PHYTOTOXICITY OF WASTEWATER CONTAINING CADMIUM (Cd) USING OLEANDER

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ABSTRACT

This study was aimed to investigate the ability of *N.oleander* to remove Cadmium (Cd) from wastewater. A prolonged toxicity test was performed in a single exposure and run for 65 days with various concentrations of Cd. Plants were grown in sand medium and irrigated with simulated wastewater contaminated with Cd, using different concentrations (0, 10, 25, 50, 75 mg/L), which were chosen based on previous preliminary test. The results of physical observation of the plants didn’t show any withering symptoms. The Cd concentration in plants increased, while in water decreased. The results of plant analysis showed that Cd concentration in plant shoots (stems and leaves) was higher than that in roots for almost all exposure doses along the test duration. The concentration of Cd in water decreased significantly from the first week of the test and become (0 mg/l) on day-35 for 10 and 25 mg/l exposure doses, while exceeded the permissible limits for 50 and 75 mg/l exposure doses and were 0.14 and 0.91 mg/l, respectively. Wet weight and dry weight of Oleander decreased with increasing Cd concentration level except for 10 mg/l exposure dose where the plant wet weight and dry weight increased at the end of the test. Bioaccumulation factor (BAF) and Translocation Factor (TF) was found to be greater than 1, indicating that Oleander is a successful hyperaccumulator for Cd.

Keywords: phytoremediation, toxicity test, heavy metals, Bioaccumulation factor, Translocation factor

مجلة العلوم الزراعية العراقية -2020 :51 (4):1231-1238 زكي ورضا

أختبار سمية المياه الملوثة بالكادميوم على نبات الدفلة

سهير لؤي زكي
باحثه

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المستخلص

تم اجراء اختبار السمية من خلال تجارب التعرض للجرعة الواحدة والتي استمرت لمدة 65 يوم لمعرفة مدى قابلية نبات الدفلة على تحمل التراكيز المختلفة للكادميوم في المياه الملوثة. تم تنمية النباتات في وسط رملي وتم سقيها بالمياه المصنعة الملوثة بالكادميوم، حيث تم استعمال عدة تراكيز (0، 10، 25، 50، 75 ملغم/لتر) والتي تم اجراؤها بناءاً على تجارب أولية مسبقة. لم تظهر نتائج مراقبة السمية أي اعراض ذبول على النباتات. كما بينت النتائج ان ازدياد تراكيز الكادميوم في النبات صاحبه نقصان للملوث في الماء. كذلك بينت نتائج التحاليل التي اجريت على اجزاء النبات بأن تراكيز الكادميوم في الاجزاء العلوية للنبات (الساق والاوراق) للنبات كانت اعلى مما هي عليه في الجذور لاغلب جرعات التعرض وعلى طول فترة التجربة. اما فيما يخص تراكيز الكادميوم في الماء فقد بينت النتائج ان تراكيز الكادميوم انخفضت بشكل ملحوظ منذ الاسبوع الاول من النمذجة حيث اصبحت تراكيز الكادميوم (0 ملغم/ لتر) خلال 35 يوم من التجربة لكل من جرعتي التعرض 10 و 25 ملغم/لتر. بينما تجاوزت تراكيز الكادميوم الحدود المسموح لنظام صيانة الانهر حيث وصلت في اخر يوم من التجربة الى 0.14 و 0.91 ملغم/ لترعلى التوالي. أظهرت النباتات انخفاضاَ في الوزن الرطب والوزن الجاف لجميع جرعات التعرض ماعدا 10 ملغم/لتر، حيث أظهرت النباتات ازدياد في الوزن الرطب والجاف في نهاية التجربة. وجد ان معامل التراكم الحيوي (BAF) ومعامل النقل (TF) كان اكثر من واحد مما يدل على كفاءة النبات في المعالجة.

الكلمات المفتاحية: المعالجة النباتية، اختبار السمية، العناصر الثقيلة، معامل التراكم الحيوي، معامل النقل

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INTRODUCTION

Environmental pollution by heavy metals (HM) is considered a global concern due to their persistence and non-biodegradable nature, thus causing serious effects on water, soil and associated implications to human health and living organisms (1). Cd is a trace element that is released into the environment from various anthropogenic activities such as agricultural, industrial, mining processes and exhaust gases of transportation means (5,8). It is highly toxic elements that lifetime exposure to low-level Cd may cause severe damage to the liver, kidneys, skeletal system, and deterioration of hearing and sight. Plants are also affected by Cd exposure because of its highly soluble in water (15). Therefore, efficient treatment methods are needed to remove Cd from contaminated water. Different remediation methods have been used to remove Cd from water such as adsorption, ion exchange, oxidation-reduction, reverse osmosis, and solvent extraction. However, these methods are expensive and generate sludge that needs further treatment (16). Phytoremediation is a promising and affordable treatment method that is less expensive, safer than other treatment methods on the environment and has aesthetic value (25). There are many factors affecting on the mechanisms of phytoremediation include plant species, type of contaminants, and medium properties (23). Many plant species have been found to absorb contaminants successfully, and being able to uptake and accumulate HM from soil or solutions by phytoextracting essential metals for plant growth (Iron (Fe), Manganese (Mn), Copper (Cu), Magnesium (Mg), Zinc (Zn), Nickel (Ni) and Molybdenum (Mo)), in addition to non-essential HM ((Lead) Pb, Chromium (Cr), Mercury (Hg), Cobalt (Co), Silver (Ag), Selenium (Se) and Cd) which have unknown biological role (19). The efficiency of plants used to uptake and accumulate heavy metals in plant tissue as a hyperaccumulator can be estimated through the values of (BAF) and (TF). Hyperaccumulators are plants that are capable of accumulating HM in their shoots with concentrations greater than that of their roots. BAF represents plant ability to store contaminants in the plant, while TF factor determines metal distribution inside plant tissues (25). Oleander used in this study is an evergreen, terrestrial plant that has an aesthetic value, and can endure various environmental conditions and known to grow well in the region. These plants are not edible, thus reducing the risk of contaminants entering the food chain. The aim of this study is to determine Cd toxicity to oleander to phytoremediate simulated industrial wastewater from Cd contamination and to determine the concentration of Cd that can be taken up by oleander. The study was conducted through a prolonged phytotoxicity test.

MATERIALS AND METHODS

Experimental setup

Five Glass aquariums used in the test, each one with dimensions of (35 cm length, 35 cm width, and 30 cm height), (Figure 1). Aquariums were filled from the bottom layer to the upper layer with; (1) 6 cm of gravel with a size of Ø ≥4 mm, (2) 7cm fine gravel with a size of Ø (2-4 mm), (3) 7 cm of sand with a size of Ø (0.1-2 mm). These aquariums are equipped with tap for sampling purposes. The sand used in this experiment has a loamy sand texture and contains 31% CaCO3, 17% CaSO4, 0.8% organic matter, and pH value of 7.3. the weight of the sand used in each aquarium is 12 kg.

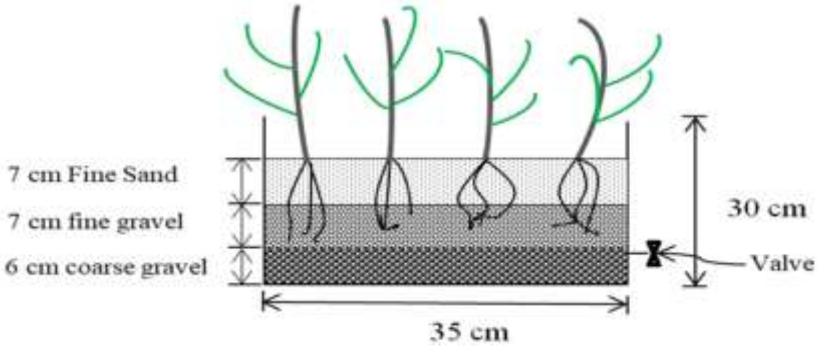

Figure 1. Schematic diagram for planting aquarium configuration

Plant selection Oleander is fast growing, has high biomass, long hairy roots, withstands temperature variation and transplanted easily into most soils. These plants were purchased from a nursery with an average height of 25m and of the same age where they are usually propagated by stem cuttings. Nine healthy oleander plants were randomly distributed in aquariums and grown under the same conditions of irrigation, soil components, temperature, and light. Plants were grown in a partially sunny area, where they were shaded using nursery net. The test continued from July to September

Preparation of synthetic wastewater

The test was conducted on a single exposure system using Cd as the contaminant at various concentrations. The Cd concentrations used in this study were 0 (control), 10, 25, 50, and 75 mg/L (hereafter denoted as C0, C10, C25, C50, and C75, respectively), prepared prior to the test using Cadmium nitrate $Cd(NO_3)_{2.}4H_2O$ (Tetenal photowerk GmbH, Germany) diluted in tap water. Five liters of simulate wastewater were added to each aquarium, and the level of simulated water in all aquariums was maintained constant at 2 cm below the soil surface to simulate subsurface flow(SSF) conditions in wetlands and water losses were compensated by adding tap water to avoid variation in HM concentrations due to evaporation and evapotranspiration.

Cd analysis

The toxicity test lasted for 65 days. Physical observation was conducted to observe the effect of Cd 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) (SensAA GBC Scientific Equipment, Malaysia). One plant was harvested on each sampling day (7, 14, 28, 49, 65 days) separated into (roots, stems, leaves) The Cd extraction from the plants was performed using wet digestion method (4). Soil samples were analyzed each (7, 14, 21, 28, 35, 49 and 65 days) to determine the accumulation of HM within soil media, total concentration of Cd in soil samples was determined using Hot aqua regia (3:1, v/v, HCl to HNO3) digestion procedure (12). The Cd levels in sand and plants were also examined by AAS.

Wet weight and dry weight

The overall health of plant growing in HM contaminated medium can be estimated throw wet weight and dry weight of the plant (10). Plant samples were harvested at the beginning and at the end of the observation. For wet weight measurements, plants were washed with tap water to remove any attached soil particles, the water then absorbed using tissue paper and weighted immediately using electrical balance (Sartorius ENTRIS 64i-1S, Germany). For dry weight (DW) measurements, Plant samples were placed in a drying oven (BINDER, Hotline International, Germany) set at 105◦C for a maximum of 2 hours and weighted.

Calculation of BAF, and TF

The plants potential in accumulating a given metal from contaminated substrate can be evaluated using BAF, it is estimated by using eq.1 (9):

$$BAF=\frac{\text{C plant}}{\text{C culture}} \qquad (1)$$

The amount of Cd transferred from roots to aerial parts is estimated using eq.2 (17):

$$TF=\frac{C\ shoot}{C\ root} \qquad (2)$$

RESULTS AND DISCUSSION

Cd concentration in water medium

The result of Cd concentration in water samples taken from the aquariums outlet shows that the concentrations of Cd decreased from the first week of the observation, (Figure 2). The concentration of Cd started to decrease from day-7, and become 0 mg/l on day-35 for exposure doses C10 and C25. At day 65, water samples taken from aquaria exposed to C50 and C75 exceeds the standard limits set for river conservation regulation No. 25 for the year 1967 and were found to be 0.14 and 0.91 mg/l, respectively.

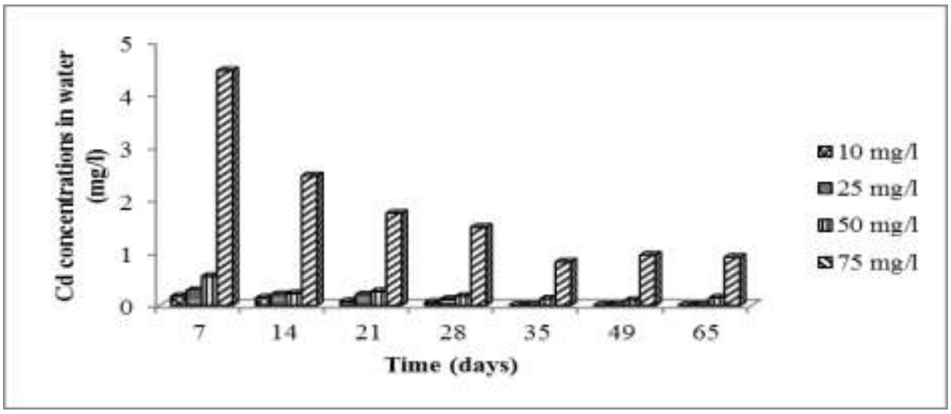

Figure 2. Cadmium concentration from effluent water

Cd concentration in plant

The Cd concentration in plant tissues increased with increasing initial concentration, which agrees with the results of other researchers (11, 20), who found that Cd concentration increased in the tissue of *Eichhornia crassipes* and *Vetiveria zizanioides*, respectively with increasing Cd concentration in solution. Cd concentrations in plant tissues increased for all exposure doses up to day-28 and decreased thereafter until the end of the test, (Figure 3). The highest Cd concentrations in plant were 65.5, 88.3, 98 and 429 mg/kg for C10, C 25, C50 and C75, respectively. For all exposure doses, most Cd concentration was higher in shoot than in roots. It had been reported by other researchers (7, 21), the shoots of *T. caerulescens* contains more Cd than roots. The physical appearances of the plants grown at each aquarium subjected to different Cd concentrations were observed for 65 days. Plants didn’t show any withering symptoms (dryness, yellowish, brownish, fall down) as shown in (Figures 4, 5). Plants use various cellular and molecular mechanisms to detoxify and hyperaccumulate HM in their tissues. Cd hyperaccumulation involves three main processes: adsorption, transportation, and translocation. Adsorption of Cd primarily occurs through the roots of the plant. Some factors like pH, humic acid, and medium are mainly responsible for effective absorption of Cd (14).

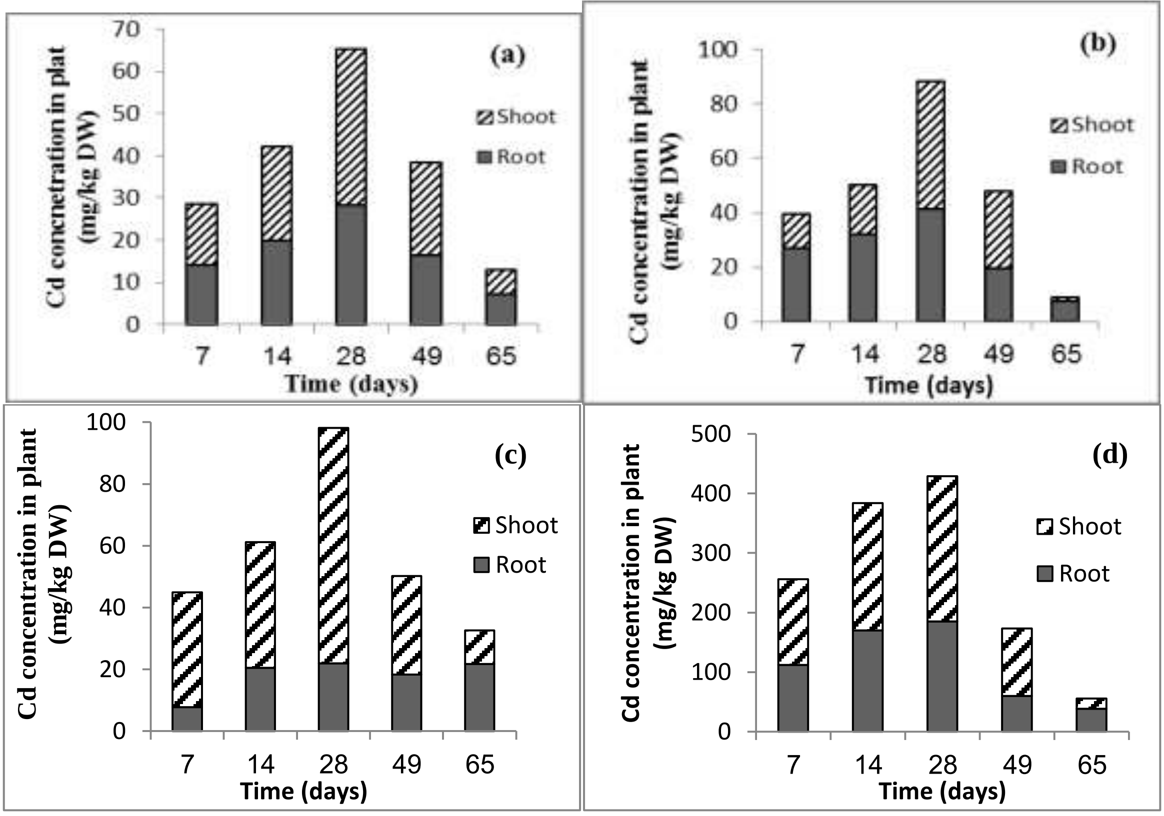

Figure 3. Cd concentrations in oleander (mg/kg) (a) C10 (b) C25 (c) C50 (d) C75

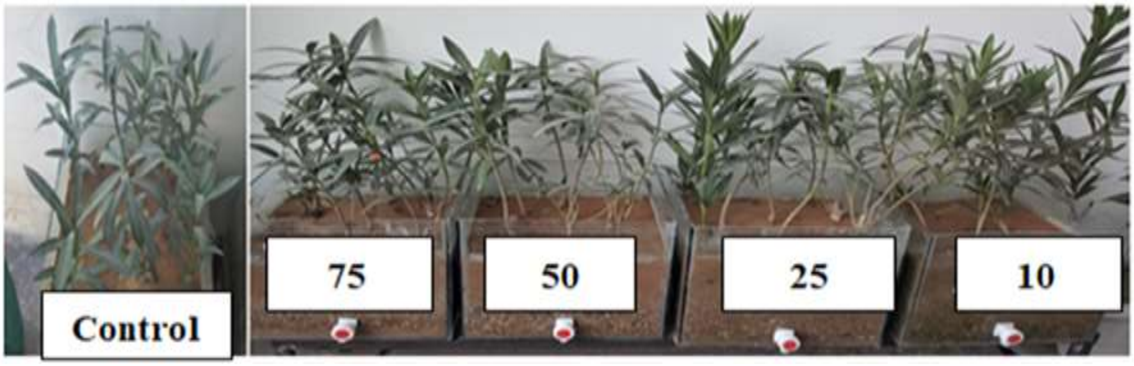

Figure 4. Plant observation at the beginning of the test

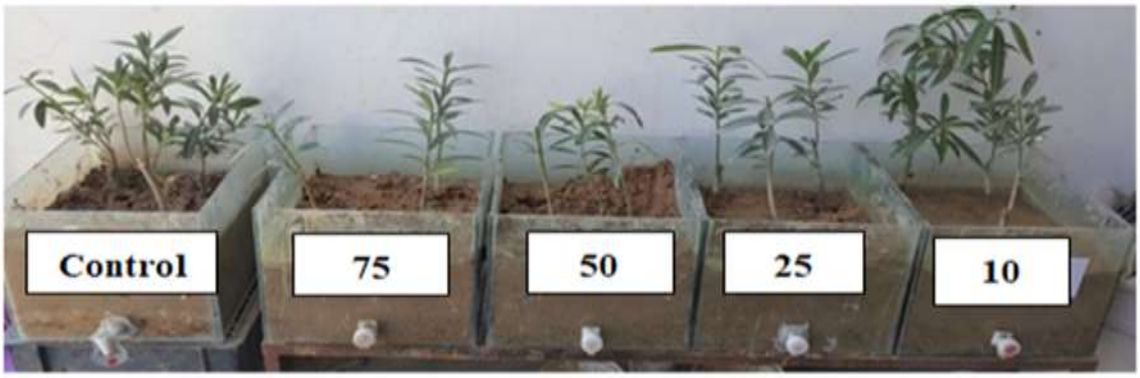

Figure 5. Plant observation at the end of the test

Cd concentration in soil medium

The results of Cd levels in the soil medium in mg/kg are shown in (Figure 6). The levels of HM in soil medium increased with increasing the initial doses for all aquariums. The results of soil analysis indicate that HM concentrations were high from the first week of the test which agrees with the findings of (23). Cd levels in soil medium increased until day-28 of observation for C10 and C25 and then gradually decreased until the end of the test. This may relate to plant uptake mechanism (23). Cd concentration in soil medium increased until day-35 for aquariums with C50 and decreased after that till the end of the test. This may also relate to plant uptake mechanism. For C75 mg/l, cd concentration was not consistent throughout the test, the highest Cd concentration was at 35-day. In general, the high level of HM in the soil medium can be attributed to the soil characteristics used in the study since the soil is gypsiferous, calcareous with high calcium carbonate content and pH, as well as, presence of clay content and to lesser extent organic matter (13).

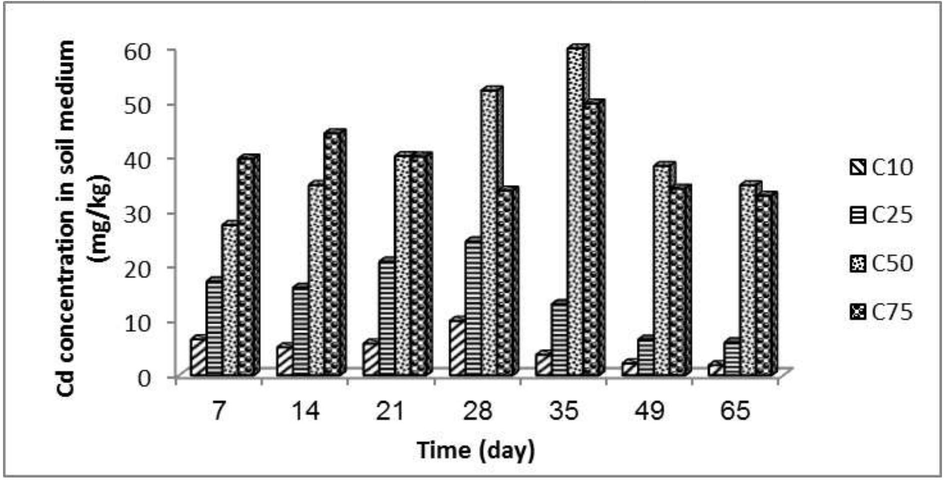

Figure 6. Cd concentration in soil medium

Plant wet weight and dry weight

The result of wet weight and dry weight measurements exhibit similar trends for all tests, (Figure 7). For all aquariums, oleander biomass decreased with increasing concentrations of Cd except for C10 where the plant shows an increase in wet weight and dry weight. Das and Goswami (12) found that the growth of *Pistia stratiotes* exposed to Cd concentrations (10, 20, and 30 mg/l) declined by increasing Cd concentration. These results also coincide with the results of other researchers (6) who found that higher doses of HM can reduce plant growth and dry biomass yield for sunflower plants subjected to different HM concentrations in simulated wastewater.

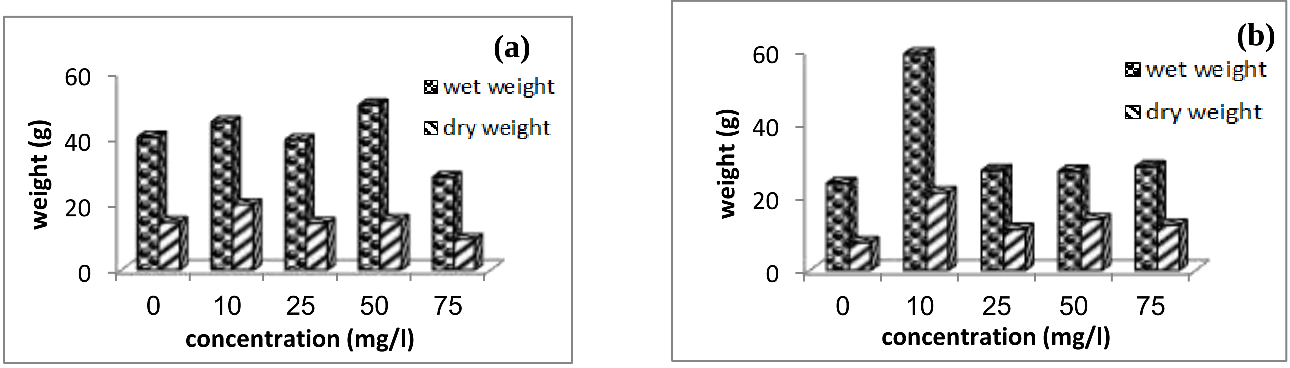

Figure 7. Wet weight and dry weight changes of oleander exposed to Cd (a) beginning of the test (b) 65 days

BAF of Cd in plant

The BCs values for most exposure doses were higher than one, except for C25, C50, C75 at the end of the test, and C50 for the day-7 (Table 1). The highest BC obtained for Cd in the present study is 6.55, 3.53, 1.96, and 5.75 for C10, 25, C50, and C75, respectively. The BC values greater than one, indicates that the oleander accumulate Cd efficiently. The differences in Cd uptake by plant for all exposure doses resulted in different BAFs values. Also, the depletion of Cd concentration in the solution -due to plant uptake- resulted in different BAFs values, in addition to physiological and biochemical factors associated with the plant. This results is similar with the study done by other researchers (3, 18, 24) in hydroponic cultures who found that water hyacinth, finger Rush, sunflower, ricinus, alfalfa, and mustard had high bioaccumulation factor (BAF) values for Cd from wastewater

Table 1. Bioaccumulation factor (BAF) of oleander

Concentration	Day-7	Day-14	Day-28	Day-49	Day-65
C10	**2.86**	**4.22**	**6.55**	**3.84**	**1.30**
C25	**1.59**	**2.01**	**3.53**	**1.92**	**0.36**
C50	**0.90**	**1.22**	**1.96**	**1.00**	**0.65**
C75	**3.41**	**5.12**	**5.72**	**2.31**	**0.74**

Translocation of Cd in oleander (TF)

TF values of Cd in N.oleander are shown in (Table 2). The TF values were greater than one starting from day-7 onwards, except for C25 for the the at 7-day and 14-day observation.These results of TF indicates that oleander can behave as a hyperaccumulator plant (2). The TF value also gave different trends for each treatment. For all aquariums, the TF values decreased at the end of the test (day-65). Higher TF values for C10 and C75 was at day-49, while C25 was at Day-49 and C50 was at day-7. The fluctuation of TF was caused by the difference of Cd concentration in root and shoot that depends on the biochemical and physiological factors contributing to heavy metal accumulation and distribution in the upper parts of the plant (22). The increased TF demonstrates the ability of the plant species to tolerate and translocate heavy metal to shoots, suggesting that the plant had stronger tolerance to heavy metal and a good potential for accumulating Cd efficiently until Day-49. TF values greater than one give evidence that high Cd was accumulated in the plant shoot, showing that oleander has a potential to hyperaccumulate Cd.

Table 2. Translocation factor of oleander

concentrations	Day-7	Day-14	Day-28	Day-49	Day-65
C10	1.002	1.11	1.30	1.34	0.78
C25	0.48	0.55	1.13	1.42	0.17
C50	4.77	1.99	3.46	1.74	0.50
C75	1.29	1.26	1.32	1.88	0.44

High concentrations of Cd significantly affected wet weight and dry weight; however, toxicity symptoms from visual observation of plant have not been noticed. It was clearly observed that oleander has the ability to tolerate high concentration of Cd concentration in water. The concentrations of HM in soil medium were high from the first week of the test which could be related to soil characteristics. The value of BAF and TF were fluctuated based on Cd concentration adsorbed by root and the concentration of Cd in the lower part and upper part of the plant.

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