Research article

Cadmium Bioaccumulation in *Amaranthus spinosus* L. Grown in Contaminated Soil of Benguet Province, Philippines

ELIZABETH T. DOM-OGEN*
Benguet State University, La Trinidad, Benguet, Philippines
Email: e.dom-ogen@bsu.edu.ph

ROMEO A. GOMEZ, JR.
Benguet State University, La Trinidad, Benguet, Philippines

Received 3 February 2022 Accepted 13 June 2022 (*Corresponding Author)

Abstract Phytoremediation has become an alternative technology for cleaning up contaminated areas by accumulation in the root and shoot systems. The study conducted a 98-day pot experiment to determine Cd accumulation and its morphological and physiological effects on *Amaranthus spinosus L.* grown in the non contaminated forest (S1) and mine contaminated (S2) soils of Benguet. Soil and plant tissue analysis were done using AAS method; quantitative descriptive method for morphological analysis; titration method for vitamin C analysis; and Kjeldahl method for protein analysis. *Amaranthus spinosus* L. grown in S1 accumulated 0.52 mg/kg Cd in the root and 0.88 mg/kg Cd in the shoot with soil Cd concentration of 3.70 mg/kg at 7.43 pH. In S2, *A. spinosus* L. had taken up 0.000012 mg/kg Cd in the roots and in the shoot systems with 0.90 mg/kg Cd soil concentration at 4.19 pH. Biological Accumulation Coefficient (BAC = 0.24) was generally weak since the equivalent is less than 1 (BAC < 1, 0.24) while translocation factor (TF = 1.69) is greater than 1 (TF>1, 1.69). For morphological characteristics comparing S1 and S2 results are the following: root length (372.33 mm and 284.70 mm); shoot length (399.33 mm and 132.33 mm); and, biomass (22.53 grams and 9.27 grams). Highly significant differences were also noted for S1 and S2 on root length, shoot length, and biomass at α = 0.01 with p – values of 0.002, 0.000, and 0.002, respectively. For the vitamin C and protein content, both (S1 and S2) were not affected. From these results, it can be concluded that *Amaranthus spinosus* L. can accumulate significant concentration of Cd in its root and shoot systems and allows Cd mobility in its system. It is then recommended that the capability of *Amaranthus spinosus* L. for Cd tolerance requires further studies.

Keywords *Amaranthus spinosus* L., bioaccumulation, phytoremediation, physiological, morphological characteristics

INTRODUCTION

Environmental sustainability issues are pressuring global concerns at present because of increasing population and industrialization. It had been realized that anthropogenic activities such as mining despite its substantial contribution to economic growth and development resulted towards environmental problems (Castillo et al., 2010). In the Philippines, it had established mining industries but problems with proper tailings disposal and policies for mitigation are not yet in place causing leakage of hazardous chemicals like cadmium, lead and copper (CPA, 2007). The presence of such contaminants in excess can lead to the reduction and inhibition of growth in plants inflicting serious morphological, metabolic, and physiological anomalies. Correspondingly, these contaminants cannot be mineralized or degraded to less toxic forms; can be leached to nearby farmlands and accumulated through the food chain; thus, requiring suitable methods for their elimination (Chen et al., 2014).

Addressing these, phytoremediation has become an alternative technology for cleaning up contaminated areas. *Amaranthus spinosus* L. (Amaranthaceae) commonly called pigweed can bioconcentrate Cd, Zn and Fe invariably high in leaf tissues (Prasad and Freitas, 2003), and shows best morphological and anatomical structure adaptation to stressful environments (Gharge and Menon, 2017).

Correspondingly, *Amaranthus spinosus* L. is a potential agent for heavy metal accumulation and translocation for Cu, Pb and Cd (Chinmayee et al., 2012) and among the dominant species growing in some mine sites in the Philippines (Malik et al., 2010).

OBJECTIVE

The study concerns the investigation of the potential ability of *Amaranthus spinosus* L. to accumulate Cd in the root and shoot system and how it responds morphologically and physiologically.

METHODOLOGY

Sample Sites

The soil sampling sites were located at Alno, La Trinidad and Antamok, Itogon, Benguet (Fig. 1). La Trinidad and Itogon are two of the 13 municipalities that comprise Benguet which, together with five other provinces, make up the Cordillera Administrative Region.Alno has an estimated land area of 958.35 ha. It is the third largest village in this municipality of La Trinidad. An area of 30.8 ha of forest and brushlands is classified as the Alno communal forest (Lumbres et al., 2014). The municipality of Itogon is the core of the Baguio Mining District. Before the World War II, large-scale mines operated, small-scale mining thrived. For Antamok, Itogon decommissioned tailings dam had been abandoned for 22 years during the collection but still a basin of mine waste for small scale miners since the tailing dam used for the study had operated for 7 years and was shut down last April 1998 (Chaloping-March, 2019).

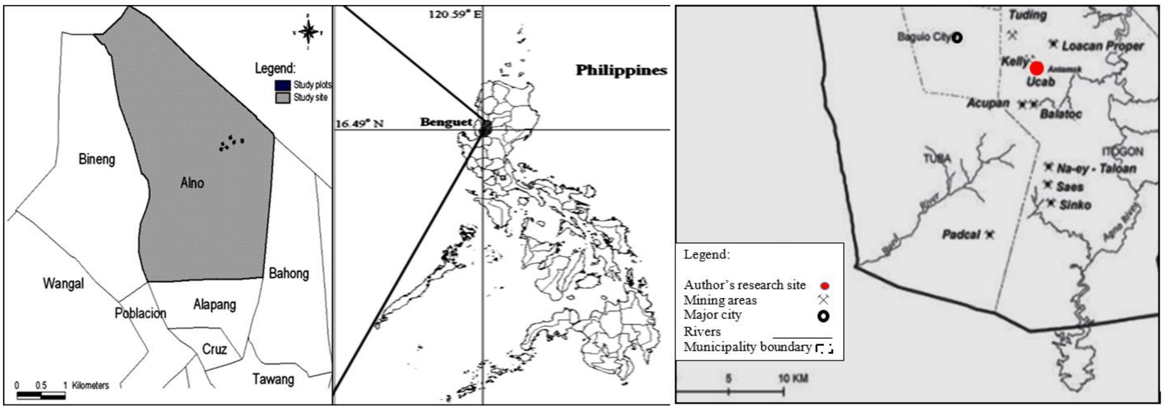

Fig. 1 Location map of Alno, La Trinidad, Benguet and of Antamok, Itogon, Benguet

Soil Sample Characteristic, pH and Cadmium Analysis

The identification of non-contaminated and contaminated soil was based on Pfeiffer et al. (1988) and the US EPA (1994) for standard maximum concentration of contaminants for toxicity characteristics. Soil samples were collected using composite soil sampling (Brady and Weil, 2000). For soil pH determination the potentiometric method was employed. The concentrations of heavy metals in soil samples were determined using Atomic Absorption Spectroscopy (AAS).

Plant Morphological Observation on the 98th Day

Plants were carefully uprooted from the plastic pots then washed properly. The plants were cut separating the root and shoot system for length measurement, and were air dried. Also, it was oven dried at 60 degrees Centigrade for biomass.

Determination of Vitamin C and Protein Content of the Plant on the 98th Day

The plants used in the morphological study were processed and analyzed separately. The vitamin C content from the plant samples was measured based on the redox titration. The total protein content from the plant samples was measured based on the Kjeldahl method.

Bioaccumulation Evaluation

Bioaccumulation and phytoextraction were evaluated in terms of Biological Accumulation Coefficient (BAC), Biological Coefficient Factor (BCF) and Trans-location Factor (TF) whereby;

BAC = [metal] shoot / [metal] soil

BCF = [metal] root and shoot / [metal] soil

TF = [metal] shoot / [metal] root

Treatment of Data

The data were tabulated, interpreted, and analyzed using comparative experimental research design. The t-test was used to determine the differences in the soil pH and soil Cd content of non-contaminated and contaminated soil; plant tissue Cd content analysis of each plant systems (root and shoot); and the plant morphological and physiological changes in *Amaranthus spinosus* L. on the 98th day.

RESULTS AND DISCUSSION

Cadmium Bioaccumulation in the Root and Shoot System of Amaranthus spinosus Linn

Table 1 shows the mean Cd concentration in the root and shoot systems of *Amaranthus spinosus* L. planted on non-contaminated (S1) and contaminated (S2) soils. The mean Cd content for the S1 in the root and shoot systems was non-detectable as represented by the value of 0.000012 mg/kg dry weight while for S2 in the root and shoot systems were 0.520000 and 0.880000 mg/kg dry weight, respectively.

Table 1 Mean cadmium concentration on the root and shoot of *Amaranthu spinosus* L.

Plant organs	Non-contaminated soil (S1) mean (mg/kg)	Contaminated soil (S2) mean (mg/kg)	P - value
Root	0.000012	0.520000	0.002**
Shoot	0.000012	0.880000	0.003**

*Legend:** highly significant at α = 0.01*

Using t- test at α = 0.01, the accumulation of cadmium in the root and shoot systems of *A. spinosus* L. comparing S1 and S2 is highly significant with a p = value of 0.002 and 0.003, respectively. This suggests that with 0.90 mg/kg in S1 mean Cd concentration, absorption and accumulation from the soil to the roots and translocation to the shoot system did not occur while at 3.70 mg/kg mean Cd concentration in S2 allows to some limited extent of Cd absorption and accumulation by the root from the soil then translocated to the shoot system.

According to Pantazis et al. (2007), the uptake of Cd by root from the soil depends on the soil properties like Cd concentration and level of organic matter aside from pH. Dada (2019) reported that *A. spinosus* L. accumulated 5.24 mg/kg in the root and 3.03 mg/kg in the shoot in soil of pH of 8.4 and 16.10 mg/kg soil Cd concentration. Moreover, Carrion and Mendoza (2019) result shows that *A. spinosus* L. accumulated 0.55 mg/kg Cd in the shoot system with 7.49 soil pH and 2.02 mg/kg Cd soil concentration.

Bioaccumulation Evaluation of *Amaranthus spinosus* L.

Bioaccumulation evaluation of *Amaranthus spinosus L.* were based from the computed value of Biological Accumulation Coefficient (BAC), Biological Coefficient Factor (BCF) and Translocation Factor (TF). The BAC value is 0.24, BCF is 0.14 and TF value is 1.69. The BAC (0.24) and BCF (0.38) values were generally weak in *A. spinosus* L. (Table 2) since their equivalents are less than 1 (BAC & BCF <1) while Translocation Factor (TF) is greater than 1 (TF > 1 = 1.69). The TF value (TF > 1) indicates capability to accumulate and transport cadmium in its organs at 3.70 mg/kg soil Cd concentration and 7.43 soil pH. With the value of 1.69 $TF_{Cd,}$ it could be deduced that the translocation of Cd from the roots to the shoot system of *Amaranthus spinosus* L. was relatively efficient but it cannot be identified as an excluder, accumulator or extractor.

Table 2 BAC; TF; and BCF of *Amaranthus spinosus* L.

Accumulation characteristics	Equivalent
BAC	0.24
TF	1.69
BCF	0.38

Cadmium Bioaccumulation Effect on Morphological Characteristics of *Amaranthus spinosus* L.

The morphological characteristics of *Amaranthus spinosus* L. measured after 98 days of growth are summarized in Table 3. These characteristics, with the paired mean measurements obtained from those planted in S1 and S2 soils respectively, include: root length (372.33 mm and 284.70 mm); shoot length (399.33 mm and 132.33 mm); and, biomass (22.53 grams and 9.27 grams).

Table 3 Morphological characteristics of the *Amaranthus spinosus* L. on the two sites

Characteristics	Non-contaminated soil (s1) mean	Contaminated soil (s2) mean	P - value
Root length (mm)	372.33	284.70	0.002**
Shoot length (mm)	399.33	132.33	0.000**
Biomass (grams)	22.53	9.27	0.002**

*Legend: ** highly significant at α = 0.01*

Highly significant differences were noted for S1 and S2 on root length, shoot length, and biomass at α = 0.01 with p – values of 0.002, 0.000, and 0.002, respectively. *Amaranthus spinosus* L. planted at 0.90 mg/kg in S1 are longer in root and shoot length and had higher biomass as compared to *Amaranthus spinosus* L. at 3.70 mg/kg soil concentration of S2; the latter had short root and shoot length and had less biomass.

The morphological results of *Amaranthus spinosus* L. in S1 having longer root and shoot length and higher biomass as compared to S2 having short root and shoot length and less biomass can be due to the nil and greater amounts of Cd root and shoot accumulation after the 98-day growth, respectively. Other observations for S2 that are not found in S1 were thicker stem diameter, delayed development, and leaf chlorosis.

These morphological observations in S2 are the results of plants ability to adjust to its contaminated environment by delaying or arresting mitotic division (Baran et al., 2010) affecting the length development of the root. The short roots will be deprived of nutrient demand and supply because its source is limited only to its immediate surroundings resulting to short shoot system because of plant nutrient deficiency. Moreover, the presence of Cd in the plant system inhibits photosynthesis hampering growth and development resulting to plant decreased biomass (He et al., 2015).

Effect on the Vitamin C and Protein Content of *Amaranthus spinosus* L.

The vitamin C content in organs of *Amaranthus spinosus* L. planted in both S1 and S2 soils is 2.40%. The plant protein content, on the other hand, is at 5.18% (S1) and 4.73% (S2). Statistically, there were no significant differences in the vitamin C (2.40% at 0.90 mg/kg – S1 and 2.40% at 3.70 mg/kg – S2) and protein (5.18% at 0.90 mg/kg – S1 and 4.73% at 3.70 mg/kg – S2) contents of *Amaranthus spinosus* L. in both soil samples were noted. This is supported by the p-values obtained comparing S1 and S2 for the physiological characteristics at 1.000 and 0.684 respectively (Table 4). The highly significant differences of the soil Cd concentrations of S1 and S2 apparently did not at all affect the vitamin C and protein contents of plants grown in both S1 and S2. The result may be due to the plants ability to maintain its metabolic activities by storing Cd in the cell wall and vacuole of the root and the shoot systems, thereby continuing growth and development.

Table 4 Mean vitamin C and protein content of the *Amaranthus spinosus* L. plant organs on the two sample sites

Physiological characteristics	Mean (%)		P - value
	Non-contaminated soil (s1)	Contaminated soil (s2)	
Vitamin C content	2.40	2.40	1.000^{ns}
Protein content	5.18	4.73	0.684^{ns}

Legend: ns - not significant at α = 0.01

Vitamin C and protein are important factors for growth and development since protein responds to a wide variety of stresses within the plant body while vitamin C plays an important role in the adaptation and ultimate survival of plants (Dinakar et al., 2008). The result of the study was similar to the study of Jibril et al. (2017) on lettuce where soil Cd concentrations of 0, 3, 6, 9 and 12 mg/kg have no significant effect in flavonoids, vitamin C and proline. This was confirmed by Wang et al. (2009) on Cd toxicity on N metabolism in leaves of *Solanum nigrum* in 6.5 soil pH where the concentration did not decrease significantly in soil Cd concentration at 6, 12 and 24 mg/kg.

CONCLUSION

Phytoremediation is a low cost and environmental friendly method to eliminate soil contaminants. *Amaranthus spinosus* L. had been used since literatures suggest that it has the capability to extract heavy metals and can survive to adverse conditions. The findings of the study shows that there is a significant accumulation of Cd in *Amaranthus spinosus* L. on contaminated soil (root-0.52 mg/kg and shoot-0.88mg/kg) and non-detectable Cd accumulation in non-contaminated soil. *Amaranthus spinosus* L. plants in non-contaminated soil are taller compared to those in contaminated soils which are short. Given the morphological characteristics of the plants, it follows that those in S1 have higher biomass as those in S2 having lower biomass. Also, vitamin C and protein content are not affected by the amount of Cd in two sample sites. Therefore, *Amaranthus spinosus* L. can survive in the environment with 3.70 mg/kg soil Cd concentration at 4.19 pH.

REFERENCES

Baral, M., Ankur, D., Chakraborty, S. and Chakraborty, P. 2011. Pharmacognostic studies on stem and leaves of *Amaranthus spinosus* Linn. International Journal of Applied Biology and Pharmaceutical Technology, 2 (1), 41-47.

Brady, N.C. and Weil, R.R. 1990. The nature and properties of soil (14th ed.). Pearson, ISBN 978-0133254488, Boston, USA.

Castillo, J.P., Verdu, M. and Valiente-Banuet, A. 2010. Neighborhood phylodiversity affects plant performance. Ecology, 91 (12), 3656-3663, Retrieved from DOI https://doi.org/10.1890/10-0720.1

Chaloping-March, M. 2019. Antamok, Launchpad of industrial mining in the Philippines. In Chaloping-March, M. (Ed.), Social Terrains of Mine Closure in the Philippines, 80-99, Routledge, London, UK.

Chen, L., Lon, S., Li, X., Won, Y., Chen, J. and Lui, C. 2014. Interaction of Cd-hyperaccumulator *Solanum nigrum* L. and functional endiphyte *Pseudomonas* sp. Lk9 on soil heavy metals uptake. Soil Biology and Biochemistry, 68, 300-308, Retrieved from DOI https://doi.org/10.1016/j.soilbio.2013.10.021

Chinmayee, M.D., Masesh, B., Pradesh, S., Mini, I. and Swapna, T.S. 2012. The assessment of phytoremediation potential of invasive weed *Amaranthus spinosus L.*. Applied Biochemistry and Biotechnology, 167 (6), 1550-1559, Retrieved from DOI 10.1007/s12010-012-9657-0

Cordillera Peoples Alliance (CPA). 2007. Case study on the impacts of mining and dams on the environment and indigenous peoples in benguet, Cordillera, Philippines. Retrieved from https://www.studocu.com/ph/document/technological-university-of-the-philippines/engineering-economics/case-study-in-benguet-from-internet /11224770

Dada, O.E. 2019. Cadmium tolerance and phytoremediation strategies of selected tropical plants cultivated on industrial dump site under the influences of two mycobionts. West African Journal of Applied Ecology, 27 (2), 106-125, Retrieved from https://www.ajol.info/index.php/wajae/article/view/192383

Dinakar, N., Nagajyothi, P.C., Suresh, S., Udaykiran, Y. and Damodharam, T. 2008. Phytotoxicity of cadmium on protein, proline and antioxidant enzyme activities in growing *Arachis hypogaea* L. seedlings. Journal of Environmental Science, 20 (2), 199-206, Retrieved from DOI https://doi.org/10.1016/S1001-0742(08) 60032-7

Gharge, S. and Menon, G. 2017. Morpho-anatomical adaptation in some herbs growing near Ulhas river polluted with industrial effluent. International Journal of Botany Studies, 2 (4), 43-48, Retrieved from http: //www.botanyjournals.com/archives/2017/vol2 /issue4/ 2-4-18

He, S., He, Z., Yang, X., Stoffella, P.J. and Baligar, V.C. 2015. Soil biogeochemistry, Plant physiology and phytoremediation of cadmium-contaminated soils. In Sparks, D.L. (Ed.), Advances in Agronomy, 134, 135-225, Academic Press, Cambridge, USA.

Jibril, S.A., Hassan, S.A., Ishak, C.F. and Wahab, P.E.M. 2017. Cadmium toxicity affects phytochemicals and nutrients elements composition of lettuce (*Lactusa sativa* L.). Hindawi Advance Agriculture, 1-7, Retrieved from DOI https://doi.org/10.1155/2017/1236830

Lumbres, R.I.C., Palaganas, J.A., Micosa, S.C., Laruan, K.A., Besic. E.D., Yun, C.W. and Lee, Y.J. 2014. Floral diversity assessment in alno communal mixed forest in Benguet, Philippines. Landscape and Ecological Engineering, 10, 361-368, Retrieved from DOI https://doi.org/10.1007/s11355-012-0204-5

Malik, R.N., Husain, S.Z. and Nazir, I. 2010. Heavy metal contamination and accumulation in soil and wild plant species from industrial area of Islamabad, Pakistan. Pakistan Journal of Botany, 42 (1), 291-301, Retrieved from http://www.pakbs.org/pjbot/PDFs/42(1)/PJB42(1)291.pdf

Pantazis, V., Kalavrouziotis, I. and Deligiannakis, I. 2007. Cu-Zn accumulation on soil plant system irrigated with wastewater. Proceedings in IWA Facing Sludge Diversities, Challenges, Risks and Opportunities. Antalya, Turkey, 673-680.

Pfeiffer, E.M., Freytag, J., Scharpenseel, H.W., Miehlich, G. and Vicente, V. 1988. Trace elements and heavy metals in soil and plants of the southeast asian metropolis Metro Manila and of some rice cultivation provinces in Luzon, Philippines. Hamburger Bodenkundliche Arbeiter, Band 11, Hamburg, Germany.

Prasad, M.N.V. and De Oliveira Freitas, H.M. 2003. Metal hyperaccumulation in plants, Biodiversity prospecting for phytoremediation technology. Electronic Journal of Biotechnology, 6 (3), 285-321, Retrieved from http://www.ejbiotechnology.info/index.php/ejbiotechnology/article/view/v6n3-6/617

U.S. Environmental Protection Agency (U.S. EPA). 1994. Test methods for evaluation of solid wastes, Physical, chemical methods (3rd Edition), Final Update 3A. CD ROM SW-846.

Wang, H., Zhao, S.C., Liu, R.L., Zhou, W. and Jin, J.Y. 2009. Changes of photosynthetic activities of maize (Z*ea mays* L.) seedlings in response to cadmium stress. Photosynthetica, 47 (2), 277-283, Retrieved from DOI https://doi.org/10.1007/s11099-009-0043-2