ISSN No: - 2456 – 2165

# Morphological and Physiological Effects of B. Vulgaris Towards Heavy Metal Toxicity

Wan-Rafiekal WAR, Hazandy AH, Mohd-Kamil I
Institute of Tropical Forestry and Forest Products,
Universiti Putra Malaysia UPM
Serdang, Selangor, Malaysia
wanrafiekal@gmail.com

**Abstract—Urbanization, industrialization, and phenomenal growth in population are the factors for increasing environmental pollution. Since the beginning of the industrial revolution, pollution of biosphere by toxic metals has accelerated dramatically. Bamboo is known as a highly useful plant but it also play valuable environmental role. Its complex root system is good for water filtration, removing nutrients and dangerous contaminants such as heavy metals. This study aimed at investigating the potential of Bambusa vulgaris to clean up heavy metal contaminants through phytoremediation. The plants were grown up in lysimeter pot for three months in the presence of heavy metal Cu, Zn and Fe with different concentration 100, 200, 300 ppm respectively to quantify the toxic effects of these metals on several different morphological growth and physiological parameter. The results indicated that the leachates status showed low concentration of heavy metal after being infiltrated throughout the study, furthermore morphological and physiological performance of B. vulgaris responded negatively towards higher concentration of heavy metal. These heavy metals are considered to be essential for plant growth at low concentration, but visible symptoms of phytotoxicity observed on plants growth performance, reduction in chlorophyll content, chlorophyll fluorescence and photosynthetic efficiency which were significantly decreased after certain level of concentration and time. Thus, we can conclude that B. vulgaris is excellent to clean up heavy metal but weak to tolerate with high concentration of heavy metal contaminants.**

**Keywords—** B. Vulgaris, Phytoremediation, Morphological, Physiological, Phytoxicity, Chlorophyll Content, Chlorophyll Fluorescence, Photosynthetic Efficiency.

## I. INTRODUCTION

Environmental contamination with heavy metals is a global disaster that is related to human activities like mining, smelting, power transmission, energy and fuel production, intensive agriculture, melting operations and sludge dumping. Heavy metal contamination to soil and water poses a major environment and health problem. Metals are naturally present in the pedo-geochemical background of soils at various levels and they are essential to plants, but may be toxic at higher concentrations. Heavy metals ions, when present at an elevated level in the environment, are excessively absorbed by roots and trans located to shoot, can lead into impaired metabolism and reduced growth. Metals such as Cu, Zn, Cd, Cr, Ni and Pb are known to be serious environmental pollutants. Heavy metal contamination to soil and water poses a major environment and health problem. Besides that, excessive metal concentrations in contaminated soils result in reduction in microbial activity, soil fertility, and yield loss. Metals accumulate in soil due to anthropogenic contamination through fertilizer, organic manure applications, irrigation, industrial and municipal wastes, and wet and/or dry deposits. [1]

Terrestrial plants have an extensive root system and an advanced uptake mechanism in their continuous search for water and mineral resources. Bamboo for example, a terrestrial plant that is known to have several merits, including high stress tolerance to various factors, high growth and biomass production [2] which probably shows good abilities for water filtration and nutrient absorption. It can take up nutrients such as nitrogen, phosphorous or heavy metals and some of them are locked in the root system. Planted alongside rivers, creeks and ditches and holding dams, bamboo can catch the excess nutrients in the runoff water thus preventing from entering nearby streams. Hence, it suitable for disposal of effluents and reduce of waste water pollution. Recently, some researchers reported on the heavy metal tolerance and accumulation in some bamboo species [3]. In perspective of these features, bamboo was used as main materials for the study of the effects of heavy metal towards its growth and the potential utilisation in plants using phytoremediation to improve water and nutrient supplies, towards providing an environmentally compatible method for wastewater disposal.

## II. MATERIALS AND METHODOLOGY

### *A. Materials*

This study done at the nursery of Faculty of Forestry, Universiti Putra Malaysia, Serdang, Selangor where the principle researcher is currently based, had some facilities that can be utilise for effective implementation of the project. The study was conducted in a covered place and the duration for the data collection is 3 months from the end of May 2014 until August 2014. The average daily temperature varied from about 27-300C and the relative humidity from 60-71%. From these study, we know the ability species of bamboo to clean up heavy metal contaminants. Apart from that, a significant treatment*time interaction was apparent for bamboo growth such as diameter of bamboo culm, number of shoots and plant

ISSN No: - 2456 – 2165

height and their physiological parameter such as chlorophyll content, chlorophyll florescence and gaseous exchange also will be identified.

*a). Bamboo*

In this study, bamboo used is B. vulgaris which is the species chosen according to the ones that grows close to the water area such as pond, lake and river. A total of 32 saplings were obtained from Forest Research Institute Malaysia (FRIM) Nursery, Selangor, Malaysia. After stabilization for 1 month, uniform bamboo plant species that were grown on same substrate will carefully selected and transplanted into a lysimeter a plastic pot that was filled with soil and sand proportion 3:2. The pots were arranged in according to the Completely Randomized Design (CRD). This study was designed with four treatment (control, 100 ppm, 200 ppm and 300 ppm) each replicate 8 times. This is because, in order to reduce errors during data collection. Meanwhile, randomization was carried out by using random number generator to prepare every treatment to have equal chances of being assigned to any experimental unit. Bamboo represents some advantage in its planting and utilization as they need same level of treatment, maintains green all the year and requires less maintanance. Plants were harvested 90 days or three months after the heavy metal treatment application.

*b). Lysimeter*

Lysimeter are devices used to collect soil water in order to measure chemical characteristics of water which has leached through the soil profile. The lysimeter design give an alternative method for collecting agrochemical leachate. These devices used to compare best management practices, gain a better understanding of the infiltration properties and to identify and quantify potential pollutant sources.

*c). Heavy Metal Solution Treatment*

After the plant can fully adapt to their soil medium and environment, the treatment were applied to each plants. Each treatment consists of different heavy metals copper, zinc and iron mix with the water to reach certain level concentration 100 ppm, 200 ppm and 300 ppm respectively. The volume of treatment before and after application had been measured. The water was filled up until it reached certain level to ensure the concentration of heavy metal remained constant. These plants were then covered with plastic to control the transpiration via soil medium and to avoid data bias.

*B. Methodology*

*a). Growth Morphological Data*

Plant growth and physiological parameters were determined during treatment. Growth data diameter and height of bamboo plant was taken every two weeks throughout the study. B.vulgaris main culm diameters were recorded with a digital vernier caliper (Mitutoyo UK Ltd., Hampshire, UK) 10 cm above the collar and heights were measured with a measuring tape and meter ruler. New bamboo shoots emerged from the base of culm, total number of and branches leaves also will also be counted. The changes on plant conditions were being observed throughout the study and measurement of root length were taken after plants have been harvested. Total average for the all the measurement will be calculated to identify the growth performance of bamboo.

*b). Physiological Data*

- *Chlorophyll Content*

Data for the chlorophyll content were collected fortnightly until study end. The chlorophyll content was measured using chlorophyll meter, SPAD-502. The equipment is a simple, saves time, spaces, and resources which is one of portable diagnostic tool that measures the greenness or relative content of leaves. [4] Three reading were taken from randomly selected leaves from each level treatment and the averages of these values were recorded.

- *Chlorophyll Fluorescence*

After one month planting, data for the chlorophyll fluorescence were collected. These data collections were repeated every two weeks until study end. Chlorophyll fluorescence measurements were conducted using chlorophyll fluorimeter, HandyPEA (Hansatech CF Model, UK). Three out of eight replicates from each treatment level was selected randomly according to different heights which were highest, middle, and lowest. For each selected trees, three fully expanded leaves were selected from 2/3 of horizontal stand and vertical branches. The selected leaves were maintained in darkness for 5 to 10 minutes before taking the data using leaf clips. Those leaf clips were placed with shutter in closed position for dark adaption. Chlorophyll fluorescence parameters such as minimal fluorescence (Fo), maximal fluorescence (Fm), variable fluorescence (Fv= Fm- Fo) and maximal quantum yield of photosystem (PS) II photochemistry (Fv/Fm) were calculated using the software supplied by the manufacturer.

- *Gas Exchange*

Three fully expanded leaves were selected for each seedling. Five of gas exchange parameters which were net photosynthesis rate (Anet), stomata conductance (Gs), transpiration rate (E), intercellular CO2 (Ci) and leaf to air vapor pressure deficit (VpdL) were measured using LiCor 6400, Portable Photosynthesis System. This open-type photosynthesis system was equipped with a standard 3cm x 2cm broadleaf cuvette. Calibrations for flow meter and CO2 zero values had been made before the gas exchange measurements. The CO2 concentration then was set at 360 molm-2s-' in order to avoid the effect of environmental fluctuating conditions. The cuvette irradiance, temperature, and relative humidity also were set at 650 µmol photons m-1s-1(saturating irradiance), 250C, and 40% respectively. Measurements were taken from 7:30 a.m. to 11:30 a.m. to avoid the midday reduction in photosynthesis.

*C. Statistical Analysis*

A completely randomized design conducted in these experiments with 8 replicates. Statistical analysis was carried out using the statistical software SPSS22.0 by repeated measures two ways ANOVA to identify differences within time, treatment and their interaction and one way ANOVA with post hoc test by Tukey's HSD at P≤ 0.05 significance level to elucidate further the different significance between treatment. All the data collected will be evaluated and shown in the amount or percentages. Comparison of the results will be conducted and obtained data will be presented in the form of tables and graphs.

## III. RESULTS

*A. Morphological Attributes*

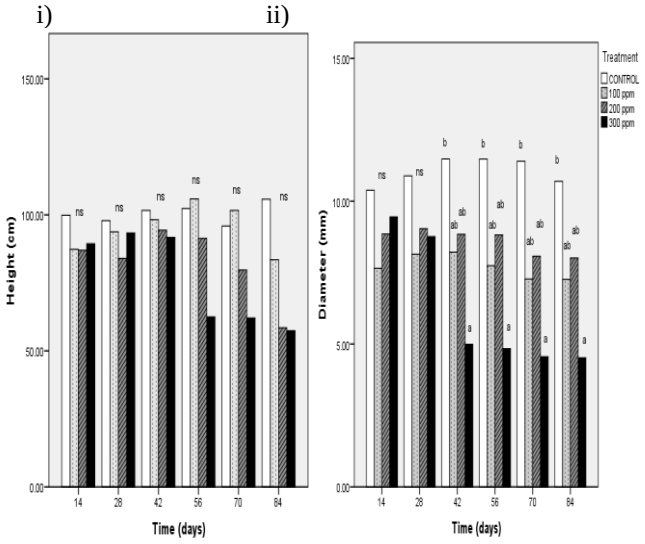


Figure 1: I) Height and Ii) Diameter of *B.* Vulgaris During Treatment *Values with Different Letters Indicate Significant Differences for All Means Comparison Within Treatment By Turkey's HSD

Table 1: Summary of Repeated Measures Two Ways ANOVA Within Time And Treatment on the Height and Diameter of Culm

*B. Vulgaris*

| Source of variation | Df | Mean Square | F value |
|---|---|---|---|
| **Height** | | | |
| Time | 5,35 | 1604.021 | 3.714** |
| Treatment | 3,21 | 6059.729 | 0.848ns |
| Time*Treatment | 15,105 | 906.363 | 2.510** |
| **Diameter** | | | |
| Time | 5,35 | 13.340 | 4.799** |
| Treatment | 3,21 | 199.036 | 3.568* |
| Time*Treatment | 15,105 | 10.930 | 3.208*** |

* Significant different at $p \leq 0.05$

Figure 1 illustrates that height of treated plants decreased rapidly with increasing heavy metal concentration compared to control. B. vulgaris in control shows the highest height whereas 200 ppm treatment shows the lowest for the first two weeks. The height gradually increased in control whereas in contrary, 300 ppm treatment exhibits the reduced trend till the end of time duration. Meanwhile for 100 ppm and 200 ppm treatments the height increases slightly however after 56 days being planted in the presence of heavy metal, it shows reduction until the end of experiment. The highest mean of height 105.88 cm recorded in control meanwhile the lowest 57.38 cm in 300 ppm treatment. The results summary of repeated measures two ways ANOVA within time and treatment on the height and diameter of culm B. Vulgarisdetermined that mean diameter differed significantly within treatment period (F(5,35)=4.799, p<0.01). Other than that for diameter the growth of B. vulgaris culm differed in treatment at F (3,21)=3.568, p<0.05. There was highly significant in response between time and treatment where F (15,105)=3.208, p<0.001.

As shown in Figure 1, diameter of treated plants decreased gradually with increasing heavy metal concentration compared to control. B. vulgaris in control shows the highest diameter whereas 100 ppm treatment shows the lowest for the first two weeks. The significant decline was observed for all treatment except for control which initially increases however after 42 dayS, it was decrease significantly until the end of experiment. The highest mean of diameter 11.48 mm recorded in control meanwhile the lowest 4.52 mm in 300 ppm treatment. The results summary of repeated measures two ways ANOVA within time and treatment on the diameter of B. vulgaris culm determined that mean diameter differed significantly within treatment period (F(5,35)=4.799, p<0.01). Other than that for diameter the growth of B. vulgaris culm differed in treatment at F (3,21)=3.568, p<0.05. There was highly significant in response between time and treatment where F (15,105)=3.208, p<0.001.

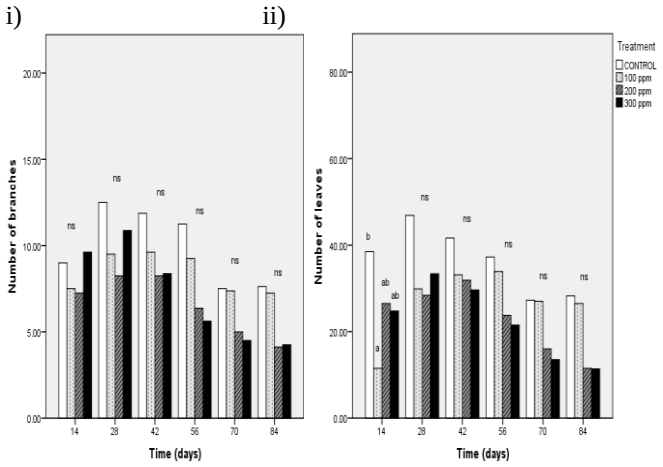


Figure 2: Number of I) Branches and Ii) Leaves *B.* Vulgaris During Treatment *Values with Different Letters Indicate Significant Differences For All Means Comparison Within Treatment By Tukey's HSD

ISSN No: - 2456 – 2165

Table 2: Summary of Repeated Measures Two Ways ANOVA with in Time and Treatment on Number of Branches and Leaves of *B.* Vulgaris

| Source of variation | Df | Mean Square | F value |
|---|---|---|---|
| **Branches** | | | |
| Time | 5,35 | 103.012 | 11.185*** |
| Treatment | 3,21 | 108.132 | 0.773ns |
| Time*Treatment | 15,105 | 10.807 | 1.647ns |
| **Leaves** | | | |
| Time | 5,35 | 1361.872 | 5.733*** |
| Treatment | 3,21 | 1811.408 | 1.174ns |
| Time*Treatment | 15,105 | 247.691 | 1.363ns |

* Significant different at $p \leq 0.05$
** Significant different at $p \leq 0.01$
*** Significant different at $p \leq 0.001$
ns Not Significant

Figure 2 summarizes that, number of branches decreased gradually with increasing heavy metal concentration. B. vulgaris in 300 ppm treatment shows the highest number of branches whereas 200 ppm shows the lowest for the first two weeks.The results show an apparent increment initially however after day 42 rapid drop was observed for all treatment till the end of time duration. The highest number of branches 12.5 recorded in control meanwhile the lowest 4.13 in 200 ppm. Table 2 shows the summary of repeated measures two ways ANOVA within time and treatment on number of B. vulgaris branches. Number of branches was not significantly affected by heavy metal treatment ($F(3,21)=108.132$, $p>0.05$) and interaction between time and treatment ($F(15,105)=10.807$, $p>0.05$). In oppose by time, there is highly significant found for these parameter where $F(5,35)=103.012$, $p<0.001$.

As shown in Figure 2, number of leaves decreased gradually with increasing heavy metal concentration. B. vulgaris in control shows the highest number of leaves whereas 100 ppm treatment shows the lowest for the first two weeks. Expressively, significant increase was observed initially however after 42 days number of leaves remained in B. vulgaris plants were decreased in all treatment till the end of study duration. The highest mean number of leaves 46.88 recorded in control meanwhile the lowest 11.38 in 300 ppm treatment. Table 2 shows the summary of repeated measures two ways ANOVA within time and treatment on the number of B. vulgaris leaves. The test between time effect yielded $F(5,35)=1361.872$, $p<0.001$, indicated that the mean for each treatment was highly significant difference. However, there is no significant difference found for number of leaves within treatment ($F(3,21)=1811.408$, $p>0.05$) and time*treatment ($F(15,105)=247.691$, $p>0.05$).

Physiological Attributes

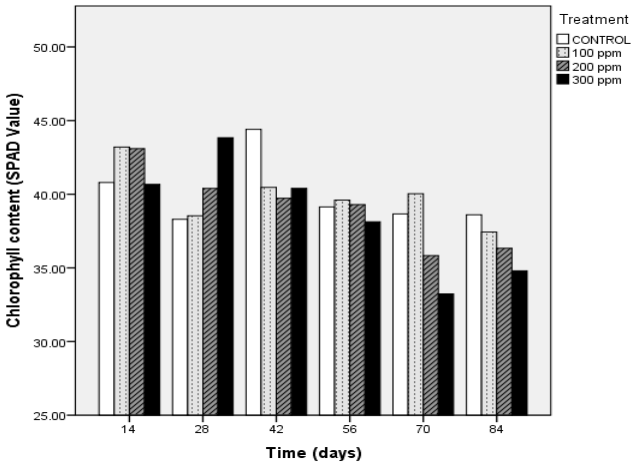


Figure 3: Mean Chlorophyll Content of *B. Vulgaris* between Treatments.

Table 3: Summary of Repeated Measures two Ways ANOVA Within Time and Treatment on Chlorophyll Content of *B.* Vulgaris

| Source of variation | df | Mean Square | F value |
|---|---|---|---|
| Time | 5,10 | 56.650 | 17.132*** |
| Treatment | 3,6 | 8.615 | 0.428ns |
| Time*Treatment | 15,30 | 13.399 | 1.294ns |

* Significant different at $p \leq 0.05$
** Significant different at $p \leq 0.01$
*** Significant different at $p \leq 0.001$
ns Not Significant

Figure 3 summarizes that exposure of *B. vulgaris* to high heavy metal concentration resulted to the reduction of chlorophyll content. The SPAD value shows the highest chlorophyll content in 100 and 200 ppm treatment compared to control and 300 ppm treatment for the first two weeks. However the amount of chloropyll content showed a depleted trend after 42 days until the end of study duration in response to heavy metal exposure. The highest mean of chloropyhl content 44.4 recorded in control and the lowest 33.23 in 300 ppm treatment.. The results summary of repeated measures two ways ANOVA within time and treatment on chlorophyll content of *B. vulgaris*in Table 3indicated that time ($F(5,10)=56.65$, $p<0.001$) had a highly significant effect on chlorophyll content. In contrast, no significant were found at all for content of chloropyll when comparison were made in treatment ($F(3,6)=8.615$, $p>0.05$) and both interaction ($F(15,30)=13.399$, $p>0.05$).

ISSN No: - 2456 – 2165

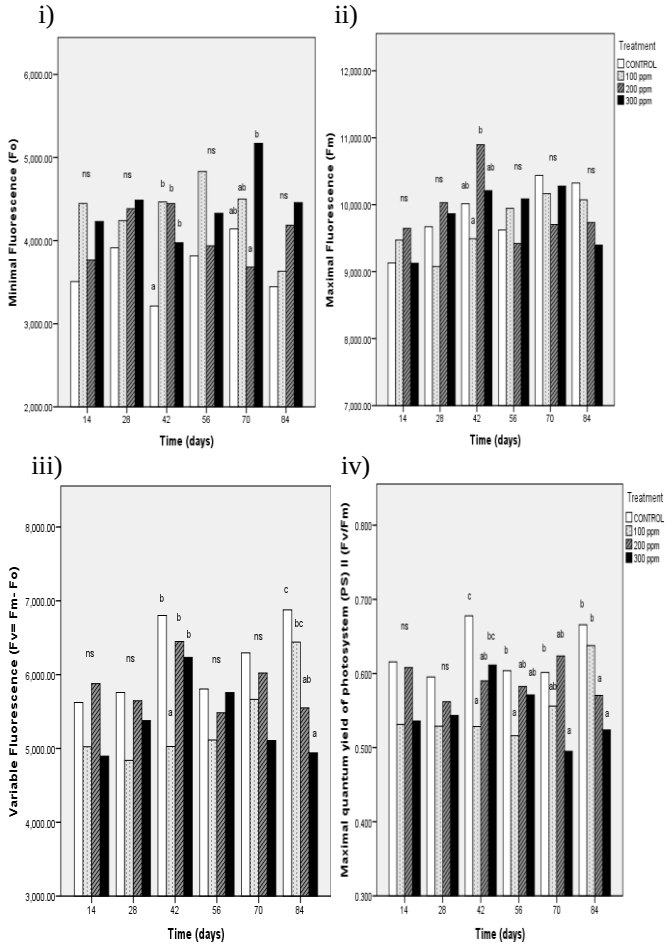


Figure 4 (I-Iv) : Mean Values Over Time Of Chlorophyll Fluorescence Parameters I) $F_o$ii) $F_m$ Iii) $F_v$ And Iv) $F_v/F_m$ Of *B. Vulgaris* In Different Treatment *Values With Different Letters Indicate Significant Differences For All Means Comparison Within Treatment By Tukey's HSD

Table 4: Summary of Repeated Measures Two Ways ANOVA Within Time and Treatment on The *B. Vulgaris* Chlorophyll Fluorescence Paramaters $F_o$, $F_m$, $F_v$, $F_v/F_m$

| Source of variation | df | Mean Square | F value |
|---|---|---|---|
| **$F_o$** | | | |
| Time | 5 | 1121844.675 | $1.316^{ns}$ |
| Treatment | 3 | 6473100.054 | $17.147^{***}$ |
| Time*Treatment | 15 | 1254572.591 | $2.286^{**}$ |
| **$F_m$** | | | |
| Time | 5 | 3425241.663 | $3.822^{**}$ |
| Treatment | 3 | 409636.302 | $0.315^{ns}$ |
| Time*Treatment | 15 | 1590074.302 | $1.798^{*}$ |
| **$F_v$** | | | |
| Time | 5 | 3477635.594 | $5.914^{***}$ |
| Treatment | 3 | 6473100.054 | $7.820^{***}$ |
| Time*Treatment | 15 | 1254572.591 | $2.249^{**}$ |
| **$F_v/F_m$** | | | |
| Time | 5 | 0.012 | $2.245^{ns}$ |
| Treatment | 3 | 0.071 | $17.642^{***}$ |
| Time*Treatment | 15 | 0.011 | $2.110^{*}$ |

* Significant different at $p \leq 0.05$
** Significant different at $p \leq 0.01$
*** Significant different at $p \leq 0.001$
$^{ns}$ Not Significant

As shown in Figure 4, $F_o$ in *B.vulgaris* leaves seemed differently affected by increasing heavy metal concentration. For the first two weeks, the minimal fluorescence 100 ppm treatment shows the highest mean value compared to control. The results show significant irregular changes but along the treatment period until the end of the duration, the highest mean of minimal fluorescence always observed in 300 ppm treatment meanwhile the lowest found in control. The highest reading of minimal fluorescence 5170.6 recorded in 300 ppm treatment and the lowest 3241.9 in control.. The results summary of repeated measures two ways ANOVA within time and treatment on minimal fluorescence *of B. vulgaris* in Table 4 shows there are significant differences in minimal fluorescence within treatment ($F(5,24)=6473100.054$, $p<0.001$) and heavy metal treatment interaction with time ($F(15,120)=1254572.591$, $p<0.01$). In spite of, there are no significant differences between treatments on the minimal fluorescence over time of experiment ($F(5,40)=1121844.675$, $p>0.05$).

Figure 4 illustrates that the value of $F_m$ in *B.vulgaris* leaves seemed affected by increasing heavy metal concentration. $F_m$ value for control and 100 ppm treatment with necessary heavy metal content gradually increase compare to the higher metal concentration 200 and 300 ppm treatment which slightly increase first but decline significantly until the end of study. The highest mean of maximal fluorescence 10895.2 recorded in 200 ppm treatment and the lowest 9077.8 in 100 ppm in the middle of treatment. At the end of treatment period, differ with $F_o$, the highest mean of maximal fluorescence found in control meanwhile the lowest in highest concentration 300 ppm treatment. Table 4 shows the summary of repeated measures two ways ANOVA within time and treatment on maximal fluorescence of *B. vulgaris*. Maximal fluorescence was not significantly affected by treatment ($F3,24)=409636.302$, $p<0.01$). In a contrary, time was significant ($F(5,40)=3425241.663$, $p<0.01$) to minimal fluorescence same goes interaction between time and treatment which $F(15,120) = 1590074.302$, $p\leq0.05$.

As shown in Figure 4, the value of $F_v$ in *B.vulgaris* leaves seemed affected by increasing heavy metal concentrations. $F_v$ value for all treatment initially increase however after day 42, only control and 100 ppm treatment rise significantly compare to the severe treatment which exhibit significant decline until the end of treatment period.The highest mean of variable fluorescence 6877.8 recorded in control and the lowest 4838.0 in 100 ppm treatment. At the end of study, control shows the highest mean of variable fluorescence meanwhile the lowest in concentration 300 ppm. Meanwhile, Table 4 shows the summary of repeated measures two ways ANOVA within time and treatment on variable fluorescence of *B. vulgaris*. Highly significant difference found on variable fluorescence through the time of study $F(5,40) = 3477635.594$, $p\leq0.001$) and among heavy metal treatment $F(3,24)= 8687930.881$, $p\leq0.001$)

ISSN No: - 2456 – 2165

whereas interaction between them shows significant difference at F(15,120) = 2056765.381, p≤0.01).

The of $F_v/F_m$ ratio a known stress marker seemed affected by increasing heavy metal concentrations. In general, based on the measurement obtained during first six weeks, the ratio remained almost unaffected regardless of the heavy metal concentration however after day 42, only control and 100 ppm treatment rise significantly compare to the severe treatment which exhibit significant decline until the end of treatment period. The highest ratio of maximal quantum yield photosystem (PS) II 0.68 recorded in control and the lowest 0.50 in 300 ppm treatment. Table 4shows the summary of repeated measures two ways ANOVA within time and treatment on maximum yield of photosystem II of *B. vulgaris*. The yield was not significantly affected throughout time but significantly affected by treatment (F(3,24)=0.077,p<0.001) and interaction with time (F(15,120)=0.012,p<0.001).

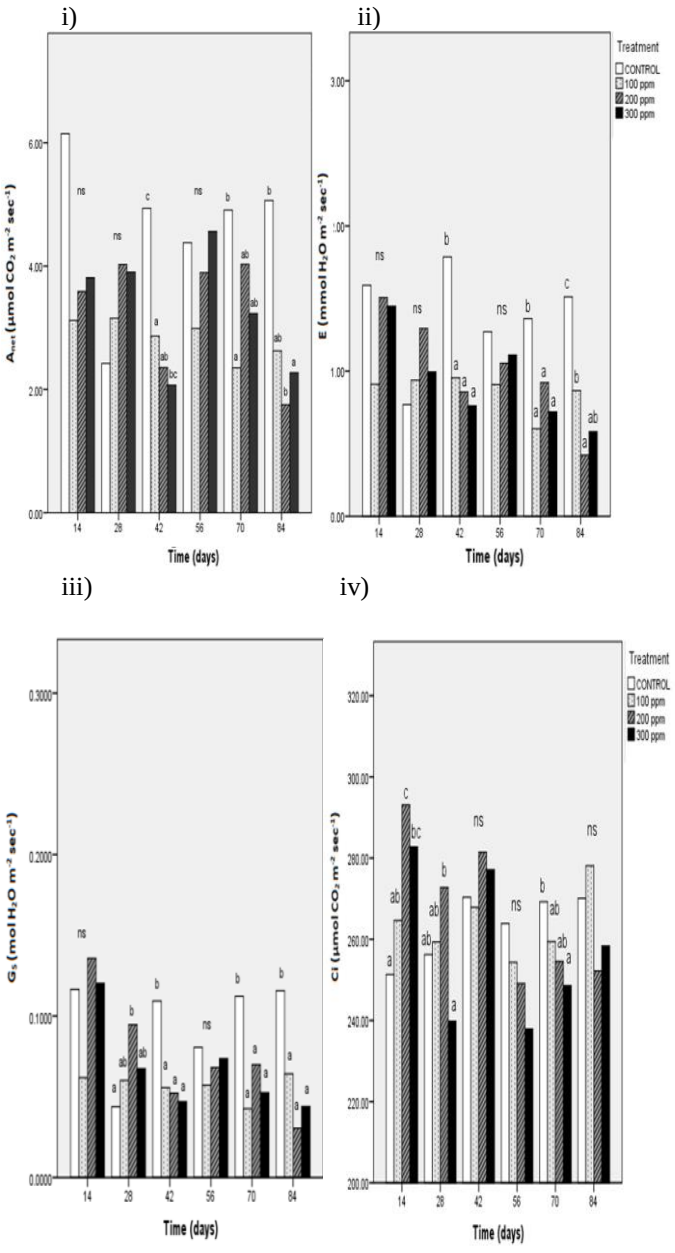


Figure 5 (I-Iv) : Mean Values Over Time of Gaseous Exchange Paramaters I) $A_{net}$ii) E Iii) $C_i$ And Iv) $G_s$ Of *B. Vulgaris* In Different Treatment *Values with Different Letters Indicate Significant Differences for All Means Comparison Within Treatment By Tukey's HSD

Table 5: Summary of Repeated Measures Two Ways ANOVA Within Time and Treatment On the *B. Vulgaris* Gaseous Exchange Paramaters $A_{net}$, $C_i$, E, And $G_s$.

| Source of variation | df | Mean Square | F value |
|---|---|---|---|
| **$A_{net}$** | | | |
| Time | 5 | 8.693 | 3.495** |
| Treatment | 3 | 32.659 | 12.362*** |
| Time*Treatment | 15 | 7.710 | 3.537*** |
| **E** | | | |
| Time | 5 | 1.209 | 4.448** |
| Treatment | 3 | 2.874 | 10.345*** |
| Time*Treatment | 15 | 0.738 | 3.078*** |
| **$G_s$** | | | |
| Time | 5 | 0.011 | 3.889** |
| Treatment | 3 | 0.015 | 5.578** |
| Time*Treatment | 15 | 0.006 | 2.484** |
| **$C_i$** | | | |
| Time | 5 | 3064.427 | 7.019*** |
| Treatment | 3 | 902.560 | 2.493ns |
| Time*Treatment | 15 | 1435.316 | 2.478** |

* Significant different at p ≤ 0.05
** Significant different at p ≤ 0.01
*** Significant different at p ≤ 0.001
ns Not Significant

Figure 5 summarizes that exposure of *B. vulgaris* to high heavy metal concentration resulted to the reduction of net photosynthesis rate. Control shows the highest mean value whereas 100 ppm treatment shows the lowest mean for the first two weeks. The results indicated that treated plants led to significant decline compared to control. In addition, a reduced trend observed in net photosynthesis rate in respect to the period of *B.vulgaris* exposure to heavy metal treatment. The deleterious effect of $A_{net}$ become more pronounced after 42 days of heavy metal treatment especially in severe treatment 200 and 300 ppm treatment.shows the. The results summary of repeated measures two ways ANOVA within time and treatment on photosynthesis rate of *B. vulgaris*in Table 5 determined that photosynthesis rate differed highly significant within treatment at F (3,24)=32.659, p<0.001 and also interaction between time and treatment where F (15,120)=7.710, p<0.001. Other than that there was significant difference between treatment period at F(5,40)=8.693, p<0.01.

Similar with photosynthesis, transpiration rate in *B. vulgaris* seemed affected by increasing heavy metal concentrations. A significant decline observed after 42 days untill the end of treatment period at the treated plant compared to control.The highest transpiration rate 1.79 recorded in control and the lowest 0.42 in 200 ppm treatment.Table 5 shows the summary of repeated measures two ways ANOVA within time and

treatment on transpiration rate of *B. vulgaris.* Transpiration rate was differed significantly throughout the time at $F(5,40)= 4.448, p<0.01$. Despite of that, the test within treatment and interaction time*treatment yielded $F(3,24)=2.874$, $p<0.001$ and $F(15,120) = 0.0738$, $p<0.001$ respectively. Those indicated that transpiration rate was highly significant differrently within treatment and interaction between time and treatment.

As shown in Figure 5, stomatal conductance in *B. vulgaris* affected similarly to photosyntesis by the exposures of plants to heavy metals treatments. Particularly, a reduced trend observed in highest concentration 200 and 300 ppm treatment after 42 days. On the other hand the mean value of stomatal conductance in control and 100 ppm treatment at the end of treatment period remains almost unaffected compared with their reading at first two weeks. The highest mean of stomatal conductance 0.14 and the lowest 0.03both recorded in 200 ppm treatment.Table 5 shows the summary of repeated measures two ways ANOVA within time and treatment on stomatal conductance of *B. vulgaris.* Specifically, the results indicated that stomatal conductance shows significant different at $p\leq0.01$ within time, treatment and time*treatment.

Figure 5 summarizes that exposure of *B. vulgaris* to high heavy metal concentration resulted to the reduction of intercellular $CO_2$. The results show 200 and 300 ppm treatment exhibit the highest in intercellular $CO_2$ whereas control and 100 ppm treatment as the lowest at the first two weeks. On the contrary, after being expose to the high concentration of heavy metal in growth media, a significant reduction of intercellular $CO_2$ observed in the two highest treatment compared to control and 100 ppm at the end of treatment period. The highest mean of intercellular $CO_2$ 293.1 recorded in 200 ppm treatment and the lowest 237.9 in 300 ppm treatment.Table 5 shows the summary of repeated measures two ways ANOVA within time and treatment on intercellular $CO_2$ of *B. vulgaris*. Intercellular $CO_2$ proves a highly significant different through the time of study $F(5,40) = 7.019, p<0.001$ and differed significantly with interaction between time and treatment at $F(15,120) = 1435.316$, $p<0.001$. Nevertheless, intercellular $CO_2$ not significantly affected by the metal treatment.

## IV. DISCUSSIONS

### A. *Effects on Morphology*

The highest total height and culm diameter of the *B. vulgaris* plants were recorded in control. *B. vulgaris* plants in control also produced most number of branches and leaves. This shows that B. vulgaris exhibited the best growth in terms of height, number of leaves and basal diameter for control. However, the *B .vulgaris* plants in treatment 300 ppm exhibited the lowest height and culm diameter. Furthermore, *B. vulgaris* plants in 300 ppm treatment also recorded the lowest number of branches and leaves. This is a clear indication that the *B. vulgaris*is unable to grow optimally in soil with very high amounts of heavy metal treatment. The growth or increase in the culm height and diameter of *B. vulgaris* was found in the first 2 – 8 weeks, which likely occurred due to the plants attempting to acclimatize themselves to their new growing medium. Improvement in the growth parameters of *B. vulgaris* is due to the micronutrient contribution from heavy metal. [5] These results prove that *B. vulgaris* has the ability to accumulate metals. After 8 weeks, *B. vulgaris* planted in 200 and 300 ppm heavy metal treatment exhibited the worst growth performance, indicating that a highest concentration of heavy metal would be the least ideal growth media for the plant, maybe due to higher toxicity from heavy metal content and soil acidity [6]. High metal concentrations in the growth media of plants would normally restrict germination and negatively affect the roots, shoots and leaf growth of the plants. [7]

### B. *Physiological Toleration Rates In Leaves*

The content of chlorophyll was reduced by the presence of Cu, Zn and Fe in the growth medium especially by the highest treatment. The decline in chlorophyll content is believed to be a consequence of the substitution of the heavy metal in chlorophyll. This prevents photosynthetic light harvesting in the affected chlorophyll molecules and results in breakdown of photosynthesis. From the observation throughout the experiment period, the plants which being exposed to heavy metals have changed its colours from bright green to light green, yellow or brown while the control remained the colour of green. This shows that the heavy metals affected the chloroplast negatively. The reduction in chlorophylls is in parallel with the toxicity symptoms observed in B. vulgaris plants.

Chlorophyll fluorescence is very useful to study various fundamental aspects of photosynthesis. It is indicative of the photosynthetic activity and status of the device of photosynthesis. When plants previously adapted to darkness are illuminated, the intensity of chlorophyll fluorescence kinetics shows highly dependent photochemical reactions of photosynthesis [8]. Measurement of chlorophyll fluorescence is a potential indicator of photosynthetic efficiency in plants and be proved as a rapid, non-invasive, and reliable method to assess photosynthetic performance under environmental stress. [9] [10] and allows the location of primary site of damage induced by environment stress.There was significant change of FO, FM and FV under different stresses which decreased drastically gradually levelled off with increasing concentration, indicating that heavy metal had inhibitory effect on the photochemical activity of *B. vulgaris*, and it is usually be explained as decrease of number of the closed PSII reaction centres, which do not participate in electron transport. [11]

### C. *Gas Exchange Changes*

Generally, gas exchange results strongly support the decline in morphological growth. The morphological growth decline trends were observed for each and every parameter taken in gas exchange, chlorophyll content as well as in transpiration and photosynthesis rates. Gas exchange is an activity that never stopped. Rates of gas exchange in plant differ with another and this is affected by the limiting factors such as light, carbon dioxide, temperature, oxygen and water (Robert et al.,1971).The parallel change of Anet (photosynthesis rate), Gs (stomatal conductance) and Ci (intercellular $CO_2$

concentration) in this study provided evidence that the photosynthetic responses of B. Vulgaris to excess heavy metal might be mainly due to the alteration of the pigment contents and stomatal conductance under heavy metal stress. Abiotic stresses could damage plant cells directly or indirectly through the formation of reactive oxygen species (ROS). [12]

Photosynthesis is a highly sensitive process significantly affected by heavy metals in several of plant species. The degree of heavy metals effect on photosynthesis depends on the growth stage, plant conditions as well as on the duration of stress. Heavy metal application was shown to affect photosynthetic functions directly or indirectly. There was a clear decreasing tendency for photosynthesis rate and stomatal conductance. Reduction in net photosynthesis rate was strongly correlated with depressed growth. This implies that inhibition of plant growth can be partially attributed to the reduction of carbon assimilation under stress. [13] The reduction of photosynthesis may be the consequences of stomatal closure [14] [15] and/or non-stomatal inhibition of photosynthesis. [16] According to [17]), lower Anet accompanied by lower Gs and lower Ci at control and low concentration of heavy metal (100 ppm) might be mainly ascribed to stomatal closure, which restricts $CO_2$ entry into leaves. Whereas, lower Anet accompanied by lower Gs and higher Ci at high concentration of heavy metal (200,300 ppm) may be attributed to non-stomata limitation, including changes in leaf biochemistry that results in inhibition or down regulation of photosynthesis. Stomatal closure is likely the first defense of heavy metal stress.

## V. CONCLUSION

Heavy metals copper zinc and iron are considered to be essential for plant growth due to the positive response in morphological and physiological at low concentration however brings phytotoxicity effects at high level of concentration and time. *B.vulgaris* performance was increased at early weeks and in low concentration metal level indicated it's tolerance to the heavy metals, making it a prospective phytoremediator species. The degree of heavy metals effect on plants depends on the growth stage, plant conditions as well as on the duration of stress besides the level of contaminated environment. Thus we can conclude that B. vulgaris is excellent to clean up heavy metal but weak to tolerate with high concentration of heavy metal contaminants. It has a potential to be a phytoremediator plant and also can be grown on moderately contaminated soil when put up with a growth reduction but it still needs to be further evaluated to optimize its potential.

## REFERENCES

[1]. Doelsch, E., Masion, A., Moussard, G., Chevassus-Rosset, C., & Wojciechowicz, O. (2010). Impact of pig slurry and green waste compost application on heavy metal exchangeable fractions in tropical soils. Geoderma,155(3): 390-400.

[2]. Gratani, L., Crescente, M. F., Varone, L., Fabrini, G., & Digiulio, E. (2008). Growth pattern and photosynthetic activity of different bamboo species growing in the Botanical Garden of Rome. Flora-Morphology, Distribution, Functional Ecology of Plants, 203(1): 77-84.

[3]. Truyens, S., Jambon, I., Croes, S., Janssen, J., Weyens, N., Mench, M., & Vangronsveld, J. (2014). The effect of long-term Cd and Ni exposure on seed endophytes of Agrostis capillaris and their potential application in phytoremediation of metal-contaminated soils. International journal of phytoremediation, 16(7-8): 643-659.

[4]. Netto, A. T., Campostrini, E., de Oliveira, J. G., & Bressan-Smith, R. E. (2005). Photosynthetic pigments, nitrogen, chlorophyll a fluorescence and SPAD-502 readings in coffee leaves. Scientia Horticulturae, 104(2): 199-209.

[5]. Majid, N. M., Islam, M. M., Abdul Raff, R., Ahmadpour, P., & Abdu, A. (2012). Assessment of heavy metal uptake and translocation in Dyera costulata for phytoremediation of cadmium contaminated soil. Acta Agriculturae Scandinavica, Section B-Soil & Plant Science, 62(3), 245 250.

[6]. Mangkoedihardjo, S. (2008). Jatropha curcas L. for phytoremediation of lead and cadmium polluted soil. World Applied Sciences Journal, 4(4): 519-522.

[7]. Parisa, A., M.N. Azmi, A. Abdu, A.H. Hazandy and K.S. Daljit et al., (2010). Uptake of heavy metals byJatropha curcas L. Planted in soils containing sewage sludge. American Journal of Applied Sciences, 7(10): 1291-1299.

[8]. Juneau, P., & Popovic, R. (1999). Evidence for the rapid phytotoxicity and environmental stress evaluation using the PAM fluorometric method: importance and future application.Ecotoxicology, 8(6): 449-455.

[9]. Krause, G. H., & Weis, E. (1991). Chlorophyll fluorescence and photosynthesis: the basics. Annual review of plant biology, 42(1): 313-349.

[10]. Schreiber, U., Bilger, W., & Neubauer, C. (1995). Chlorophyll fluorescence as a nonintrusive indicator for rapid assessment of in vivo photosynthesis. In Ecophysiology of photosynthesis . Springer Berlin Heidelberg. 49-70.

[11]. Tóth, S. Z., Schansker, G., & Strasser, R. J. (2005). In intact leaves, the maximum fluorescence level ($F_m$) is independent of the redox state of the plastoquinone pool: a DCMU inhibition study. Biochimica et Biophysica Acta (BBA)-Bioenergetics, 1708(2): 275-282.

[12]. Andrade, L. R., Farina, M., & Amado Filho, G. M. (2004). Effects of copper on Enteromorpha flexuosa (Chlorophyta) in vitro. Ecotoxicology and environmental safety, 58(1): 117-125.

[13]. Lovelock, C. E., & Ball, M. C. (2002). Influence of salinity on photosynthesis of halophytes. In Salinity: environment-plants-molecules (pp. 315-339). Springer Netherlands.

[14]. Downton, W. J. S., Grant, W. J. R., & Robinson, S. P. (1985). Photosynthetic and stomatal responses of spinach leaves to salt stress. Plant Physiology,78(1): 85-88.

[15]. Youssef, T. (2007). Stomatal, biochemical and morphological factors limiting photosynthetic gas exchange in the mangrove associate Hibiscus tiliaceus under saline and arid environment. Aquatic Botany, 87(4): 292-298.

[16]. Jeranyama, P., & DeMoranville, C. J. (2008). Gas exchange and chlorophyll content of cranberry under salt stress. In IX International Vaccinium Symposium810 (pp.753-758).
[17]. Farquhar, G. D., Ehleringer, J. R., & Hubick, K. T. (1989). Carbon isotope discrimination and photosynthesis. Annual review of plant biology, 40(1):503-537.