INNSPUB

International Journal of Biosciences | IJB |
ISSN: 2220-6655 (Print), 2222-5234 (Online)
http://www.innspub.net
Vol. 16, No. 5, p. 222-231, 2020

RESEARCH PAPER OPEN ACCESS

Heavy metal phytoremediation potential of *Brassica chinesis* (Pechay)

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Key words: *Brassica chinesis*, Phytoremediation potential, Heavy metal, Cadmium, Lead.

http://dx.doi.org/10.12692/ijb/16.5.222-231 Article published on May 28, 2020

Abstract

In order to make the environment healthier for human beings, contaminated water bodies and land need to be cleansed to make them free from heavy metals and trace elements. This study aimed to determine the heavy metal phytoremediation potential of Brassica chinesis (pechay). This study used a completely randomized experimental research design and was conducted at PengueRuyu Tuguegarao City from June 21, 2017 to October 6, 2017. The plant was cultivated in a hydroponics system. Three hydroponics solutions were prepared namely the control, hydroponics solution spiked with cadmium and the hydroponics solution spiked with lead. The plants were grown in these hydroponics solution for ninety days. After which, the concentration of the heavy metals in the hydroponics solution, roots and shoot system of the plant was determined with the use of Flame Atomic Absorption Spectrophotometer at the Department of Science and Technology [DOST]Regional Standards and Testing Laboratory Regional Office 2 in Tuguegarao City, Cagayan. The phytoremediation potential of *Brassica chinesis* were described in terms of the bioconcentration factor (BCF) and translocation factor (TF).Results of this study revealed that *Brassica chinesis* is a potential metal excluder for lead where most of the heavy metals are deposited at the roots of the plant. Moreover, it has also the phytoremediation potential as shown by its high value of BCF and low value of TF. Lastly, it was found out that the plant is a potential phytoremediator both for cadmium and lead.

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Introduction

Heavy metal contamination in soil and water is a widely recognized global problem because of its high toxicity to biotic communities, bioaccumulation and biomagnification in food chains (Bang *et al.*, 2015; Batvari *et al.* 2008; Kamala-Kannan *et al.*, 2008; Batvari *et al.,* 2012) and unlike organic substances, heavy metals are essentially nonbiodegradable and therefore accumulate in the environment (Ali, Khan and Sajad, 2013). As mentioned by Khan *et al.*, (2010), the accumulation of heavy metals in soils and waters poses risk to the environmental and human health. These elements accumulate in the body tissues of living organisms (bioaccumulation) and their concentrations increase as they pass from lower trophic levels to higher trophic levels (a phenonmenon known as biomagnification).

World Health Organization [WHO] (2011) defined heavy metal as metal of high specific gravity, especially; a metal having a specific gravity of greater than or equal to five. These are metallic elements with high atomic weights; (e.g. mercury, chromium, cadmium, arsenic, and lead); [that] can damage living things at low concentrations and tend to accumulate in the food chain. In addition to the negative effects of accumulation of heavy metals on human beings, ecosystems and other natural resources (United States Environmental Protection Agency [USEPA]).

Heavy metal contamination of the aquatic environment is increasingly becoming common in many developing countries, where there has been linked to several anthropogenic processes including artisanal gold mining, (Donkor *et al.*, 2005; Gbogbo, 2017) electronic waste processing (Caravanos, *et al.*, 2011; Atiemo, Ofosu, Aboh & Kuranchie, 2012) industrial processes (Armah, Obiri, Yawson & Pappoe, 2010) domestic sewage discharges (Flanko *et al.*, n.d.) and agricultural activities (Gbogbo, 2017). While most of these activities and contamination occur in the terrestrial environment, the metals are transferred to rivers and sea through fluvial processes, predisposing both fresh and marine water fisheries to risk of heavy metal accumulation (Vega, Covelo, Andrade & Marcet, 2004; Blight and Fourie, 2005).

Regarding their toxicities, the most problematic heavy metals are Hg, Cd, Pb, As, Cu, Zn, Sn, and Cr (Wright, 2007; Ghosh, 2010). Two substances are classified by World Health Organization as chemicals of major public health concern are lead (Pb) and Cadmium (Cd). These metals have been extensively studied and their effects on human health regularly reviewed by international bodies such as the WHO (Jarup, 2003).

In order to make the environment healthier for human beings, contaminated water bodies and land need to be cleansed to make them free from heavy metals and trace elements (Dixit, *et al*, 2015) which is considered to be a challenging job with respect to cost and technical complexity (Barcelo and Poshcenreider, 2003).Coagulation and flocculation using organic coagulants from plants are becoming more popular to decrease thehazards of using inorganic coagulants which can leave trace amounts of heavy metals for preliminary water treatment (Nozaleda, 2019). On the other hand, the treatment of toxic and hazardous heavy metal contaminated wastewater generally includes the conventional remediation technologies which can be and the plant-based bioremediation technologies which have been collectively termed as phytoremediation (Alberto and Sigua, 2013).

The conventional remediation technologies comprise both ex-situ and in-situ techniques and the treatment can be done by using physical and chemical method. Most of these conventional remediation technologies are costly to implement and cause further disturbance to the already damaged environment (Ghosh and Singh, 2005). Generally, the physical and chemical clean- up methods suffer from limitations like high cost, intensive labor, irreversible changes in the soil properties and disturbance of native soil microflora while chemical methods can create secondary pollution (Ali, Khan &Sajad, 2013). Garbisu and Alkorta (2001), defined phytoremediation as an emerging cost effective, non-intrusive, aesthetically pleasing, and low-cost technology using the remarkable ability of plants to metabolize various elements and compounds from the environment in their tissues. Phytoremediation technology is applicable to a broad range of contaminants, including metals and radionuclides, as well as organic compounds like chlorinated solvents, polychlori-biphenyls, polycyclic aromatic hydrocarbons, pesticides/insecticides, explosives and surfactants. This technology directly uses green plants to degrade, contain, or render harmless various environmental contaminants, including recalcitrant organic compounds or heavy metals. Plants are especially useful in the process of bioremediation because they prevent erosion and leaching that can spread the toxic substances to surrounding areas (Macek, 2003; USEPA, 2004).

Plants that are used in heavy metal phytoremediation are collectively known as hyperaccumulator. Hyperaccumulator describes a number of plants that belong to distantly related families but share the ability to grow on metalliferous soils and to accumulate extraordinarily high amounts of heavy metals in the aerial organs, far in excess of the levels found in the majority of species, without suffering phytotoxic effects.

In a review paper entitled “Potential of Brassicaceae Burnett (Mustard family; Angiosperms) in Phytoremediation of Heavy Metals” by Pantola and Alam in 2014, few of the members of *brassicaceae*(mustard family) have potentials for phytoremediation due to their well-known natural tolerant against various environmental stresses including heavy metals.

Hence, the present study was undertaken to determine the heavy metal phytoremediation potential of *Brassica chinesis*(pechay).

Statement of the problem

Generally, this study aimed to determine the heavy metal (lead and cadmium) phytoremediation potential of *Brassica chinesis* (pechay). Specifically, it sought to answer the following questions:

1 What is the concentration in parts per million of the following heavy metal present in the hydroponics solution, roots and shoots system of *Brassica chinesis* (pechay).

a) Lead (Pb)

b) Cadmium (Cd)

2. What is the bioconcentration factor and translocation factor of heavy metals in *Brassica chinesis*?

3. Is there significant difference on the heavy metal concentration found in the hydroponics solution, roots and shoots system of the plants?

4. Is there significant difference on the BCF and TF of the samples?

Methodology

Experimental design

A completely randomized design was used for this experiment. The experiment was conducted to investigate the ability of the hydroponically grown *Brassica chinesis* to absorb heavy metals without showing signs of toxicity. Triplicate of each sample were prepared to determine the confidence level of the data that is reported.

The germination of the pechay seeds, the culturing of these plants in hydroponics solution and the drying of the plants was conducted at PengueRuyu, Tuguegarao City. The digestion of the plant sample and the analysis of the heavy metal concentration in the roots system and shoots system of *Brassica chinesis*, hydroponics solution and control at Department of Science and Technology Regional Office 2 while the soil used in the seed bed was analyzed at the Cagayan Valley Integrated Agricultural Laboratory.

Germination of seedlings

*Brassica chinesis*seeds were taken from the Department of Agriculture. Seedlings were allowed to germinate in the seed bed composed of 9 kilograms of soil and 3 kilograms of manure for ten days. After ten days, seedlings were transferred to the seedling plug of the hydroponics solution that was spiked lead and cadmium.

The soil that was for the seed bed was subjected for heavy metal analysis last March 27, 2017 that ensured that the soil was not contaminated with the heavy metal under study.

Preparation and growing plants in hydroponics solution spiked with heavy metals

The preparation of hydroponics solution was aided with Simple Nutrient Addition Program (SNAP) A solution and Simple Nutrient Addition Program (SNAP) B solution that was based on the demonstration of Dr. Primitive Jose A. Santos – SNAP co-developer from the University of the Philippines Los Baños, Institute of Plant Breeding (https://www.youtube.com/watch?v=9y5YdUC4).

The general procedures were as follows: A prototype styrobox was covered by a plastic sheet lining that has suggested dimensions of 20 by 30 inches and has a thickness of 0.003 inches. This box held the seedling plug and the nutrient solution.

Six equidistant slits were created on an eight-ounce styrocup with the aid of a small saw. This styrocup was filled with decomposed covering of the grains of rice which supported the seedlings in place.

The styrobox was filled with 10 liters of de-ionized water and the seventy-five milliliters of SNAP A solution was added and mixed to it. The solution was mixed thoroughly then, seventy-five milliliters of SNAP B solution was added to it and was stirred carefully. Then 20 ml of 1000 ppm standard solutions of lead and cadmium were added separately to the assigned boxes. Holes were created on the lid of the styrobox which will serve as seedling plugs of the styrocups. The seedlings were transferred to the styrocups and be grown hydroponically for ninety days. Three separate hydroponics solutions were prepared. One of which was spiked with 2 ppm of lead, another was spiked with 2 ppm of cadmium. Another unspiked solution for the control. The lead and cadmium use in spiking the hydroponics solutions were taken from the Central Analytical Chemistry and Biotechnology Laboratory (CABLab) of CSU-Andrews Campus. *Brassica chinesis* (pechay) were allowed to grow in the solution for ninety days.

Collection of plant sample

Plants that have grown for in ninety (90) days in the hydroponics solution spiked with 2 ppm of heavy metals were collected and were placed in polyethylene bags for analysis (Tuliao, 2016). The number of leaves of each plant were properly taken into account.

Collection of water sample

One liter of water sample from each hydroponic solution were taken and kept in polyethylene bottles. The water sample was filtered and 10 ml of hydrochloric acid were added to the solution to preserve and sequester the heavy metals in the solution before it was transported to the laboratory (DOST RO2 Standards).

Preparation of plant samples for AAS

The plant samples were washed with de-ionized water and air dried. Afterwards, each plant was cut into roots system and shoots system. Roots system and shoots system were ground separately with nitric acid pretreated porcelain mortar and pestle, sieved and eventually, three replicates of ground roots and three replicates of ground leaves were prepared from 2ppm lead spiked plants, 2ppm cadmium spiked plants and control.

One gram-sample from each replicate was ashed in muffle furnace for six hours. Afterwhich each of the sample was moistened and three milliliters of concentrated nitric acid was added to it. The solution was heated on a hot plate to evaporate excess nitric acid. The solution was allowed to cool and it was filtered into a 50-mL volumetric falsk using whatman no. 40 filter paper. The filtrate was made to mark with de-ionized water. (Official Methods of Analysis of AOAC International 20th edition, 2016. Method 972.25)

Preparation of water samples for AAS

Nine replicates of fifty milliliter of water sample was prepared out of the filtrate. Three replicates were taken from the hydroponic solution spiked with 2 ppm of lead and another three were taken from the solution spiked with 2 ppm of cadmium. The same number of replicates were prepared from the control hydroponics solution. Three milliliters of concentrated nitric acid were added to each replicate (protocol from Department of Science and Technology).

Data gathering procedure

The leaves of each *Brassica chinesis*were counted manually during the collection of the plants. The concentration of the heavy metal lead and cadmium in the roots system and the shoots system of *Brassica chinesis* as well as in the hydroponics water were determined through FAAS.

The concentration of the heavy metal in the solution was determined by using the Beer-Lambert Law or also known as the Beer's Law. This principle shows the linear relationship between absorbance and concentration of absorbing species. Experimental measurements are usually made in terms of transmittance (T) which is defined as $T = I/I_o$; where I is the intensity after it passes through the sample and I_o is the initial light intensity. The relation between absorbance (A) and transmittance (T) is then computed using A= -log T.

Modern absorption instruments can usually display the data as either transmittance, % transmittance, or absorbance. An unknown concentration of analyte can be determined by measuring the amount of light that a sample absorbs and applying the Beer's law. The calibration curve is drawn using the concentration and absorbance data for a set of standards and together with absorbance of the sample, it is use to read off the concentration of the species in the sample as shown in the example below.

The bioconcentration factor (BCF) was obtained by the dividing the concentration of the heavy metals found in the roots system of *Brassica chinesis*to that of the corresponding water sample where this roots system will be taken. BCF can be computed using the formula:

BCF= (P/E) Iwhere

I – denotes the heavy metal

BCF- is the bioconcentration factor and is dimensionless

P – is the heavy metal concentration in plant tissues (mg kg^{-1} dry wt.)

E – is the heavy metal concentration in the water (mgL^{-1})

A larger ratio implies better phytoaccumulation capability

On the other hand, translocation factor can be derived by dividing the concentration of the heavy metals found in the root system to that of the concentration of heavy metals found in the shoots system. It can be calculated using the equation:

TF = $(F_r/F_s)I$where

I – denotes the heavy metal

TF – is the translocation factor and is dimensionless

F_r -heavy metal concentration in the roots system (mg kg^{-1}dw)

F_s –heavy metal concentration in the shoots system (mg kg^{-1}dw)

A larger ratio implies poorer translocation capability

Atomic Absorption Spectrometry (AAS)

Atomic absorption analysis involves measuring the absorption of light by vaporized ground state atoms and relating the absorption to concentration. Specifically, a detector measures the wavelengths of the light transmitted by the sample and compares them to the wavelengths that originally passed through the sample. A signal processor then integrates the changes in the wavelength which appear in the read out as peaks of energy absorption at discrete wavelengths. In order to tell how much of a known element is present in a sample, one must first establish a basis for comparison using known quantities. It can be done by producing a calibration curve. For this process, a known wavelength is selected, and the detector will measure only the energy emitted at that wavelength. However, as the concentration of the target atom in the sample increases, absorption will also increase proportionally. Thus, one runs a series of known concentrations of some compound, and records the corresponding degree of absorbance, which is an inverse percentage of light transmitted. A straight line can then be drawn between all of the nown points. From this line, one can extrapolate the concentration of the substance under investigation from its absorbance. The use of special light sources and specific wavelength selection allows the quantitative determination of individual components of a multielement mixture. (http://web.nmsu.edu/~esevosti/report.htm)

Data analysis

The results of the study were analyzed with the use of mean for the mean concentration of the heavy metals, one sample t-test was utilized to test the difference of concentration in the hydroponics solution and at the roots and shoot system of the plant. Paired sample t-test was used to test the difference between the concentration, bio concentration factor and translocation factor of the heavy metal in the control and experimental group. The five percent level of significance was used. Data was processed using SPSS v20.

Results and discussion

Heavy metal concentration in parts per million in Brassica chinesis

Table 2 showed the concentration of Lead and Cadmium in the water, roots and shoots system of the plants under the control group. It can be gleaned on the table that lead is highly absorbed at the roots of the plant and is least concentrated at the hydroponics solution where the plants were grown. However, it is worthy to note that the concentration of the heavy metal cadmium did not vary wherein it showed a heavy metal concentration of <0.1 ppm in the water, roots and shoots sytem of the plant.

This implies that *Brassica chinesis*has the ability to transport heavy metal particularly lead to the different parts of its body. On the other hand, heavy metal cadmium was not significantly transported to any body part of the plants under the control group.

Table 1. Concentration of the heavy metal lead and cadmium in the roots system and the shoots system of *Brassica chinesis*and in the hydroponics water.

Sample	Concentration (ppm)	Absorbance
Blank	0.00	0.00
Standard 1	1.00	0.17
Standard 2	2.00	0.34
Standard 3	3.00	0.48
Standard 4	4.00	0.65
Standard 5	5.00	0.83
Sample	?	0.58

Table 3 showed the concentration of heavy metals in the solution, roots and shoots system of the plants when they are spiked with lead and cadmium (which was the experimental group of the study). Consistent with the result showed in the previous table, the heavy metals were mostly deposited at the roots of the plants both for the two heavy metals.

This finding revealed that *Brassica chinesis*is a potential metal excluder for lead. This was supported by the results of the study conducted by Gisbert *et al.*, (2006) wherein they observed that these *Brassica* species behaved as Zn and Pb excluders, able to maintain an almost constant level of these metals in the shoots, up to a certain level of toxicity. Metal excluders accumulate heavy metals from substrate into their roots but restrict their transport and entry into their aerial parts (Sheoran *et al.*, 2011; Malik and Biswas, 2012). Such plants have low potential for metal extraction but may be efficient for phytostablization (Lasat, 2002).

Table 2. Heavy metals absorbed by *Brassica chinesis*under control group.

Sample code	Parameter	Sample description	Concentration (in ppm)	Mean
PbCtrl$_w$	Lead	Water	0.42	
PbCtrl$_r$	Lead	Roots	77.30	27.53
PbCtrl$_s$	Lead	Shoot system	4.86	
CdCtrl$_w$	Cadmium	Water	<0.1	
CdCtrl$_r$	Cadmium	Roots	<0.1	<0.1
CdCtrl$_s$	Cadmium	Shoot system	<0.1	

Lastly, the heavy metals were least concentrated in the hydroponic solution where the plants were grown. This implies that *Brassica chinesis*has the ability to transport heavy metal from its environment (which is the hydroponics solution) to the different parts of its tissue.

Brassica chinesis'bioconcentration Factor and translocation factor

Table 4 presented the bioconcentration factor and translocation factor of *Brassica chinesis.* The bioconcentration factor (BCF) and the translocation factor (TF) are quantitative measure of the phytoremediation potential of a plant wherein the greater the value of the BCF the better is the phytoaccumulation ability of the plant while reverse is true for the TF, lesser value implies better phytoaccumulation ability. Moreover, it was also shown that the plant had a high a bioconcentration factor and low value of translocation factor. It was known that high value of BCF and low value of TF implies better phytoremediation potential.

Table 3. *Brassica Chinesis'* heavy metal concentration in parts per million under experimental group.

Sample code	Parameter	Sample description	Concentration (in ppm)	Mean
PbEx$_w$	Lead	Water	0.06	
PbEx$_r$	Lead	Roots	80.83	98.06
PbEx$_s$	Lead	Shoot system	17.17	
CdEx$_w$	Cadmium	Water	0.53	155.55

The standard for hyperaccumulators has not been defined scientifically (Nazir *et al.*, 2011); however, individual authors or research groups have defined hyperaccumulator (Ali, Khan & Sajad, 2013).

Study of Baker and Brooks, (1989) have shown that the nominal threshold criteria or the range of accumulation (mg/kg) and reference for element cadmium being hyperaccumulated by plant is 100 while Haque *et al.* provided an evidence that lead is hyperaccumulated at 1000 mg/kg dry weight (as cited by Krzciuk and Galuszka, 2015; Ali, Khan &Sajad, 2013).

Thus, basing on these criteria *Brassica chinesis* is a potential hyperaccumulator of both lead and cadmium. TF values greater >1 indicate that the plant is effective in translocating metals form roots to shoot, and the TF values <1 indicate the ineffectiveness of plants in translocating the elements.

It has been established that TF varied according to the type of plant, substrate, and concentration of pollutants. (Tang *et al.*, 2009; Bang *et al.,* 2015). Several works have been published comparing the performance of several *Brassica* species to toxic concentrations of heavy metals. Hernandez-Allica *et al.*, (2008) made an extensive study regarding the heavy metal tolerance of different species (including several varieties of *B. campestris*, *B. rapa*, *B. napus*, *B. oleracea*and *B. carinata*), confirming that they have high levels of tolerance mainly to Zn, and less to Pb and Cd, the metals under study.

Table 4. Bioconcentration Factor (BCF) and Translocation Factor (TF) of *Brassica chinesis*.

Treatment	Parameter	Bioconcentration factor (BCF)	Translocation factor(TF)
Control	Lead	195.62	15.91
	Cadmium	2.00	1.00
Experimental	Lead	1633.33	4.71
	Cadmium	879.49	1.26

Difference on the Heavy Metal Concentration on the Hydrponics Solution, Roots and Shoot System

The following table (5) shows the test of significant difference on the heavy metal concentration in the hydroponics solution (water), roots and shoot system of the plant both under the control and experimental group.

Table 5. Test of significant difference on the heavy metal concentration on the hydrponics solution, roots and shoot system.

Treatment Group	t-value	p-value	Decision
Control	1.086	0.327	Accept H_o
Experimental	2.049	0.096	Accept H_o

Results of one sample t-test of control and experimental yielded a t-value of 1.086 and 2.049, respectively whose associated probability values are 0.327 and 0.096 correspondingly, which is higher than the level of significance set, 0.05; thus, the null hypothesis is accepted. This result could mean that the amount of heavy metal that can be absorbed by the roots of *Brassica chinesis* do not significantly differ with the amount of heavy metal that can be absorbed by the shoot system of plant.

Thus, the capacity of the roots to accumulate heavy metal is approximately comparable with the capacity of the leaves to absorb heavy metals.

Table 6. Test of significant Difference on the BCF and TF of the Samples.

Variable	Treatment group	t-value	p-value	Decision
BCF	Control	1.021	0.493	Accept H_o
	Experimental	3.333	0.186	Accept H_o
TF	Control	1.134	0.460	Accept H_o
	Experimental	1.730	0.334	Accept H_o

Difference on the bioconcentration factor and translocation factor of the samples

The table (6) below showed the results of the test of significant difference on the bioconcentration factor and translocation factor of the plant sample in order to evaluate the plant's heavy metal phytoremediation potential. It has showed that there is no significant difference on the bioconcentration factor of the plants. It was also found that there was no variation in the translocation factor of plant. This further confirms that *Brassica chinesis*is a potential phytoremediator for both cadmium and lead. This observation could be result of the ability of the plant to absorb both heavy metals.

Conclusionand recommendations

In the light of the results and findings of this study, it can be concluded that *Brassica chinesis*(pechay)is a potential metal excluder for lead, hyperaccumulator and potential phytoremediator of cadmium and lead heavy metals due to its high value of bioconcentration factor and low value of translocation factor. The results of the study can be utilized by using *Brassica chinesis* as a phytoremediator for areas where heavy metal contamination is evident. Furthermore, a study to compare the phytoremediation potential of *Brassica chinesis*grown in soil and in hydroponics system is also recommended. Increasing the amount biomass of the plant used in the study especially if the instrument used in the determination of heavy metal is the atomic absorption spectrophotometer is also worthwhile to consider.

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