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Absorptive capacity of selected plant varieties of *Brassica oleracea* on water soluble mercury from growing media

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Abstract

Heavy metal contamination of soils has become a serious environmental concern today specially that relevant reports have increased over the past years. Given that plants have natural ability to absorb toxicants from soil, profiling of specific species has been determined for phytoremediation purposes. This is an alternative method of reducing amounts of toxic substances and their effects to the environment. This study aimed to scientifically investigate the capacity of selected varieties of *Brassica oleracea* to absorb concentrations of Mercury from its growing media. Using T-test with equal variances, the results showed that there was no significant difference with the 10, 20 and 30-day monitoring of the germinated crops of broccoli, cabbage and cauliflower in terms of their height in millimeters. In addition, with the experimentally introduced 0.01M of laboratory grade $HgCl_2$, Hg uptake of 0.0951, 0.0965 and 0.1982 ppm for broccoli, 0.1038, 0.1436 and 0.2780 ppm for cabbage and 0.1366, 0.1246 and 1239ppm for cauliflower resulted from metal analysis using Atomic Absorption Spectrometry. Except for cauliflower – observed to have not grown well with -0006x, both broccoli and cabbage had a positive linear regression slope with 0.0052x and 0.0087x respectively. Data indicates that the crops used have a positive result on Hg absorption. It is recommended to completely monitor the growth and absorption until the crops are fully grown, and explore abilities of phytoremediation of other plant species.

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Introduction

Plants are the target of wide range pollutants that vary in concentrations, speciation, and toxicity. These pollutants enter the plant systems through the soil or through the atmosphere (Tangahu *et al.,* 2011). Chemicals like iron, lead, mercury, copper, zinc, cadmium, aluminum, cyanides, acids, and alkalies are present in industrial wastes and read the soil either directly with water or indirectly through air. They accumulate in the soil as they degrade very slowly by soil and water bacteria. Consequently, they have a very deleterious effect on the plant growth stunting their growth and reducing the yield and size of fruit. Their degradation products may be absorbed by the plants from where they reach (Ashraf *et al.,* 2014). Unlike organic substances, heavy metals are basically nonbiodegradable and therefore, accumulate in the environment (Schutzendubel, 2002).

Rapidly expanding industrial areas, mine tailings, disposal of high metal wastes, leaded gasoline and paints (Kamal *et al.,* 2010). Land application of fertilizers, animal manures, sewage sludge, pesticides, waste water irrigation, coal combustion residues, spillage of petrochemical and atmospheric deposition were the primary cause of soil contamination (Wuana and Okiemen, 2011). The heavy metals waste from industries deposited in soil will just increases concentration over period of time for most metals do not undergo chemical degradation. Some heavy metals have bio-importance as trace elements but, the biotoxic effects of many of them in human biochemistry are of great concern (Duruibe *et al.,* 2007). In fact, results of the study of Nahmani and Lavelle (2001) showed that heavy metals reduce the overall density of earthworms and other macrofauna populations. Due to the increasing problems with the contaminated soil, remediation has become a trend nowadays. It could be physical, biological or chemical means; these are some of the processes that are useful and effective in decontamination of the soil. Phytoremediation techniques are frequently listed among the best demonstrated available technologies (BDATs) for remediation of heavy metal-contaminated sites (Wuana and Okiemen, 2011).

Trace metals like zinc, copper, are vital for plant growth and human and animal nutrition but can end in phytotoxicity and zootoxicity concerns when accumulated in excess in soil and in plant others such as cadmium, arsenic, mercury, and lead are not essential for either plant or human and animal and pose threat when they enter the food chain (Singh *et al.,* 2011; mc Bride 2007). Schutzendubel (2002) describe heavy metal metals based on their chemical and physical properties.

Three different molecular mechanisms of heavy metal toxicity can be notable: production of reactive species by autoxidation and Fenton reaction (Fe, Cu), blocking of essential functional groups in biomolecules (Cd, Hg), and displacement of essential metal ions from biomolecule.

This study has employed true experimental research design which has aimed to compare the early plant growth increment of the selected plant species of the selected plant species of *Brassica oleracea* with the Hg treated and nontreated growing media. It also compares the rate of Hg absorption of the plant varieties. The results provide information on the absorptive capacity of the three varieties of *brassica; capitata, botrytis and italica*. This can be a basis in providing awareness to the society about the potential of these selected plants to accumulate heavy metals which can cause impairment to health once taken. This can also beneficial to the farmers for it provide alternative and low-cost methods in soil remediation because phytoremediation is prominent for low installation and maintenance costs compared to other remediation methods.

Materials and methods

Research Design

This study has employed true experimental research design which has aimed to compare the early plant growth increment of the selected plant species of the selected plant species of Brassica oleracea with the Hg treated and nontreated growing media. It also compared the rate of Hg absorption of the plant varieties.

Research Setting

The experimental set up was situated in Malanang, Opol Misamis Oriental Philippines. The seeds were purchased from a local agricultural supply, and plant germination were conducted in the same setting. A seedling bed was made so that the crops were placed properly. Further chemical analysis was done at Mindanao State University - Naawan Research Division, Naawan, Misamis Oriental Philippines using atomic absorption spectroscopy.

Crop Selection

Certain members of the Asteraceae and Brassicaceae plant families have been previously identified as hyperaccumulator plants, and it can be inferred that members of these families have genetic and physiological capacity to accumulate, translocate, and resist high amounts of metals (Ramirez-Andreotta *et al.*, 2013). For this research, three varieties *Brassicaceae oleracea* namely *oleracea italica* (broccoli), *oleracea capitata* (cabbage) *and oleracea botrytis* (cauliflower) are germinated and grown to determine in an experimentally mercury salt polluted growing media. The crops were selected from the same genus so that significant comparison can be extracted based on possible results in individual absorption capacity. Although there are other few varieties of *Brassica oleracea,* the specific crops previously mentioned are three of the most grown and commercially available locally.

Dilution of Mercury (II) Chloride as Experimental Toxicant

Analytical grade Mercury (II) chloride ($HgCl_2$) was a diluted to 0.01M concentration. 100mL experimental toxicant which was introduced to all samples of the experimental set up where a total of 2.7 liters of 0.01M $HgCl_2$ was utilized for 27 experimental growing media.

Sampling Procedure

Crop seeds of *Brassica oleracea italica, Brassica oleracea capitata and Brassica oleracea botrytis* were commercially purchased from a local agricultural supply and garden soil sample was purchased from a garden supply in Bulua, Cagayan de Oro City. The set up was done in three replicates to ensure that plant samples will have ample of space in terms of their distance of the neighboring plant. 0.01M of Mercury (II) Chloride was used as the experimental toxicant and 100mL of the diluted reagent will be introduced to 27 experimental pots. Three pots for each type of crop was also utilized for the controlled set up to quantitatively compare relevant early growth increment of the experimentally polluted and non-polluted crops. The treated set up was dispensed with the same amount of prepared reagent, twenty seven - the total number of experimental pot for the three varieties of *Brassica oleracea* with their replicated pots and three sets of sample collection. Three sets of monitoring based on the number of days from the date the crops were planted. The first set of samples was collected during the tenth, twentieth and thirtieth day for the second and third set of samples respectively. All samples were measured in terms of height using a ruler. Plant samples are washed properly using distilled water and then air dried for approximately 7 days. Air dried samples from the first batch of sample collection were secured in an airtight container for metal analysis.

Metal Analysis

The research samples from each batch which were air dried were sent to the Research Division of Mindanao State University at Naawan for metal analysis. The samples were first oven dried at 60°C for 30 minutes and were weighed using an analytical balance. The dried samples have undergone size reduction up to 2mm and were digested using aqua regia, an acid mixture of HCl and HNO_3 in a ratio 3:1. The digested samples were filtered using Whatman filter paper and were prepared together with the standard solution of an analytical grade Mercury salt. The samples were analyzed using an Atomic Absorption Spectrophotometry (AAS AGILENT 4200) which has a detection limit of 1ppb for metal concentration.

Statistical Analysis

The data from the height measurement of the early growth of the plants were analyzed with T-test with equal variance using Microsoft Excel 2016. Likewise, with the same computer application, the data on the concentration of Mercury absorbed by the plant species in 10, 20 and 30 days were graphed and analyzed with linear regression.

Results and discussion

According to Seed Savers Exchange in 2017, good vernalization of *Brassica oleracea* can be around 10 degree Celsius for about 10 to 12 weeks. This can be a factor on the slow growth of the crops experimentally in a warm area. Given the circumstance, the results still support that species of Brassica family is a good absorber of Mercury from soils.

The result of the present study showed that the growth increment between the controlled and experimental species of the three varieties of *Brassica oleracea* has no significant difference in terms of height in millimeter after 10, 20 and 30 days respectively.

Table 1. Summary of Plant Growth.

Plant	Days	Mean	Variance	Critical Value	T – Value	Conclusion
Bras.oleracea italia Broccoli	Ctrl 10	25.400	2.800	1.771	0.095	No Significant Difference
	Exp 10	20.400	61.600			
	Ctrl 20	40.167	10.967	1.771	0.191	
	Exp 20	43.889	92.361			
	Ctrl 30	37.500	27.500	1.812	0.005	
	Exp 30	49.167	54.167			
Bras. oleracea capitata Cabbage	Ctrl 10	23.625	71.411	1.761	0.207	
	Exp 10	19.875	87.839			
	Ctrl 20	36.571	27.952	1.734	0.122	
	Exp 20	40.385	54.590			
	Ctrl 30	39.571	29.619	1.745	0.381	
	Exp 30	38.636	45.455			
Bras. oleracea botrytis Cauliflower	Ctrl 10	28.875	56.125	1.753	0.062	
	Exp 10	22.889	57.861			
	Ctrl 20	40.167	69.767	1.771	0.015	
	Exp 20	32.111	21.111			
	Ctrl 30	38.000	57.500	1.833	0.195	
	Exp 30	33.167	94.167			

Using one tail T-Test and 0.05 level of significance, after 10 days, the critical value for broccoli is 1.771 greater than the t-value of 0.095. While cabbage has a critical value of 1.761 which is greater than the t-value of 0.207 and cauliflower has the critical value of 1.753 greater than the t-value 0.062. The growth increment after 20 days, the critical value for broccoli is 1.771 which is greater than the t-value 0.191. While the cabbage pair has the critical value of 1.734 greater than the t-value 0.122. Moreover, the critical value of cauliflower is 1.771 greater than the t-value 0.015. The data also revealed after 30 days there is no significant difference in growth between the controlled and experimental group of the three species of Brassica oleracea in terms of height. The critical value for broccoli is 1.812greater than the t-value 0.005. Comparatively, cabbage has the critical value of 1.746 greater than the t-value 0.122. While cauliflower pair has a critical value 1.833 is greater than the t-value 0.195.

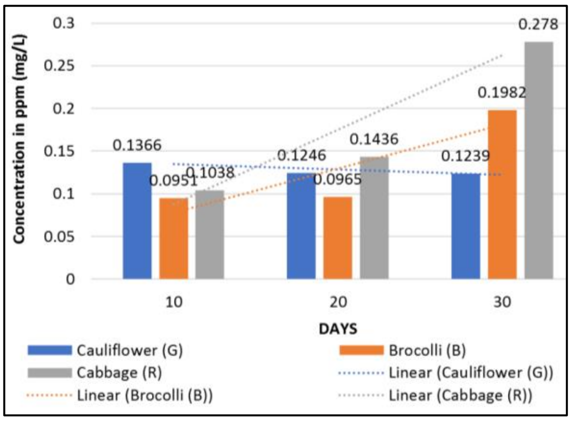

Fig. 1. Comparison of the Rates of Hg Absorption.

Fig. 1 presents the comparison of the rate of absorption of mercury by the selected plant species of *Brassica oleracea*. Both broccoli and cabbage had an increasing uptake of Hg concentration while cauliflower had slightly decreasing uptake. The graph shows that between the plant species that have been experimentally investigated, the cabbage plant has the highest rate of Hg uptake and has a concentration of 0.2780ppm in 30 days. This is followed by the broccoli plant with a total uptake of 0.1982ppm and 0.1239ppm for cauliflower plant which is observe to have performed the least uptake after 30 days. Plants have a natural ability to take up toxic materials from soil. However, Cd, Pb and Hg are always toxic at any level of the trophic pyramid (Gruca *et al.,* 2006). The data gathered from the scientific investigation revealed that the selected plant species of *Brassica oleracea* have absorbed notable amount of Mercury from its growing media.

Table 2. Rate of Absorption of Plants.

Plant	Uptake Concentration In ppm (mg/L)			Slope Value in Linear Regression	R Squared	Trend
	After 10 Days	After 20 Days	After 30 Days			
Bras.oleracea italia Broccoli	0.0951	0.0965	0.1982	0.0052	0.7602	increasing
Bras. oleracea capitata Cabbage	0.1038	0.1436	0.278	0.0087	0.9105	increasing
Bras. oleracea botrytis Cauliflower	0.1366	0.1246	0.1239	-0.0006	0.7912	slightly decreasing

According to the International Standards for Heavy Metals on Foods, the maximum permitted concentration of Hg in both solid and liquid foods in 0.5ppm (mg/kg) or 6.765 ppm (mg/L) using the density of Mercury. Although the latest accumulated Hg concentration from the experimental set up is 0.2780ppm, 0.1982ppm and 0.1239 ppm for cabbage, broccoli and cauliflower respectively, a positive value of the slope of the regression implies that the plant species will still continue to accumulate Hg as they mature especially that the plant samples collected are still too young.

Conclusion

The selected species of *Brassica oleracea* exhibited high uptake capacity and expected to absorb more as these plants grow and mature. The growth increment of the plant species contaminated with heavy metals in terms of height has no significant difference with the normal and healthy ones. With this, the researchers also concluded that proper and precautionary measures should be implemented to the public since no notable indications and differences can be observed in the contaminated plants.

Recommendation

The study recommends to consider the nitrate content of the plant sample which need to follow and ensure safe levels of nitrate in plants. Conduct the study longer than 30 days and in an appropriate cool environment and monitor growth and metal absorption until the maturation of the crops. Soil sampling and analysis must be included to the method in determining the critical levels nutrients in soil.

Recommendation for other method of analyzing metals from the samples such as inductive coupled plasma mass spectrometry (ICP-MS) which is capable of detecting metals and several non - metals up to parts per quadrillion. Existing plant diversity such as leafy vegetables should be explored for accumulation of various metals and its potential to accumulate heavy metals. The study highly suggests for the future researcher review and research on the proper disposal of chemical wastes as it is hazardous to the environment.

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