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Responses of *Lantana Camara* Linn. Callus Cultures to Heavy Metals Added to the Culture Media

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Abstract

Heavy metals represent a growing threat for ecosystems worldwide. In Jordan, several studies have searched the amounts of heavy metals in soils at roadsides of high ways and soils of litter dumps. The conducted studies found that these soils contained worrying levels of heavy metals that have exceeded in many cases the average world safe limit. For example, lead (Pb) level was 79 mg/ kg in soils closed to some highways while the world safe limit is only (25 mg/kg). In other studies, cadmium (Cd) concentration was (5.9 mg/kg) while the world safe limit for Cd is only (0.03 mg/kg). Also in some litter dumps in Jordan, soil chromium (Cr) average content was (6.9 mg/kg) while the world safe limit was only (0.1 mg/kg). *Lantana camara* Linn. is a flowering plant that has recently attracted the attention of researchers due to its novel phytoremediation powers against some heavy metals, which encouraged some countries to grow them in roadsides of highways and litter dumps. Tissue culture is an excellent approach for studying responses of *Lantana camara* to heavy metals without interference of other factors. In addition, tissue culture is the main method for producing elite plants lines from callus and cell suspension cultures with superior characteristics by genetic transformation. In this study, callus cultures were induced from leaf discs *in vitro.* Then, responses of *Lantana camara* callus cultures to different concentrations (0.0, 0.12, 0.2, 0.3 mg/L) of Cr, Pb, or Cd were monitored under *in vitro* environmentally controlled conditions to exclude the interference of any other factor. The obtained results revealed that callus growth and quality were found to decrease in response to adding heavy metals to the media at all concentrations. Meanwhile, all callus cultures recorded full survival rates (100%) by the end of the experiments and resumed growth after being transferred to normal growth conditions. Amounts of the three experimented heavy metals (Cr, Pb, and Cd) were found to reach the maximum in callus cultures (0.096, 0.109, 0.0193 mg/ kg), when pregrown on Murashige and Skoog media (MS media) supplemented with (0.3 mg/L), while maximum Biological Absorption Coefficient (BAC) values of (0.41, 0.6, 0.30) were recorded in callus cultures pregrown in media with (0.12 mg/L) of either Cr, Pb, or Cd, respectively. More work is still needed to improve BAC values obtained in *Lantana camara* callus cultures. Also, the produced callus cultures can be used in future research for preparing cell suspension cultures to be introduced to genetic transformation to produce *Lantana camara* plant lines with hyper accumulation powers against these contaminants.

Key words: *Lantana camara* Linn., Callus, *In vitro*, Heavy metals..

1. Introduction

Heavy metals act as natural components in the earth's crust (Laghlimi *et al*., 2015). Meanwhile, due to the anthropogenic activities such as industrial effluents, fuel production, mining, and agricultural chemicals, heavy metals are presented nowadays as a major source of toxicity that threatens the health of more than 10 million people in many countries (Jadia and Fulekar, 2009).The problem with heavy metals is due to the fact that these elements cannot be degraded into non -toxic forms, so they would remain in the ecosystem for many years (Jabeen *et al*., 2009, Waoo *et al.*, 2014, Waoo *et al*., 2015,). Cd, Cr, Hg and Pb present the most common category of heavy metals, due to their presence in the in the ecosystems and bioaccumulation in the food chain, which made them extremely dangerous to most biological systems (Jadia and Fulekar, 2009). In Jordan, several studies were conducted to investigate soil content of these contaminants in side roads of high ways and litter dumps. For example, El-Radaideh *et al.* (2018) found that Cr, Pb and Cd reached levels of 16, 11, 79 mg/kg respectively along Irbid-North Shooneh highway which are higher than world safe limit of each element (0.1, 5.9, 25.0 mg/kg, respectively). This was in agreement with Howari *et al.* (2004) who reported that soils along North Shuna-– Aqaba Highway contained 5 mg/kg Cd and 79 mg/kg Pb. Additionally, Mashal *et al*. (2017) found that soils of roadsides of different sites of Al Hashmiyya City contained heavy metals (Pb, Zn, Cr and Cu) above threshold levels. Moreover, Daabes *et al*. (2013) reported in their study about soils of three litter dumps in

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Jordan (Ghabawi, Akaider and Russeifah) that the average concentrations of Cr (6.5 mg/ kg) and Cd (4.5 mg/ kg) were high and exceeded local and international standard limits.

Phytoremediation is a process where green plants remove pollutants from the environment before transforming them into biological forms (Doran, 2009). Via phytoremediation, pollutants are up taken by the plants before being translocated, and stored within plant biological systems (Subhashini, and Swamy 2013). Meanwhile, plant species differ in their phytoremediation potentials according to their root depth, type of contaminants, and regional climate (Laghlimi *et al*., 2015).

For many years, studies were conducted in field or *in vivo* to investigate plant responses under environments contaminated with heavy metals, which was encountered by environmental, microflora and translocation barriers that made such studies very difficult (Doran, 2009). For this reason, plant tissue culture was presented as a reasonable and complementary approach for such studies, as via tissue culture most obstacles that might encounter such research types are eliminated, which would promote a deeper understanding for the mechanism of how plant cells detoxify such pollutants (Doran, 2009). Additionally, via *in vitro* techniques including callus and cell suspension cultures, it is possible to study genes responsible for their ability of accumulating heavy metals and to produce biotransformed plants with super phytoremediation powers that would present a revolutionary solution for heavy metals management strategies.

Lantana camara Linn. (also known as Wild Sage, Surinam Tea Plant and Spanish flag) is a widely spread flowering ornamental plant, and is a member of Verbenaceae family (Kalita *et al.,* 2012). Besides its aesthetic importance, *Lantana camara* Linn. has recently attracted the attention of researchers due to its novel phytoremediation powers against many types of heavy metals (Jusselme *et al.,* 2012; Mkumbo *et al.,* 2012; Waoo *et al.,* 2014; Waoo *et al.,* 2015). For example, in Mkumbo *et al.,* (2012) study, phytoremediation potential was investigated in *L. camara* plants grown in soil artificially contaminated soil with 500 or 1000 mg of Pb mg/ kg soil, where *Lantana camara* was found to be very promising as it was able to uptake 10% of 1000 mg/ kg of Pb per year. Additionally , Waoo *et al*. (2014) confirmed phytoremediation potential of *Lantana camara* plants collected from polluted areas in India, as these plants were found to be able to accumulate and tolerate high concentrations of different heavy metals types (Cd, Ni, Cr and Pb), mainly in their leaves and shoots. Moreover, Waoo *et al*. (2015) went more deep in their phytoremediation investigations about this plant, as they studied responses of *Lantana camara* microplants grown under *in vitro* conditions in MS medium supplemented with different concentrations of Cr, and they found that the microplants were able to maintain high survival rates in MS media up to 35 mg/L Chromium, while their survival and growth decreased drastically at higher concentrations. However, Waoo *et al*. (2015) had used only microplants as plant material in their research, and didn't measure the amount of Cr that had actually accumulated inside the *in vitro* grown microplants of *Lantana camara*.

From this prospective, our study aimed to investigate responses of *Lantana camara* callus cultures to addition of Pb, Cr and Cd to the culture media. Moreover, for our knowledge this is the first research that uses callus cultures as a source of plant material for studying *Lantana camara*. responses to heavy metals, hopping that this approach would be a platform for future genetic and biotransformation studies at cell concentration that might produce *Lantana camara* plants with super accumulation powers.

2. Materials and Methods

2.1. In vitro establishment of plant material

Vegetative buds of *Lantana camara* Linn. were collected from a single plant growing at University of Jordan campus (Amman, Jordan). Buds were surface sterilized under running tap water before being soaked in tap water supplemented with few drops of a commercial detergent in addition to (1.0 g) of a fungicide (Benomyle) for 15 min. Next, the buds were washed again under running tap water and then soaked in 40% sodium hypochlorite for 20 min under the laminar air flow cabinet. Then, buds were rinsed with sterile distilled water and soaked in 70% ethanol (v/v) for 30 seconds before being rinsed again with sterile distilled water for three times. The buds were then inoculated in 4.4 g/L of MS (Murashige and Skoog, 1962) media premix (Sigma Aldrich Murashige and Skoog basal salt mixture) plus 1 ml\L MS vitamin mixture (Sigma Aldrich Murashige and Skoog Vitamin Powder 1000X) in addition to 0.1 M sucrose and 8.0 g/L bacto agar. The cultures were then kept under growth room consisting of a daily regime of 24±1 ℃ and 16/8 (light/dark) photoperiod of 45–50 μmol $m^{-2}s^{-1}$ irradiance. Subculturing was performed every four weeks to fresh MS media till enough plant material was obtained for the experiments.

2.2. Callus Establishment:

Callus was induced from leaf discs excised from the *in vitro* grown microshoots of *Lantana camara* Linn (5.0 mm diameter). The leaf discs were sub-cultured into Petri dishes containing solid MS media supplemented with either of (0.0, 1.0, 2.0 mg/ L) thidiazuron (TDZ) or 2,4-dichlorophenoxy acetic acid (2,4-D). Each treatment consisted of 10 replications with 2 leaf discs/ replicate. Cultures were then maintained under complete dark condition at 24±1 ℃. Data on callus formation and development were collected after two months including; callusing percentage, callus fresh weight, color and texture.

2.3. Callus Multiplication

Induced calli were transferred into Petri dishes containing fresh hormone free MS solid media for 5 days under dark to remove any hormonal carryover effect. Then, calli (0.7 mg) were transferred into MS media + 0.1 M sucrose and 2.0 mg/L 2,4-D (2,4-dichlorophenoxy acetic acid) in combination with different concentrations (0.5, 1.0 mg/L) of either Kinetin or Benzylaminopurine (BAP). The control treatment consisted of callus establishment media that yielded best callus establishment results in section (2.2) which consisted of a solid MS media plus 0.1 M sucrose and (2.0 mg/ L) 2,4-D. Each treatment consisted of 10 replications with 2 calli/ replicate. Data was collected after one month for callus fresh weight, color and texture.

2.4. Heavy Metals Experiments

Before heavy metals experiments, calli were transferred into fresh hormone free MS solid media for 5 days under dark to remove any hormonal carryover effect as described earlier in section 2.3. Next, callus multiplication media (control) that yielded best callus multiplication results in section (2.3) [MS media supplemented + 0.1 M sucrose + 2.0 mg/L 2, 4-D + 1.0 mg/L Kinetin] was prepared and poured into 250 ml glass bottles before being autoclaved. After media sterilization, each heavy metal type (chromium (Cr), lead (Pb) and cadmium (Cd) at concentrations of (0.0, 0.12, 0.2, 0.3 mg/L) was infiltered aseptically into each media bottle, under continuous stirring before being poured into sterile Petri dishes. Next, calli (0.7 mg) were transferred into each treatment with ten replications/ treatment with two calli/ replicate. Data was collected after one month for callus fresh weight, color and texture. Also, samples of calli (0.7 mg) were transferred into to the control multiplication media with ten replications/ treatment and two calli/ replicate, to see if there were any carry over effect on callus growth resulting from preexposure to the experimented heavy metals, and data for fresh weight was taken after one month.

For heavy metals analysis, acid digestion of dried calli was carried out according to Jones (1984), where 20 samples were taken from each treatment, and 0.5g of each callus sample was heated with 5 ml of 70% HNO3 and 1.5 ml of 60% HClO4 until the brown fumes disappeared. Next, the solution was cooed down and 1:1 diluted with 5ml HCl (density 1.18 g/ml). The diluted solution was then filtered and diluted with distilled water up to 25ml.

Determination of Cr, Cd and Pb were done by Inductively Coup lead Plasma–Optical Emission Spectrometry (ICP-OES) equipped with 40.68 MHz operating frequency generator, 1,800 L/mm t67 holographic grating which allows a wide range analysis from 160–800 nm and up to 6 pm resolution. This method meets the EN655011, IEC801-2, IEC801-3 and IEC801-4 EMC standards. The minimal detection limits and the R^2 for standard curves are listed in the Table 1. Then, data was recorded for concentration of each heavy metal absorbed in the callus cultures, while plant ability to uptake heavy metals from the media (Biological Absorption Coefficient (BAC) was determined as a ratio of element concentration in callus to element concentration in the growth media (Cheraghi *et al*., 2011).

Table 1. The minimal detection limits and the R^2 for standard curves of Cr, Pb, and Cd using Inductively Coupleadlead Plasma–Optical Emission Spectrometry (ICP-OES) technique

Element	Detection limit (ppb)	R^2
Cr	2	0.9918
Cd	0.5	0.9996
Pb	10	0.9980

2.5. Experimental Design

Treatments of the experiments were arranged in a completely randomized design (CRD) and each treatment was replicated ten times with 2 explant/ replicate.

Data recorded from each treatment was analyzed separately according to the analysis of variance (ANOVA) using (SPSS, version 17) analysis system. Means were separated and standard errors (SE) were extracted according to the Tukey's HSD test (Honest Significant Difference 22T) at a probability concentration of 0.05.

3. Results and Discussion

3.1. Callus Establishment

The obtained results indicated that, callus establishment was a function of growth regulator type and concentration in the culture media. In leaf discs treated with TDZ under complete dark conditions, callusing responded positively to increasing concentration of TDZ to reach maximum callusing rate (70%) and fresh weight (1.465 g) at concentration of 2.00 mg/L (Table 2). Meanwhile, the quality of the resulted callus was poor, as the callus was brown with a hard texture (Table 2). On the other hand, using 2,4-D, as a source of growth regulator enhanced all callus induction parameters (Table 2, Figure 1 B). Full callusing rate and maximum fresh weight (2.05 g) were obtained at concentration of 2.00 mg/L 2, 4-D under complete dark conditions (Table 2, Figure 1B). This was a combined with high quality of white friable callus. Successful callus induction from leaf discs and shoot tips of *Lantana camara* L. was reported by (Saxena *et al.*,2013), where greenish compacted callus was obtained when the explants were treated with 0.02 mg/L 2, 4-D under light. Also, Veraplakorn (2016) was able to induce callusing from *Lantana camara* leaf discs using 40.0 µmol/L of 1-naphthalene acetic acid (NAA) combined with 40.0 µmol/L BA under light. So, our approach for successful callusing in *Lantana camara* Linn. leaf discs was completely different from the previous study, as 2.00 mg/L 2,4-D was used for callus induction under complete dark conditions.

Table 2: Effect of growth regulator type and concentration on *in vitro* establishment of *Lantana camara* Linn. callus cultures from leaf discs under complete dark conditions.

Concentration (mg/L)	Callus %	Fresh weight (g)	Color	Texture
Thidiazuron (TDZ)				
C* (0.0)	00.0±0.0	00.0±0.0 c	-	-
1.0	31.25±4.762	0.65±0.092 b	Brown	Hard
2.0	70±2.961	1.465±0.033 a	Brown	Hard
(2,4-D)				
C (0.0)	00.0±0.0	00.0±0.0 c	-	-
1.0	75±4.051	1.70±0.041 b	White	Friable
2.0	100.0±0.0	2.050.033 a	White	Friable

*C: represents control treatment (solid hormone free MS media+ 0.1 M sucrose). Values represent means ± standard error. Data for each growth regulator was statistically analyzed separately. Means within columns for each growth regulator having different letters are significantly different according to Tukey HSD at P≤0.05.

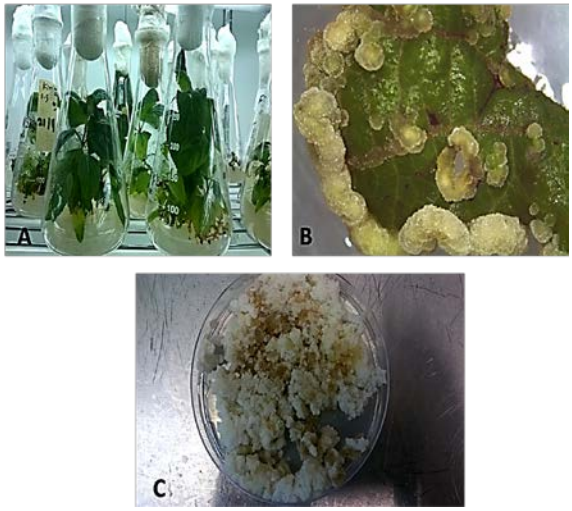

Figure 1. *In vitro* grown *Lantana camara* Linn. A: Microshoots grown in hormone free MS media + 0 .1 M sucrose. B: Establishment of callus cultures from leaves discs in MS media + 2.0 mg/L 2,4- D + 0 .1 M sucrose. C: Callus multiplication in MS media + 2.0 mg/L 2,4-D + 1.0 mg/L Kinetin + 0 .1 M sucrose.

3.2. Callus growth

A tremendous increase in callus fresh weight was obtained on media supplemented with kinetin in combination with 2.0 mg/L 2, 4-D compared to those grown in either the control or BAP treatments (Table 3, Figure 1C). Also, our results revealed that the increase in fresh weight responded positively to the increase in kinetin concentration in the media to reach a maximum weight of (5.92 g) at kinetin concentration of 1.0 mg/L (Table 3). Moreover, quality of the resulted callus was excellent in kinetin treatment at all concentrations, as white friable callus was obtained, while callus turned brown when exposed to BAP treatments (Table 3, Figure 1C). Our results were in complete harmony with those reported by Raj *et al.* (2015) and Slazak *et al.* 2015), as kinetin in combination with 2,4-D were found to be excellent formula for callus growth in *Securinega suffruticosa* and *Viola uliginosa*. On the other hand, in Veraplakorn (2016) study, callus multiplication was best in *Lantana camara* L. when cultured on MS media supplemented with 40.0 µmol/L (NAA) + 40.0 µmol/L BA under light. Meanwhile, in another related study, Zatimeh *et al*. (2017) tried different hormones in combination with 2.0 mg/L 2, 4-D for multiplication of *Peganum harmala* L. callus cultures under dark conditions, where they found that (1.5 mg/L) TDZ in combination with 2.0 mg/L 2, 4-D was a great prescription for maximum callus diameter and fresh weight (3.98 cm, 2.88 g). This might lead to a conclusion that, despite using 2.0 mg/L 2, 4-D in combination with different hormones for callus multiplication, the response of callus cultures to these combinations is plant type dependent.

Table 3: Effect of growth regulator type and concentration in combination with 2,4-D on growth and quality of *Lantana camara* Linn. callus cultures.

Concentration (mg/L)	Fresh weight (g)	Color	Texture
Kinetin			
C*	2.03± 0.0194 c	White	Friable
0.5	4.65± 0.0133 b	White	Friable
1.0	5.92 ± 0.119 a	White	Friable
Benzylaminopurine (BAP)			
C	2.03 ±0 .019 b	White	Friable
0.5	2.21 ± 0.125 b	Brown	Friable
1.0	3.14 ± 0.179 a	Brown	Friable

*C: represents control treatment (solid MS media+ 0.1 M sucrose + 2, 2,4-D). Values represent means ± standard error. Data for each growth regulator was statistically analyzed separately. Means within columns for each growth regulator having different letters are significantly different according to Tukey HSD at P≤0.05.

3.3. Effect of Heavy Metals

3.3.1. Effect of Chromium

The obtained data revealed that growing callus cultures in media supplemented with Chromium (Cr) for one month had a negative impact on callus growth and quality. A significant decrease in callus fresh weight was recorded as Cr concentration increased in the media to reach a minimum record of (2.13 g) at Cr concentration of (0.3 mg/L) (Table 4). This was a combined with a decline in color quality, as cultures turned creamy compared with the whitish healthy callus obtained in the control treatment (Table 4, Figure 2A, B). Cr was reported to have inhibitory influence on plant growth even at low rates (Sundaramoorthy and Ganesh, 2015; Waoo *et al*., 2015). This was attributed to the fact that Cr at high rates, like any other heavy metal, would interfere with metabolic reactions in plants and would lead to growth retardation or death (Schmidt 2003; Jadia and Fulekar 2009). Moreover, Waoo et al.,(2015) found that adding Cr in a form of Chromium Sulphate (CrSO4) to the culture media had significantly decreased the shoot length and quality of *Lantana camara in vitro* grown plantlets, while survival was drastically declined when (CrSO4) concentration exceeded 35 mg/L.

Meanwhile, full survival rates were obtained by the end of Cr experiment, and most callus cultures had recovered from the side effects of Cr one month after being subcultured into fresh control media, as an increase in fresh weight was recorded in all cultures (Table 4). This might indicate that *Lantana camara* L. callus cultures have the ability to withstand living under such contaminated environment without altering their survival and regrowth potentials. Moreover, Cr was detected in the dried callus cultures exposed to Cr at all concentrations and the maximum amount of Cr (0.069 mg/kg) was detected in cultures exposed to (0.3 mg/L) Cr (Table 4), which indicated that callus cultures were found to be able to accumulate Cr from chromium contaminated media without any losses in their survival rates (Table 4). Meanwhile, the biological absorption coefficient (BAC) decreased with increasing Cr concentration in the media to reach a minimum value of (0.23) in cultures pre-exposed to (0.3 mg/L) Cr compared to (0.41) recorded in callus

pretreated with (0.12 mg/L) Cr (Table 4), which implies that callus ability to uptake Cr from the media became more weak as Cr concentration increased in the culture media (Table 4). This would act as a defense mechanism adopted by living cells of plants growing in environment where high concentrations of heavy metals exist to prevent absorption of high amounts of heavy metals that would enable the plant to avoid exposure to toxicity hazard or death. Meanwhile, according to Mkumbo *et al.* (2012), a plant with (BAC value > 1) is described as suitable for phytoextraction of heavy metals from soil, but this applies only at whole plant concentration and not at concentration of callus cultures as it is the case in our study.

Table 4. Effect of Chromium (Cr) concentrations on fresh weight, color and texture of *Lantana camara* Linn. callus cultures, in addition to phytoremediation potential of the plant material.

Concentration (mg/L)	FW1(g)*	Color	Texture	FW2(g)	Cr^Y concentration (mg/ kg dry weight)	BAC^Z
C *	5.86±0.081 a	White	Friable	5.94±0.072 a	-	-
0.12	3.58 ± 0.065 b	Creamy	Friable	4.82±0.151 b	0.0493± 0.012 b	0.41± 0. 051 a
0.2	2.60±0.058 c	Creamy	Friable	4.39±0.091 c	0.0687± 0.018 a	0.34± 0.016 b
0.3	2.13±0.044 e	Creamy	Friable	3.78 ±0.073 d	0.06948± 0.022 a	0.23± 0.012 c

*C: represents control treatment (solid MS media+ 0.1 M sucrose +1 mg/L kinetin + 2 mg/L 2,4-D). FW1: callus fresh weight recorded after growth for one month in media supplemented with each type and concentration of heavy metal. FW2: callus fresh weight recorded one month after being subcultured into control media. Y: concentration of the heavy metal in the callus cultures in dry weight bases. Z: BAC represents the Biological Absorption Coefficient. Values represent means ± standard error. Means within columns for each heavy metal type having different letters are significantly different according to Tukey HSD at P≤0.05.

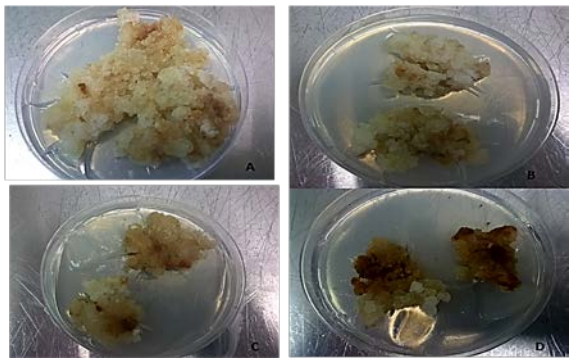

Figure 2. Effect of heavy metals on growth and quality of *Lantana camara* L. callus cultures grown in either of A: control, B: 0.3 mg/L Cr, C: 0.3 mg/L Pb or D: 0.3 mg/L Cd.

3.3.2. Effect of Lead

Adding lead to the culture media resulted in a drastic decline in callus fresh weight and quality. The lowest fresh weight was recorded in callus exposed to (0.3 mg/L) Pb, while red spots appeared in all callus clumps treated with Pb at all concentrations (Table 5, Figure 2C). Although Pb is described as nonessential element in most vital biological reactions in plant cells, still it is reported as hazardous or lethal to most livings even when absorbed at very small amounts (Boonyapookana *et al*. (2005). At plant concentration, Pb was found to be responsible for necrosis, growth stunting and reduction in biomass production in sunflower, tobacco, and vetiver plants grown in hydroponic environment supplemented with different concentrations of Pb (Boonyapookana *et al*. (2005), which agrees with our results. Meanwhile, full survival rates were recorded in all callus cultures of our study, and they resumed growth after being subcultured into lead free fresh media but at a lower extent in those exposed to either (0.2 or 0.3 mg/L) Pb (Table 5). Moreover, amount of Pb detected in callus cultures increased with increasing Pb concentration in the media to reach the maximum (0.1095 mg/ kg) at Pb concentration of (0.3 mg/L). On the other hand, BAC values declined with increasing Pb concentration to reach the minimum (0.37) at (0.3 mg/L) Pb compared to (0.60) BAC recorded in cultures pretreated with (0.12 mg/L) Pb (Table 5). In another related study, Mkumbo *et al.* (2012) assessed phytoextraction potential of several plant species *in vivo* against Pb, where they found that *Lantana camara* seedlings recorded low BAC (0.4) compared to values recorded in *Tephrosia candida* and *Tagetes minuta (L.)* (2.55 and 1.0) respectively, which made these two species most suitable for phytoextraction of Pb in the gold mining areas of Tanzania. However, BAC value (0.4) recorded in *Lantana camara* seedlings grown in Pb polluted soil (318.9 mg/kg dry weight) in Mkumbo *et al*. (2012) study was so close to BAC (0.45) obtained in our study when callus was pregrown in media supplemented with (0.2 mg/L) Pb.

Table 5. Effect of Lead (Pb) concentrations on fresh weight, color and texture of *Lantana camara* Lin. callus cultures, in addition to phytoremidation potential of the plant material.

Concentration (mg/L)	FW1(g)	Color	Texture	FW2(g)	Pb^Y concentration mg/kg dry weight)	BAC^Z
C *	5.86±0.081 a	White	Friable	5.94±0.072 a	-	-
0.12	2.72 ± 0.083 b	Reddish white	Friable	3.97 ± 0.086 b	0.0716± 0.011 c	0.60± 0.016 a
0.2	1.97 ± 0.084 c	Reddish white	Friable	3.25 ± 0.081 c	0.0895± 0.013 b	0.45± 0.01 bc
0.3	1.83 ± 0.069 c	Reddish white	Friable	2.93 ± 0.099 c	0.1095± 0.015 a	0.37± 0.021 c

*C: represents control treatment (solid MS media+ 0.1 M sucrose +1 mg/L kinetin + 2 mg/L 2,4-D). FW1: callus fresh weight recorded after growth for one month in media supplemented with each type and concentration of heavy metal. FW2: callus fresh weight recorded one month after being subcultured into control media. Y: concentration of the heavy metal in the callus cultures in dry weight bases. Z: BAC represents the Biological Absorption Coefficient. Values represent means ± standard error. Means within columns for each heavy metal type having different letters are significantly different according to Tukey HSD at P≤0.05.

3.3.3. Effect of Cadmium

A significant decline in callus fresh weight was recorded in response to Cadmium addition to the media at all concentrations (Table 6). However, the decline in fresh weight was most severe at Cd concentration of (0.3 mg/L) where callus color turned brown (Table 6, Figure 2D). Meanwhile, all callus cultures were back to grow normally when transferred to normal heavy metal free culture media, but cultures precultured in either (0.2 or 0.3 mg/L) Cd supplemented media were still suffering from the negative impacts of prexposure to Cd (Table 6). Toxic concentrations of Cadmium were reported to interfere with most primary biological processes such as, photosynthesis, carbohydrate metabolism, in addition to enzymatic activities that would either decline growth or cause plant death (Javed and Greger, 2011). Moreover, Cd was not detected in callus dried samples at concentration of (0.12 mg/L), while the highest records for Cd concentrations (0.0913 mg/ kg) and BAC values (0.30) were found in callus pregrown in (0.3 mg/L) Cd supplemented media. In another related study about evaluation of phytoremediation potential in several plant species grown in Cd contaminated soil, it was found that the highest values for bioconcentration factor (BCF: concentration of heavy metal in shoots / concentration of heavy metal in soil) in French marigold, Impatiens, Garden verbena, and Scarlet sage plants exceeded (1) and ranged from 1.75 to 5.68, which made these plants hyper accumulators for Cd (Lin *et al*., 2010)

Table 6. Effect of Cadmium (Cd) concentrations on fresh weight, color and texture of *Lantana camara* Lin. callus cultures, in addition to phytoremediation potential of the plant material.

Concentration (mg/L)	FW1(g)	Color	Texture	FW2(g)	Cd^Y concentration (mg/ kg dry weight)	BAC^Z
C*	5.86±0.081 a	White	Friable	5.94±0.073 a	-	-
0.12	3.07 ± 0.075 b	Creamy	Friable	4.37 ± 0.081 b	-	-
0.2	2.43 ± 0.066 c	Brown	Friable	3.82 ± 0.055 c	0.0587± 0.023 b	0.29± 0.028 a
0.3	1.79 ± 0.032 e	Brown	Friable	3.61 ± 0.066 c	0.0913± 0.011 a	0.30± 0.012 a

*C: represents control treatment (solid MS media+ 0.1 M sucrose +1 mg/L kinetin + 2 mg/L 2,4-D). FW1: callus fresh weight recorded after growth for one month in media supplemented with each type and concentration of heavy metal. FW2: callus fresh weight recorded one month after being subcultured into control media. Y: concentration of the heavy metal in the callus cultures in dry weight bases. Z: BAC represents the Biological Absorption Coefficient. Values represent means ± standard error. Means within columns for each heavy metal type having different letters are significantly different according to Tukey HSD at $P \leq 0.05$.

4. Conclusions

Callus induction and multiplication were obtained successfully from *Lantana camara* L. *in vitro* grown leaf disc. Successful callus induction would facilitate using this callus as a source of cells which can be used in future studies to study and to specify genes responsible for phyto remediation powers in *Lantana camara* plants and to produce genetically transformed cell lines with super accumulation capabilities. Also, the obtained results showed that, although exposing callus cultures to different types and concentrations of heavy metals (Cr, Pb, and Cd) had negatively affected callus growth and color, callus cultures were able to withstand the exposure of heavy metals at all concentrations, as they maintained their survival potential and resumed growth after being subcultured into normal growth media. Also, callus cultures were able to absorb and accumulate different amounts of the experimented heavy metals types from the culture media at all concentrations. This indicates that survival and accumulation powers of *Lantana camara* delicate callus cultures against the tested heavy metals can be introduced to further studies to be improved if proper *in vitro* breeding protocols are applied. However, more work is still needed to test the response of *Lantana camara* callus cultures to higher and even toxic concentrations of each heavy metal and to find ways to improve BAC values obtained in *Lantana camara* L. callus cultures. This can be achieved by means of plant genetic biotransformation, where callus can be used as a raw material for massive production of elite hyperaccumulators against such annoying contaminants.

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