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Heavy metal content in the leaves of *Crassocephalum crepidioides* due to the application of various types of manure

To cite this article: M W Lestari 2021 *IOP Conf. Ser.: Earth Environ. Sci.* **733** 012001

Heavy metal content in the leaves of *Crassocephalum crepidioides* due to the application of various types of manure

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Abstract. In Indonesia, vegetable farmers use manure to increase production. In fact, one of the sources of heavy metal pollution on agricultural land is the use of manure. This study, therefore, aims to determine the content of heavy metals in the leaves of *Crassocephalum crepidioides*, due to fertilization, involving the use of several types of manure. Furthermore, it is possible to apply the results in the establishment of dose limiting policies for vegetable crops, in an attempt to prevent dangers to the consumer. The tested sources consisted of chicken, goat, and cow manure, as well as a control (without manure), and each was applied at a dose of 20 tons/Ha. Meanwhile, the third leaf of the upper part of the plant was analyzed using Atomic Absorption Spectrophotometer (AAS), to determine Cu, Pb, Zn and Cd. The results showed the leaves of *Crassocephalum crepidioides* fertilized with 20 tons/Ha of chicken manure, goat manure and cattle manure had low heavy metal (below the set threshold of 50, 100-400, and 5-30 ppm for Pb, Zn and Cd, respectively). However, at a dose of 20 tons/Ha, the leaves pastored with goat and cow manure had a high content of Cu (209.78 and 530.90 ppm) than control and chicken manure (67,92 and 92,68 ppm), which are higher than the established threshold. Meanwhile, those fertilized with chicken compost, had better area and plant height value, compared to the goat and cow sources.

1. Introduction

The contamination of agricultural lands by heavy metals must be prevented to realize the availability of food that safe for human health. This occurs most commonly on farms, due to the application of fertilizers (cages or chemicals) and pesticides [1]. Furthermore, its content in manure affects soil quality, contaminate crops and poses health risks [2, 3, 4, 5, and 6]. However, the content of heavy metals vary greatly between the types of manure, and the category of animals and livestock [7]. Previous reports indicate that donkey sources possess the lowest amount of Zn, Cu, Pb, Ni, and Mg, while pig has the highest Fe content and the least Mn, Cd and Ca [2]. Furthermore, very low concentrations of Fe are found in chicken manure (852.3 mg Kg^{-1}), as well as dirty Mn (375.0 mg Kg^{-11}), and the least significant concentration of Zn was recorded in horse manure (94.3 mg Kg^{-1}), while other wastes ranged from 294.5 to 697.6 mg Kg^{-1} [8]. Conversely, goat manure source had almost no Cd, in comparison with others.

This study, therefore, aims to determine the effect of dosage (20 tons Ha^{-1}) of manure used by Indonesian farmers on Cu, Pb, Zn, and Cd contamination in plants. The indicator used was

Crassocephalum crepidioides, an indigenous vegetable, widely used in the community. Furthermore, the results serve as a useful reference point to the government to assist in the determination of policies, regarding limitations on the use of manure for vegetable farming. Meanwhile, some countries implemented national regulations, which establish a threshold amount for application [9].

2. Materials and Methods

2.1. Time and place
The experiment was conducted in the garden of the Faculty of Agriculture, University of Islam Malang, from September to November 2018 (dry season). The soil was alluvial, and at an altitude is 516 m above sea level, located in 112º06 '- 112º07' east longitude and 7º06 ' - 8º02 'north latitude. Furthermore, the temperature used was 20º - 29ºC, at a humidity of 78 - 86% and an average rainfall of 1,883 mm. year^{-1}. The heavy metal analysis was carried out in the Faculty of Nature and Science Laboratory, University of Islam Malang. Meanwhile, before sowing, the heavy metals content in soil was analyzed to ensure it was not more than the threshold.

2.2. Research methods
The experimental configuration involved a randomized complete block design, consisting of treatments, which include manure of chicken, cow, and goat, at a dose of 20 ton Ha^{-1}, respectively and a control (without manure). These were applied one week before sowing, by immersion into the planting medium, presented in a plastic bag, and the entire treatment was repeated three times, with each contained five samples. *Crassocephalum crepidioides* were seeded in 10 kg polyethylene bags, and its leaves were harvested 45 days after sowing for the analysis of its heavy metal content.

2.3. Analytical procedure for heavy metal analysis
The analysis of Cu, Pb, Zn, and Cd involved the use of atomic absorption spectrometry (AAS), which was conducted on the third leaf of the upper part of the plant, collected as the sample, through the following stages: The distilled water, 25 ml (70% high purity HNO_3 and a ratio of 3: 1 HCl) and 5 ml of 30% H_2O_2 were mixed in a 100 ml empty beaker and heated to 80°C, followed by the formation of a clear solution [10]. This was then cooled, filtered, and made up to 50 ml with ionized water, which was stored at room temperature for further analysis. The heavy metal assay was conducted by measuring 1 g of the sample into a 100 ml beaker, containing acid wash, to which 25 ml of water and 5 ml of 30% H_2O_2 were added [10]. Subsequently, the mixture was digested at 80°C, followed by the formation of a clear solution, which was cooled, and the digested sample filtered and diluted to 50 ml with deionized water. Determination and filtration targeting of heavy metals in the sample, including Cu, Pb, Zn, and Cd, was achieved by absorption of atoms, using spectrophotometers (model: Buck 200A). This instrument was calibrated using a standard solution, made manually from each heavy metal.

Blanks and quality control sources were conducted on five samples of leaves, in an attempt to detect contamination and irregularities, and on average, each blank analyte recorded <5% of the element. Furthermore, the precision and accuracy were assured through a repetition of the procedure, and the percentage of acceptable recovery (86.5% ± 0.005 to 96.4 ± 0.003) was obtained from the vegetable samples that were digested with spikes.

3. Results and Discussion
Before sowing, the result of the heavy metal analysis showed the media to have minimal metal contents, which were below the threshold. Table 1 shows the media used in this research as follows. The results of the analysis showed that applying chicken, goat manure and cattle (Figure 1) at 20 tons/Ha produced *Crassocephalum crepidioides* with the heavy metal content of Pb (31,34; 32,11; and 29,82 ppm), Zn (134,80; 166,19; and 72,19 ppm), Cd (7,83; 7,13; and 7,43 ppm) and Cu (92,68; 209,78; and 530,90 ppm). While the Pb, Zn, and Cd values were still below the set threshold (50, 100-400, and 5-30 ppm for Pb, Zn and Cd, respectively), some of the Cu content were over the threshold (goat and cow manure).

Table 1. Heavy metals in soil media and plants.

Type of heavy metal	Concentration (ppm)*)	Threshold in soil (ppm)[11]	Threshold in plant (ppm)[11]
Cu	31.1	2 - 100	20 – 100
Zn	3.2	10 - 300	100 - 400
Pb	3.4	2 - 200	50
Cd	2.5	0.1 – 7.0	5 - 30

Source: *) Laboratory analysis before sowing

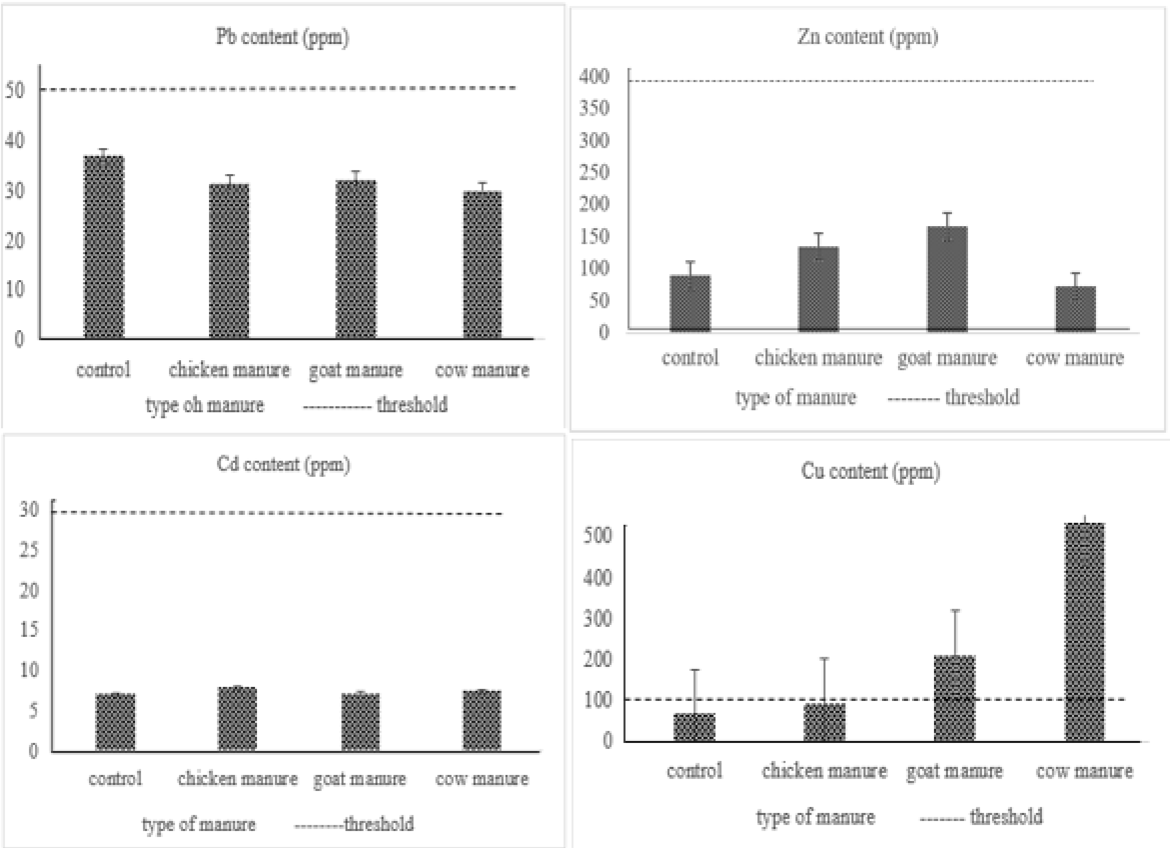

Figure 1. The heavy metal content of *Crassocephalum crepidioides* leaves under various types of manure application.

Crassocephalum crepidiodes is included in the group of Cu hyper-accumulator plants, which are capable of absorbing the element at a specific concentration, exceeding the usual rate of other plants. This is illustrated through the phenomenon observed in this research that it grows normally and showed no symptoms of poisoning as commonly seen in the leaves of other plants that obtain the application of cow manure (Figure 2).

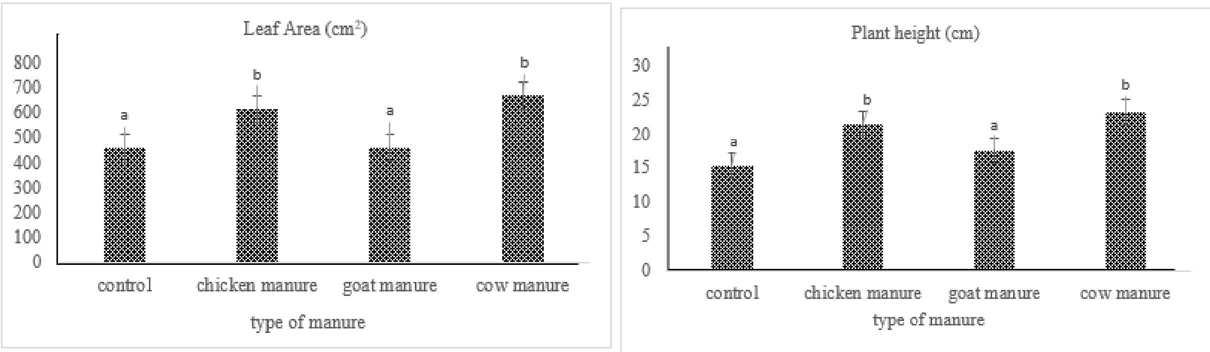

Figure 2. Effect of various types of manure application on *Crassocephalum crepidioides* growth (The notification letters indicated significant differences between types of manure, according to Duncan test 5%).

The high level of Cu in the leaves of *Crassocephalum crepidioides* was due to the high doses of goat and cow manure in the soil. This element was absorbed by the roots, transported to the leaves and therefore used as photosynthetic material. Furthermore, the movement of ions in the xylem tissue flows upwards, through the mass of water, due to transpiration [12]. Alloway et al., showing the dose as safe for fertilization [13]. Conversely, the use of cattle and goat manure elicited a high content of Cu (up to 209.78 and 530.90 ppm, respectively), exceeding the limit, while the chicken source treatment had a lower Cu content of about 93.67 ppm, which is below the threshold (20-100 ppm).

The difference in the number of Cu elements content in manure was due to several factors, including the type of animal, their diet and age [7]. Chicken feed involves the use of more vegetable ingredients, such as rice bran, cornflour, and others, while Cu is often added meals given to ruminants, including cattle and goats [14, 15], therefore, their manure administration causes elevated levels of the metal in the leaves of *Crassocephalum crepidioides*, rather than those sourced from chicken.

Cu maintains body functions and initiates optimal developmental performance in poultry [16], and it is also regarded as a growth promoter in the aspect of breeding [17]. Furthermore, they are normally used in animal feed at concentrations in excess of the nutritional requirements for the prevention of diarrheal disease, and also as an alternative to in-feed antibiotics [18, 19 and 20].

Heavy metals are known to be micro-elements essential for plant survival. However, an increased concentration induces the inhibition of various processes during metabolism [21, 22]. Furthermore, photosynthetic reactions, both photochemical and biochemical, are probably hampered by their presence, especially Cu [23], because in excess quantity, it is capable of reducing plant height, a number of tillers and leaves, as well as rice production. Meanwhile, symptoms caused by its toxicity include blackish-brown plant stalks and stems, the plants appear to be shorter, and there is also a marked reduction in the number of tillers and leaves [24]. However, these indications did not appear on *Crassocephalum crepidiodes,* as normal growth, better leaf area and plant height were observed, although it was fertilized using cow manure, containing a high amount of Cu in its leaves.

4. Conclusions

In Indonesia, there are no obstacles to getting chicken manure and the price is also cheap. The use of manure from chicken sources, at a dose of 20 tons/Ha is recommended for fertilizing *Crassocephalum crepidioides,* while those from cow must be reduced to ensure safety for human consumption

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