PHYTOREMEDIATION OF SYNTHETIC WASTEWATER CONTAINING COPPER BY USING NATIVE PLANT

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ABSTRACT

This study was aimed to assess the efficiency of *N.oleander* to remove heavy metals such as Copper (Cu) from wastewater. A toxicity test was conducted outdoor for 65-day to estimate the ability of *N.oleander* to tolerate Cu in synthetic wastewater. Based on a previous range-finding test, five concentrations were used in this test (0, 50, 100, 300, 510 mg/l). The results showed that maximum values of removal efficiency was found 99.9% on day-49 for the treatment 50 mg/l. Minimum removal efficiency was 94% day-65 for the treatment of 510 mg/l. Water concentration was within the permissible limits of river conservation and were 0.164 at day-35 for the 50 mg/l treatment, decreased thereafter until the end of the observation, and 0.12 at day-65 for the treatment 100 mg/l. the concentrations of water samples exceeded the permissible limits for 300 and 510 mg/l throughout the observation. Bioaccumulation factor (BAF) for *N.oleaner* was found to be greater than one for all the treatments. Higher translocation factor (TF) were 1.65, 1.73, 2.61 and 2.34 mg/l for 50, 100, 300 and 510 mg/l, respectively. This study revealed that *N.oleander* can tolerate and treat Cu concentration in wastewater.

Keywords: toxicity, heavy metals, *N.oleander*, bioaccumulation factor (BAF), translocation factor (TF).

المعالجة النباتية للمياه المصنعة الملوثة بالنحاس بأستعمال نبات محلي

سهير لؤي زكي
باحثه

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المستخلص

لغرض تقييم كفاءة نبات الدفلة في ازالة العناصر الثقيلة كعنصر النحاس من المياه الملوثة، تم اجراء اختبار السمية في الظروف الحقلية لمدة 65 يوم لغرض معرفة امكانية تحمل النبات لعنصر النحاس في المياه المصنعة الملوثة بالنحاس. تم أستعمال خمسة تراكيز مختلفة في الاختبار (0، 50، 100، 300، 510 ملغم/لتر) والتي تم اختيارها بناءأ على تجارب مسبقة لغرض تحديد مديات النحاس المستخدمة في هذا الاختبار. بينت النتائج ان اعلى كفاءة ازالة كانت 99.9 % لدى 35 يوم من التجربة للحوض الملوث بتركيز50 ملغم/ لتر. اقل كفاءة ازالة كانت 94% لدى 65 يوم من التجربة للحوض الملوث بتركيز 510 ملغم/ لتر. كانت تراكيز المياه المعالجة ضمن محددات تصريف المياه الملوثة للانهار بالنسبة للتركيز 50 ملغ/ لتر حيث بلغت 0.164 ملغ/ لتر لدى 35 يوم من التجربة واستمرت بالتناقص لغاية نهاية التجربة، كذلك الحال بالنسبة للتركيز 100 ملغم/ لتر حيث بلغت 0.12 ملغم/ لتر لدى 65 يوم من التجربة، بينما تجاوت الحدود المسموح بها لكل من التركيزين 300 و 510 ملغم/لتر. كانت قيم معامل التراكم الحيوي لكل الاحواض اعلى من واحد. بلغ معامل النقل للنبات 1.65، 1.73، 2.61 و 2.34 ملغم/لتر للاحواض ذات تراكيز 50، 100، 300، 510 ملغم/لتر على التوالي. أظهرت الدراسة أن نبات الدفلة لديه القدرة على تحمل واستصلاح المياه الملوثة بالنحاس.

الكلمات المفتاحية: السمية، العناصر الثقيلة، الدفلة، معامل التراكم الحيوي، معامل النقل

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INTRODUCTION

Heavy metal contamination of water is one of the most important environmental problems throughout the world. These elements arises from natural or man-made activities through industrial and agricultural practices without proper control for their emission and discharge to the surrounding which have resulted in contamination of water and soil around the world (8). These elements are known to be carcinogenic and cause serious health issues to humans and noxious effects on animals, microorganisms, and plants because they are non-biodegradable and highly toxic even at low concentrations (6). Heavy metals are categorized as either non-essential or essential to biological activities. Non-essential ones are those elements that have an unknown biological role for all organisms such as Cd, Pb, Cr, As and Hg (11). Essential metals are needed in trace amounts by living organisms and have an important role in many physiological and biochemical functions such as Fe, Mn, Cu, Ni and Zn. several methods have been used to treat heavy metals from water such as solvent extraction; membrane filtration; precipitation by chemical reagents; coagulation; adsorption and ion exchange (1). However, these methods are economically unfeasible due to their high cost and generate additional waste to get rid of. Phytoremediation is inexpensive and ecofriendly treatment strategy which involves using plants to remove various organic and inorganic contaminants from water taking advantage of their abilities to absorb pollutants by their roots and transport it to their aerial parts (12). Cu is an essential element for living organisms and plays a significant role in various physiological processes, though it can cause detrimental health effect when exceeds allowable limits (1). In humans, severe health concerns are related to excessive Cu intake such as kidney damage, vomiting, intestinal irritation cramps, or even death for long term exposure (18). In plants, the exposure to high Cu concentrations may cause leaf chlorosis, stunting, growth retardation, photosynthesis disruption and oxidative stress (4). Plants with fibrous root and higher biomass are thought to be more suitable for phytoremediation of heavy metals from water. Terrestrial plants usually produce long fibrous root systems with large surface area for metal sorption (14). The aim of this study was to determine the ability of *N. oleander* to tolerate and uptake Cu from wastewater.

MATERIALS AND METHODS

Experimental setup

The phytotoxicity experiment was conducted outdoor, in order to accommodate field conditions, and continued for 65 days from October 2018 to January 2019. Nine glass pots used in the test, each one with dimensions of 35cm x35cm x30cm (LxWxD), equipped with a bottom tap for sampling purposes. Each pot was filled with 6 cm layer of gravel ≥4 mm size placed in the bottom, intermediate layer of 7cm fine gravel with a size (2-4 mm), while the upper layer where 7 cm of sand with a size (0.1-2 mm) with an overall weight of 12 kg, (Figure 1). The characteristics of the sand used in the test are as follows: 17% $CaSO_4$, 31% $CaCO_3$, 0.8% organic matter, and pH value of 7.3 with a loamy sand texture. Five pots were planted (each of which transplanted randomly with eight healthy plants) and designated as: T_{PC}; T_{P1}; T_{P2}; T_{P3}; T_{P4} corresponding to Cu treatments of 0, 50, 100, 300, 510 mg/l concentrations, respectively. Four pots were kept unplanted for comparison and designated as: T_1, T_2, T_3 and T_4 to represent Cu treatments of 50,100, 300, 510 mg/l concentrations, respectively (Figure 2). Tpc control pot was used in order to compare the results of plants in contaminated planted pots to observe the effect of Cu toxicity. The concentrations used in this experiment were based on the result of the visual observation of the physical status of plant growth during a preliminary test conducted prior to the toxicity test.

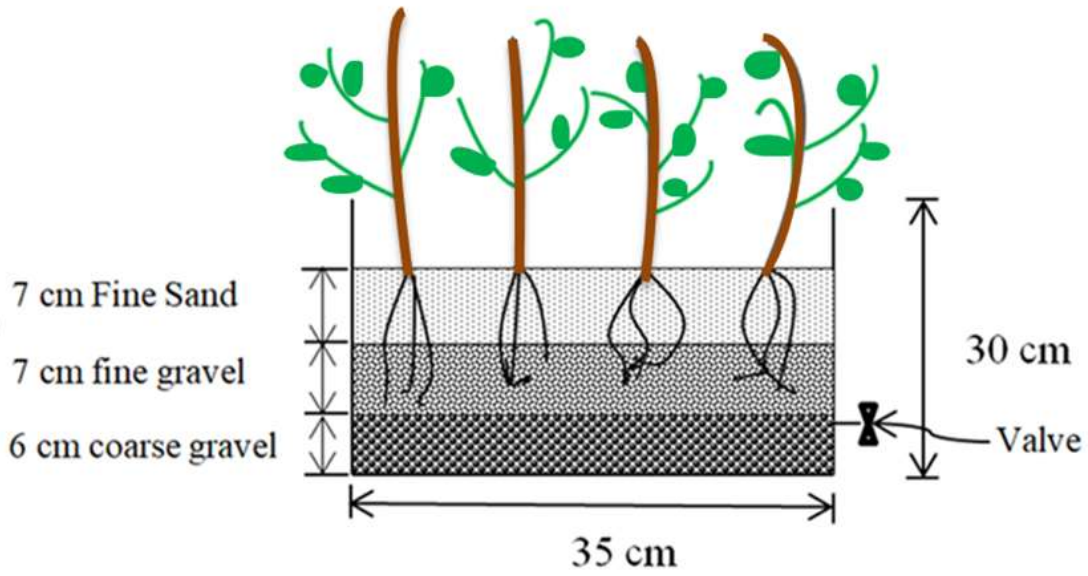


Figure 1. Schematic diagram for planting aquarium configuration

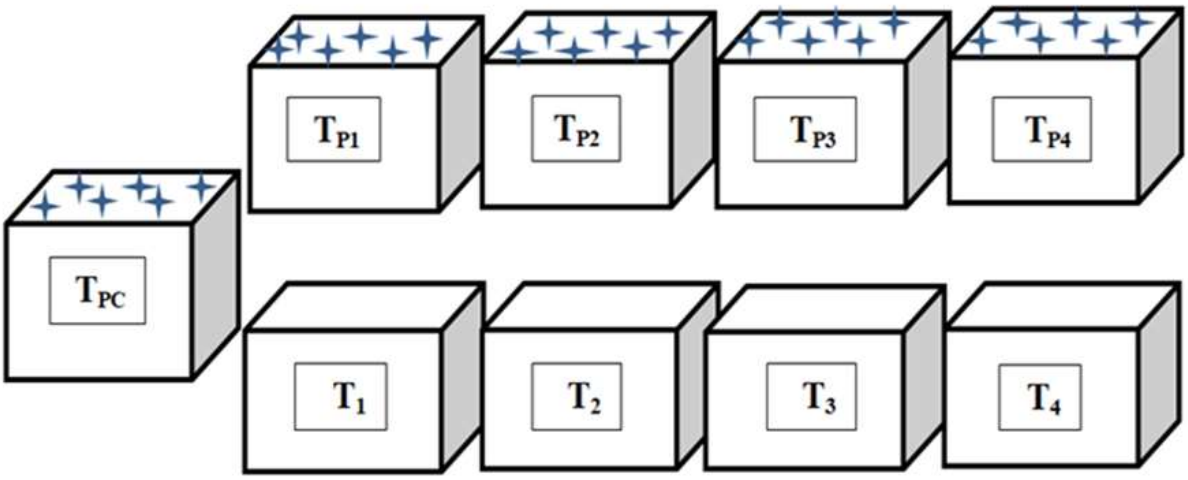


Figure 2. Schematic diagram of the experiment set-up

Figure 3. ***Nerium oleander*** **image**

Plant selection

Nursery grown *Nerium oleander* of the same age and average height of 25 cm has been used in the study. It is an ornamental, evergreen plant found in most parts of Iraq and can be found along the streets and highways of Baghdad city, (Figure 3).

Preparation of synthetic wastewater

The experiments were conducted in a single exposure system using Cu as the contaminant at various concentrations. The Cu levels used in this study were 0, 50, 100, 300 and 510 mg/L, prepared using Copper nitrate Cu (NO3)2.3H2O from (BDH Chemicals Ltd, UK) diluted in tap water. Five liters of the synthetic wastewater was added to each pot, and the level of water in all pots was maintained constant at 2 cm below the soil surface to simulate a subsurface flow system (SSF) similar to that used in a constructed wetland. Water losses were compensated by adding (≈0.5 L) tap water for approximately 1-2 time/week to avoid variation in Cu concentrations due to evaporation and evapotranspiration.

Cu analysis in water, plant, and soil

The toxicity experiments continued for 65 days. Physical observation was conducted to observe the effect of Cu contamination level on the plant compared to the control. The water samples were taken after (7, 14, 21, 28, 35, 49 and 65 days) and analyzed using Atomic Absorption Spectrometry (ASS). One plant was harvested on each sampling day (7, 14, 28, 49, 65 days) separated into (roots, stems, leaves). Cu extraction from the plants was performed using wet digestion method 6HNO_3: 2H_2O_2 adopted by (3). Soil samples were analyzed at 7, 14, 21, 28, 35, 49 and 65 days, total concentration of Cu in soil samples was determined using aqua regia digestion procedure (7). The Cu concentrations in plant and sand samples were also analyzed using AAS.

Determination of wet weight and dry weight

The overall health of plant growing in heavy metals contaminated medium can be estimated through plant biomass (5). Plant biomass could be obtained from the measurement of plant wet and dry weight. Plant samples were harvested at the end of the observation. For dry weight (DW) measurements, Plant samples were placed in a drying oven (BINDER, Hotline International, Germany) set at 105˚C until reaching constant weight for a maximum of 2 hours (19), and weighted using electrical balance (Sartorius ENTRIS 64i-1S, Germany).

Calculation of removal efficiency, BAF, and TF

The efficiency of *N.oleander* to remove Cu from water was calculated using eq.1 (13):

$$\%Removal\ efficiency = \frac{(C_0 - C_t)}{C_0} \times 100 \quad (1)$$

Where C_0= initial Cu concentration in wastewater (mg/l), C_t= Cu concentration with time (mg/l).

The potential of plants in accumulating a given metal from contaminated substrate can be evaluated using BAF, it is estimated by using eq.2 (13):

$$BAF = \frac{C_{in\ plant}}{C_{water\ medium}} \quad (2)$$

The amount of Cu transferred from roots to aerial parts is estimated using eq.3 (15)=:

$$TF = \frac{c_{shoot}}{c_{root}} \quad (3)$$

RESULTS AND DISCUSSION

Cu concentration in water medium

The results of Cu concentration for all treatments in planed and unplanted pots are represented in (Figure 4a, b). The concentrations of the effluent water for both planted and unplanted pots showed a similar trend of Cu removal from the first week up to the end of the observation day-65. However, in planted pots, Copper removal was more pronounced due to plant uptake. Minimum Cu concentrations were 0.042 and 0.12 mg/l for T_{P1} and T_{P2}, respectively at the end of the observation and were within the permissible limits set for river conservation 0.2 mg/l (according to the Iraqi standard river limits No.25/1967). For T_{P3} and T_{P4}, Cu concentrations exceed the limits and were found to be 9.811 and 30.524 mg/l, respectively. This indicates the importance of oleander plant as a water phytoremediator plant for considerable high Cu concentrations polluted water up to 100 mg/l. water samples taken from the outlet of all unplanted treatment showed that Cu concentrations were higher than standards at the end of the observation and were 1.482 mg/l, 6.303 mg/l, 18.697 mg/l and 45.62 mg/l for T_1, T_2, T_3, and T_4, respectively. The high concentration of the effluent of unplanted treatments may also enhance the role of *N.oleander* as a good phytoremediation plant. For T_{P1}, Cu concentration decreased as much as 97.5% on day-7, the reduction became 99.3% on day-28 and then reach 99.9% on day-49. Cu concentration with T_{P2} decreased to 91.6% on day-7, and became 98.9% on day-28 and reached 99.9% on the day-65. For T_{P3}, the reduction of Cu concentration on day-7 was 93.2%, became 94.8% on day-28 and reached 96.7% on day-65. For T_{P4}, Cu removal efficiency on day-7 was 90.4%, became 92.9% on day-28, and reached 94.0% on day-65.

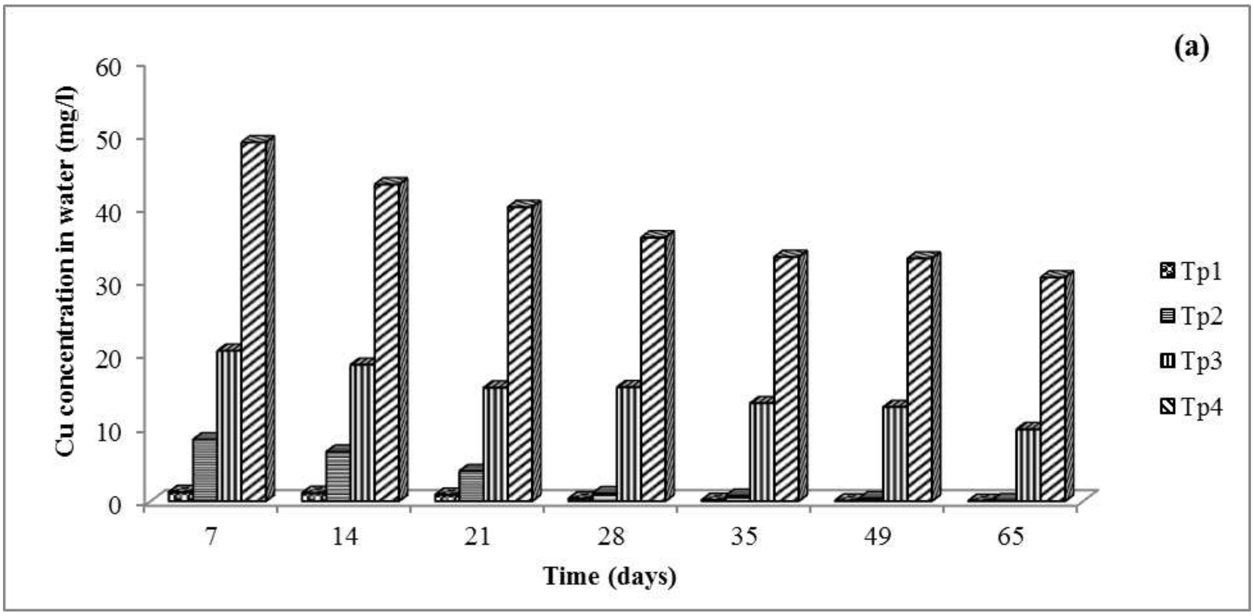


Figure 4 a. Cu concentration in water medium with plant

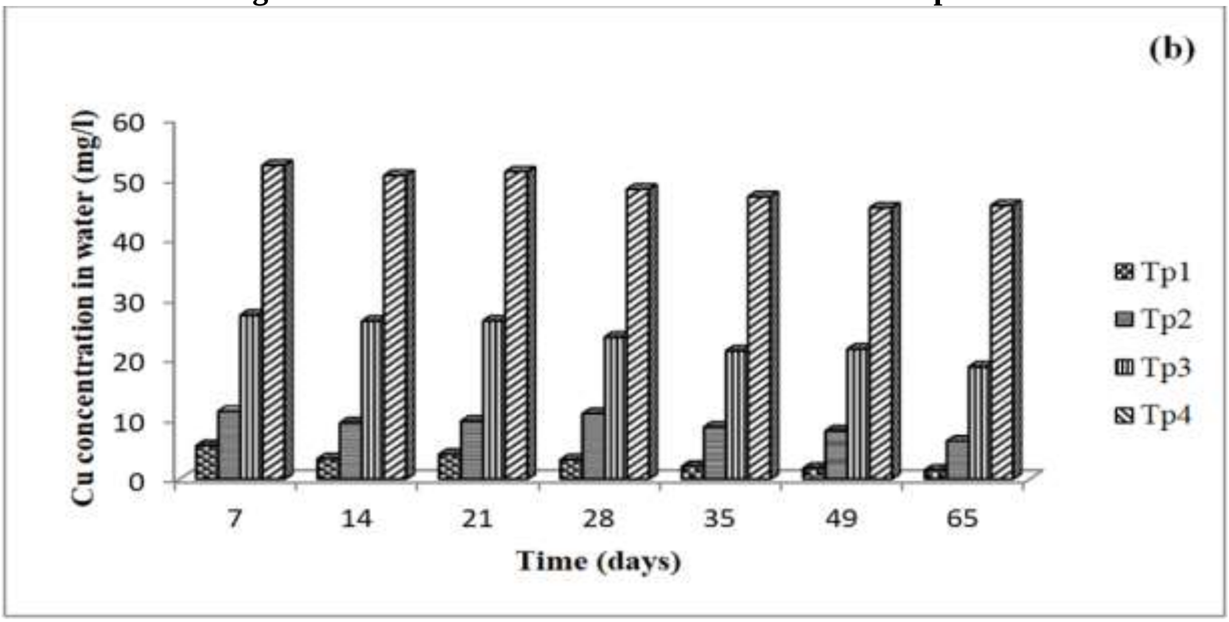


Figure 4b. Cu concentration in water medium without plant

Cu concentration in plant

Cu concentration in *N.oleander* tissues for all the treatments are shown in (Figure 5). The concentration increased up to day-28 for T_{P1} and T_{P2} and continues to decrease till the end of the observation. For T_{P3} and T_{P4}, Cu concentration in plant tissues increased up to day-14 and continues to decrease to the end of the observation. The highest Cu concentrations in plant were 337.6 mg/kg, 354 mg/kg, 433 mg/kg and 484 mg/kg for T_{P1}, T_{P2}, T_{P3} and T_{P4} respectively. From these results, one can conclude that the highest plant uptake of *N.oleander* reached at 28-day to remediated Cu polluted water up to 100 mg/l, while for higher concentrations up to 510 mg/l Cu polluted water reaches highest uptake at half of this period 14-days. (Figure 6a) shows the images of *N.oleander* at the beginning of the phytotoxicity test. Plants images at the first week of observation are shown in (Figure 6 b). Leaves chlorosis was observed at T_{P2} from the first week of observation. At higher concentrations of T_{P3}, T_{p4} plant withering symptoms were started from day-7 observation. Figure 6 c depicts the images of plants for the day-28. Effect of toxicity increased gradually until the end of the observation (Figure 6 d). Many researchers (9,10) reported that Copper toxicity may cause leaf chlorosis; oxidative stress; reactive oxygen species (ROS) formation and Growth retardation.

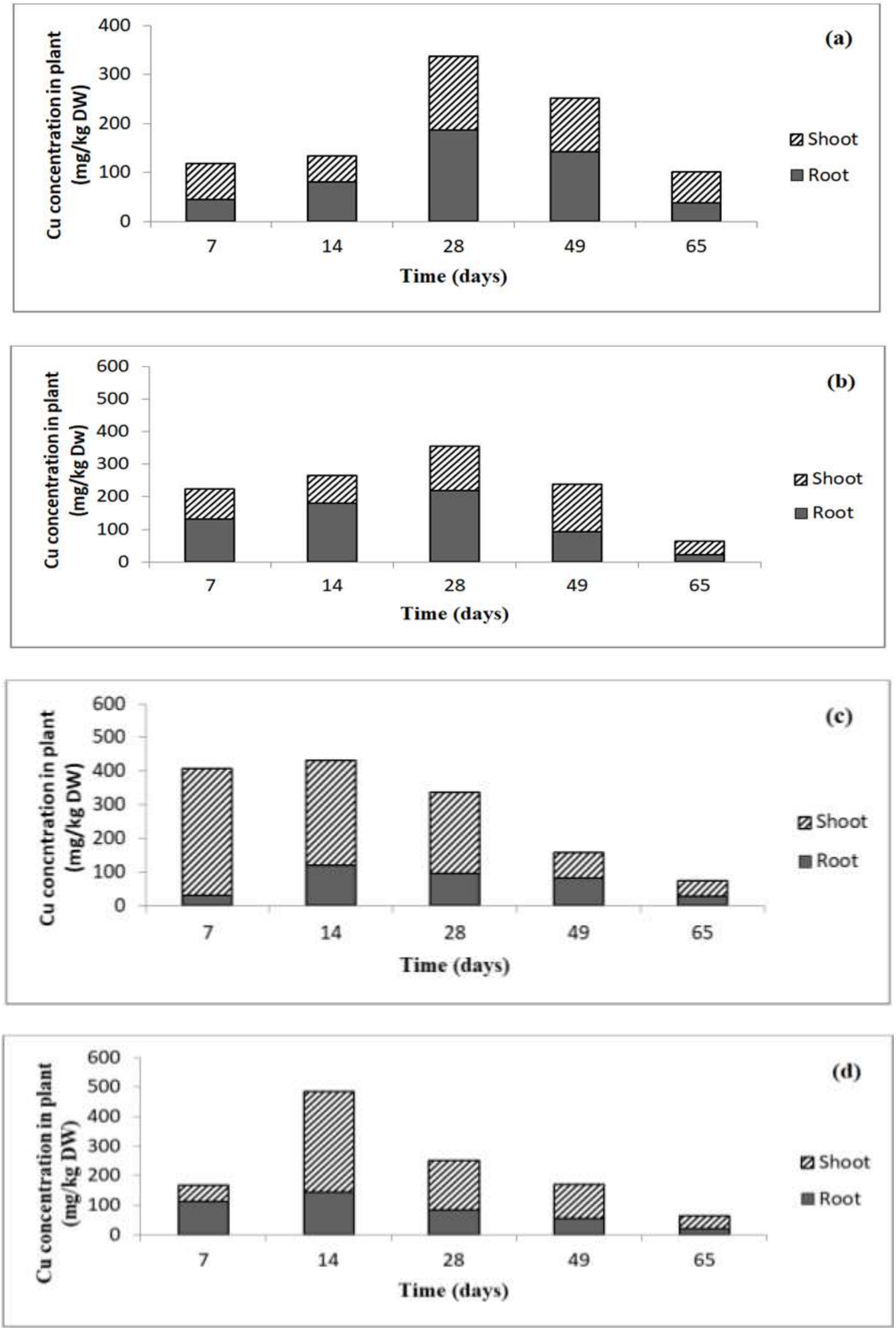
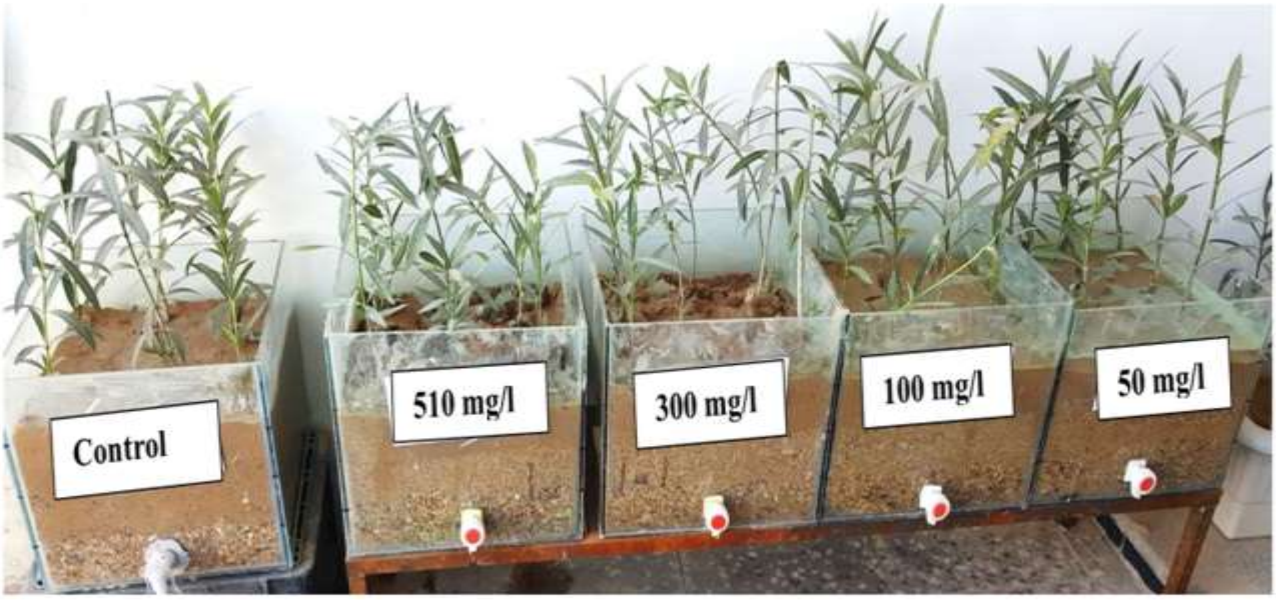
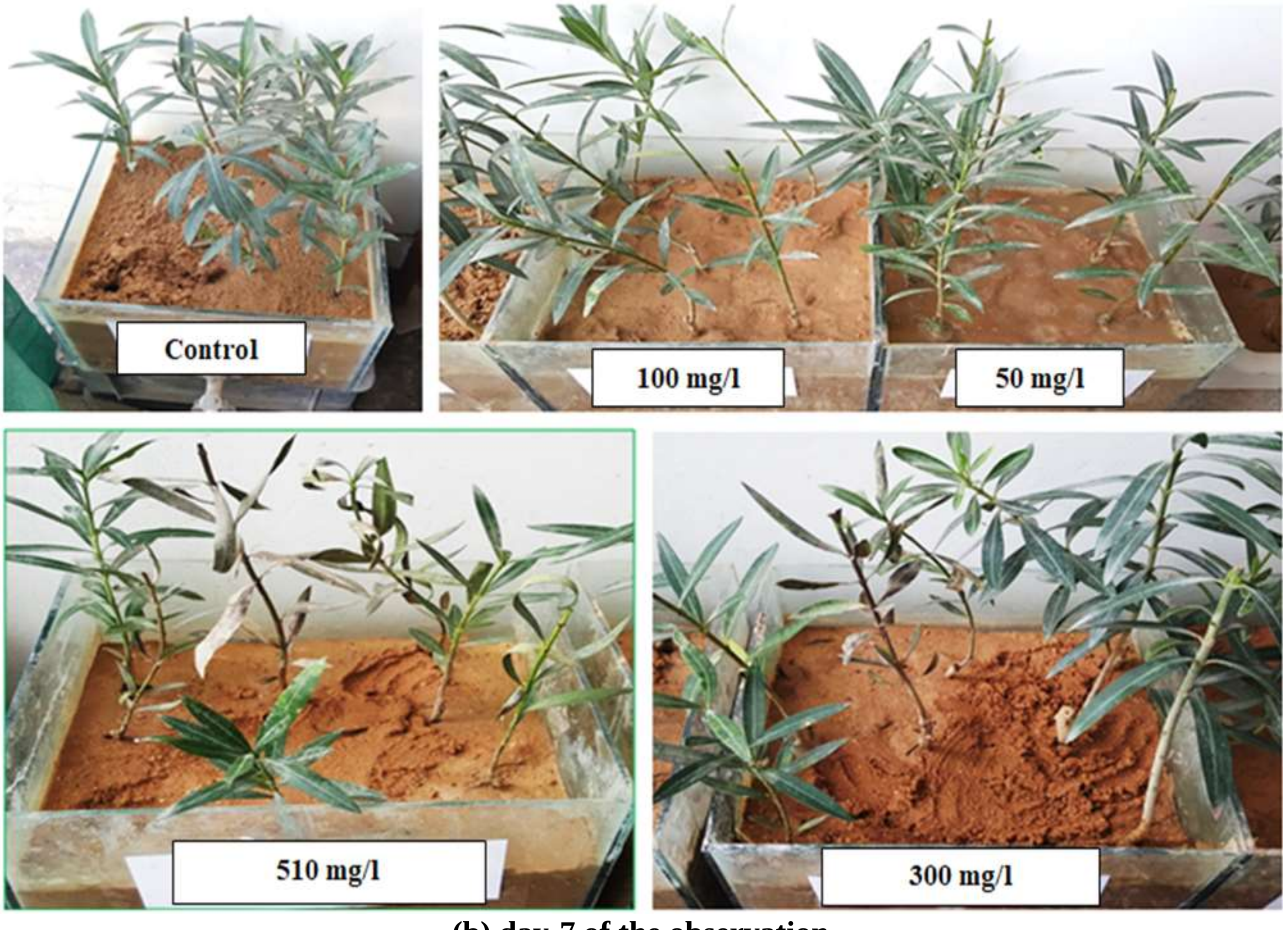


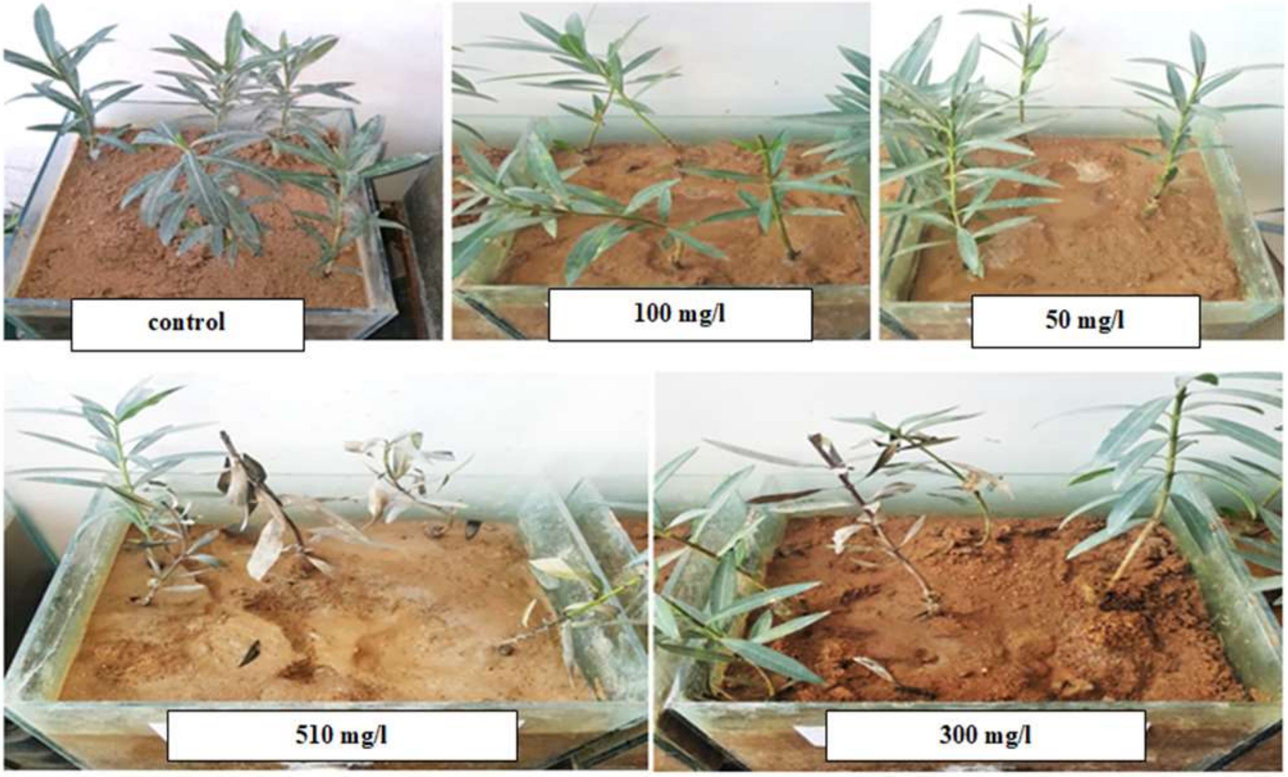
Figure 5. Concentrations of Cu in *N.oleander* tissues (a) T_{P1} (b) T_{P2} (c) T_{P3} (d) T_{P4}



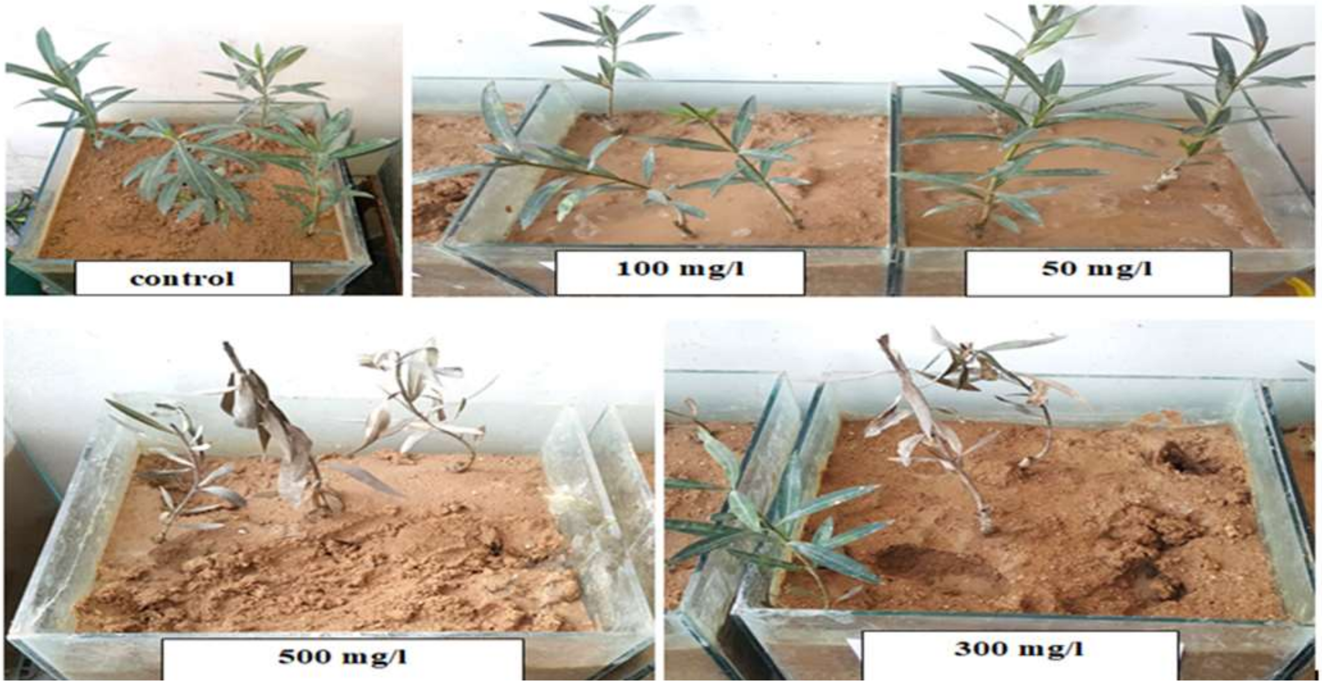
(a)Plants at the beginning of the observation



(b) day-7 of the observation



(c) day-28 of the observation

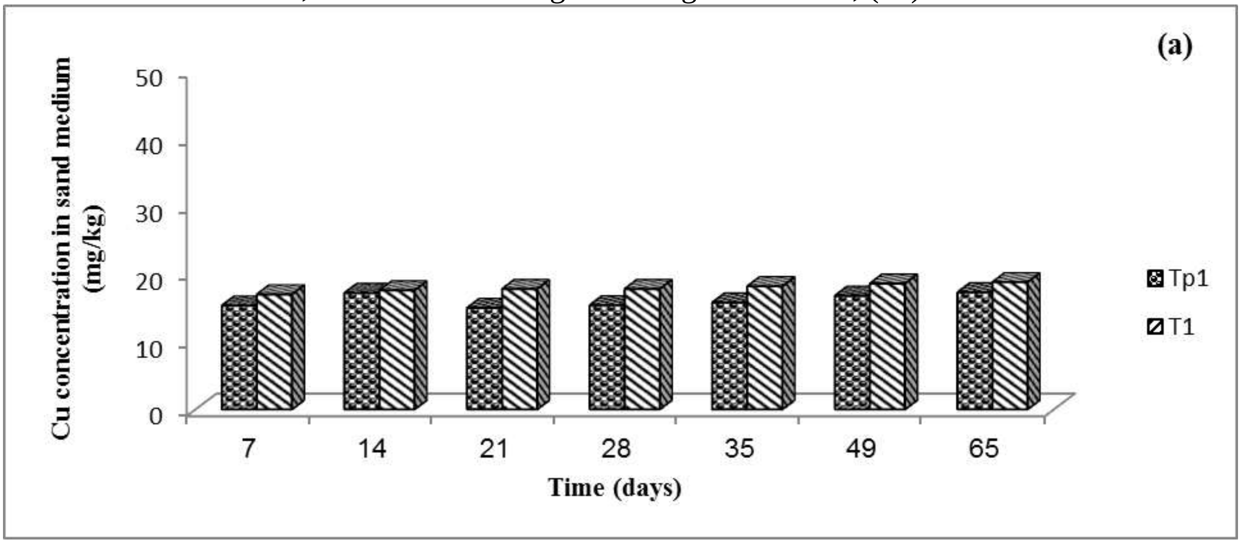


(d) End of the observation

Figure 6 (a-d). ***N.oleander*** **during phytotoxicity test**

Cu concentration in soil medium

Cu levels in soil for both planted and unplanted pots are depicted in (Figure 7). The concentration of heavy metals in soil medium increased with increasing the added doses for all aquaria with and without plants. This agrees with the findings of Tangahu *et al.*, (20). The results of Cu concentration in planted pots generally increased for day-14 of observation for most exposure doses and were 17.2, 35.8, 102.8 and 200 mg/kg for 50, 100, 300 and 510 mg/l, respectively and decreased until the end of the experiment. This phenomenon could be related to plant uptake mechanism (20). For unplanted pots, the concentration of Cu in soil was almost stable for each treatment and no significant variation noticed for all pots throughout until the end of the observation. This stability is probably due to soil water reaction and equilibration without interference of plant. The highest Cu concentrations recorded for soil in unplanted pots were 18.8 and 37.5, 108.7 and 187 mg/l for 50, 100, 300 and 510 mg/l, respectively. In general, high Cu concentration in the soil medium could be attributed to high Cu affinity to soil medium, also soil characteristics play a significant role, (16).



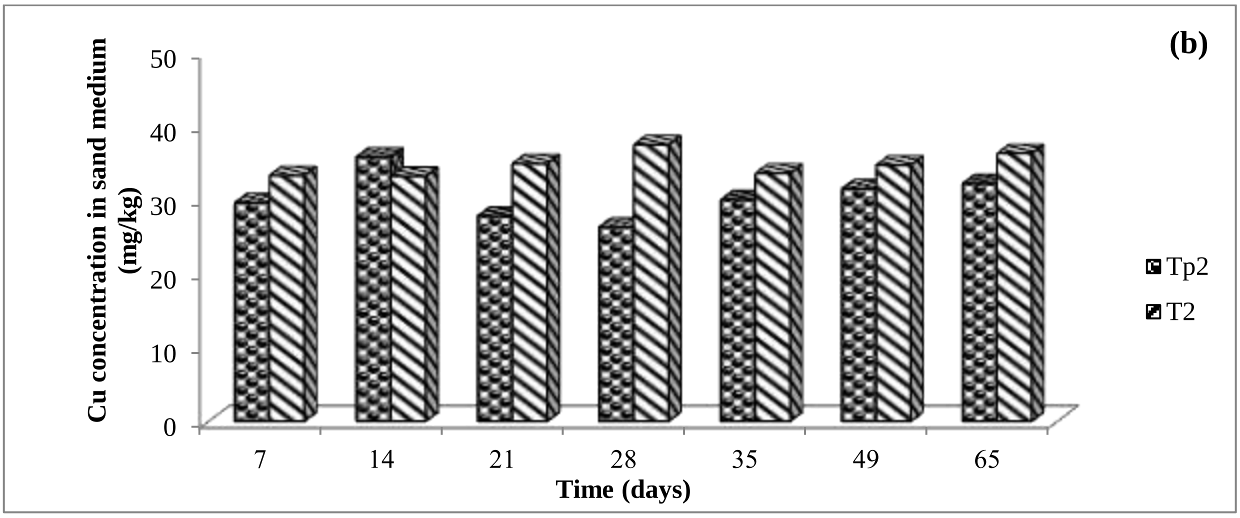
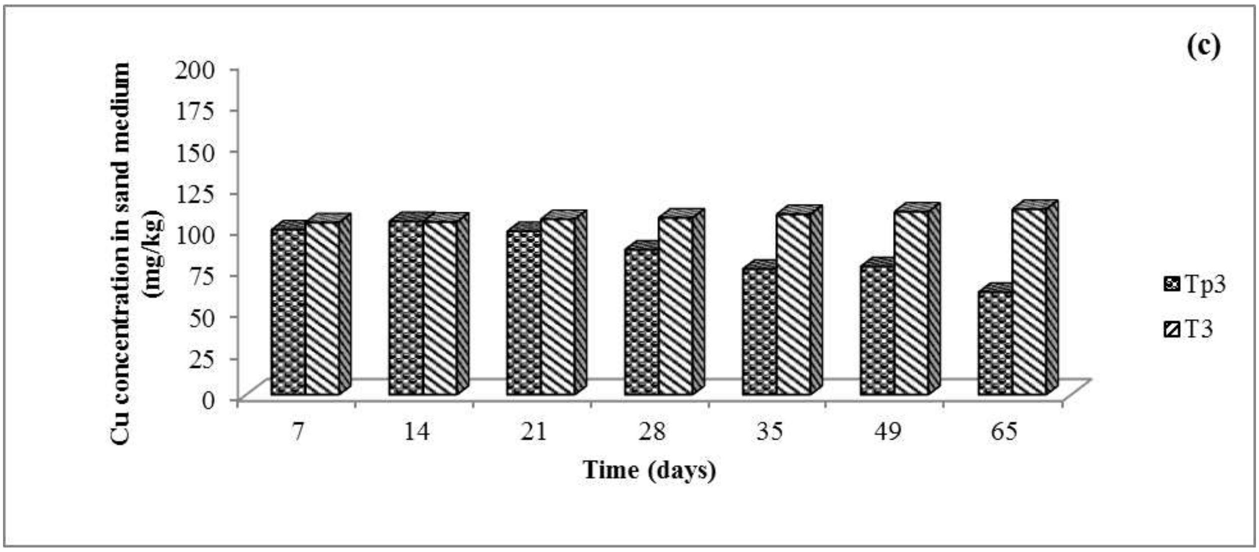


Figure 7(a-d). continue



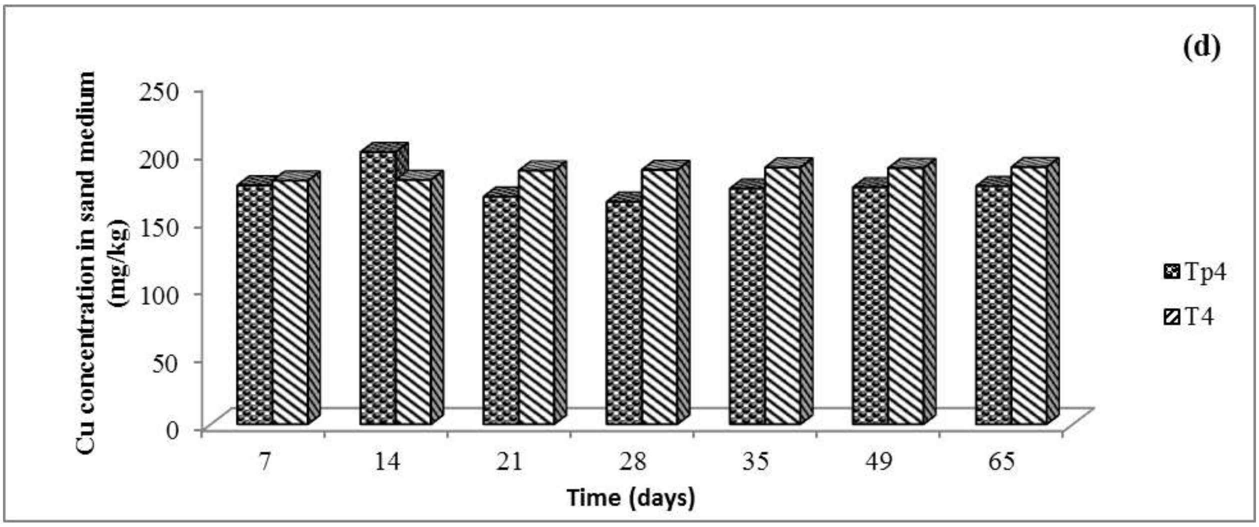


Figure 7(a-d). continue

Figure 7. (a-d). Cu concentration in soil medium

Effect of Cu on wet weight and dry weight

Plant wet weight and dry weight is an important parameter used to estimate their ability to tolerate heavy metals (22). The wet weight and dry weight measured at the end of observation decreased along with increasing Cu concentration in water, a decrease in dry weight of 11, 37, 57 and 85% compared with the control was found when Cu concentrations were 50, 100, 300 and 510 mg/l, (Figure 8).

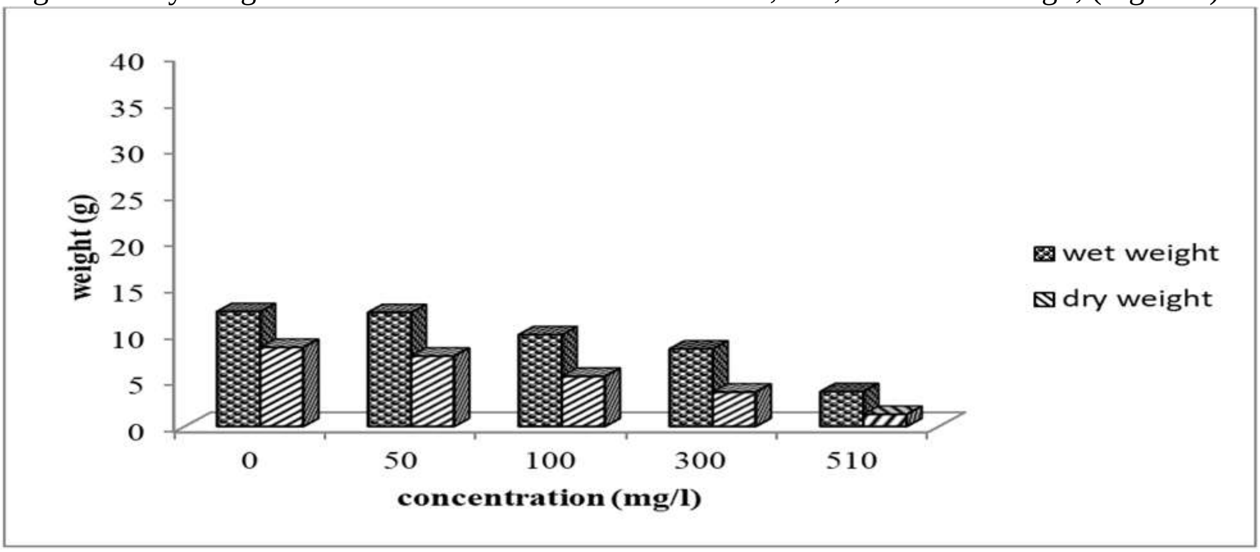


Figure 8. Plant dry weight at the end of the observation

BAF and TF of Cu in *N.oleander*

Referring to Cu concentrations in water and plant (Figure 4a, 5), BAF was calculated as shown in (Table 1). The BAF values for all treatments were higher than one which indicates that *N.oleander* has the capacity to adsorb Cu from the water medium, translocate and store it in the above ground parts (2). The initial concentration effect on Cu uptake by plants in each treatment resulted in different trends of BAF values over the different treatments. Most of the TF values of Cu by *N.oleander*, as shown in (Table 2), are greater than one especially with higher concentrations TP3 and TP4, this may relate to Cu availability in water at higher concentrations (21). In addition, the variation of TF values could be caused by the difference of Cu concentration in different plant parts that depends on the biochemical and physiological factors that participate in heavy metal accumulation and distribution in the morphological parts of the plant (17).

Table 1. BAF values for *N.oleander*

Treatment	Day-7	Day-14	Day-28	Day-49	Day-65
TP1	95.08	120.2	938	3533.8	2412.7
TP2	26.6	39.2	329.5	605.4	536
TP3	19.9	23.3	21.7	12.4	7.52
TP4	3.4	11.2	7	5.14	2.1

Table 2. TF values for *N.oleander*

Treatment	Day-7	Day-14	Day-28	Day-49	Day-65
TP1	1.64	0.64	0.81	0.76	1.65
TP2	0.70	0.47	0.61	1.59	1.73
TP3	11.89	2.61	2.53	0.95	1.68
TP4	0.49	2.34	2.01	2.00	2.13

The results of the experiments performed with oleander grown in synthetic wastewater with increasing concentrations of Cu revealed that Cu removal by *N.oleander* decreased with increasing initial concentration added. The results of Cu concentrations in water were within acceptable river discharge criteria for both T_{P1} and T_{P2}. Higher removal efficiency obtained at T_{P1}, so this concentration will be applied in the future for horizontal subsurface flow constructed wetlands experiments. The plant developed no visible toxicity symptoms for Tp1, while chlorosis appeared at T_{P2}, and some plants in T_{P3} and T_{P4} showed withering symptoms. Plant biomass is an important parameter used to assess their tolerance to heavy metals. The plants exposed to single Cu concentration in water caused some reduction in the growth of *N.oleander*. BAF values were higher than one for all the treatments. TF values fluctuated during the observation, the main Cu accumulation was in the plant shoot, particularly for higher concentrations. *Nerium oleander* is obviously suitable for phytoremediation applications of water polluted with Cu.

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