IJBPAS, December, 2017, 6(12): 2514-2525

ISSN: 2277–4998

International Journal of Biology, Pharmacy and Allied Sciences (IJBPAS)

'A Bridge Between Laboratory and Reader'

www.ijbpas.com

NUTRIENT DYNAMICS OF WASTEWATER SOLUTION AS MEDIUM IN HYDROPHONIC CULTIVATION OF *Brassica rapa chinensis*AND ITS HEAVY METAL ACCUMULATION POTENTIAL

JAIME AGUSTIN A. SANTOS[1], MA. ELLENITA DE CASTRO[1*], RICH MILTON R. DULAY[2]AND NADINE ADELLIA LEDESMA[3]

1: Department of Biology, College of Science, De La Salle University, Taft Avenue, Manila, Philippines

2: Department of Biological Sciences, College of Arts and Sciences, Central Luzon State University, Science City of Munoz, Nueva Ecija, Philippines

3:Agrivet Sciences Institute, De La Salle Araneta University, San Jose Del Monte, Bulacan, Philippines

*Corresponding Author: ma.ellenita.decastro@dlsu.edu.ph

Received 25th March 2017; Revised 16th April 2017; Accepted 20th July 2017; Available online 1st Dec. 2017

ABSTRACT

Plants of Family Brassicaceae are known as one of the most edible group of plant which are usually consumed as vegetables by humans. However, they are also known to hyperaccumulate nickel and other heavy elements. In the present study, a preliminary assessment of the heavy metals present in the wastewater solution was determined to test its ability of support the growth of *Brassica rapachinensis* (pechay). The wastewater used was collected from the De La Salle University Wastewater Treatment Facility.Three different dilutions of wastewater were used as treatments namely 5% wastewater, 15% wastewater, and 25% wastewater, respectively.It was found out that the nutrient compositions in the three wastewater concentrations significantly varied with one another. In addition, the concentration of the nutrients increased overtime indicating that other factors may have influenced the amount of nutrients present in the solution. Among the heavy metals tested, zinc, copper, and iron were detected in the wastewater solution, whereas cadmium and lead were found in any of the varying concentrations of wastewater used in the study. The plant tissue was found to contain considerable amount of these heavy metals

but the concentration is below the threshold level to consider *B. rapa chinensis* as a hyperaccumulator.

Keywords: *Brassica rapa chinensis,* nutrient dynamics, wastewater, hyperaccumulation

INTRODUCTION

Heavy metal contamination is now considered one of the most serious environmental problems and its management requires a unique approach since due to the difficulty involved in its clean up **[1]**. Heavy metal pollution sources can be traced to multiple origins and eventually they can be discharged in several outlets and water systems, both fresh and marine. Wastewater pollution comes from the discharge of agricultural, municipal, and industrial waste products and the contamination of high concentrations heavy metals to aquatic environments poses a serious threat due to the toxicity, long persistence, and bioaccumulation in the food chain. Some of the biologically important heavy metals which are also considered micronutrients include copper, zinc, and iron. Their presence is vital for the survival of most biological systems but the large concentration could in turn result to detrimental effects.

There is an increasing trend in land, surface waters, and groundwater contamination due to military, industrial and agricultural events or simply due to ignorance, lack of vision, or carelessness **[2]**. This global event is due to the buildup of various toxic pollutants, such as radionucleocides, metals, and organic contaminants in soil, surface, and ground water. Various remediation technologies were developed that resulted in an isolation, immobilization, toxicity reduction, physical separation and extraction of some of the most delicate heavy metals known to man **[3]**. However, most of these technologies are often expensive and are only applicable to small areas, thus limiting its potential for rehabilitation and environmental clean-up. To make matter worst, some of these conventional approaches to remediation may even lead to soil infertility, making it unsuitable for agricultural and other activities. For these reasons, an alternative approach was deemed necessary that will not only reduce cost but also makes use of available resources in the surroundings.

Phytoremediation, an emerging technological advancement uses metal-accumulating plants which are specifically selected and engineered for environmental cleanup **[4]**. This technology have three modalities, namely, phytoextraction or the use of metal-accumulating plant to remove toxic metals from soil, rhizofiltration which refers to the use of plant roots to remove toxic metals from polluted waters and phytostabilization, the use of plants to eliminate the bioavailability of toxic metals in soils.

Brassica rapa chinensis is a member of Family Brassicaeae, which is known for their capacity to absorb nickel above threshold level. The high potential of *Brassicaceae* for biofumigation and phytoremediation of zinc and lead from soil was already proven in several accounts. The research carried out using white cabbage for phytoremediation and biofumigation of soils proved the ability of brassicas to bioaccumulate heavy metals and can be used to reduce the level of contaminants in the soil, and thus for clean-up and rehabilitation to prepare soils for another season of cultivation **[5]**. Several works has already been done to establish the potential of *B. rapa chinensis* for heavy metal uptake but mostly were done using soil as a substrate. The*B. rapa chinensis'* potential for phytoremediation using a wastewater contaminated with heavy metals is of great interest hence, the present work provides preliminary basis for the capability of the test plant to absorb and possibly hyperaccumulate toxic heavy metals via hydroponic set-up.

MATERIALS AND METHOD

Source of Waste Water and Treatment Preparation

The wastewater was obtained from Wastewater Treatment Facility of De La Salle University.All the wastewater used were collected at the same time and was brought in the laboratory to prepare the different treatment dilutions namely, T1- 5% wastewater + 95% distilled water, T2- 15% wastewater + 85% distilled water, and T3- 25% wastewater + 75% distilled water. Hoagland solution, the diluent, served as the control. Each treatment was replicated 10 times.

Test Plant and Treatment Exposure

*B. rapachinensis*seeds were first grown in a coco peat until they sprouted. Upon reaching 5cm in height, germinants were transferred in a hydroponic solution containing commercially-available Hoagland solution to acclimatized the plantin a water-based substrate and ensure that plants received adequate supply of nutrients prior to exposure in the wastewater. After two weeks, germinants were individually transferred in an improvised hydroponic container made of 1.5 liter bottle. The bottle was cut from the neck as to create a cradle for the coco peat to allow the seed to grow then, the cut end of the bottle was flipped over and the mouth of the bottle was wrapped in mesh to allow the passage of liquid to the coco peat and the eventual roots of the plant. The bottom half of the bottle was filled with wastewater solution. All the bottles were handsprayed with black paint to ensure that no photosynthetic activity will occur during the course of the study. The hydroponic bottles were then placed in a controlled environment provided with fluorescent lights, plastic wrap, PVC pipes, metal mesh, and curtains.

Nutrient analysis of wastewater solutions

Three (3) representatives per treatment were selected for the nutrient content analyses which were done before and after the conduct of the experiment. All the analyses were done at the Philippine Institute of Pure & Applied Chemistry, Ateneo De Manila University. Among the elements detected include nitrate, phosphate, calcium, magnesium, potassium and manganese.

Analysis of heavy metal uptake in wastewater and plant tissues

The heavy metal present in the wastewater and plant tissues of *B. rapa chinensis* at the start and at the end of the experimentation were both analyzed using Atomic Absorption Spectrophotometer (AAS) available at the Chemistry Department, De La Salle University. Water samples were filtered and placed in individual containers while the plant tissues were acid digested using nitric acid and hydrochloric acid in 3:1 ratio.The samples were atomized by the graphite tube atomizer and then subjected to optical radiation. The data from the detector were analyzed.

Statistical Analysis

Data were presented as means ± standard deviation. Analysis of variance (ANOVA) was used to compare outcome measures in the different treatment levels. Student's t-test was also used to compare the different treatments. All statistical analysis was calculated at a 5% level of significance.

RESULTS AND DISCUSSION

Nutrient Content of the Wastewater Solution

To assess the dynamics of nutrients present in the wastewater solutions, the initial and final nutrient contents were analyzed and the results are presented in Table 1. Surprisingly, the initial concentration of the nutrient in the three treatments already vary hence it cannot be assumed that the overall response of the test species was solely attributed to the nutrient solution used. Particularly for the nitrate, the initial concentration range decreases from 30, 31 and 32 for 5%, 15%, and 25% wastewater, respectively. As for the calcium, magnesium, potassium and manganese, the trend was rather unpredictable which initially increased then decrease at the end of the experimentation. Statistical analysis showed that the initial nutrient concentration across treatment was not significantly different from one another. Same trend was observed in the final nutrient content. It was also observed that there was an increased in all the elements tested overtime. For instance, nitrate increased from 37 mg/L to 58 mg/L, 30 to 85 mg/L and 32 to 63 mg/L for 5%, 15%, and 25% wastewater, respectively. The observed trend is likely connected to the concept of eutrophication which may led to anoxic water samples due to the possible oxygen consumption of microorganisms present **[6]**. The eventual death of some bacteria might have caused the increase in nitrate present in the tested samples.Potassium on the other hand, had initial and final values ranging from 8.2 to 39 mg/L, 7.3 to 23 mg/L and 7.8 mg/L to 22 mg/L for 5%, 15%, and 25% wastewater, respectively. According to Goto et al. **[7]**, the critical dissolved oxygen in a hydroponic setup had a reading below 2.1 mg/L and this likely affected the amount of the potassium in the wastewater. With regards to the initial and final concentration, ANOVA showed that nutrients did not vary overtime, indicating that the growth of the plant might be affected by other factors beyond the nutrient present in the wastewater.The overall increase in nitrate might also be a plausible explanation for the death of the test plants in various treatments used and not necessarily the presence of heavy metals.Sethy and Gosh, **[8]** reported that plants are able to develop various strategies and coping mechanisms for heavy metal stress. Since Hoagland solution was used as the control, it is expected that plants received adequate supply of nutrient needed for plant growth. Assessment of the nutrient present was only done at the termination of the study for the control set-up. In particular, the following values were generated: 900 mg/L for nitrate, 83mg/L for phosphate, 0.12mg/L for magnesium and 22mg/L for potassium. No wastewater was added to the control plants. It was evident that all the plants grown in the control did not only survived but also exhibited vigorous growth.

Table 1: Nutrient content of control and the varying concentrations of wastewater

Nutrients	Content (mg/L)						
	Control	5% wastewater		15% wastewater		25% wastewater	
		Initial	Final	Initial	Final	Initial	Final
Nitrate	900.00	37.00	58.00	30.00	85.00	32.00	63.00
Phosphate	83.00	1.40	6.30	ND	ND	ND	ND
Manganese	0.12	19.00	24.00	17.00	24.00	18.00	19.00
Magnesium	22.00	5.30	7.20	4.40	4.40	4.70	5.60

Potassium	**86.00**	**8.20**	**39.00**	**7.30**	**23.00**	**7.80**	**22.00**
Calcium	**160.00**	**0.04**	**0.13**	**0.02**	**0.03**	**0.02**	**0.03**
ND; not detected							

Heavy Metal Content of Wastewater Solution

Plants in general, have two ways of responding to increased concentration of heavy metals. First, plants may exhibit growth inhibition, structure damage, a decline of physiological and alteration of important biochemical activities. Alternatively, plants may use their resistance properties against toxic effects and for detoxifying heavy metal pollution, such as combining heavy metals by proteins and expression of detoxifying enzyme and nucleic acid **[9]**.The result of this study showed varying response of *B. rapa chinensis* in the presence of heavy metals in wastewater.Zinc toxicity is considered a medical condition involving an intake or overexposure to zinc. Ingestion of 225 mg and upwards can cause nausea, vomiting, pain, and diarrhea **[10]**. In fact, it is recommended that daily intake should be set at 15 mg/day as suggested by the Food Nutrition Board of the United Stated of America is 15 mg per day **[11]**. The initial concentration of zinc in the wastewater was found to be either too low to be read or completely absent. However, after growing of the test plant, the concentration increased in all the treatments with average values of 0.16ppm, 0.05ppm, 0.07ppm, and 0.19ppm for 5%, 15%, and 25% wastewater and control, respectively (Figure 1). The most evident increase was again in the control followed by 5%wastewater. Statistical analysis however showed that the initial and final concentrations of zinc did not significant different from one another.

Copper is considered toxic if taken in amount beyond 1.3 milligrams per liter, according to the maximum contaminant level of U.S. Environmental Protection Agency **[2]**. Excessive free copper will lead to increased oxidative stress in the body, caused by impaired zinc homeostasis combined with impaired antioxidant enzyme function **[12]**. The highest amount of copper observed in the wastewater samples was found in the control plant with an average concentration of 0.11ppm, a value which is considerably far from toxic levels of copper.There was also an observed increasing trend in the amount of copper in the wastewater treatments with the following range of values (Figure 2): 5% wastewater (0.02 to 0.04ppm), 15% wastewater (0.00 to 0.005ppm), and 25%

wastewater (0.003 to 0.01ppm). The control plant had the highest copper content with 0.11ppm (compared to 5%: 0.017ppm, 15%: 0.02ppm, and 25%:0.019). Unlike zinc, statistical analysis showed that there was a significant difference between initial and final copper concentrations.

The toxicity of iron in humans is based on the presence of iron in blood, that is, 350–500 µg/dL (when converted it is 3 ppm) concentration of iron in blood is already considered toxic. The level of iron in the water samples showed that the control was the highest at 1.91ppm (191 µg/dL), while the rest of the treatments reached low values of not more than 1.4 ppm (140 µg/dL). The data indicate that in terms of iron toxicity the *B. rapa chinensis* grown in these setups was able to tolerate the presence of iron and it is therefore safe to consume by humans. The amount of iron increased in the final concentration of wastewater in majority of the treatments used in the study. In particular, the following values were generated from the analysis: control-0.49ppm, 5% -0.15ppm, 15%-0ppm, and 25%-:0.18ppm) as compared to the initial concentration as follows: control- 0.18ppm, 5% - 0.05 ppm, 15% - 0.004 ppm and 25% -0.006ppm, respectively (Figure 3). The increasing trend was most evident in the control set-up which could be explained by the adequate supply of nutrients needed by plant growth were essentially supplied by the Hoagland solution used. Surprisingly, only 15% wastewater exhibited decreasing trend. Statistical analysis showed that the change from initial and the final values are significantly different.

On the other hand, both lead and cadmium were not detected in the different treatments used.Higher pH > 6.5 significantly reduced the quantity of readily soluble forms of metals in the soil and limits their uptake and accumulation by plants. The pH level of all the water samples used in the experiment were observed to be below 6.5. Similar to the results of Gruca-Królikowska and Waclawek**[13]**, the pH level in the experiment might be too low thereby hindering the uptake of soluble forms of metals like zinc, iron, and copper. In this regard, plants in an acidic environment can take up substantial amounts of these metals, even from soils, (in this study wastewater solution) that are only moderately polluted.

In a similar experiment done in a contaminated soil,*B. rapa chinensis*was grown in contaminated soil containing known concentration of cadmium, chromium, copper, nickel, lead, and zinc,it was surprisingly noticeable that the plant only accumulated cadmium and lead. This was done with and without additional use of soil mobilizers. The distribution of the heavy metals was found to be the highest in the leaves, followed by the stems, roots, fruit shell, and lastly the seeds **[14]**. In the present study, the uptake of lead or cadmium was not observed possibly because there was no lead and cadmium present in the wastewater collected from De La Salle University.

From the abovestated results, it must be noted however that although *B. rapa chinensis*are able to uptake iron, zinc, and copper in considerable amount, it is not that the threshold limits thus the phytoremediation potential cannot be established at this point.The increased level of essential heavy metals (Fe, Cu, and Zn) from initial to final concentrations could likely contribute to the findings of this study.

Heavy Metal Uptake by the plant tissues of *B. rapa chinensis*

According to Pulford and Watson **[15]**, the process of phytoremediation makes use of green plants and their ability to accumulate or degrade contaminants. One of the ways phytoremediation is carried out is through phytoextraction, wherein plants accumulate heavy metals in their aboveground organs. In the present study, the presence of heavy metals in the plant tissues of the test plant was determined to correlate to the metals detected in the wastewater solution.The highest amount of heavy metal uptake in all wastewater treatments was zinc. It was also observed that there was higher zinc in the control compared to the three wastewater concentrations used (Figure 4). Statistical analysis however, provedthat there was no significant difference in the zinc content of *B. rapa chinensis* across treatments. This shows a strong property for the uptake of zinc for the *B. rapa chinensis* plant. In this study, plant grown in hydroponic wastewater was observed to uptake heavy metals into their aboveground organs, based on the concentration of iron, zinc, and copper analyzed in the plant samples. Metals observed to be taken up were iron, zinc, and copper **[13]**.

Copper was found in the plant samples grown in varying concentrations of wastewater solutions (Figure 5). The highest value was recorded in the control with a concentration reaching up to 0.12 ppm thereby confirming the ability of the *B. rapa chinensis* plant as hyperaccumulator of copper. Statistical analysis showed that the copper content of the plant tissues across treatments did not significantly differ from one another.

The iron uptake of the plant was evident as all plants in each treatment were able to show traces of iron. The values range from 0.3 ppm up to 1.8 ppm, the highest value being in the control plants (Figure 6). Similar to the copper content, statistical analysis also showed that the three treatments are not significantly different from one another in terms of iron content, indicating that the test plant can tolerate varying concentrations of this metal present in a wastewater substrate.

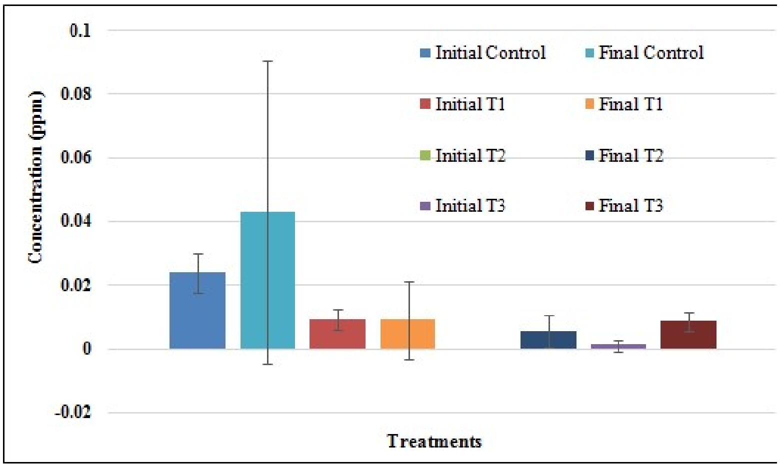

Figure 1: Initial and final zinc content of control and wastewater solution grown with *B. rapa chinensis.*

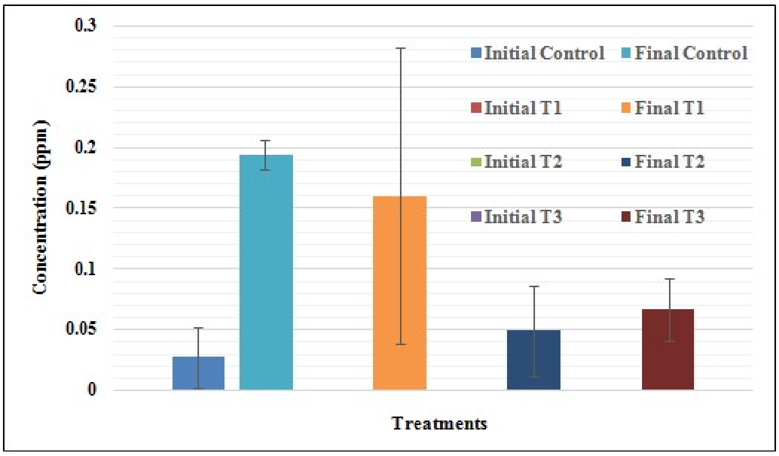

Figure 2: Initial and final copper content of control and wastewater solution grown with *B. rapa chinensis.*

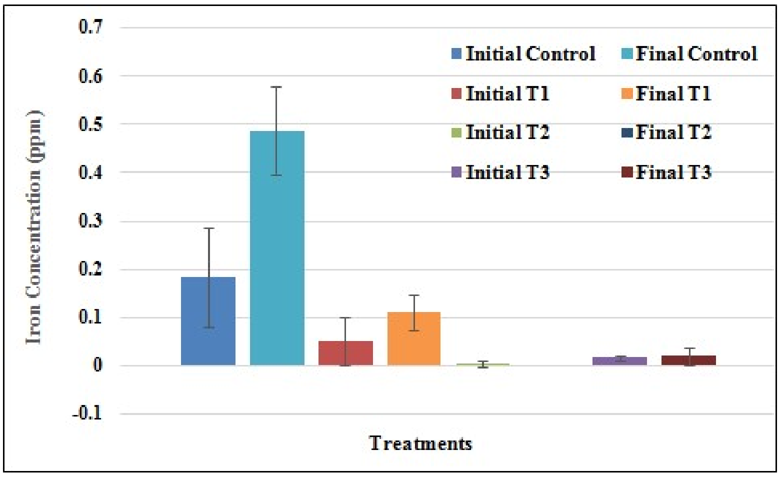

Figure 3: Initial and final iron content of control and wastewater solution grown with *B. rapa chinensis.*

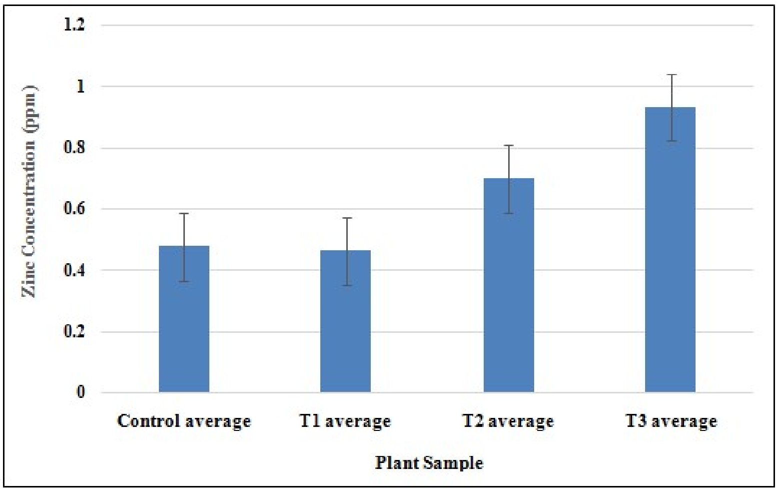

Figure 4: Zinc content of plant tissue of *B. rapa chinensis*grown in control and varying concentrations of wastewater solution

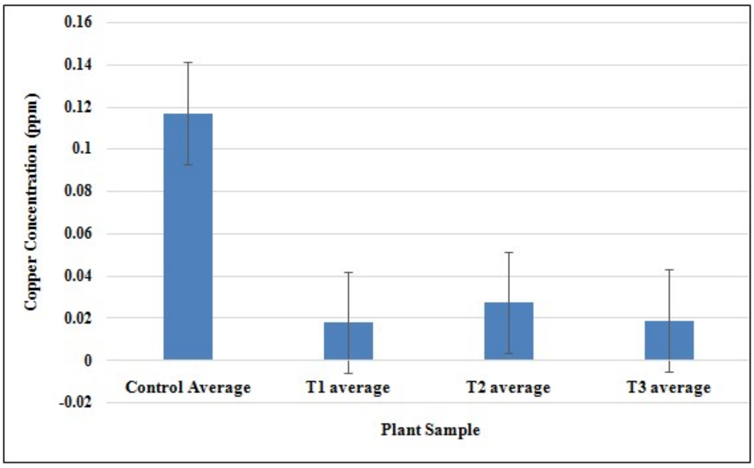

Figure 5: Copper content of plant tissue of *B. rapa chinensis* grown in control and varying concentrations of wastewater solution

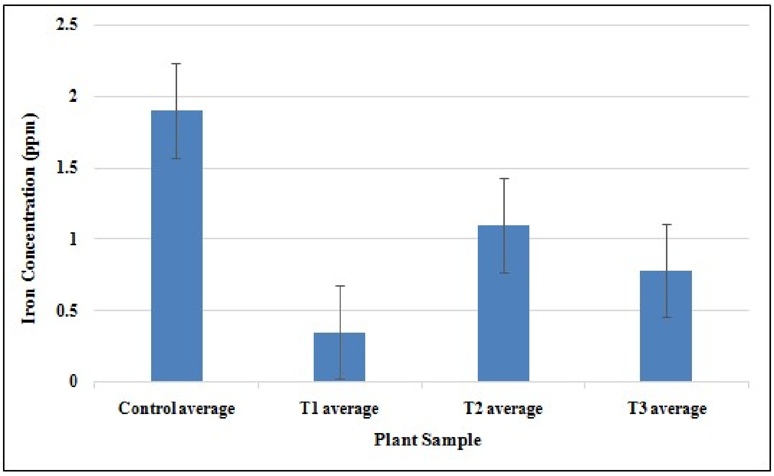

Figure 6: Iron content of plant tissue of *B. rapa chinensis* grown in control and varying concentrations of wastewater solution.

CONCLUSION

In conclusion, the present work demonstrated the successful growth of plants in varying concentration heavy metals in a hydroponic wastewater setup. *B. rapa chinensis*grown beyond 50 days may contain considerable amount of iron, copper and zinc but unable to phytoremediate heavy metals present in the hydroponic wastewater environment. The concentrations of heavy metals in the plant tissues were observed to be higher in lower wastewater concentrations.

REFERENCES

[1] Singh AK, Singh DP, Singh VN. Removal of Zn (II) from water by adsorption on China clay. Environmental Technology, 1998, 9.10, 1153-1162.

[2] U.S. Environmental Protection Agency. Introduction to phytoremediation. Pub.No. 600/R-99/ 107. Cincinnati, OH: U.S. Environmental Protection Agency. 2000, 104.

[3] Mulligan CN, Yong RN, Gibbs BF. Remediation technologies for metal-contaminated soils and groundwater: an evaluation. Engineering Geology, 2001, 60(1), 193-207.

[4] Salt DE, Prince RC, Pickering IJ, Raskin I. Mechanisms of cadmium mobility and accumulation in Indian mustard. Plant Physiol.,1995, 109, 1427-1433.

[5] Szczygłowska M, Piekarska A, Konieczka P, Namieśnik J. Use of Brassica Plants in the Phytoremediation and Biofumigation Processes. International Journal of Molecular Sciences, 2011, 12(11), 7760–7771.

[6] Nikulina A, Dullo WC. Eutrophication and heavy metal pollution in the Flensburg Fjord: A reassessment after 30 years. Mar Pollut Bull. 2009, 58(6), 905-915.

[7] Goto E, Both AJ, Albright LD, Langhans RW, Leed AR. Effect of dissolved oxygen concentration on lettuce growth in floating hydroponics. Acta Hortic., 1996, 440, 205-210.

[8] Sethy SK, Ghosh S. Effect of heavy metals on germination of seeds. Journal of Natural Science, Biology, and Medicine, 2013, 4(2), 272–275.

[9] Cheng S. Effects of Heavy metals on plants and resistance mechanisms. Environmental Science and Pollution Research, 2003, 10(4), 256-264.

[10] Fosmire GJ. Zinc toxicity. Am. J. Clin. Nutr., 1990, 51 (2): 225–7.

[11] Ciubotariu D, Ghiciuc CM, Lupușoru CE. Zinc involvement in opioid addiction and analgesia – should zinc supplementation be recommended for opioid-treated persons? Substance Abuse Treatment, Prevention, and Policy, 2015, 10(1).

[12] Sandstead HH. Requirements and toxicity of essential trace elements, illustrated by zinc and copper. Am. J. Clin. Nutr. 1995, 61 (3 Suppl), 621S–624S.

[13] Gruca-Królikowska S, Wacławek W. Metal environment. Part. II. Effects of heavy metals on plants. Chemistry − Teaching − Ecology – Meteorology, 2006, 11(1-2): 41-56.

[14] Van Ginneken L, Meers E, Guisson R, Rutterns A, Elst K, Tack FMG, Vangroncveld J, Diels L, Dejonghe W. Phytoremediation for heavy metal-contaminated soils combined with bioenergy production. J Environ Eng Landscape Manage, 2007, 15(4), 227–236.

[15] Pulford ID, Watson C. Phytoremediation of heavy metal-contaminated land by trees–a review. Environment International, 2003, 29(4), 529–540.