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Research Article

Heavy Metal Accumulation in Lettuce (*Lactuca Sativa L.*) Amended with Different Amounts of Fresh Broiler Poultry Manure

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Abstract

Fresh broiler manure is a natural, locally available and relatively cheap material that vegetable growers can obtain. The use fresh of poultry manure is associated with the introduction of heavy metals into leafy vegetables and, consequently these heavy metals enter the human body through the food chain. On the other hand, no research has been done specifically on fresh broiler manure and its use in green house vegetable production. Lettuce (*Lactuca sativa L.*) is one of the widely used salad vegetable crops in Swaziland. This experiment was carried out in the green house at Luyengo campus, Horticulture farm, University of Swaziland to assess the extent of varied amounts of fresh broiler manure on heavy metals accumulation in lettuce plant tissue. The cultivar used was crisp head. The amounts of fresh broiler manure used were 0, 20, 40 and 60 tons per hectare. The design of the experiment was a randomized complete block with three replications. Data on fresh mass, dry matter yield and the content of some heavy metals were collected. The results showed that lettuce grown in soil amended with fresh broiler manure had contents of some heavy metals which were above toxic levels for human consumption, for cadmium, iron, zinc and, manganese except for copper which was below the permissible limit set by the World Health Organization. Increasing applications of fresh broiler manure resulted not only in increased in yield of lettuce, but accumulation of heavy metals in lettuce leaves. At the safest level of application (20t/ha) if fresh broiler manure were used combined with synthetic fertilizer in the greenhouse cost can be reduced whilst increasing yield, reducing pollution and re-using waste. Further studies maybe carried out to investigate the possible outcomes.

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Introduction

Lettuce (*Lactuca sativa*) is an annual plant of the aster or sunflower family, asteraceae. It is most often grown as a leafy vegetable, but sometimes for its stem and seeds. Lettuce was first cultivated by the ancient Egyptians who turned it from a weed, whose seeds were used to produce oil, into a plant grown for its leaves. Lettuce spread to the Greeks and Romans, the latter of whom gave it the name "*lactusa"*, from which the English "lettuce" is ultimately derived. By 50 AD, multiple types were described and lettuce appeared often in medieval writings, including several herbals. Europe and North America originally dominated the market for lettuce, but by the late 1900s the consumption of lettuce had spread throughout the world [1].

Poultry manure is one of the commonly used forms of manure mainly because of its availability from local poultry farms; it has been used for centuries because of its high nitrogen content [2]. The frequent clean out of many poultry houses makes poultry manure available in sufficient quantities and on timely basis to supply most fertilizer needs [2], Poultry litter is made up of raw poultry manure and bedding material like sawdust, wood shavings, grass cuttings, banana leaves or rice hulls. This combination provides a good source of NPK including heavy metals [3]. The general objective of this study was to improve food security by providing information on the use of fresh broiler manure to produce healthy leafy vegetables especially lettuce in the greenhouse environment in Swaziland.

The specific objectives of the experiment were:

1. To evaluate different amounts of broiler manure (20t/ha, 40t/ha, 60t/ha) application on the level of heavy metal accumulation in lettuce plant tissues.
2. To find how much fresh broiler manure to apply to maximize production without posing possible harm to human health and the plant itself.

Swaziland imports 80% of fruits and vegetables it consumes from neighboring South Africa. As an economy, Swaziland faces a tough challenge to increase food production and food security by increasing production locally. To realize this vision, it is about time that greenhouse vegetable production be taken to a commercial level. In green houses, broiler manure at the correct application rate may be used to increase production whilst cutting down costs and reducing waste without causing harm to the environment and human health. The use of poultry manure has increased; however, the potential of heavy metal contamination has not been studied.

Materials and Methods

Experimental site

The experiment was conducted at the Horticulture Farm green house at the University of Swaziland, Faculty of Agriculture Luyengo campus. The area is found in the Middleveld of Swaziland, 21034'S and 31012'E at an altitude of 750m. The annual precipitation is about 800mm, with most rainfall occurring between October and April. The average summer maximum temperature is 27˚C and 15˚C in winter. The soils are mostly sandy loam [4].

Plant material

Lettuce seedlings, fresh broiler manure was collected from the University farm, it was the analyzed for its nutrient content in the Crop Production Department particularly nitrogen, phosphorus and potassium before being applied to the experimental rows. This then will be the basis of their application rate.

Experimental design

Fresh broiler manure was applied as treatment in the pots at 20t/ha, 40t/ha, 60t/ha and at 0t/ha as a control treatment. The experiment was laid in a Randomized Complete Block Design (RCBD) with four treatments each replicated six times in each replication plot. Plot layout in the experimental Green house is shown in table 1. Fresh broiler manure rates/amounts used are shown in table 2.

Block 1		Block 2		Block 3	
40t/ha	0t/ha	60t/ha	20t/ha	0t/ha	60t/ha
60t/ha	20t/ha	0t/ha	40t/ha	20t/ha	40t/ha

Table 1: Layout of experiment.

Treatment Code	Treatment Description	Rate of Application
1	Control	0t/ha
2	Broiler manure	20t/ha
3	Broiler manure	40t/ha
4	Broiler manure	60t/ha

Table 2: Application rates of the treatments.

The soil in the experimental rows/plots and fresh broiler manure were randomly sampled and analyzed at the Malkerns Research Station, for fertilizer recommendation before the start of the experiment (Table 3).

Texture	pH	Exchangeable Acidity (meq/100g)	N (mg/kg)	P (mg/kg)	K (mg/kg)
Manure	7.9	0.39	165	1965	1900
Soil	6.2	0.24	6.7	39	100

Table 3: Physical and chemical characteristics of broiler manure and garden soil.

Data Collection

The whole lettuce head was cut at the base of the soil from each pot four weeks after planting using a scalpel and put in a brown paper bag then weighed for fresh mass weight in the laboratory. The leaves were then chopped and put into brown paper bags and oven dried at 65˚C for three days after which they were weighed for dry mass. The leaves of the lettuce head were then ground to powder using a Christy and Norris laboratory grinder (Greifensee, Switzerland). After grinding all material belonging to a particular pot was packed separately for storage in moisture proof container. The ground plant material was then ready for and analysis.

Parameter	Mn	Fe	Zn	Cd	Cu
Wave length (nm)	279.5	248.3	213.9	228.8	234.8
Slit width (nm)	1.0	2.0	1.0	0.5	0.5
Lamp current (mA)	10	30	5	4	4.0

Table 4: The operating parameters for AAS.

Cu	Cd	Mn	Fe	Zn
0.5	0.5	1.0	0.5	0.5
1.0	1.0	2.0	1.0	1.0
2.0	2.0	3.0	2.0	2.0
3.0	3.0	4.0	3.0	3.0
4.0	4.0	5.0	4.0	4.0

Table 5: Standard solutions used in AAS (ppm).

Determination of the basic properties of the soil

Determination of the soil pH: The method outlined by Mclean [5], was used in the determination of the pH of the soil used in this study. Duplicate 10g samples of the soil were weighed into 50 ml beakers and 20 ml of 0.01M $CaCl_2$ solution was added. The mixture was then swirled for 5 minutes with a glass rod and allowed to equilibrate for 30 minutes before pH measurements. The pH measure was standardized against a pH 4 and 7 buffer solutions, the pH of each soil sample was then measured with a Hanna pH meter (model 2011) by immersing the pH electrodes in to the supernatant solution.

Determination of pH of fresh manure used in this study: The method outlined below was used in the determination of the pH of the fresh broiler manure used in this study. Duplicate 5g samples oven dried manure was weighed into 100ml beakers and 20ml of 0.01M $CaCl_2$ solution was then added. The mixture was swirled for 5 minutes using a glass rod and allowed to equilibrate for 30 minutes. The pH was measured with a Hanna pH meter (model 2011) by immersing the pH electrodes in to the mixture.

The determination of exchangeable Ca, Mg and K: The content of Ca, Mg and K was determined following the method of Lanyon and Heald [6]. Duplicate 10g soil sample were weighed into 250 ml Erlenmeyer flasks and 90ml of 1N ammonium acetate solution was added. The flasks was then placed on a shaker and shaken for 10 minutes. The content of the flask were allowed to settle and the filtered through Whatman no. 42 filter paper into 100ml volumetric flask, the content of the flask were made to volume with the extracting solution. The amounts of Ca and Mg in the extract were measured by atomic absorption spectrophotometry using a Varian Techtron atomic absorption spectrophotometry (model AA200 Felsted, Dunwom, UK). Potassium was measured by flame photometry using a Jenway flame photometer (model PFP7) following the model proposed by Knudsen et al., [7].

Determination of exchangeable Aluminium (Al): In the determination of Al the method outlined by Olsen and Sommers [8], was followed. Duplicate 5g soil samples were weighed into 250ml flasks and 50ml of 1N KCL solution was added. The content of the flask was shaken for 30 minutes then allowed to settle and filtered through a Whatman no. 42 filter paper. The content of the Al in the extract was measured by titration, against a 0.01 M NaOH solution.

Determination of available phosphorus in the soil: The method outlined by Olsen and Sommers [8], was followed, duplicate 2g soil sample was weighed into 50ml centrifuge tubes and 20ml of Bray-1 solution was added. The tubes were then mounted onto mechanical shakers and shaken for5 minutes. The content of the tubes were centrifuged for 10 minutes on a Kubota centrifuge (model 2010 Tokyo Japan). The supernant solution was filtered through a No. 42 Whatman filter paper into 50ml volumetric flasks. The amount of phosphorus in the extract was determined calorimetrically following the ammonium molybdate blue method. Phosphorus in the extract was measure using a Biochrom spectrophotometer (model Libra S12 Tokyo, Japan) at a wave length of 730nm.

Lettuce analysis: Elemental analysis of a compost sample requires that the organic fraction of the sample be destroyed; leaving the heavy metals either in solution or in a form that is readily dissolved. The approaches for destroying organic material and dissolving heavy metals fall into two groups. The wet digestion by acid mixtures prior to elemental analysis and the dry-ashing, followed by acid dissolution of the ash [9]. A modified method outlined by Thomas et al., was used, to determine the concentrations of Cd, Cu, Mn, Fe and Zn in the final solutions was determined by an Atomic Absorption Spectrometer (AAS) available in the university chemistry laboratory.

Nitric digestion method: One gram of sample was placed into a 250ml digestion tube and 10ml of concentrated nitric acid was added to the sample then heated for 45 min at 90˚C in a boiler. The temperature was increased to 150˚C for at least 8 hours until a clear solution was obtained Then 5 ml of concentrated HNO_3 was added to sample at least three times, digestion occurred and reduced the volume to about 1ml.Interior wall of digestion tube were washed with little distilled water, when removing swirled the tube throughout the digestion to keep the wall clean and prevent loss of sample. Sample was cooled, then 5ml of 1% HNO_3 was added to sample, finally solution was filtered with Whatman no. 43 filter paper and transferred to a 25 ml volumetric flask by adding distilled water. Standard solutions of 0.5 ppm, 1.0 ppm, 1.5 ppm, 2.0 ppm, 3.0 ppm and 4.0 ppm, were prepared from a 1000 ppm stock solution of Fe, Zn, Mn, Cu and Cd using the formula: C1V1 = C2V2. With a known Concentration (C2) and Volume (V2) required, the volume of 1000 ppm stock solution was calculated and made necessary dilutions to make V2. Standards were run in the Atomic Absorption Spectrometer (AAS) to determine their absorbance from which calibration curves were plotted. Standard solutions used in AAS are shown in tables 4 & 5.

Data analysis: Collected data was analyzed using MSTAT-C statistical package [10]. Data was subjected to Analysis of Variance (ANOVA). Where statistical differences were detected, mean separation was done by Duncan's New Multiple Range Test (DNMRT) at 5 % probability level [11].

Results

Fresh mass yield

There were significant (P<0.05) differences in fresh yield of lettuce plants. Lettuce plants which had been fertilized by higher amounts of fresh broiler manure (60t/ha) produced highest yield (203.3g) compared to those applied with lower amounts (20t/ha) and the control (0t/ha) showing the lowest fresh mass yield (23.50g) (Figure 1).

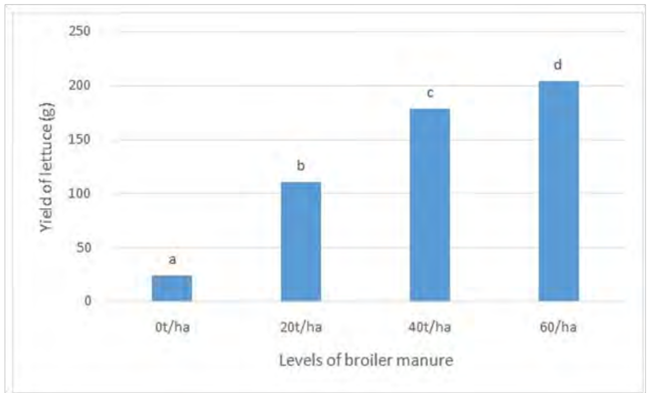

Figure 1: Fresh mass of lettuce (*Lactuca sativa*) plants amended with various amounts of fresh broiler manure. Bars with same letters not significantly different from each other at P=0.05. Mean separation by DNMRT.

Dry mass

There were significant (P<0.05) differences in dry mass of lettuce plants among treatments, (0t/ha, 20t/ha, 40t/ha) and (60t/ha). Plants amended with (60t/ha) were found to have the highest mean dry mass, followed in decreasing order by plants with (40t/ha) then (20t/ha). The lowest dry mass was obtained from the control (0t/ha) treated plants (Figure 2)

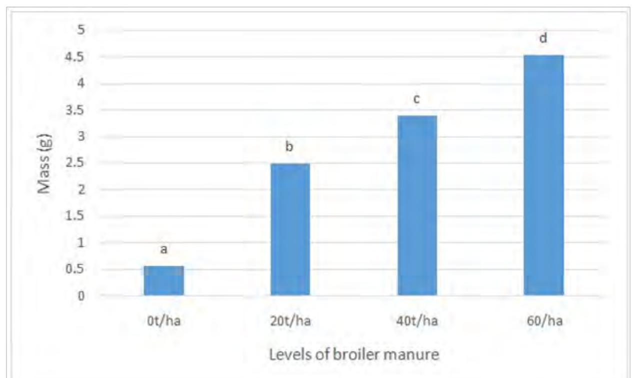

Figure 2: Dry mass of lettuce (*Lactuca sativa*) plants amended various amounts of fresh broiler manure. Bars with same letters not significantly different from each other at P=0.05. Mean separation by DNMRT.

Manganese content

There were significant (p<0.05) differences in manganese content among treatments, with plants amended with (20t/ha) having the highest content followed in decreasing order by (40t/ha), (60t/ha) and the control (0t/ha) being the lowest (Figure 3). The content of manganese increased significantly as the application rate increased from (0t/ha) to (20t/ha), but decreased with the increasing amount of fresh broiler manure applications (Figure 3).

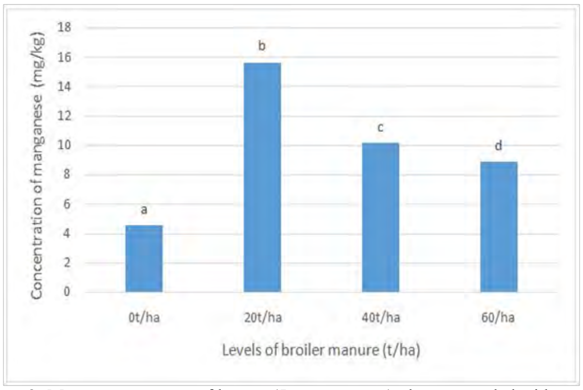

Figure 3: Manganese content of lettuce (*Lactuca sativa*) plants amended with various amounts of fresh broiler manure. Bars with same letters not significantly different from each other at P=0.05. Mean separation by DNMRT.

Iron content

Lettuce plants showed a significant (P<0.05) difference among the treatments applied in relations to iron content in plant tissue. Plants fertilized with (60t/ha) fresh broiler manure had the highest mean iron content (74.7mg/kg) followed in decreasing order by 40t/ha (66.80mg/kg), 20t/ha (60.77mg/kg) and lastly 0t/ha (49.17) (Figure 4).

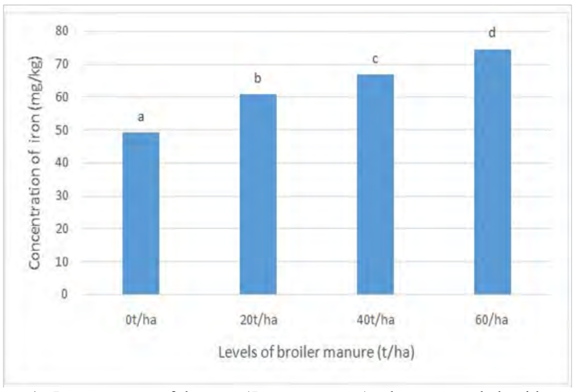

Figure 4: Iron content of lettuce (*Lactuca sativa*) plants amended with various amounts of fresh broiler manure. Bars with same letters not significantly different from each other at P=0.05. Mean separation by DNMRT.

Zinc content

There were significant (P<0.05) differences in zinc content (mg/kg) among treatments. Lettuce plants amended with highest (60t/ha) fresh broiler manure had the highest zinc content (95.10mg/kg), followed in decreasing order by 40t/ha (80.84mg/ha), 20t/ha (19.28mg/kg) and lastly 0t/ha (7.137mg/ha) (Figure 5).

Cadmium content

There were significant (P<0.05) differences across treatments applied; 0t/ha, 20t/ha, 40t/ha and 60t/ha. Lettuce plants amended with the highest amounts (60t/ha) were found to have the highest cadmium content (1.90mg/kg) mean, followed in decreasing order by plants amended with 40t/ha, 20t/ha and lastly, 0t/ha (1.27, 0.70 and 0.40mg/kg respectively) (Figure 6).

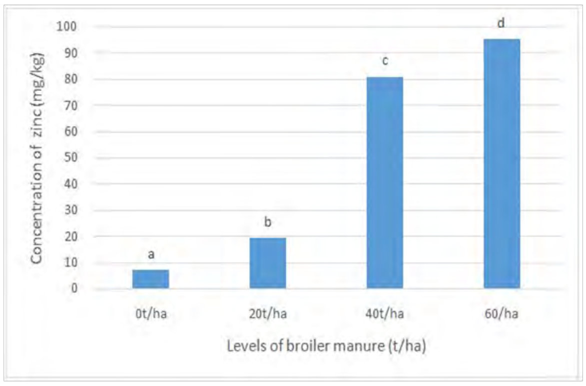

Figure 5: Zinc content of lettuce (*Lactuca sativa*) plants amended with various amounts of fresh broiler manure. Bars with same letters not significantly different from each other at P=0.05. Mean separation by DNMRT.

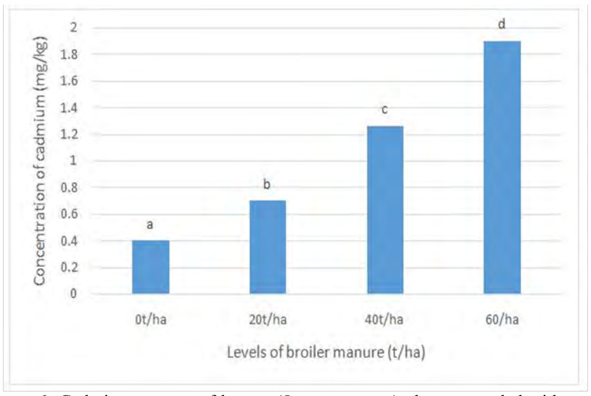

Figure 6: Cadmium content of lettuce (*Lactuca sativa*) plants amended with various amounts of fresh broiler manure. Bars with same letters not significantly different from each other at P=0.05. Mean separation by DNMRT.

Copper content

There were significant (P<0.05) differences in copper content in lettuce plant samples among treatments. The highest copper content (20.34mg/kg) was obtained from lettuce plants provided with the highest (60t/ha) amount of fresh broiler manure followed in decreasing order by 40t/ha, 20t/ha and lastly 0t/ha (2.30mg/kg). Figure 7 has been included to show trend of the element.

Discussion

There were significant differences in fresh yield and dry mass of lettuce among treatments. These depended on the amounts of fresh broiler manure applied. Plants which were amended with higher levels of fresh broiler manure (60t/ha and 40t/ha) resulted in higher yields compared to those applied with lower amounts, this may be due to the relatively higher amounts of available potassium and phosphorus per kilogram in the fresh broiler manure. According to Rao [12], the soil could be enriched due to application of higher amount of organic materials which tend to release relatively large amounts of nitrogen into the soil before planting which subsequently boost yield. A study done by Xu et al. [13], showed that vegetables grown with higher levels of organic manures grew better and resulted in higher yields than those grown in lower amounts.

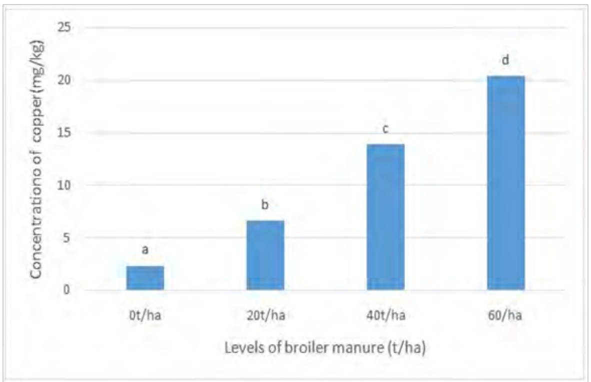

Figure 7: Copper content of lettuce (*Lactuca sativa*) plants amended with various amounts of fresh broiler manure. Bars with same letters are not significantly different from each other at P=0.05. Mean separation by DNMRT.

There was a slight increase in Mn content in lettuce upon application of poultry manure and the content of this element decreased at the 40t/ha rate of poultry manure application. Compared to the increase from the control (0t/ha) to 20t/ha there was little increase of manganese content with increasing amount of fresh broiler manure application. The content of manganese was found to be below the WHO [14] permissible limit in all the treatments. This suggests that fresh broiler manure may not contain harmful amounts of manganese.

There was a significant increase in Cd content in lettuce with increasing rate of fresh broiler manure application. The highest amount of cadmium was obtained at the highest rate of application of this material (60t/ha), while the control recorded the least. The Cd content in lettuce was found to be above the World Health Organization WHO [14], permissible limit of 1mg/kg for plants amended with 60t/ha and 40t/ha. At the lowest application rate (20t/ha) cadmium content was found to be below the health hazard level. This means that the broiler manure applied above 20t/ha used in this study had elevated levels of Cd. This element is extremely toxic to humans and animals and its accumulation in the human body is linked to a number of ailments.

The content of iron in the plant material significantly increased with increased rates of application of fresh broiler manure. The content of iron was found to be below the permissible limit of 100mg/kg in all the treatments including the control. This may eliminate one more heavy metal fear in broiler manure; however, it does not guarantee the safe use of fresh broiler manure in vegetable production due to the possible accumulative effect of the heavy metals in the human body.

There was a significant increase in zinc content with increasing rate of fresh broiler manure application. This might be due to high content of zinc in the manure used in this study. The content of Zn in the control was 7.13mg/kg which is below the WHO permissible limit [14] and the content of this material increased to 95.10mg/kg at the highest amount of fresh broiler manure application (60t/ha). This suggests that vegetable produce grown in soils amended with fresh broiler manure may not be safe for human consumption since it is above the WHO permissible limit [14].

There was an increase in the content of copper in lettuce grown in soil amended with fresh broiler manure but the content of Cu was below the WHO permissible limit of 30mg/kg in all the treatments including the control [14]. This however, does not guarantee the safe use of poultry manure in vegetable production.

Conclusion

Under the conditions of this study and the soil used while conducting the experiment, it can be concluded that leafy vegetables, such as lettuce accumulate many heavy metals when grown in soils treated with fresh broiler manure. In most cases the heavy metal accumulation in the lettuce leaf tissues increased with the increase in fresh broiler manure application.

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