

***In vivo and in vitro* studies on heavy metal tolerance in
Sesbania grandiflora L.**

***Sesbania grandiflora* L. للمعادن الثقيلة خارج وداخل الجسم الحي
دراسات عن تحمل نبات السيسبان**

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Abstract

Several experiments were carried out to study heavy metal tolerance in tissue cultures or whole plants of *S. grandiflora*., Callus was induced and maintained on modified Murashige and Skoog, 1962 medium (MS) supplemented with (0.5)mg/l benzyl adenine and (2)mg/l 2,4-phenoxy acetic acid . Heavy metals (Cd, Co, Cu, Cr or Zn) were added to the culture medium at different concentrations as contamination agents. In order to asses the effect of these heavy metals on seed germination; seeds were sown in soil contaminated with different concentrations of heavy metals for 3 weeks. Atomic Absorption Spectrophotometer was used for analysis of samples taken from whole plants and callus cultures. Results showed that callus fresh weight decreased with increasing heavy metal concentration in cultural medium. Germination percentages and plant heights increased over time. However, a reduction occurred in these parameters with increasing heavy metal levels. Percentages of metals accumulated in calli were (0.001, 0.011, 0.012 and 0.013%) at (0.0, 0.05, 0.075 and 0.1)mg/l Cd respectively; (0.001, 0.008, 0.016 and 0.006%) at (0.0, 0.1, 0.25 and 0.5)mg/l Co respectively; (0.001, 0.020, 0.034 and 0.015%) at (0.0, 0.075, 0.2 and 0.5)mg/l Cu respectively; (0.001, 0.013, 0.012 and 0.010%) at (0.0, 0.25, 0.4 and 0.5)mg/l Cr respectively and (0.027, 0.051, 0.059 and 0.056%) at (0.0 , 0.75, 1.0 and 1.5)mg/l Zn respectively. Percentages of metals accumulated in whole plants were (0.08, 0.55, 1.11, 0.83 and 0.44%) at (0.0, 1.0, 2.0, 3.0 and 4.0)mg/Kg soil Cd respectively; (0.11, 0.22, 0.55, 0.47 and 0.44%) at (0.0, 15.0, 30.0 45.0 and 60.0)mg/Kg soil Co respectively; (0.01, 0.10, 0.57, 0.58 and 0.72%) at (0.0, 25.0, 50.0, 75.0 and 100.0)mg/Kg soil Cu respectively. (0.08, 0.80, 1.28, 1.31 and 0.88%) at (0.0, 25.0, 50.0, 75.0 and 100.0)mg/Kg soil Cr respectively and (0.06, 1.11, 1.20, 1.83 and 2.22%) at (0.0, 100.0, 200.0, 300.0 and 400.0)mg/Kg soil Zn respectively.

Keywords: *Sesbania grandiflora*, heavy metals tolerance, tissue cultures.

المستخلص

نفذت عدة تجارب لغرض دراسة تحمل نبات السيسبان *Sesbania grandiflora* للعناصر الثقيلة على مستوى النبات الكامل ومزارعه النسيجية. استحث الكالس وأديم على وسط موراشيغ و سكوج, 1962 المحور والمجهز ب (0.5) ملغم/لتر من البنزل الدين و (2) ملغم/لتر من حامض 2,4 داي مثيل فينوكسي باستعمال الفلق كمصدر لنشوء الكالس. أضيفت تراكيز مختلفة من العناصر الثقيلة (Cd, Co, Cu, Cr, Zn), إلى الوسط الغذائي كعوامل تلوث لغرض دراسة تأثير هذه الملوثات من العناصر الثقيلة على مستوى الخلية والنبات الكامل. زرعت بذور نبات السيسبان في تراكيز مختلفة من هذه العناصر لمدة 3 أسابيع. استخدم جهاز الامتصاص الذري في تقدير النسب المئوية للعناصر الممتصة من قبل النبات الكامل والكالس. اذ بلغت في الكالس (0.011, 0.001, 0.012 و 0.013%) عند اضافة التراكيز (0.0, 0.05, 0.075 و 0.1) ملغم/لتر على التوالي من الكاديوم؛ (0.001, 0.008, 0.016 و 0.006%) للتراكيز (0.0, 0.1, 0.25 و 0.5) ملغم/لتر على التوالي من الكوبالت؛ (0.001, 0.020, 0.034 و 0.015%) للتراكيز (0.0, 0.075, 0.2 و 0.5) ملغم/لتر على التوالي من النحاس؛ (0.001, 0.013, 0.012 و 0.010%) للتراكيز (0.0, 0.25, 0.4 و 0.5) ملغم/لتر على التوالي من الكروم؛ (0.027, 0.051, 0.059 و 0.056%) للتراكيز (0.0, 0.75, 1.0 و 1.5) ملغم/لتر على التوالي من الخارصين. اوضحت النتائج نقصان وزن الكالس بزيادة تراكيز العناصر الثقيلة في الوسط الزراعي مع زيادة في نسبة إنبات البذور وارتفاع النبات مع زيادة مدة التعريض (الأيام) و انخفضت بزيادة كافة تراكيز العناصر الثقيلة قيد الدراسة. أما النسب المئوية للعناصر الممتصة والمتجمعة في النبات الكامل فكانت (0.08, 0.55, 1.11, 0.83 و 0.44%) عند اضافة (0.0, 1.0, 3.02.0 و 4.0) ملغم/كغم تربة على التوالي من الكاديوم؛ (0.11, 0.22, 0.55, 0.47 و 0.44%) ل (0.0, 15.0, 30.0, 45.0 و 60.0) ملغم/كغم تربة على التوالي من الكوبالت؛ (0.01, 0.10, 0.57, 0.58 و 0.72%) ل (0.0, 25.0, 50.0, 75.0 و 100.0) ملغم/كغم تربة على التوالي من النحاس؛ (0.08, 0.80, 1.28, 1.31 و 0.88%) ل (0.0, 25.0, 50.0, 75.0 و 100.0) ملغم/كغم تربة على التوالي من الكروم؛ (0.06, 1.11, 1.20, 1.83 و 2.22%) ل (0.0, 100.0, 200.0, 300.0 و 400.0) ملغم/كغم تربة على التوالي من الخارصين.

Introduction

Soils normally contain low levels of heavy metals. Excessive levels can be hazardous to man, animals and plants. The term heavy metal, however, is often broadly applied to include other potentially hazardous elements. The most important heavy metals involved in soil contamination are arsenic (As), cadmium (Cd), chromium (Cr), nickel (Ni), and selenium (Se) [1].

Phytoremediation has been defined as the use of green plants and their associated micro-organisms in optimized agronomical conditions to remove harmless contaminants, including organic compounds and toxic metals. Phytoremediation is a technology that aims to provide a cheap, soft and safe treatment applicable to contaminated sites". It is based on the following considerations: Short term effectiveness, reduction in the toxicity and mobility [2].

Tissue culture is a powerful tool that gives the possibility to grow millions of cells under controlled conditions, and to get physiological information about the behavior of the plant cells under stress conditions [3]. Plant tissue culture and molecular genetics have opened new avenues in plant improvement. Screening and selection at the plant cell level has established plant clones with increased tolerance or resistance in plants to various environmental stresses like salt, heat, cold, drought, disease, insects, heavy metals and herbicides [4]. Cell lines tolerant to elevated levels of salt in the medium have been selected in *Brassica juncea* [5]. Cell lines resistant to elevated concentrations of aluminum have been selected in *Nicotiana plumbaginifolia* [6].

The genus *Sesbania* belongs to the family fabaceae, which includes important plants that are used for treatment of contaminated soils. It is known for exceptionally fast growth rates as well as a very high affinity for association with nitrogen-fixing bacteria. It has several potential uses including forage, fuel wood, pulpwood, fences, medicines, shade for other crops and soil improvement [7].

The aim of this study was to assess the ability of *S. grandiflora* tissue cultures and whole plants to accumulate heavy metals in their tissues and hence using the plant for phytoremediation purposes.

Materials and Methods

Sesbania grandiflora seeds were collected from one of the fields located in Sayafia village 20 Km south of Baghdad. MS [8] medium was used. Sucrose 30 g/l, Myoinositol 100 mg/L and the plant growth regulators 2, 4-D and BA at different concentrations were added. The pH of the medium was adjusted to 5.8, and then 7g/l of the agar type (Agar-Agar) was added to the medium, placed on a hot plate magnetic stirrer till boiling. Aliquots of 10 ml were dispensed into (8 × 2.5) cm culture vessels. Culture media were sterilized by autoclaving at 121°C under (1.04 Kg/cm²) pressure, for 15 min. Glassware and other instruments either autoclaved or placed in electric oven (180-200) °C for 2 hrs [5]. The medium was left at room temperature to cool and become ready to culture explants.

S. grandiflora seeds were surface sterilized according to Purohit [9]. Briefly, they were rinsed with continuous shaking in 1.75% NaOCl for 5 min., and then rinsed three times with sterilized DDH₂O. Seeds were submerged in DDH₂O for 24 hrs, for imbibition. Embryos were dissected and discarded then cotyledons were cultured on MS medium for callus induction.

Combinations of plant growth regulators were prepared and added to the cultural medium to determine the most effective one for callus induction. Cotyledons were placed onto MS medium containing different concentrations of 2, 4-D and BA. The response of cotyledons to auxin and cytokinin combinations was evaluated after 25 days in culture. The initiated callus was removed from the explants using forceps and scalpel, then pieces weighting 35 mg were subcultured onto fresh medium supplemented with the same combinations of 2,4-D and BA. Callus fresh weight was determined using sensitive balance, then oven dried at 60°C for 24 hrs for callus dry weight measurements and for heavy metals concentration measurements. Callus pieces (35 mg each) were placed on the surface of culture medium supplemented with different concentrations of heavy metals that prepared as solutions included; Cd (0.0, 0.05, 0.075 and 0.1); Co (0.0, 0.1, 0.25 and 0.5); Cu (0.0, 0.075, 0.2 and 0.5); Cr (0.0, 0.25, 0.4 and 0.5) and Zn (0.0, 0.075, 0.2 and 0.5) mg/l. The experiment was continued for 25 days. *S. grandiflora* seeds were germinated on different concentrations of heavy metals. These were Cd (0.0, 1.0, 2.0, 3.0 and 4.0); Co (0.0, 15.0, 30.0, 45.0 and 60.0); Cu (0.0, 25.0, 50.0, 75.0 and 100.0); Cr (0.0, 25.0, 50.0, 75.0 and 100.0); and Zn (0.0, 100.0, 200.0, 300.0 and 400.0) mg/Kg soil. This experiment was accomplished at (July-December), in pots with a diameter of 16 cm using clay silt soil containing: sand 140g, clay 640g, silt 220g. Each pot was containing 2Kg of soil that contaminated with heavy metals. Heavy metals were

mixed with small amount of soil, then the contaminated soil was mixed with the bulk soil to make 2Kg of contaminated soil. These pots were placed under field conditions. Seeds started germination after 5 days. Germination percentage and seedling height were recorded daily for 20 days.

Samples of the plant parts were harvested (roots, stems, leaves, flowers), and subjected to analysis. Callus pieces were also harvested then dried at 60°C for 24 hrs and subjected to analysis. A quantity of 0.25g of the whole plant samples or callus sample was weighed and placed in the digestion apparatus, and then 10ml of concentrated H₂SO₄ was added and heated till boiling for 2 hrs until the color of the sample turned black. The solution was cooled, and 1.5ml of HClO₄ was added, reheated until the solution became clear. The volume was completed to 50ml using a volumetric flask. The concentrations of heavy metals in this solution were measured using Atomic Absorption Spectrophotometer [10].

Results and Discussion

Table (1) shows that the percentage of callus induction decreased with increasing of BA concentrations reaching 22.2, 100 and 66.6% in the combinations of (2mg/l 2,4-D and 0.1mg/l BA), (2mg/l 2,4-D and 0.5mg/l BA), (2mg/l 2,4-D and 1.0 mg/l BA) respectively. These percentages fluctuated with increasing 2,4-D concentrations which were (33.3, 55.5, 33.3 and 22.2)% for combination of (2.5mg/l 2,4-D and 0.1mg/l BA), (2.5mg/l 2,4-D and 0.5mg/l BA), (2.5mg/l 2,4-D and 1.0mg/l BA), (2.5mg/l 2,4-D and 1.5mg/l BA) respectively. No callus induction was reported on untreated cotyledon explants, and in the interaction between low concentrations of 2, 4-D and BA.

Table (1): Percentages of *S. grandiflora* explants showed callus initiation on MS medium supplemented with different concentrations of 2,4-D and BA after 25 days (n= 9)

2,4-D (mg/l)	BA (mg/l)				
	0.0	0.1	0.5	1.0	1.5
0.0	*	*	*	*	*
0.5	*	*	*	*	*
1.0	*	*	*	*	*
2.0	*	22.2	100	66.6	22.2
2.5	*	33.3	55.5	33.3	22.2

(*) No callus induction occurred

Calli were inoculated into the same combinations of plant growth regulators used for callus induction to determine the appropriate one for callus maintenance (Table 2). The combination of 2.0mg/l 2,4-D and 0.5mg/l BA produced more callus fresh weight (615.57mg) than any other ones (fig. 1). Dry weight of callus cultures initiated from cotyledon explants on MS medium is shown in Table (3). Maximum dry weight occurred in the combination of 2.0mg/l 2, 4-D and 0.5mg/l BA (190.10mg). Lower dry weight recorded as (100.01, 111.01, 95.81 and 60.10) mg in combinations of (0.1mg/l

BA and 2.5mg/l 2, 4-D), (0.5mg/l BA and 2.5mg/l 2,4-D), (1.0mg/l of BA and 2.5mg/l of 2,4-D) and (1.5mg/l BA and 2.5mg/l 2,4-D) respectively.

The effect of different concentrations of Cd on callus fresh weight was determined as shown in (fig. 2). There was a progressive decline in callus fresh weight with increasing Cd concentration. Cadmium is a non essential element that negatively affects plant growth and development. It is relatively mobile in plants since it influences mineral nutrition [11]. According to the results stated above, callus was induced on cotyledon explants then maintained for many subcultures on MS medium containing 0.5mg/l BA and 2mg/l 2,4-D for subsequent experiments

Table (2): Callus fresh weight (mg) grown on MS medium supplemented with different combinations of 2,4-D and BA, after 25 days, the initial callus fresh weight is 35 mg (n= 9)

2,4-D (mg/l)	BA (mg/l)				
	0.0	0.1	0.5	1.0	1.5
0.0	*	*	*	*	*
0.5	*	*	*	*	*
1.0	*	*	*	*	*
2.0	*	190.01	615.57	522.05	200.95
2.5	*	241.01	443.50	211.91	197.21

(*) Callus showed significant deterioration



Fig (1): Callus grown on MS medium supplemented with 2.0mg/l 2, 4-D and 0.5mg/l BA, after 25 days in culture

Induction and maintenance of callus cultures in *S. grandiflora* seems to favor high levels of 2,4-D and lower level of BA. Auxin is generally included in a culture medium to stimulate cell growth, induction of somatic embryogenesis and stimulate growth of shoot apices, shoot tip cultures and cotyledons [9]. Cytokinin is very important for cell division in the presence of the auxin. So, the interaction between the cytokinin and the

auxin led to cell division and callus induction. It is also prevents the oxidation of the natural auxin IAA [12].

Fig. (3) Represents the effect of different concentrations of Co on callus fresh weight. The highest callus fresh weight obtained in control treatment reached up to (58.6) mg, then started to decrease with increasing Co concentration reaching (43.7, 41 and 38.2) mg for the media containing (0.10, 0.25 or 0.50) mg/l Co.

Table (3): Callus dry weight (mg) grown on MS medium supplemented with different concentrations of 2,4-D and BA for 25 days (n= 9)

2,4-D (mg/l)	BA (mg/l)				
	0.0	0.1	0.5	1.0	1.5
0.0	*	*	*	*	*
0.5	*	*	*	*	*
1.0	*	*	*	*	*
2.0	*	52.59	190.10	145.52	89.05
2.5	*	100.01	111.01	95.81	60.10

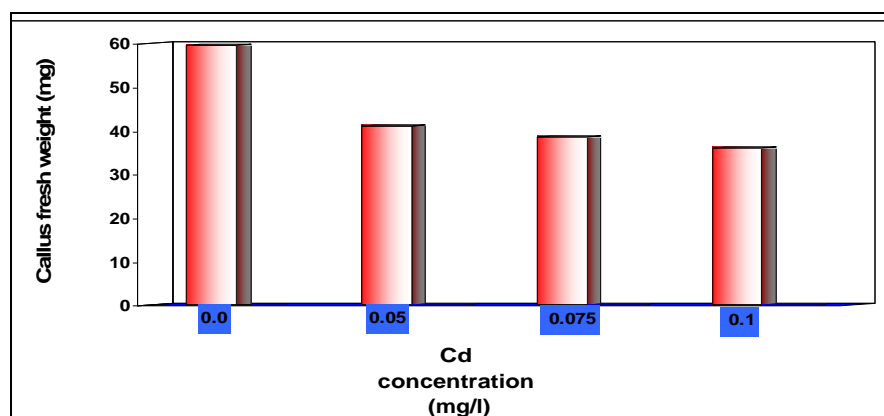


Fig (2): Effect of different concentrations of Cadmium on callus fresh weight grown on MS medium supplemented with 0.5mg/l BA and 2mg/l 2, 4-D, after 25 days. The initial callus fresh weight is 35mg (n=9)

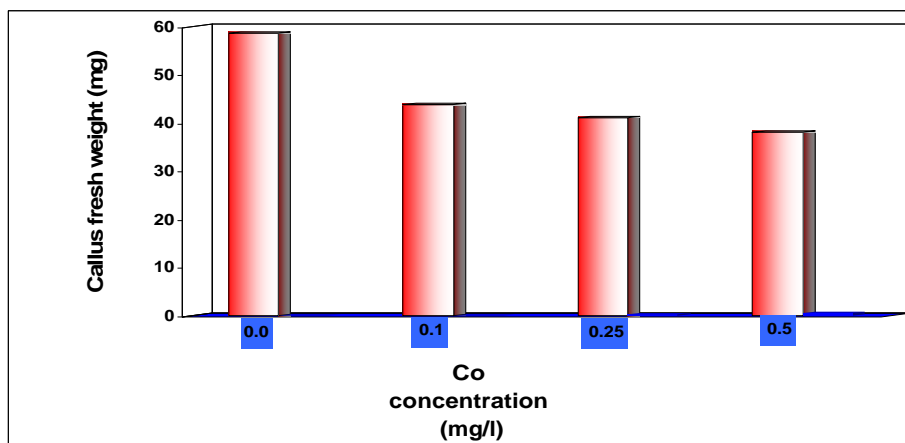


Fig (3): Effect of different concentrations of Cobalt on callus fresh weight grown on MS medium supplemented with 0.5mg/l BA and 2mg/l 2,4-D, after 25 days. The initial callus fresh weight is 35mg (n=9)

Cobalt is considered as transition element, plays an important role in plants as cofactor absorbed from soil or media by tissues or plants [13]. Callus fresh weight decreased with increasing of Cu concentration (fig. 4). Maximum callus fresh weight was reported in (0.0) mg/l Cu reached (62.4) mg then decreased at (0.075 and 0.2) mg/l of Cu recording (56.8 and 51.1) mg respectively. While the lowest callus fresh weight was recorded in (0.5) mg/l Cu reached (40) mg. Copper is essential for plant growth and development, since it interacts with many enzymes and proteins. While elevated concentrations of Cu leads to toxicity symptoms and inhibition of plant growth [14].

The highest callus fresh weight recorded was (62) mg in callus cultures grown on Cr free medium. Meanwhile callus fresh weight decreased at (0.25, 0.40 and 0.5) mg/l of Cr reached (57.1, 49.2 and 42.5) respectively fig. (5).

Fig.(6) exhibits that the highest callus fresh weight (61)mg appeared in control treatment. A decline in callus fresh weight with increasing Zn concentration reached (51.5, 46.1 and 39.2) mg for (0.75, 1.00 and 1.50) mg/l of Zn respectively. High levels of Zn lead to toxicity symptoms and inhibition of plant growth. Toxicity led to an inhibition of cell activity or disruption of its structure. In addition, excess Zn may stimulate the formation of free radicals, perhaps resulting in oxidative stress [15].

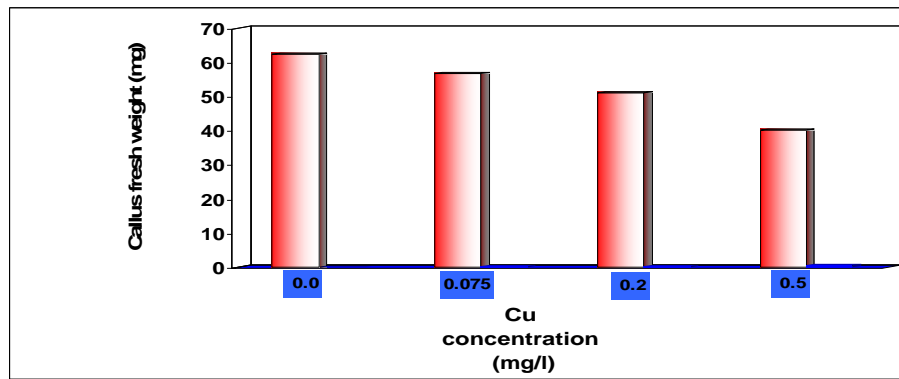


Fig (4): Effect of different concentrations of Copper on callus fresh weight grown on MS medium supplemented with 0.5mg/l BA and 2mg/l 2, 4-D, after 25 days. The initial callus fresh weight is 35mg (n=9)

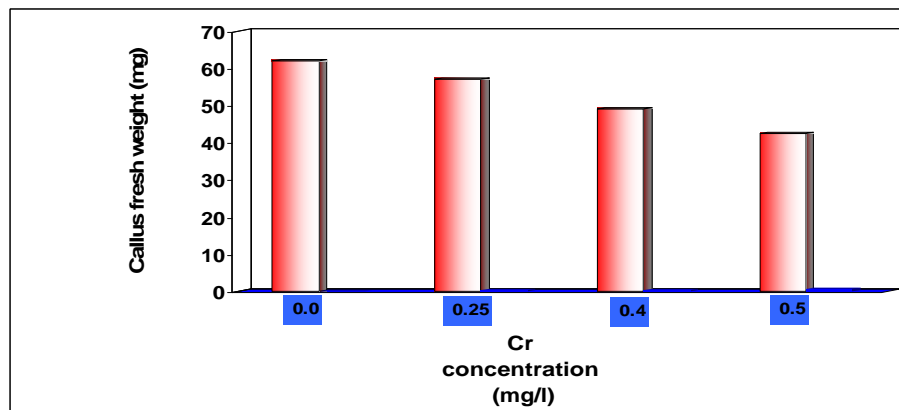


Fig (5): Effect of different concentrations of Chromium on callus fresh weight grown on MS medium supplemented with 0.5mg/l BA and 2mg/l 2, 4-D, after 25 days. The initial callus fresh weight is 35mg (n=9)

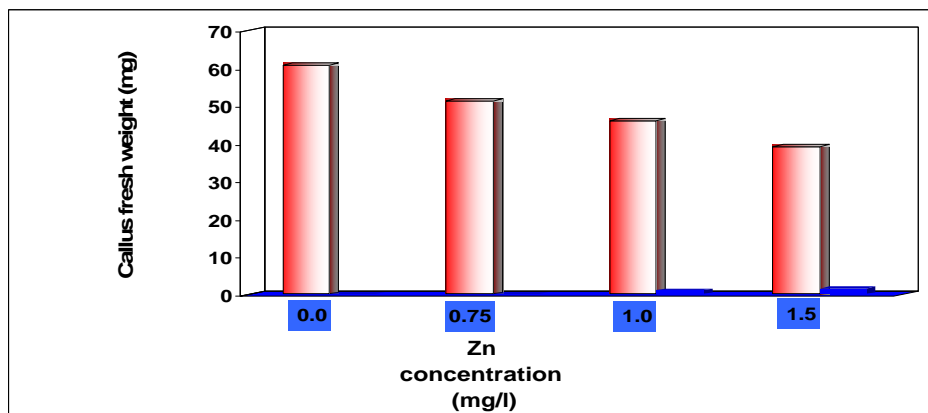


Fig (6): Effect of different concentrations of Zinc on callus fresh weight grown on MS medium supplemented with 0.5mg/l BA and 2mg/l 2, 4-D, after 25 days. The initial callus fresh is 35mg (n=9)

The concentration of Cd accumulated in callus cultures increased with increasing of Cd concentration (Table 4). This is in agreement with Duxbury, (1985) [16] who classified

Cd as an element of intermediate toxicity. While the concentrations of heavy metals absorbed by callus increased by increasing of heavy metals concentrations reaching (0.25, 0.2, 0.25 and 1.0)mg/l of (Co, Cu, Cr and Zn) respectively.

Table (4): Concentrations of heavy metal ions absorbed by callus culture of *S. grandiflora* grown on MS medium supplemented with 2mg/l 2,4-D and 0.5mg/l BA, after 25 days

Treatment	Heavy metal concentration (mg/l)	Absorbed concentration (ppm)
Control	0.0	2
Cd	0.05	110
	0.075	120
	0.10	130
Control	0.0	10
Co	0.1	80
	0.25	160
	0.5	60
Control	0.0	100
Cu	0.075	200
	0.25	340
	0.50	150
Control	0.0	1.5
Cr	0.25	130
	0.4	120
	0.5	100
Control	0.0	150
Zn	0.75	450
	1.0	560
	1.5	340

This amount decreased (0.5, 0.5, 0.4 and 1.5)mg/l with increasing Co, Cu, Cr and Zn concentrations respectively. This result is in agreement with Benavides *et al.*, (2005)[17] who found that Cobalt, Copper, Chromium, Zinc, and Nickel are toxic elements to plant tissue at high concentrations. In order to calculate the germination percentage of germinated seeds subjected to heavy metals under field conditions. Germination started at day 6 in all treatments reaching to (87.1%) in control.

Germination declined with increasing Cd level over time course. At the end of day 20, germination percentages reached to (99.7, 92.6, 84, 70.6 and 52%) for the Cd concentrations (0, 1, 2, 3 and 4) mg/Kg soil respectively fig. (7).

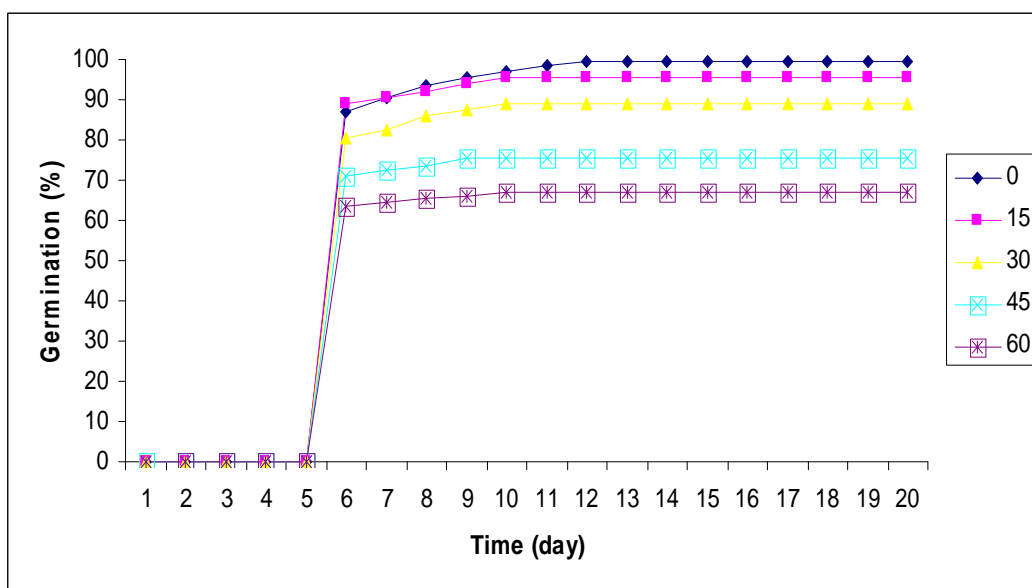


Fig (7): Germination percentages of *S. grandiflora* seeds germinated for 20 days on different concentrations of Cd (mg/Kg soil)

Maximum plant height was recorded in control at the end of day 20 reached 32.6cm fig. (8). Plant height decreased with increasing Cd concentration recording (25, 20.6, 16.6 and 11.6) cm at (1, 2, 3 and 4) mg/Kg soil respectively. Cd is considered as a heavy metal or metal trace element frequently applied to agricultural land as a fertilizer [1]. High amounts of Cd in plants lead to damage the photosynthetic apparatus, inhibition of Rubisco activity in Calvin cycle, decreased seed germination, affection of photosystems I and II. The levels of total chlorophyll and carotenoids are reduced [18].

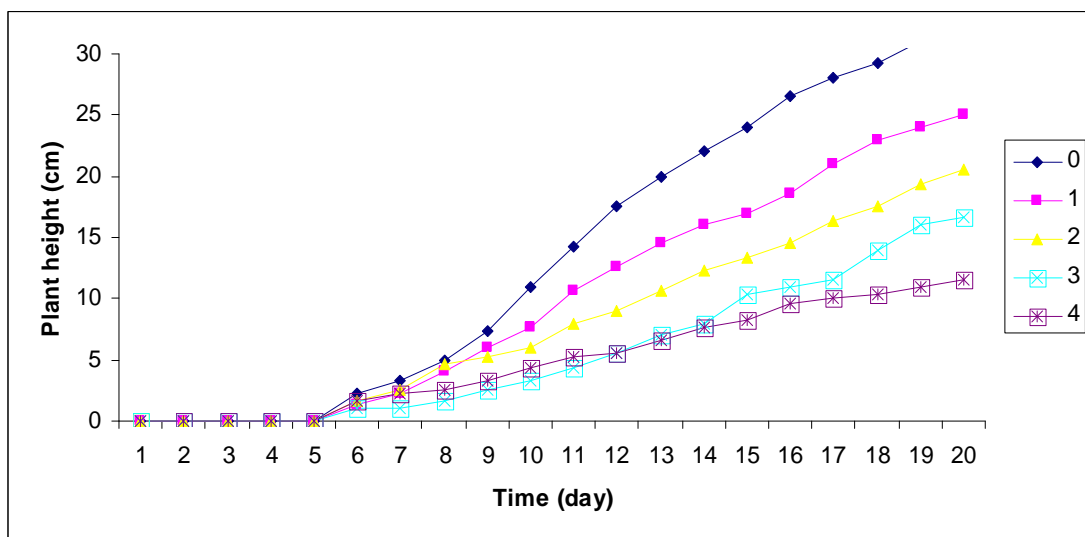


Fig (8): Effect of different concentrations of Cd (mg/Kg soil) on plant height after 20 days growth in pots

Fig. (9) shows that the highest germination percentage of germinated seeds was 99.7% recorded in control treatment at day 20. Germination percentages decreased with

increasing Co concentration reaching (95.4, 89, 75.6 and 67%) at Co concentrations (15, 30, 45 and 60) mg/Kg soil respectively.

Cobalt is a kind of trace element and heavy metal found in soil that can be incorporated into the active site of urease and render enzyme inactive. Excess Co induces yield reduction and an inhibition in assimilates production in leaves, and even inhibits the export of photo assimilates to roots and causes toxicity to plants [19].

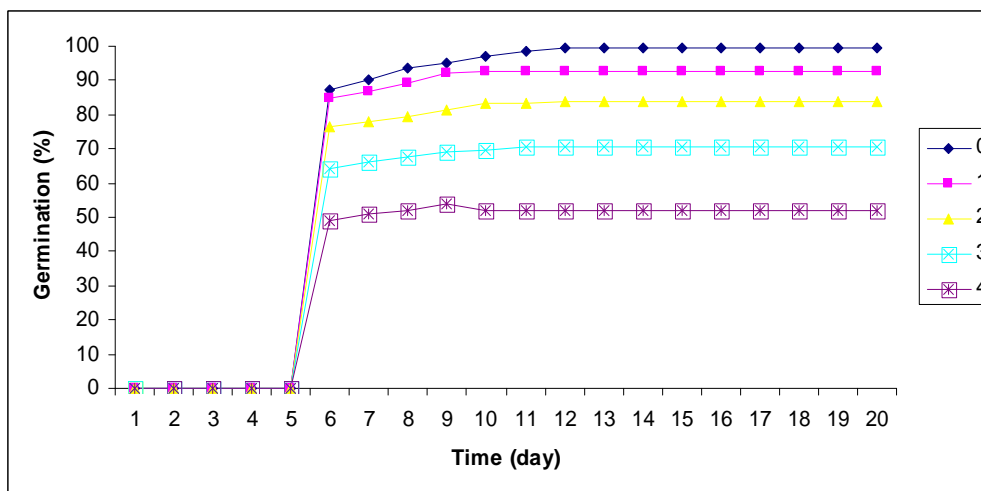


Figure (9): Germination percentages of *S. grandiflora* seeds germinated for 20 days on different concentrations of Co (mg/Kg soil)

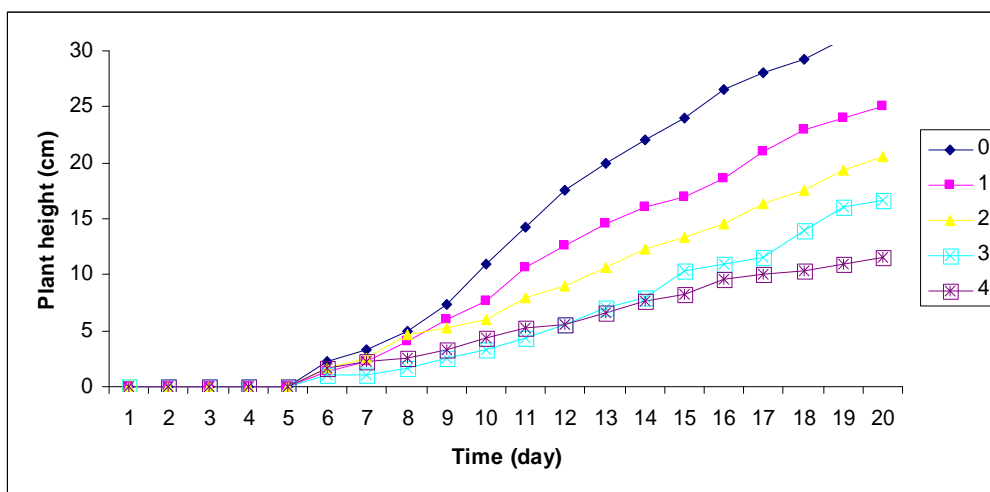


Fig (10): Effect of different concentrations of Co (mg/Kg soil) on plant height after 20 days growth in pots

Germination percentages decreased with increasing Cu concentrations fig. (11). the highest germination percentage was recorded in control (99.7%). The germination percentages decreased at (25, 50, 75 and 100) mg/Kg soil of Cu reaching (89, 84.6, 77.6 and 71.6%) respectively. Fig. (12) Shows that plant height decreased with increasing Cu concentration and time. Maximum plant height was (32.6) cm recorded in control then decreased at (25, 50, 75 and 100) mg/Kg soil reaching (27.6, 25, 21.6 and 18) cm

respectively. Cu is essential plant micronutrients. High levels of Cu are toxic to plants because of binding to soil organic matter and become unavailable to plant [1].

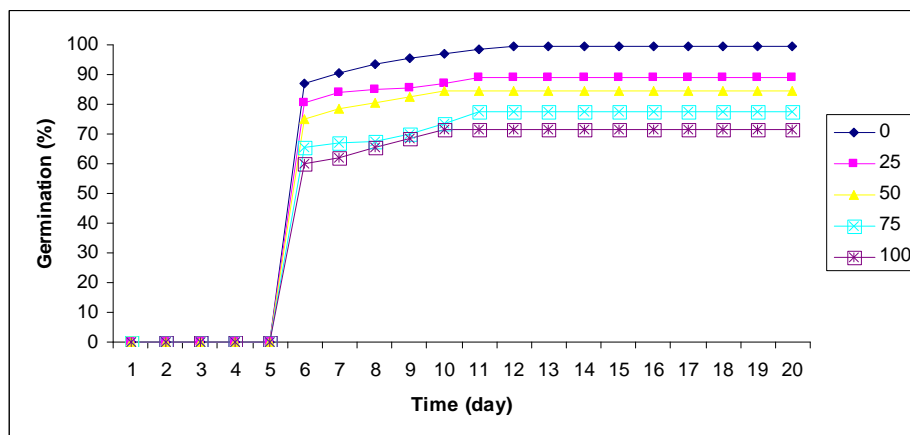


Fig (11): Germination percentages of *S. grandiflora* seeds germinated for 20 days on different concentrations of Cu (mg/Kg soil)

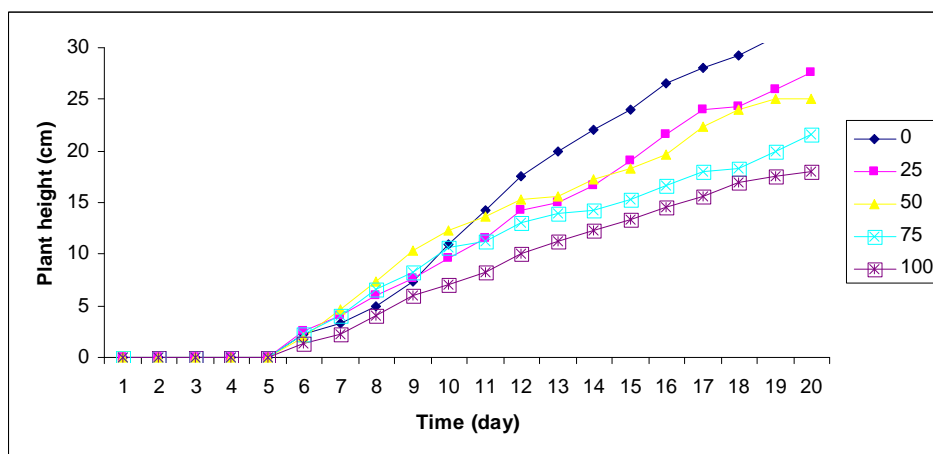


Fig (12): Effect of different concentrations of Cu (mg/Kg soil) on plant height after 20 days growth in pots

Germination percentages of seeds exposed to different Cr concentration are shown in fig.(13). Germination started at day 6. Maximum germination percentage was recorded in control treatment reaching (99.7%), then declined at (25, 50, 75 and 100) mg/Kg soil reaching (80, 78.5, 74.1 and 63.5) % respectively at day 20.

Fig. (14) Shows that the plant height increased gradually recording maximum height in control (32.6) cm. While the lowest plant height recorded (10) cm at (100) mg/Kg soil. Chromium is a naturally occurring element found in rocks, animals, plants and soil. It is present in the environment in several different forms. The most common form is chromium (III) occurs naturally in the environment and is an essential nutrient, but high amounts of Cr lead to toxicity in plant, animals and human.

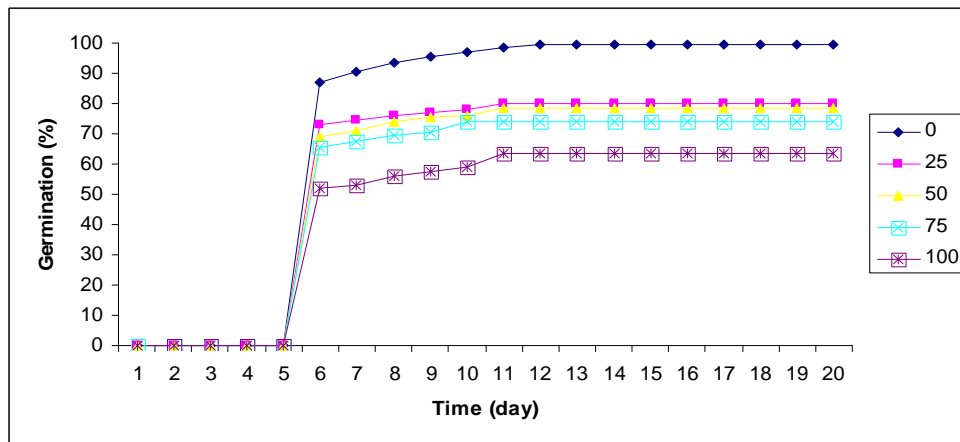


Fig (13): Germination percentages of *S. grandiflora* seeds germinated for 20 days on different concentrations of Cr (mg/Kg soil)

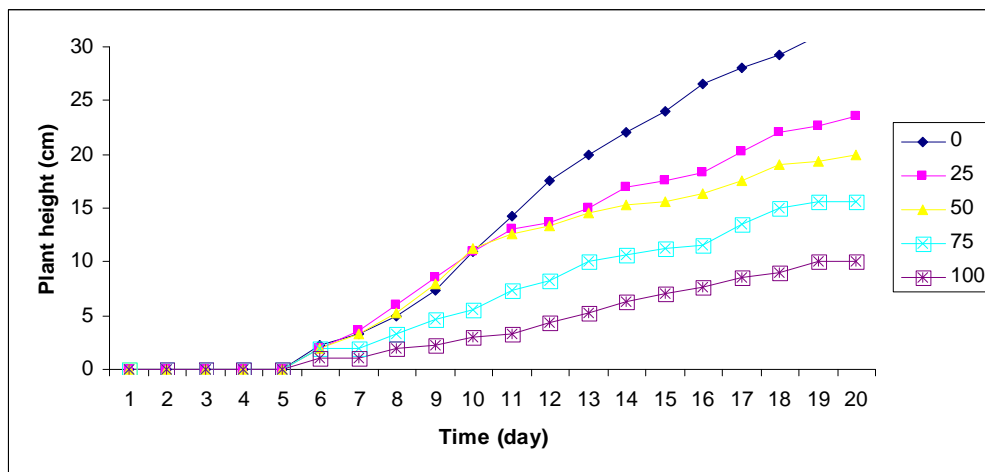


Fig (14): Effect of different concentrations of Cr (mg/Kg soil) on plant height after 20 days growth in pots

Treatment with Zn fig. (15) Resulted in a decline in seed germination. Maximum germination percentage was recorded in control treatment reaching (99.7%). While reached to a minimum (38.4%) at (400) mg Zn/Kg soil. These percentages increased with decreasing Zn concentration reaching (50.6, 59.2 and 67.4%) at (300, 200 and 100) mg/Kg soil respectively.

Plant height increased over time reaching (32.6) cm in control treatment at day 20 (fig. 16), then decreased with increasing Zn concentration reaching (26, 21.5, 18.5 and 13.5)cm at (100, 200, 300 and 400)mg/Kg soil respectively. Zinc is an essential element for both plants and animals. It plays an important role in several plant metabolic processes, it activates enzymes and is involved in protein synthesis, carbohydrates, nucleic acids and lipids metabolism. Excessive accumulation in plant tissues causes alterations in vital growth processes such as photosynthesis and chlorophyll biosynthesis [20].

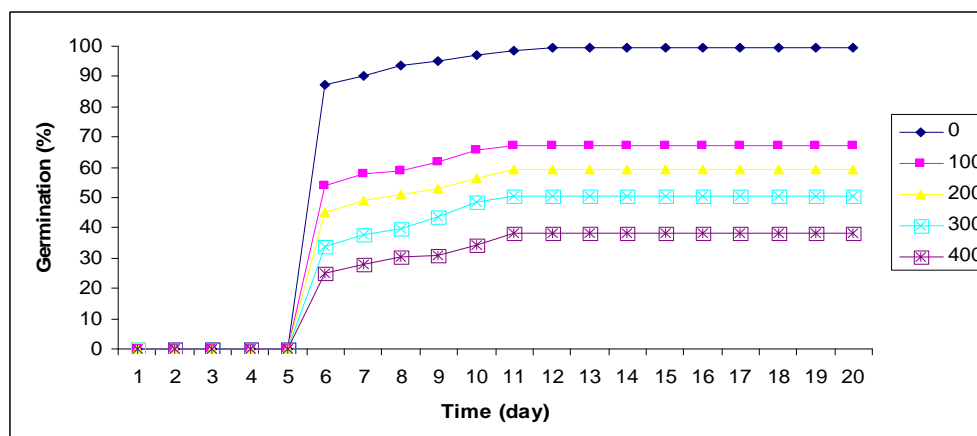


Fig (15): Germination percentages of *S. grandiflora* seeds germinated for 20 days on different concentrations of Zn (mg/Kg soil)

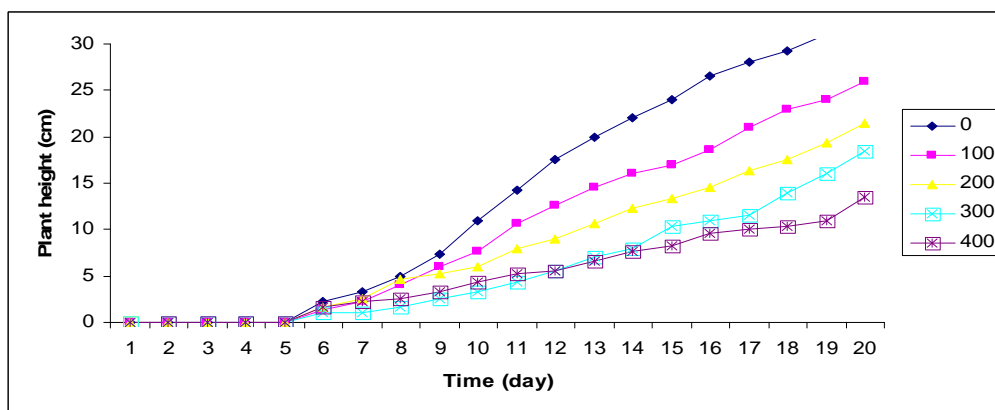


Fig (16): Effect of different concentrations of Zn (mg/Kg soil) on plant height after 20 days growth in pots

There are two mechanisms responsible for metal transport from soil particles to plant roots: mass flow, and diffusion, that make the soluble ions move from soil to root surfaces as sediments in tissues and therefore prevent growth [21].

Plants evolved several effective mechanisms for tolerating high concentrations of heavy metals. Because of their charge, metal ion can not move freely across the cellular membranes, which are lipophilic structures. Therefore, ion transport into cells must be mediated by membrane proteins with transport functions, known as transporters. Transmembrane transporters possess an extra cellular binding domain to which the ion attach just before the transport, and a transmembrane structure which connects extra cellular media. The binding domain is receptive only to specific ions and is responsible for transporter specificity. The transmembrane structure facilitates the transfer of bound ions from extra cellular space through the hydrophobic environment of the membrane into the cell [22]. As shown in table (5) the highest concentration of Cd accumulated was measured at (2) mg/Kg soil reaching (1110) ppm. The progressive decrease in amount of Cd absorbed by plant occurred at (3 and 4) mg/Kg soil reaching (830 and 440)ppm respectively. Lowest level of Cd were accumulated in control treatment. The absorption of heavy metals is severely affected at high concentrations due to cell toxicity

and ultimately cell death. The heavy metal must mobile into the soil solution, for the plants to accumulate them [22]. The bioavailability of metals is increased in soil through several means in which plants achieve it by secreting phytosidophores into the rhizosphere to chelate and absorb. Both acidification of the rhizosphere and exudation of carboxylates are considered potential targets for enhancing metal accumulation [14]. Metals are first bound by the cell wall with a low affinity and selectivity. Membrane transporter systems are likely to play a central role in these processes. The application of powerful genetic and molecular techniques has now identified a range of gene families that are likely to be involved in transition metal transport. These include the Heavy Metal ATPases (HMAs), the Nramps, the cation diffusion facilitator (CDF) family [13].

Table (5): Concentrations of heavy metals ions absorbed by *S. grandiflora* whole plant

Heavy metal	Heavy metal concentration (mg/Kg)	Absorbed concentration (ppm)
Control	0.0	80
Cd	1	550
	2	1110
	3	830
	4	440
Control	0.0	110
Co	15	220
	30	550
	45	470
	60	440
Control	0.0	250
Cu	25	460
	50	620
	75	760
	100	890
Control	0.0	100
Cr	25	800
	50	1280
	75	1310
	100	880
Control	0.0	720
Zn	100	1110
	200	1350
	300	1830
	400	2220

The concentration of Co absorbed by the whole plant increased with increasing of Co level until reaching (30)mg/Kg soil recording (550) ppm. Then a decline in Co absorbed by plant was recorded with increasing Co concentration reaching (470 and 440) ppm at (45 and 60)mg/Kg soil respectively. The amount of Co accumulated by plant recorded at (0 and 15) mg/Kg soil was (110 and 220) ppm respectively table (5). Maximum amount

of Cr absorbed by whole plant occurred at (75) mg/Kg soil reaching (1310) ppm. A decline in the concentration of Cr accumulated in plant tissue at (100) mg/Kg soil and higher reaching to (880)ppm. Levels of recorded Cr were (1280, 800 and 80) at (50, 25 and 0) mg/Kg soil respectively. High levels of Cd, Co and Cr lead to toxicity of the plant so can't be absorbed by plant at high concentrations. These results are in agreement with [3] who found that Ni, Co, Va, Cr, Cd are toxic elements with high levels in soils. The same table shows that the amount of Cu accumulated by *S. grandiflora* whole plant tissue increased with increasing Cu concentrations. Cu increase with increasing Cu in soil because its necessary element for plant.

Maximum values of Zn absorbed by whole plant tissue were recorded at (400) mg/Kg soil reaching (2220)ppm. While the lowest occurred in control treatment reaching (720)ppm then started to increase at (100, 200 and 300) mg/Kg soil reaching (1110, 1350 and 1830)ppm respectively. Zinc increased with increasing Zn in soil since it is a necessary element for plant growth and development. Cu and Zn are essential plant micronutrients and important to support cell growth, so the percentages of these two heavy metals absorbed by *S. grandiflora* plants increased with increasing of heavy metals concentrations. Plant possesses potential cellular mechanisms that may be involved in detoxification of heavy metal. These include: reduced uptake or efflux pumping of metals at the plasma membrane; chelation of metals by peptides such as phytochelatins; the repair of stress damaged proteins [14]. It is concluded from this study that *S. grandiflora* callus cultures and the whole plant level are tolerant to the studied heavy metals compared to other plants which their tolerance to heavy metals was documented in the literature.

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