



# PHYTOEXTRACTION OF ARSENIC AND BORON AND ITS EFFECT ON GROWTH PARAMETERS OF *TARGETES ERECTA* L.

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## ABSTRACT

*Tagetes erecta* L. was used to explore the use of hydroponics technique for phytoremediation. The treatment of heavy metals such as Arsenic and Boron was provided to the plants of marigold. Various experiments conducted have shown the use of marigold as a plant species with hyperaccumulator capacity. In the present study the plants were given treatments of heavy metals for a period of 20 days. Growth analysis was carried out at the interval of 5 days and the data was recorded. This paper has focused on changes in the growth parameters in proportion to the heavy metal treatment provided. Here, hydroponics technique was used as an alternative to the soil media.

**Key words:** Arsenic, Boron, UV-visible spectrophotometer, *Tagetes erecta* L.

## INTRODUCTION

Environmental pollution is one of the major problems faced by the human society (Hazrat and Khan, 2017). The pollution by heavy metals is one of the greatest issues (Hazrat *et al.*, 2013; Hashem *et al.*, 2017). The environment is polluted by various pollutants including organic, inorganic and organometallic elements, isotopes of radioactive compounds, toxic gases etc. (Walker *et al.*, 2012). Inorganic pollutants include different domestic, agricultural and industrial wastes. The different types of inorganic wastes penetrate our surrounding by various natural and anthropogenic activities. Metallurgical processes, mining of mineral ore, smelting and waste generated from industries by different types of chemical processes are the examples of anthropogenic activities. Each of these sources add harmful matter in the environment. The pollutants which can be degraded biologically in the environment are termed as organic pollutants. The organic pollutants are found naturally in the environment itself, but some of anthropogenic sources can also promote organic pollutants. Among all, the heavy metals pose serious threat to mankind. Heavy metals are elements which are naturally occurring, having the properties of metallic element, metalloids or metals (Aka and Akuma, 2012). They have high atomic weight and density (Briffa *et al.*, 2020). They are highly toxic even when present at very low concentration range. Heavy metals are sometimes termed as "Trace metals or Micronutrients" (Duffus, 2002). These heavy metals can occur in a variety of forms from ionic, compound to complexes form. The heavy metals used in the present study for phytoremediation are Arsenic and Boron. Both the metals are highly toxic at higher concentrations. The objectives of the paper include treatment of *Tagetes erecta* plant with selected heavy metals, evaluating the growth parameters and analysis of the heavy metal contaminated water solution.

## METHODOLOGY

### Plant Selected for The Study-

The plants of marigold (*Tagetes erecta* L.) were bought from Bhagwati nursery, Ahmedabad. All the plants were washed thoroughly with tap water followed by distilled water to get soil - less plants for the experimental work. The plants were then placed in the jars seeded with Hoagland nutrient medium. Following this the plants were given the heavy metal treatments. The whole experimental work was carried out in triplicates. In the current study 1 set was kept as a control set and 4 sets were given treatments of the heavy metals.

The heavy metals used for the treatment purpose were Arsenic and Boron respectively. Two different concentrations were preferred for the treatment i.e., 50 ppm and 100 ppm. After every 5 days interval the growth parameters were noted down from each set. The last step of experiment included analysis of the treated water solution using UV-Visible spectrophotometer.

#### **Methodology for growth analysis-**

Following the heavy metal treatment to the plants, various growth parameters were measured including shoot length, root length, fresh weight and dry weight. After every 5 days the data were recorded.

#### **Preparation of Nutrient medium-**

The plants require nutrient medium for the growth in hydroponics technique. The nutrient medium used in this experiment was Hoagland nutrient solution.

#### **Preparation of heavy metal solution-**

The heavy metal selected for the experiment were Arsenic and Boron.

#### **Preparation of heavy metals stock solution-**

##### **Preparation of Arsenic stock solution-**

The stock solution of Arsenic was prepared by addition of 0.17 gm of Sodium Arsenite ( $\text{NaAsO}_2$ ) in distilled water to make total of 100 ml stock solution.

##### **Preparation of Boron stock solution-**

The Boron stock solution was prepared by adding 5.71 gm of Boric Acid in distilled water to make 100 ml of boron stock solution.

##### **Preparation of working standard solution-**

Two different solutions were prepared with concentration 50 and 100 ppm respectively.

#### **Methodology for growth analysis-**

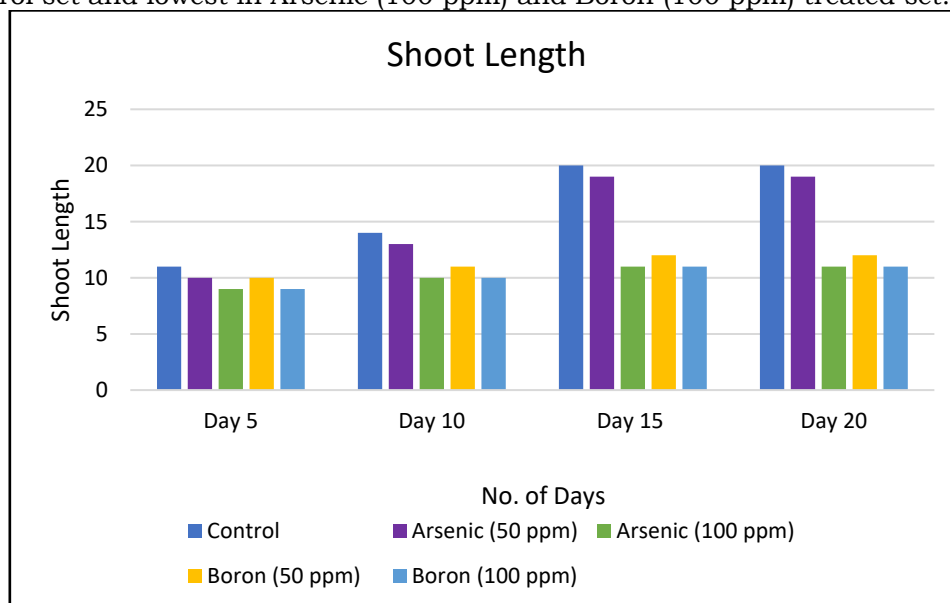
Following the heavy metal treatment to the plants various growth parameters were measured including shoot length, root length, fresh weight and dry weight. After every 5 days the data were recorded.

#### **Methodology for UV-visible spectrophotometer-**

The methodology for determining heavy metal concentration in the solutions was followed according to Udeagbara *et al.*, 2019 along with some modifications.

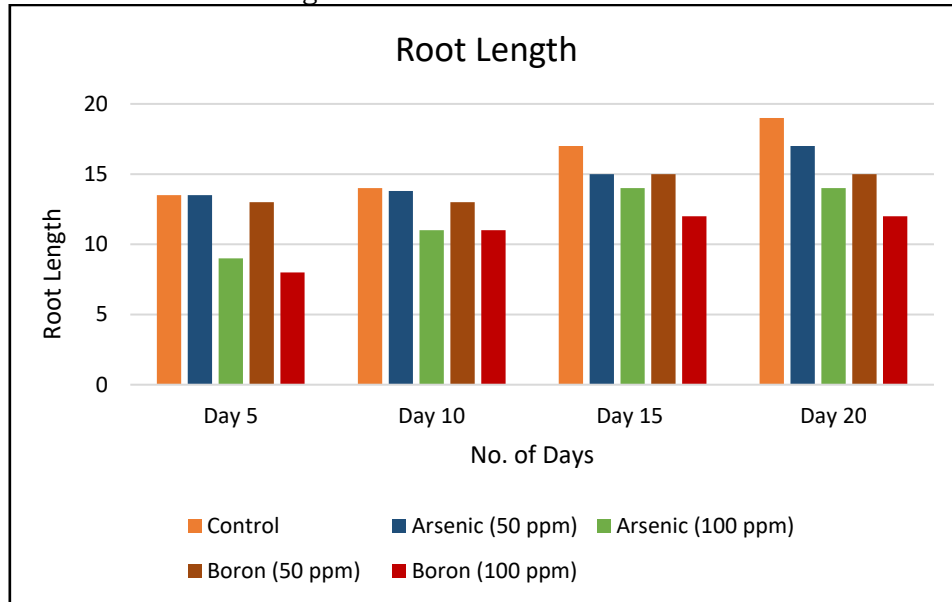
#### **Growth Parameters-**

The graph presented in **Figure 1** indicates changes observed in the length of shoot after the treatments given to the saplings of marigold. The X-axis represents the number of days and Y-axis represents the shoot length (in cm). The length of the shoot was observed highest in the Control set and lowest in Arsenic (100 ppm) and Boron (100 ppm) treated set.



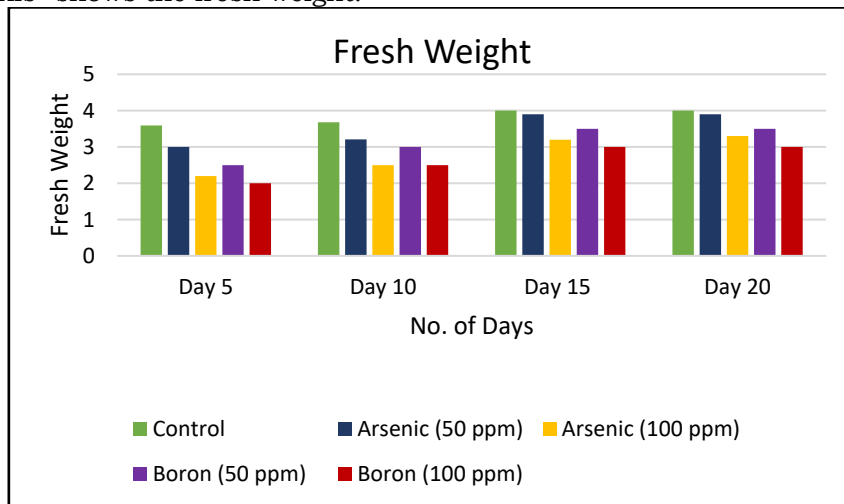
**Figure 1- Graph showing the comparative data of length of shoot.**

Among the morphological features the next parameter that was observed after the shoot length was root length. The graph of root length illustrated below shows that X- axis represents no. of days and Y- axis represents the root length (in cm). As presented in the **Figure 7** it was observed that the control set showed, excessive increase in the length followed by Arsenic (50 ppm) treated set. The Arsenic (100 ppm) and Boron (100ppm) treated set showed least increase in the length of root.



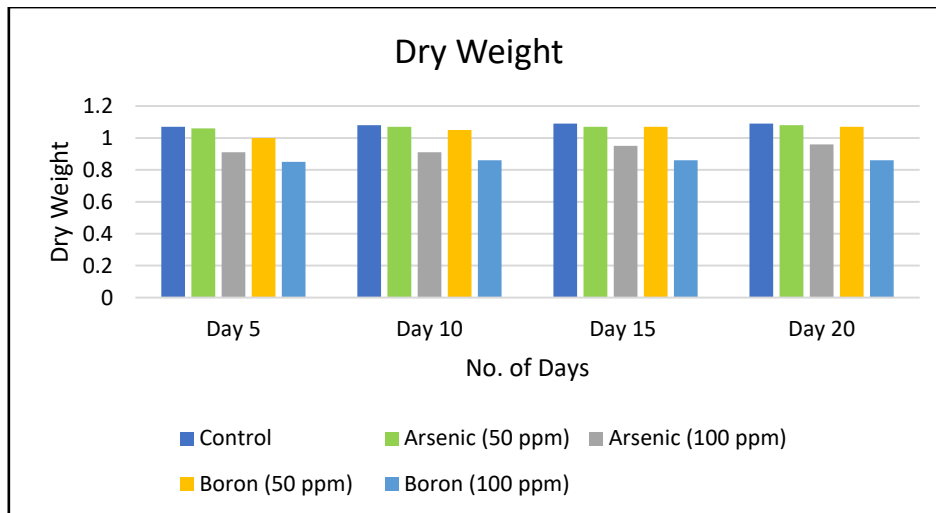
**Figure 2- Graph showing the comparative data of growth parameter of root length.**

After the root length, the next parameter observed was the fresh weight. In the graph represented in **Figure 3**, it is clearly observed that the result of fresh weight is as follows- Control > Arsenic (50 ppm) > Boron (50 ppm) > Arsenic (100 ppm) > Boron (100 ppm). Therefore, the best result was obtained from control set. The X- axis represents the no. of days and Y axis- shows the fresh weight.



**Figure 3- Graph showing the comparative data of fresh weight.**

The last parameter considered in the morphological characteristics was the dry weight of plant represented in the **Figure 4** The result obtained was as follows- Control > Arsenic (50 ppm) > Boron (50 ppm) > Arsenic (100 ppm) > Boron (100 ppm). The X- axis shows the no. of days and Y- axis shows the dry weight of plant. The best results were obtained from the control set. The least dry weight was observed of Boron (100 ppm) treated set.



**Figure 4- Graph showing the comparative data of growth parameter dry weight.**

**Discussion-**

Marigold (*Tagetes erecta*) is an ornamental plant with various economic and medicinal benefits. The flowers yield aromatic oil and is used in perfume industry (Gopi *et al.*, 2012). The flowers are used for religious purpose. The plant is also known for its capacity to absorb heavy metal from the environment by different methods. One of the approaches is by “Phytoremediation”. Many experiments have been conducted for observing the potentiality of *Tagetes erecta* in phytoremediation. Some examples of the experiments have been described below.

The experiment conducted by Reed *et al.*, 2013 evaluated various ornamental plants for Arsenic accumulation. The plants investigated were *Iris savannarum*, *Panicum virgatum*, *Tithonia rotundifolia*, *Coreopsis lanceolata*, *Helianthus annuus* and *Tagetes erecta*. All the plants were grown in soil-less media using hydroponics. Among all the selected ornamental plants for study marigold (*Tagetes erecta*) was one of them. The Arsenic treatment provided to plants ranged from 0.0 mgL<sup>-1</sup> which was used as control set to 5.25 mgL<sup>-1</sup> concentration. The results obtained by Reed *et al.*, 2013 showed that the root and shoot dry weight of the *Tagetes erecta* exhibited small or no distinct change in the concentration from 0.0 mgL<sup>-1</sup> Arsenic i.e., control set to 5.25 mgL<sup>-1</sup> Arsenic treated set. Similar results were found in our study, the dry weight of the whole plant did not change from the 5<sup>th</sup> day to 20<sup>th</sup> day in the set treated with Arsenic (50 ppm). The observed results of Reed *et al.*, 2013 were also in contrast to the results obtained from the present study. In the current study it was observed that the set treated with Arsenic (100 ppm) showed no increase in dry weight up to 10<sup>th</sup> day and further the dry weight increased on 15<sup>th</sup> day and remained the same for 20<sup>th</sup> day.

In another experiment conducted by Bardiya *et al.*, 2017 on *Tagetes erecta* using hydroponics. The plantlets were given the treatment of Nickel (50- 250 mmol) and Lead (100-200 mmol) concentrations. The various growth parameters including root length, shoot length, fresh weight and dry weight were noted after 21 days. It was found that the control set showed poor results for both Nickel and Lead for each of the growth parameters. The growth parameters such as shoot length and fresh weight did not increase with increase in the concentration of both the metal treatments. The root length and dry weight elevated at the higher concentration of Nickel and Lead supplied in media. These observations were in contrast to the present study as the control set showed the best result in comparison to the heavy metal treated sets. The results of Bardiya *et al.*, 2017 also showed some similar observations to the present study. In the present study for shoot length parameter, it was observed that the set treated with Arsenic (50 ppm) showed increase in shoot length up to 15<sup>th</sup> day and remained the same till 20<sup>th</sup> day. Similar observations were noted for Arsenic (100 ppm) treated set. Whereas, in the Boron (50 ppm) treated set it was observed that the shoot length increased till 15<sup>th</sup> day and remained unchanged till 20<sup>th</sup> day. Similar observations were noted for Boron (100 ppm) treated set. The fresh weight in the Arsenic (50 ppm) and (100 ppm) treated set showed increase up to 15<sup>th</sup> day. Similar observations were found in the Boron (50 ppm) and (100 ppm) treated set. It was observed in the results of Bardiya *et al.*, 2017 shoot length and fresh weight did not increase with increase in the concentration of both the metal treatments. Similarly in the present study it was observed

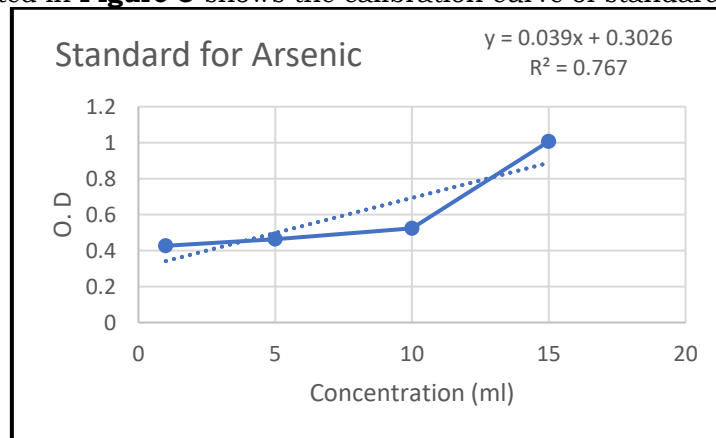
that after a certain period of time the shoot length and fresh weight did not increase for a given heavy metal treatment. Moreover, as the concentration of heavy metal increased from 50 ppm to 100 ppm the plants showed less increase in terms of fresh weight.

In the present study the root length of Arsenic (100 ppm), Boron (50 ppm) and Boron (100 ppm) treated set showed increase up to 15<sup>th</sup> day. Whereas, the root length increased up to 20<sup>th</sup> day in the Arsenic (50 ppm) treated set. The dry weight of Arsenic (50 ppm) and Boron (100 ppm) did not show any variation from the 5<sup>th</sup> to 20<sup>th</sup> day. Till 15<sup>th</sup> day increase in dry weight of Arsenic (100 ppm) and Boron (50 ppm) treated set was observed. According to Bardiya *et al.*, 2017 the root length and dry weight elevated at the higher concentration supplement of heavy metals. This observation is in contrast to the results of our present study as with the increase in concentration the dry weight and root length did not increase.

**UV Vis- Spectrophotometer-**

The plants of *Tagetes erecta* were grown for about 20 days using hydroponics. Subsequently the heavy metal treated solution of Arsenic and Boron was tested and analysed with the help of UV spectrophotometric method. The UV spectrophotometric method is used for quantitative determination of heavy metals or trace elements. It is very simple and economic substitute for the analysis of heavy metals other than AAS (atomic absorption spectrometry) (Kulkarni *et al.*, 2015). The two heavy metals treatment provided to plants were Arsenic and Boron in the present study. The evaluation of heavy metals was done using UV Vis-Spectrophotometry.

The graph presented in **Figure 5** shows the calibration curve of standard Arsenic solution.

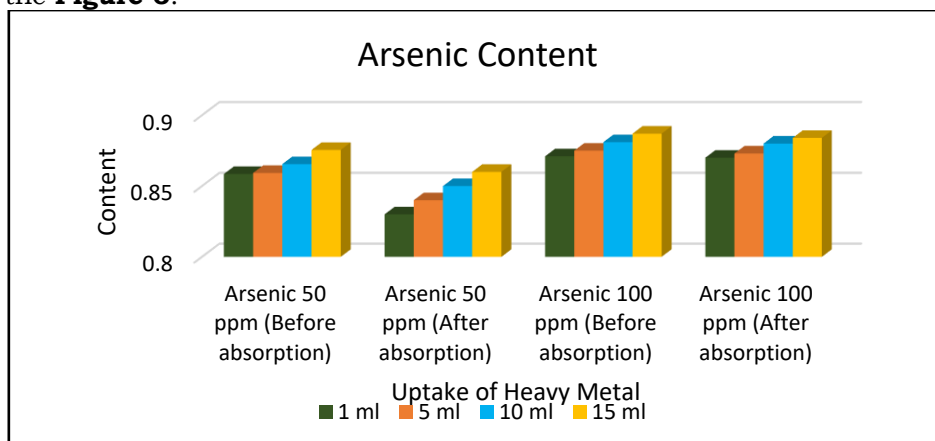


**Figure 5- Represents the graph of standard for Arsenic.**

In the experiment for determining the arsenic concentration Sodium Arsenite was used as the standard compound. The concentration was estimated at 628nm. For estimating the Arsenic concentration, the curve equation  $y = -0.037x + 0.8918$  and  $R^2 = 0.6897$  (**Figure 5**) was considered. The other two concentration range used for experiment were Arsenic (50 ppm) and (100 ppm).

**Arsenic (50 ppm and 100 ppm) solutions-**

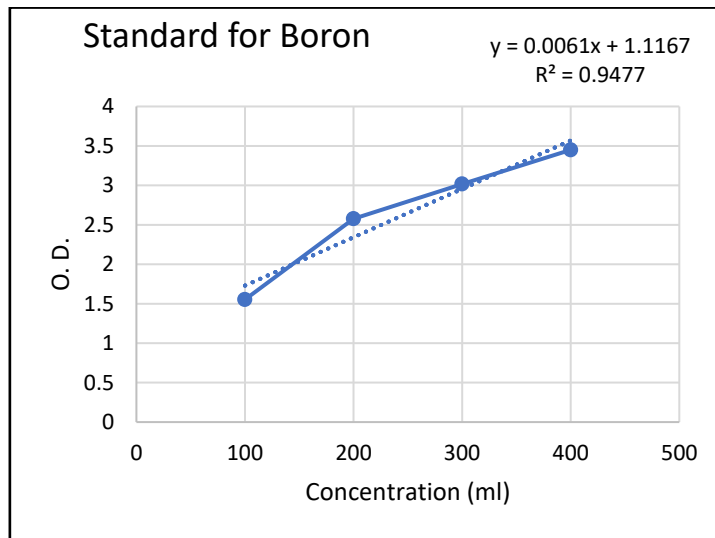
The graphs of before and after absorption of Arsenic (50 ppm) and (100 ppm) respectively are shown in the **Figure 6**.



**Figure 6 Represents the comparative graph of Arsenic (50 ppm) and Arsenic (100 ppm).**

The detection of heavy metal content in UV vis-spectrophotometer at 628nm exhibited that before absorption by the plants the content of Arsenic (50 ppm) and (100 ppm) were higher in comparison to the results displayed after absorption. Also, it was observed that the content increased proportionately as the concentration increased from 1 ml to 15 ml.

**Boron stock solution-** In the similar way the content of Boron was detected using UV Vis-Spectrophotometer at 410 nm. The graph presenting standard for Boron is presented in **Figure 7.**

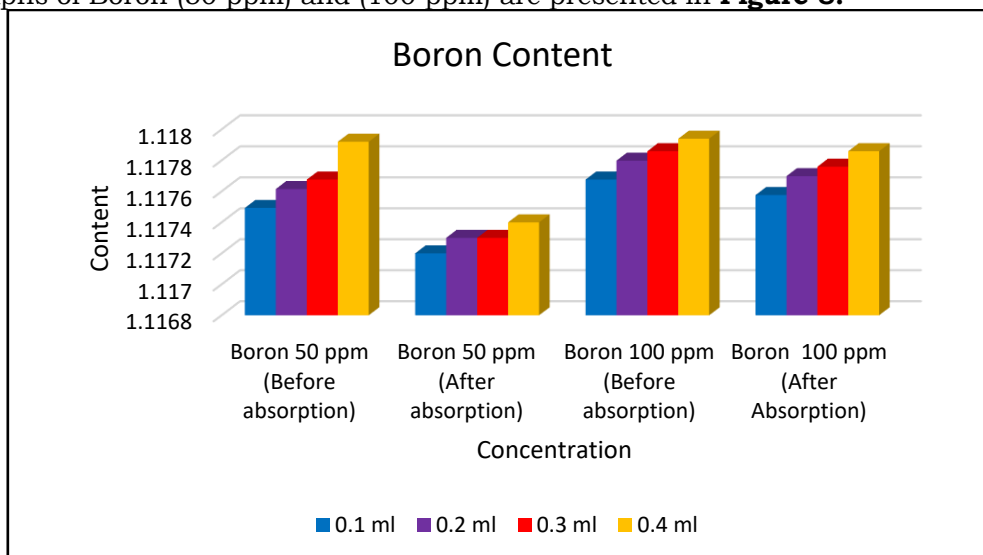


**Figure 7- Represents the standard curve of Boron.**

Boric Acid was used as standard for determining the concentration of Boron. The concentration of Boron was estimated at 410 nm. For estimation of Boron concentration, the curve equation  $y = 0.0061x + 1.1167$  and  $R^2 = 0.9477$  presented in **Fig-7** was considered.

**Boron (50 ppm and 100 ppm)-**

The graphs of Boron (50 ppm) and (100 ppm) are presented in **Figure 8.**



The detection of heavy metal content in UV vis-spectrophotometer at 410 nm exhibited that before absorption by the plants the content of Boron (50 ppm) and (100 ppm) were higher in comparison to the results displayed after absorption. Also, it was observed that the content increased proportionately as the concentration increased from 0.1 ml to 0.4 ml.



## DISCUSSION

Heavy metals are serious environmental pollutants. The removal of these metals from the environment is very essential for the protection of the ecosystem. The most effective remedy to save the environment is “Phytoremediation”. It involves the use of plant for cleaning the environment. The aim of this technique is reducing the toxic metals to non-toxic metals which can be easily absorbed by the plants. There are certain definite values set for each of the heavy metals used as standard. Standard values of various heavy metals stated by Fakhru<sup>1</sup> and Pendashteh, 2009 shows that the maximum value for Arsenic is 0.3 mg/L and for Boron it is 5mg/L. The present experiment involves utilisation of two heavy metals Arsenic and Boron. The heavy metal analysis was done by UV vis-spectrophotometer for Arsenic and Boron. Many experiments have been conducted for quantitative analysis of heavy metals. For detection of heavy metals various techniques have been adopted. Atomic Absorption Spectrometry is one of the widely used technique for the detection. This technique involves acid digestion which is risky to handle as it involves concentrated acid. So, we have used UV Vis-Spectrophotometer in our experiment. Limited experiments have been conducted till date using UV Vis-spectrophotometer with hydroponics technique in phytoremediation. But some of the experiments have been conducted such as treating wastewater collected from mining site or polluted areas using UV Vis-Spectrophotometer.

One such experiment involving the use of UV Vis-Spectrophotometer had been conducted by Udeagbara, S. G. *et al.*, (2019). The experiment performed by Udeagbara, S. G. *et al.*, (2019) was based on determination of heavy metals carried out from the field of Niger delta using UV Vis-Spectrophotometer. Five heavy metals used for the experiment such as Boron (B), Manganese (Mn), Arsenic (As), Barium (Ba), Tin (Sn). The standard absorbance values of Arsenic obtained by analysis of the experiment performed by Udeagbara, S. G. *et al.*, (2019) states that increasing the concentration of solution leads to decrease in the absorbance. In case of Boron the result obtained were just similar to that of Arsenic. In Boron, the increase in the concentration leads to rise in the content of the Boron. The results collected from the experiment conveyed that the concentrations of Arsenic exceeded than the standard value of the produced water in the experiment conducted by Udeagbara, S. G. *et al.*, (2019). The results obtained by us showed same results for standard of Arsenic. Further in our experiment apart from standard solution of Arsenic, Arsenic 50 and 100 ppm solutions were also used. The results obtained by our experiment for Arsenic 50 and 100 ppm supports the results presented by Udeagbara, S. G. *et al.*, (2019). Furthermore, the results obtained by our experiment for Boron supported the results obtained by Udeagbara, S. G. *et al.*, (2019).

## CONCLUSION

The results showed that among all the sets control showed the best result in comparison to the other sets. In our current study it was observed that the concentration of both the heavy metals were higher than the permissible limit. Regardless of the fact mentioned above, the content of both the heavy metals i.e., Arsenic and Boron declined from the solutions respectively. This concludes that the uptake of both i.e., Arsenic and Boron has been observed in the present experiment. Hence, the plants in each set were able to tolerate the heavy metal treatment even after 20 days. The plant of *Tagetes erecta* survived for 20 days after the treatments this means it was able to tolerate moderate amount of heavy metal concentration provided to each set.

## REFERENCES

- 1) Aka, C. K., Akuma, O. (2012). Removal of heavy metal from produced water using Biosorption techniques. *European Journal of Applied Engineering and Scientific Research*.1(4): 190-195.
- 2) Bardiya, K. B., Sharma, S., Mishra, Y., and Patankar, C. (2017). *Tagetes erecta* (Marigold), a phytoremediant for Ni- and Pb- contaminated area: a hydroponic analysis and factors involved. *Rendiconti. Lincei. Scienze. Fisiche e Naturali*. 28(4): 673-678.
- 3) Briffa, J., Sinagra, E., and Blundell, R. (2020). Heavy metal pollution in the environment and their toxicological effects on humans. *Heliyon*. 6(9): 1-26.
- 4) Duffus, J. H. (2002). “Heavy metals” a meaningless term? (IUPAC Technical Report)”, *Pure and Applied Chemistry*. 74(5). 793-807.



- 5) Fakhru'l, R. A., and Pendashteh, A. (2009). Review of technologies for oil and gas produced water treatment. *Journal of Hazardous materials*. 170(2-3): 530-551.
- 6) Gopi, G., Elumalai, A., and Jayasri, P. A. (2012). A concise review on *Tagetes erecta*. *International Journal of phytopharmacy research*. 3(1): 16-19.
- 7) Hazrat, A., and Khan, E. (2017). Environmental Chemistry in the twenty-first century. *Environmental Chemistry Letters*. 15(2): 329-346.
- 8) Hazrat, A., Khan, E. and Sajad, M. A. (2013). Phytoremediation of heavy metals- concepts and applications. *Chemosphere*. 91(7): 869-881.
- 9) Kulkarni, S., Dhokpande, S., Kaware, J. (2015). A review on spectrophotometric determination of heavy metals with emphasis on Cadmium and Nickel determination by UV vis- spectrophotometry. *Journal of hazardous materials*. 170(1): 530-551.
- 10) Reed, S., Silva, T. A., Dunn, C. B., Gordon, G. G., and Meerow, A. (2013). Nutrient uptake of ornamental plants exposed to Arsenic in hydroponic solution. *Journal of Agricultural Science*. 5(12): 1-13.
- 11) Udeagbara, S. G., Ogiriki, S. O., Afolabi, F., and Fakorede, O. (2019). Quantitative analysis of heavy metals in produced water from NDX011 in Niger- Delta oil field. *International journal of Petroleum and Gas Engineering Research*. 3(1): 19-33.
- 12) Walker, C. H., Sibly, R. M., Hopkin, S. P., D B. P. (2012). *Principles of Ecotoxicology*. United States: CRC Press.