <sup>ab</sup>	1.39±0.27 <sup>ab</sup>
BS <sub>20</sub>	60.91±4.30 <sup>a</sup>	9.85±0.47 <sup>a</sup>	5.36±0.35 <sup>a</sup>	2.55±0.17 <sup>a</sup>	1.51±0.27 <sup>a</sup>
CF	35.50±6.88 <sup>c</sup>	6.47±0.88 <sup>c</sup>	4.60±0.40 <sup>ab</sup>	2.14±0.07 <sup>c</sup>	1.27±0.24 <sup>bc</sup>
MPL*	99.4	73.4	0.3	2.3	0.2
LSD <sub>0.05</sub>	14.07	2.07	0.98	0.24	0.13
CV	18.83	17.33	14.69	7.16	6.53

<sup>a</sup>Means followed by different letter(s) in column are significantly different at 0.05 probability level by DMRT

\*MPL=Maximum Permissible Limits, FAO/WHO, 2001

TABLE 5 EFFECTS OF BREWERY SLUDGE APPLICATION ON HEAVY METALS (Zn, Cu, Pb, Cr AND Cd) UPTAKE BY CAULIFLOWER CURD AT DIVYAPURI, NAWALPARASI, NEPAL

Treatments	Zinc (Zn) ( $\mu\text{g g}^{-1}$ )	Copper (Cu) ( $\mu\text{g g}^{-1}$ )	Lead (Pb) ( $\mu\text{g g}^{-1}$ )	Chromium (Cr) ( $\mu\text{g g}^{-1}$ )	Cadmium (Cd) ( $\mu\text{g g}^{-1}$ )
BS <sub>0</sub>	33.81±0.34 <sup>c</sup>	4.32±0.21 <sup>b</sup>	0.27±0.03 <sup>c</sup>	1.05±0.04 <sup>b</sup>	0.32±0.01 <sup>c</sup>
BS <sub>5</sub>	35.89±1.66 <sup>bc</sup>	4.65±0.22 <sup>ab</sup>	0.30±0.07 <sup>c</sup>	1.09±0.09 <sup>b</sup>	0.37±0.25 <sup>bc</sup>
BS <sub>10</sub>	37.42±2.52 <sup>abc</sup>	4.89±0.41 <sup>ab</sup>	0.46±0.10 <sup>b</sup>	1.13±0.07 <sup>b</sup>	0.44±0.05 <sup>ab</sup>
BS <sub>15</sub>	41.17±3.37 <sup>ab</sup>	4.95±0.25 <sup>ab</sup>	0.52±0.16 <sup>ab</sup>	1.25±0.12 <sup>ab</sup>	0.44±0.06 <sup>ab</sup>
BS <sub>20</sub>	43.41±4.35 <sup>a</sup>	5.35±0.16 <sup>a</sup>	0.59±0.14 <sup>a</sup>	1.45±0.25 <sup>a</sup>	0.48±0.05 <sup>a</sup>
CF	33.74±0.54 <sup>c</sup>	4.10±0.18 <sup>b</sup>	0.44±0.02 <sup>b</sup>	1.02±0.03 <sup>b</sup>	0.33±0.01 <sup>c</sup>
MPL*	99.4	73.4	0.3	2.3	0.2
LSD <sub>0.05</sub>	5.83	0.90	0.12	0.25	0.08
CV%	10.80	10.72	19.55	14.31	13.86

\*FAO/WHO, 2001, MPL=Maximum Permissible Limits

<sup>a</sup>Means followed by different letter(s) in column are significantly different at 0.05 probability level by DMRT

significantly higher than all other treatments except in 15 Mg ha<sup>-1</sup> BS application. Cadmium (Cd) concentrations in leaf (1.51  $\mu\text{g g}^{-1}$ ) and curd (0.48  $\mu\text{g g}^{-1}$ ) were highest in 20 Mg ha<sup>-1</sup> (Table 4 and 5). The concentrations of Cd in leaf and curd obtained from 15 and 20 Mg ha<sup>-1</sup> sludge were similar.

## Discussion

Increased rate of brewery sludge application was associated with increased curd yield of cauliflower.

Thus, increased vegetable yield from sludge applied soils underlines the function of and contribution from higher macronutrients and organic matter content in the sludge (Jamil, Qacim, and Umar, 2006). A study showed that the fresh weight of lettuce (Mohammad and Athamneh, 2004) and fruit yield of cucumber (Hussein, 2009) was increased over control with increasing rates of sewage sludge application. Moreover, Akdeniz *et al.* (2006) reported that the highest yield of sorghum was obtained from the highest dose of sewage sludge application, and yield of sorghum showed no significant difference between the sewage sludge and chemical fertilizer.

The total heavy metal concentration in soil augmented

with increased level of BS application rate compared to control and chemical fertilizer. Among the heavy metals accumulation in soil, Zn concentration was highest following Cu, Pb, Cr and Cd with increased sludge rate. Kumar and Chopra (2012) reported that the concentration of heavy metals (Zn, Cu, Pb, Cr, and Cd) in soil have positive linear relationship with the paper mill sludge application. Our results were in agreement with the findings of other researchers (Wong *et al.*, 2001; Mohammad and Athamneh, 2004; Hussein, 2009) who observed that the concentration of Zn, Cu, Cd, and Pb in vegetable grown soil progressively increased with increasing rate of sewage sludge application. Topcuodlu (2005) stated that repeated application of sewage sludge and solid waste results in gradual accumulation of heavy metals in soil and subsequently different environmental problems. The heavy metals concentration in our experimental soil was below the USEPA maximum permissible limits (USEPA, 1997).

Increased rate of sludge application results in greater accumulation of heavy metals in curd and leaf tissues. Similar results were reported in earlier studies by Mohammad and Athamneh (2004), and Hussein

(2009). Singh and Agrawal (2010) reported that Cd, Cu, Pb, Cr, and Zn contents in the edible part of mungbean increased significantly with sludge application as compared to the control. Kumar and Chopra (2012) found that there was greater accumulation of Zn, Cu, Cd, Pb, and Cr in French bean plants with increased rate of paper mill sludge application, and the concentration of these elements were more in leaves than in fruits. In leaf and curd, Zn and Cu concentrations were below the maximum permissible limits whereas Cd concentration exceeded the maximum permissible limits of FAO/WHO (2001). Cr concentration in curd was below the maximum permissible limits but it exceeded the maximum permissible limits in leaf at 10, 15 and 20 Mg ha<sup>-1</sup> BS application. Pb concentration in leaf exceeded the permissible limits of FAO/WHO (2001) whereas the concentration in curd was below the limit in control and 5 Mg ha<sup>-1</sup> BS application. Principally, human beings are exposed to soil contaminants including heavy metals via food chain (soil–plant–human) (Arora *et al.*, 2008). Hence, our study underlines the risks involved in the consumption of vegetables obtained from the soils receiving brewery sludge due largely to likelihood of human exposure to heavy metals.

### Conclusion

The yield of cauliflower increased with brewery sludge application, but soil and plant tissues also incurred greater accumulation of heavy metals such as Zn, Cu, Cd, Cr and Pb with increasing rate of brewery sludge. Concentration of Cd, and Pb (except in 5 Mg ha<sup>-1</sup>) in curd of cauliflower exceeded the permissible limits of FAO/WHO (2001), although the heavy metals content in soil and sludge were within the permissible limits. Higher concentration of Cd and Pb suggest potential risks to human health through the consumption of cauliflower. Since the brewery sludge characteristics are within the permissible ranges, this sludge can be used for agriculture application. Future study should consider the application of brewery sludge on other crops and soil types, and human health risk assessment.

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