Abstract

In an attempt to enhance heavy metal tolerance in tissue cultures and intact plant of *S. grandiflora*. Several experiments were carried out.

Callus was induced on cotyledon explants and maintained on MS medium supplemented with (0.5) mg/l BA and (2) mg/l 2,4-D.

Heavy metals (Cd, Co, Cu, Cr and Zn) were added to the culture medium at different concentrations as contamination agents.

Results showed that callus fresh weight decreased with increasing heavy metals concentrations in cultural medium.

In order to asses these heavy metals on seed germination, seeds were sown in soil contaminated with different concentrations of heavy metals for 3 weeks.

Result showed that germination percentage and plant height increased over time. However a reduction occurred in these parameters with increasing of heavy metals level.

Atomic Absorption Spectrophotometer 5000 was used for analysis of samples taken from whole plants and callus cultures.

Levels of metals accumulated in callus 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.

Levels of metals accumulated in whole plant 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.

الاسم: شيماء عبد الوهاب يسر ملغاف

اسم الرسالة: دراسة تحمل نبات السيسبان *Sesbania grandiflora* L. للعناصر الثقيلة خارج وداخل الجسم الحي.

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الايميل: shaimaa_alsamare@yahoo.com

اسم المشرف:أ. د.كاظم محمد ابراهيم

اسماء المناقشين: د.حميد محمد جاسم رئيسا

د.مؤيد صبري شوكت عضوا

د.محمد شهاب حمد عضوا

1.1-Introduction

Soils normally contain low levels of heavy metals. However, in areas where industrial or municipal waste are land- applied, concentrations may be much higher. Excessive levels of heavy metals 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 are arsenic (As), cadmium (Cd), chromium (Cr), nickel (Ni), and selenium (Se) (Tucker *et al*., 2005).

Some plant species can be grown in polluted soils to remove heavy metals, and as a way of continuing agricultural production on contaminated soils (Brooks, 1997).

Phytoremediation has been defined as the use of green plants and their associated micro-organisms in optimized agronomical conditions to remove, contain or render 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" is based on the following considerations: Short term effectiveness, reduction in the toxicity, mobility, or volume of heavy metal concerned, whether implementation is feasible, cost, overall benefit to the environment (Adriano *et al.*, 1997; Chen, 1997; Iskander and Adriano, 1997).

Tissue culture is a powerful tool that give the possibility to grow millions of cells under controlled conditions, and to get preliminary physiological information about the behavior of the plant cells under stress conditions (Stefano and Edoardo, 2003).

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 enhanced tolerance to various

environmental stresses like salt, heat, cold, drought, disease, insects, heavy metals and herbicides (Tal, 1983).

Cell lines tolerant to elevated levels of salt in the medium have been selected in *Brassica juncea* (Jain *et al.*, 1991). Cell lines resistant to elevated concentrations of aluminum has been selected in *Nicotiana plumbaginifolia* (Meredith *et al.*, 1988).

The genus *Sesbania* belongs to the family fabaceae, which include 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, fuelwood, pulpwood, fences, medicines, shade trees for other crops, gums and soil improvement (Onim and Dzowela, 2006).

Due to the importance of enhancing metal tolerance for phytoremediation purposes, the aims of this study are:

1. Investigation of some heavy metal tolerance in the locally grown *Sesbania grandiflora* plants as a potential candidate for phytoremedation applications.
2. Investigation of such tolerance at the cellular level.
3. Investigation of some heavy metal accumulation in both, intact and plant tissue cultures.

1.2-Literature Review

Sesbania grandiflora L. Poir**,** Common names are English: Corkwood tree, Hummingbird tree, Scarlet wisteria tree, Sesban, Sesbania. French: Agati a grandes fleurs. Hawaiian:Ohai ke‘oke‘o. Palauan: Katurai. Samoan: Sepania. Tahitian: Afai, Ofai, Ouai, Oufai. (Heering, and Gutteridge, 1992). The genus Sesbania is important in Africa, and it has a large number of species. Some of the most important members of this subgenus *Agati* is mainly found in southern Asia and its members are more of perennial and tree types as compared to the relatively more annual and shrub types found in the genus Sesbania (Onim and Dzowela, 2006).

1.2.1-Plant Taxonomy

Kingdom : Plantae

Subkingdom : Tracheobionta — vascular plants

Division : Magnoliophyta — angiosperms, angiosperms,

plants, phanerogames, plantes a fleurs, plantes a fruits

Class : Magnoliopsida – dicots, dicotyledones, dicotyledons

Subclass : Rosidae

Order : Fabales

Family : Fabaceae

Genus : Sesbania

Species : *Sesbania grandiflora* L. Poir., (Kartesz, 2000).

1.2.2-Plant Description

Small, open-branched plant with drooping branches; 2-6 m tall (picture 1). Leaves are up to 30 cm long, leaflets 20-40 pairs. Flowers are white or deep pink, quite large, 7-8 cm long; pods are green with many seeds, 25-55 cm long, edible when young (Stone, 1970).

Picture (1-1): *S. grandiflora* L. plant (Heering and Gutteridge, 1992).

1.2.3-Plant Distribution

It is widespread in Northern Australia (possibly native), Benin, Burkina Faso, Cameroon, Chad, Cuba, Djibouti, Dominican Republic, Eritrea, Ethiopia, Gambia, Ghana, Guadeloupe, Guinea, Guinea-Bissau, Haiti, Kenya, Liberia, Mali, Martinique, Mauritania, Mauritius, Mexico, Nepal, Niger, Nigeria, Puerto Rico, Senegal, Sierra Leone, Somalia, South Africa, Tanzania, Togo, Uganda, United States of America (Evans and Rotar, 1987). It is grown in Iraq for multipurposes including, windbreakes, forage and firewood.

1.2.4-Plant Ecology

The plant is tolerant to a wide range of soils including soils that are alkaline, poorly drained, saline, and low fertility. *S. grandiflora* has some tolerance to acid soils down to pH 4.5. It is well adapted to heavy clay soils (Gutteridge, 1994).

It is best adapted to regions with annual rainfall of 2,000-4,000 mm, but has been grown successfully in semi-arid areas with 800 mm annual rainfall and up to 9 months dry season. The plant is tolerant to flooding over short periods. Its rapid early growth and erect habit usually enables *S. grandiflora* to access sunlight by overtopping neighboring plants. The large hermaphroditic flowers are pollinated by birds. *S. grandiflora* is able to produce ripe pods 9 months after planting (Gutteridge, 1994).

1.2.5-Plant Uses

Several Sesbania species are sources of livestock feed, both as fodder and hay (Onim *et al.,* 1985). Grandiflora is valued as a fodder in many regions. In south-central Lombok, Indonesia, grandiflora grown around rice fields provides up to 70 percent of the diets of cattle and goats during the annual eight-month dry season. The leaves contain as much as 8.4 percent crude protein. The plants are used to shade nurseries and some crops, and as an element of windbreaks. The leaves of the tree have various uses in the herbal medical lore (Evans, 2001).

Dry matter yield of Sesbania forage is quite high when compared to other forage legumes like *Leucaena leucocephala*, pigeon pea (*Cajanus cajan).* The dry matter forage yields of legumes after six months from planting were 8000, 5500, 3000 Kg/ha for *Sesbania*, *Leucaena* and *pigeon pea* respectively (Onim, 1986).

Nitrogen yields of these legumes were 250, 175 and 120 Kg/ha for *Sesbania, Leucaena* and *pigeon pea* respectively. The mean crude fiber content in *Sesbania* is low (13%) and the mean calcium to phosphorus ratio is high 3:8. It is therefore clear that *Sesbania spp* are forages of very high quality (Onim, 1986).

Leaves, seed pods, and flowers of grandiflora are prepared as food. The young, tender pods are cooked similarly to other green beans. In South Asia, the young leaves are chopped and sautéed, perhaps with spices, onion, or coconut milk. In the Philippines, unopened white flowers are a common vegetable, steamed or cooked in soups and stews after the stamen and calyx have been removed (Evans, 2001).

Sesbania spp have been used for many years as a source of fuelwood. Bulk density of *Sesbania* varies according to species, rate of growth, and age. Values ranging between 240-616 Kg/m^3 have been reported (NAS, 1980, 1983).

Evans, (2001) reported that *S. grandiflora* is often maintained in gardens and around crop fields for its contribution of nitrogen fixation. They are known to fix between 500 to 600 kg/ha of nitrogen per year. Falling leaflets and flowers recycle nutrients to the ground. In a green manure experiment in Maseno, Kenya, reported that *Sesbania sesban* fixed up to 250 kg N/ha in six months (Onim, 1986).

The use of *Sesbania* as green manure crop is a common practice in Southeast Asia. Several studies have shown that *Sesbania* returns into the soil as green manure between 80 and 120 kg of N within 90 days (Dargan *et al*., 1975; Bhardwaj *et al.*, 1981). *Sesbania spp* are also often used in land reclamation especially in salty (saline) and sodic soils as well as in

mining and excavation sites (Srivastava *et al.*, 1973; Malik and Haider, 1977).

1.2.6-Sources of Soil Contamination

Rural and urban soils in both industrial parks and near small factories outside the parks are affected by a wide variety of contaminants. The most serious sources of soil contamination are: Heavy metals in hazardous waste, including materials from chemical production, dyeing, electroplating and heat treatment, the production of batteries, metal treatment, mining and extractive industries, scrap yards, service stations and tanning. Hazardous organic waste materials, including those from medical centers, oil production and storage, paint and pesticide production, Corrosive metal waste materials, including those from acid/alkali factories and chemical engineering work (Chen, 2000).

1.2.7-Toxic Metals in Soil

Heavy metals are chemical elements, important environment pollutants and their toxicity is a problem of increasing significance for ecological, evolutionary, nutritional, and environmental reasons. Plants have homeostatic cellular mechanisms to regulate the concentration of metal ions inside the cell to minimize the potential damage that could result from the exposure to non essential metal ions. Heavy metals are conventionally defined as elements with metallic properties (ductility, conductivity, stability as cations and ligand specificity). The most common heavy metals contaminants are :- Cd, Cr, Cu, Hg, Pb, and Zn (Benavides *et al.*, 2005).

1.2.7.1-Cadmium-Cd

Atomic number (48), atomic mass (112.4) $g.mol^{-1}$, density (8.7) $g.cm^{-3}$ at 20ºC, melting point (321)ºC, Boiling point (767)ºC. Cadmium is a lustrous, silver-white, ductile, very malleable metal. Its surface has a bluish tinge, soft enough to be cut with a knife, but it tarnishes in air. It is soluble in acids but not in alkalis. Cadmium is used in Ni-Cd batteries, pigments, coatings and plating, and as stabilizer for plastics. Cadmium is mainly found in the earth's crust. It always occurs in combination with zinc. Cadmium also consists in the industries as an inevitable by-product of zinc, lead and copper extraction. Naturally a very large amount of cadmium is released into the environment, about 25,000 tons a year. About half of this cadmium is released into rivers through weathering of rocks and some cadmium is released into air through forest fires and volcanoes. The rest of the cadmium is released through human activities, such as manufacturing. Cadmium waste streams from the industries mainly end up in soils. It strongly adsorbs to organic matter in soils. When cadmium is present in soils it can be extremely dangerous, as the uptake through food will increase. Soils that are acidified enhance the cadmium uptake by plants. This is a potential danger to the animals that are dependent upon the plants for survival (Tucker *et al.*, 2005).

Cadmium accumulates in animal bodies, especially when they eat multiple plants. Earthworms and other essential soil organisms are extremely susceptive to cadmium poisoning. They die at very low concentrations and this has consequences for the soil structure. When cadmium concentrations in soils are high they influence soil processes of microrganisms and threat the whole soil ecosystem. Human uptake of cadmium takes place mainly through food. Doses of (20-30 mg/Kg body weight) of cadmium have resulted in human death. Cadmium is first

transported to the liver through the blood. There, it is bond to proteins to form complexes that are transported to the kidneys. This causes the excretion of essential proteins and sugars from the body and further kidney damage (Atlanta, ٢٠٠٠).

1.2.7.2-Cobalt-Co

Atomic number (27), atomic mass (58.9332) $g.mol^{-1}$, density (8.9) $g.cm^{-3}$ at 20°C, melting point (1495)°C, boiling point (2927)°C. Cobalt is a hard ferromagnetic, silver-white, hard, lustrous, brittle element. It is similar to iron and nickel in its physical properties. The element is active chemically, forming many compounds. Cobalt is stable in air and unaffected by water, but is slowly attacked by dilute acids. Cobalt is used in many alloys, in magents and magnetic recording media, as catalysts for the petroleum and chemical industries, as drying agents for paints and inks. Most of the Earth's cobalt is in its core. Cobalt is of relatively low abundance in the earth's crust and in natural waters, from which it is precipitated as the highly insoluble cobalt sulfine CoS. Although the average level of cobalt in soils is 8 ppm, there are soils with as little as 0.1 ppm and others with as much as 70 ppm. Cobalt is an element that occurs naturally in the environment in air, water, soil, rocks, plants and animals. It may also enter air and water and settle on land through wind-blown dust and enter surface water through run-off when rainwater runs through soil and rock containing cobalt. Cobalt is not destroyed in the environment. It may react with other particles or adsorbed on soil particles or water sediments. Cobalt mobilizes under acidic conditions, and ultimately end up in soils and sediments (Earnshaw and Greenwood, 1997).

On the other hand, soils near mining and melting facilities may contain very high amounts of cobalt, the uptake by animals through eating

plants cause health effects. Cobalt accumulates in plants and in the bodies of animals that eat these plants. Cobalt is widely dispersed in the humans environment as a result of breathing air, drinking water and eating food that contains cobalt. It is beneficial for humans at low levels as it is a part of vitamin B_{12}, which is essential for human health. Cobalt is used to treat anaemia in pregnant women, because it stimulates the production of red blood cells. High concentrations (42.4-317 mg/Kg body weight) of cobalt may cause death to human and may cause serious effects on human health causing asthma and pneumonia (Tucker *et al*., 2005).

1.2.7.3-Copper-Cu

Atomic number (29), atomic mass (63.546) g.mol -1, density (8.9) g.cm-3 at 20°C, melting point (1083)°C, boiling point (2595)°C. Copper is a reddish metal with a face-centered cubic crystalline structure. It reflects red and orange light and absorbs other frequencies in the visible spectrum. It is softer than iron but harder than zinc and can be polished to a bright finish. Copper has low chemical reactivity. Most copper is used for electrical equipment (60%); construction, such as roofing and plumbing (20%); industrial machinery, such as heat exchangers (15%) and alloys (5%). Copper is ideal for electrical wiring because it is easily worked, can be drawn into fine wire and has a high electrical conductivity. Copper can be released into the environment by both natural sources and human activities. Examples of natural sources are wind-blown dust, decaying vegetation, forest fires and sea spray. A few examples of human activities that contribute to copper release, mining, metal production, wood production and phosphate fertilizer production. When copper ends up in soil it strongly attaches to organic matter and minerals. As a result it does not travel very far after release and it hardly enters groundwater. Copper does not break down in the environment and because of that it accumulates

in plants and animals. On copper-rich soils only a limited number of plants has a chance of survival. Copper interrupts the activity in soils, as it negatively influences the activity of microrganisms and earthworms (Earnshaw and Greenwood, 1997).

Copper is found in many kinds of food, in drinking water and in air. Humens are therefore absorb eminent quantities of copper each day by eating, drinking and breathing. The absorption of copper is necessary, because copper is a trace element that is essential for human health. Long-term exposure to copper causes irritation of the nose, mouth and eyes and it causes headaches, stomachaches, dizziness, vomiting and diarrhoea. High uptake of copper may cause liver and kidney damage and even death. The lowest level of copper that has been toxic when ingested by human is (11 mg/Kg body weight) (Tucker *et al*., 2005).

1.2.7.4-Chromium-Cr

Atomic number (24), atomic mass (51.996) g.mol -1, density (7.19)g.cm-^{3} at 20°C, melting point (1907)°C, boiling point (2672)°C. Chromium is a lustrous, brittle, hard metal. Its colour is silver-gray and it can be highly polished. It does not tarnish in air, when heated it burns and forms the green chromic oxide. Chromium main uses are in alloys such as stainless steel, in chrome plating and metal ceramics. Chromium plating was once widely used to give steel a polished silvery mirror coating. Chromium enters the air, water and soil in the chromium(III) and chromium(VI) forms through natural processes and human activities. Crops contain systems that regulate the chromium-uptake to be low enough not to cause any harm. But when the amount of chromium in the soil rises, this can still leads to higher concentrations in crops.

Acidification of soil also influences chromium uptake by crops (Tucker *et al*., 2005).

Plants usually absorb only chromium(III). This may be the essential kind of chromium, but when concentrations exceed a certain value, negative effects can still occur. People are exposed to chromium through breathing, eating or drinking and through skin contact with chromium or chromium compounds. Chromium(III) is an essential nutrient for humans and shortages may cause heart conditions, disruptions of metabolisms and diabetes. But the uptake of large amount of chromium(III) causes health effects such as, skin rashes. Lethal dose of Chromium for human is 200µg. Chromium(VI) is hazouradous to human health, mainly for people who work in the steel and textile industry. People who smoke tobacco also have a higher chance of exposure to chromium (John, 2004).

1.2.7.5-Zinc-Zn

Atomic number (30), atomic mass (65.37) g.mol $^{-1}$, density (7.11)g.cm^{-3} at 20°C, melting point (420)°C, boiling point(907)°C. Zinc is the 23rd most abundant element in the Earth's crust. The main zinc mining areas are Canada, Russia, Australia, USA and Peru'. Zinc is a lustrous bluish-white metal. It is brittle and crystalline at ordinary temperatures, but when heated to between 110°C and 150°C it becomes ductile and malleable; it can then be rolled into sheets. It is used principally for galvanizing iron, more than 50% of metallic zinc goes into galvanizing steel, but is also important in the preparation of certain alloys, bronze. It is used for the negative plates in certain electric batteries and for roofing and gutters in building construction. Zinc metal is included in most single tablet, it is believed to possess anti-oxidant properties, which protect against premature aging of the skin and muscles of the body. Large

quantities of zinc are found in soils. When the soils of farmland are polluted with zinc, animals will absorb concentrations that are damaging to their health. Water-soluble zinc that is located in soils can contaminate groundwater. Zinc cannot only be a threat to cattle, but also to plant species. Plants often have a zinc uptake that their systems cannot handle, due to the accumulation of zinc in soils. On zinc-rich soils only a limited number of plants has a chance of survival. Zinc can interrupt the activity in soils, as it negatively influences the activity of microrganisms and earthworms (Earnshaw and Greenwood, 1997).

Zinc is a trace element that is essential for human health. When people absorb too little zinc they can experience a loss of appetite, decreased sense of taste and smell, slow wound healing and skin sores. Zinc-shortages can even cause birth defects. Although humans can handle proportionally large concentrations of zinc, too much zinc can still cause eminent health problems, such as stomach cramps, skin irritations, vomiting, nausea and anaemia (Tucker *et al*., 2005).

Very high levels of zinc can damage the pancreas and disturb the protein metabolism, and cause arteriosclerosis. Extensive exposure to zinc chloride causes respiratory disorders. Toxic doses of zinc to human is (55-70 mg/Kg body weight) (Clarkson, 1991).

1.2.8-Removal of Toxic Metals from Contaminated soil by Plants

Elevated concentrations of both essential and non-essential metals result in growth inhibition and toxicity symptoms. Plants possess a range of potential cellular mechanisms that may be involved in the detoxification of heavy metals and thus tolerance to metal stress. These include binding to cell wall and extracellular exudates; reduced uptake or efflux pumping of metals at the plasma membrane; chelation of metals in the cytosol by

peptides such as phytochelatins; repair of stress-damaged proteins; and compartmentation of metals in the vacuole by tonoplast-located transporters (Hall, 2002).

The use of plants to clean up contaminated environments is not new. About 300 years ago, plants were proposed for use in the treatment of wastewater (Hartman, 1975).

In addition, relevant applied aspects, such as the effect of agronomic practices on metal removal by plants are largely unknown. Natural occurrence of plants species capable of accumulating extraordinarily high metal levels makes the investigation of this process particularly interesting. Byers, 1935 found that plants of the genus *Astragalus* were capable of accumulating up 0.6% selenium in dry shoot biomass. At the end of the 19^{th} century, *Thlaspi caerulescens* and *Viola calaminaria* were the first plant species documented to accumulate high levels of metals in leaves (Baumann, 1985).

The term phytoremediation ("phyto" meaning plant, and the Latin suffix "remedium" meaning to clean or restore) actually refers to a diverse collection of plant-based technologies that use either naturally occurring or genetically engineered plants for cleaning contaminated environments (Cunningham *et al.* 1997; Flathman and Lanza, 1998).

Lasat *et al.*, 2000 reported that phytoremediation is an emerging technology that employs the use of higher plants for the clean up of contaminated environments. Remediation of metal contaminated soil faces a particular challenge. Unlike organic contaminations, metals cannot be degraded. Commonly, decontamination of metal-contaminated soils requires the removal of toxic metals. Phytoremediation consists of four different plant-based technologies each having a different mechanism of action for the remediation of metal-polluted soil, sediment, or water. These include: rhizofiltration, which involves the use of plants to clean various

aquatic environments; phytostabilization, where plants are used to stabilize rather than clean contaminated soil; phytovolatilization, which involves the use of plants to extract certain metals from soil and then release them into the atmosphere through volatilization; and phytoextraction, where plants absorb metals from soil and translocate them to the harvestable shoots where they accumulate. Although plants show some ability to reduce the hazards of organic pollutants, the greatest progress in phytoremediation has been made with metals. Phytoremediation is emerging as a cost-effective alternative. Several analyses have demonstrated that the cost of metal phytoextraction is only a fraction of that associated with conventional engineering technologies. In addition, because it remediates the soil *in situ*, phytoremediation avoids dramatic landscape distruption, and preserves the ecosystem. Despite these advantages, several disadvantages restrict the applicability of phytoextraction like, low plant tolerance, small size of remediating plant species, lack of cost and performance data (Lasat *et al*., 2000).

Many field and pot experiments on remediation techniques for contaminated soils include:

a. Chemical stabilization, to reduce the solubility of heavy metals by adding non-toxic materials to the soil.

b. Removal of polluted surface soil, and replacing it with clean soil.

c. Covering the original polluted soil with clean soil.

d. On-site chemical leaching, using some acid agent.

e. The dilution method, mixing polluted soil with clean soil to dilute the concentration of heavy metals.

f. Remediation by plants, using plant species (Lee and Chen 1994, Wang *et al.*, 1994a, 1994b; Chen and Lee, 1997).

1.2.9-Phytoextraction

Phytoextraction is the most commonly recognized of all phytoremediation technologies. The terms phytoremediation and phytoextraction are sometimes incorrectly used as synonyms, but phytoremediation is a concept while phytoextraction is a specific cleanup technology. The phytoextraction process involves the use of plants to facilitate the removal of metal contaminants from a soil matrix (Kumar *et al.*, 1995).

Recently, phytoextraction, the use of plants to extract toxic metals from contaminated soils, has emerged as a cost-effective, environment-friendly clean up alternative. Metal contaminated soils are notoriously hard to remediate. Current technologies resort to soil excavation and either landfilling or soil washing followed by physical or chemical separation of the contaminants. The cost of soil remediation is highly variable and depends on the contaminants of concern, soil properties, and site conditions (Lasat *et al.*, 2000).

Salt *et al.*, 1995 found that cleaning of metal-contaminated soils via conventional engineering methods can be prohibitively expensive and therefore there is a need for less-expensive clean up technologies.

In practice, metal-accumulating plants are seeded or transplanted into metal-polluted soil and cultivated using established agricultural practices. The roots of established plants absorb metal elements from the soil and translocate them to the above-ground shoots where they accumulate. If metal availability in the soil is not adequate for sufficient

plant uptake, chelates or acidifying agents may be used to liberate them into the soil solution (Huang and Cunningham, 1996; Huang *et al*., 1997; Lasat *et al.*, 1998).

After sufficient plant growth and metal accumulation, the above-ground portions of the plant are harvested and removed, resulting in permanent removal of metals from the site. As with soil excavation, the disposal of contaminated material is a concern. Some researchers suggest that the incineration of harvested plant tissue dramatically reduces the volume of the material requiring disposal (Kumar *et al*., 1995).

Comis, 1996; Cunningham and Ow, 1996 suggested that in some cases valuable metals can be extracted from the metal-rich ash and serve as a source of revenue, thereby offsetting the expense of remediation.

Phytoextraction should be viewed as a long-term remediation effort, requiring many cropping cycles to reduce metal concentrations to acceptable levels (Kumar *et al*., 1995).

The time required for remediation is dependent on the type and extent of metal contamination, the length of the growing season, and the efficiency of metal removal by plants, but normally ranges from 1 to 20 years. This technology is suitable for the remediation of large areas of land that are contaminated at shallow depths with low to moderate levels of metal- contaminants (Kumar *et al*., 1995; Blaylock and Huang, 2000).

Many factors determine the effectiveness of phytoextraction in remediating metal-polluted sites. The selection of a site that is conducive to this remediation technology is of primary importance. Phytoextraction is applicable only to sites that contain low to moderate levels of metal pollution, because plant growth is not sustained in heavily polluted soils.

Soil metals should also be bioavailable, or subject to absorption by plant roots (Blaylock and Huang, 2000).

The land should be relatively free of obstacles, such as fallen trees or boulders, and have an acceptable topography to allow for normal cultivation practices, which employ the use of agricultural equipment. As a plant-based technology, the success of phytoextraction is inherently dependent upon several plant characteristics. The two most important characters include the ability to accumulate large quantities of biomass rapidly and the ability to accumulate large quantities of environmentally important metals in the shoot tissue (Kumar *et al*. 1995; Cunningham and Ow, 1996; Blaylock *et al*. 1997; Mc Grath, 1998).

Interest in phytoremediation has grown significantly following the identification of metal hyperaccumulator plant species. Hyperaccumulators are defined as species capable of accumulating metals at levels 100-fold greater than those typically measured in common non accumulator plants (Reeves and Baker, 2000).

Some plants are "super-accumulators" of heavy metals. These species are defined as plants contain more than 0.1% (1,000 mg/kg) of copper, lead, nickel or cobalt in their dried tissues. In the case of zinc, a threshold of 1% (10,000 mg/kg) is proposed (Brooks, 1997, 1998).

Three patterns of the relationship between the bioavailability of nutrients and uptake in the crops have been proposed. In Type 1, uptake increases as the crop grows, then falls when the crop reaches maturity. This pattern is seen in the uptake of major nutrients such as nitrogen, potassium and phosphorus. Type 2 has a similar but steeper peak, and is seen with the uptake of micronutrients such as copper or zinc. In type 3, uptake is highest at the early growing stages, and falls during later stages

of growth. This pattern is seen for heavy metals such as arsenic, cadmium, chromium, lead, nickel and mercury (Wang and Liao 1999).

1.2.10-Plant Tissue Culture

Plant tissue culture techniques have many applications in life. It can be defined as the production of plants from very small parts (such as shoot tip, axillay buds, nodes, rhizomes) in sterile controlled environments (Stiff, 2006).

The advantages of plant tissue culture techniques over traditional breeding methods are outlined by Croughan, *et al*., (1981) and Gibbs, *et al*., (1989) as follows:

- **a.** To control large variation in genotypes may be obtained.
- **b.** Large numbers of individuals can be evaluated and selected using relatively little space.
- **c.** Time between generations can be reduced.
- **d.** The environment is closely controlled.
- **e.** Physiology can be studied at the cellular level.
- **f.** Cultured cells are relatively undifferentiated which reduce the complications caused by differences in morphology and stage of growth.
- **g.** Culture of plant cells on rigidly defined media permits uniform and precise treatments.

Anderson,1980 reported that four sequential stages in plant tissue culture systems are : establishment, multiplication, rooting and acclimating.

Explants (starting point for all tissues culture) from any plant structure or part, such as seeds, stems, roots, leaves, storage organs, or fruits, can be excised, disinfested, and placed on the surface of culture medium to produce callus that is as a result of wounding and in response to

hormones, either endogenous hormone or supplied in the medium (Ramawat, 2004).

A number of different culture media have been used to grow callus, but the most common is Murashige and Skoog (MS) medium, this medium rich in macroelements, nitrogen, sucrose, and certain vitamins (Murashige and Skoog, 1962; Purohit, 2003).

One major application of plant tissue culture is *in vitro* screening and selection of resistant plants which provide the variation required for a crop improvement program. The assumption is made in the selection at the cell level that the resistance to a particular set of cultural or environmental conditions is expressed in the same way in the cells as in the intact plant (Collin and Dix, 1990).

Several screening and selection methods have been used to select tolerant variants. The efficiency of certain method depends on the effectiveness of selecting desired variants out of large number of individuals. Most have used callus cultures for selecting environmental stresses tolerant cell lines (Naik and Babu, 1988).

Callus cultures have been used routinely in selection for resistance or tolerance to the selective agent in the medium. The risk that not all the cells in the callus aggregate may be uniformly exposed to the agent. Small pieces of callus may be used to overcome such problem. Cells tolerant to agent then grow out as a small mass on the side of an otherwise dead callus piece. The new growth can be easily identified and subcultured (Collin and Dix, 1990).

A few attempts have been made to use cell culture systems to screen for variants with increased tolerance to a particular stress like salt, heat, cold, drought, disease, insects, heavy metals and herbicides. Tomato plants regenerated from callus tissues resistant to toxins secreted into media displayed resistance to *Pseudomonas solanacearum* (Toyoda *et al.*, 1989).

Fungal growth in coculture with callus cultures was correlated with known field resistance of the trees to Dutch elm disease (Dormir, 1992).

Regeneration of shoots from poplar leaf explants exposed to glyphosate gave rise to glyphosate tolerant plants (Michler and Haissig, 1988). In pea, *in vitro* sensitivity of some commercial cultivars showed some correlation with field sensitivity to glyphosate (Yenne *et al.*, 1987).

Cell lines tolerant to elevated levels of salt in the medium have been selected in *Coleus blumei* (Ibrahim *et al.*, 1992). In 1991 Watad *et al.*, reported tobacco plants tolerant to elevated levels of salt in the medium.

Van Sint Jan and Bouharmont, (1992) reported that Cell lines resistant to increased concentrations of aluminium have been selected in rice. Plants regenerated from cotton cell cultures exposed to regular high temperature treatments yielded callus with an elevated tolerance to high temperatures (Trolinder and Shang, 1991). Rascio, (1977) reported tolerance to zinc in shoots of *Thlaspi caerulescens*. Cadmium tolerance of *Pisum sativum* L. reported by Becerril *et al.*, 2001.

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List of Abbreviations

Abbreviation	Full name
BA	Benzyl adenine
°C	degree Celsius
CDF	Cation diffusion facilitator
DDH_2O	Double distilled water
$H2SO_4$	Sulphuric acid
HMAs	Heavy metal ATPases
MS	Murashige and Skoog medium, 1962
n	number of replicates
ha	Hectare
NaOCl	Sodium hypochlorite
ppm	Part per million
UV	Ultraviolet (light)
wt	weight
2,4-D	2,4-diclorophenoxyacetic acid

2.1-Materials

2.1.1- Apparatus and Equipments

The following equipments and apparatus were used throughout the experimental work:

Apparatus	**Company**
Autoclave	Karl / Germany
Distillator	GFL /Germany
Accurate balance	Mettler (Switzerland)
Hot plate with magnetic stirrer	Ikamag
Incubator	Sanyo / Japan
Laminar air flow cabinet	ESCO
Micropipettes	Brand / Germany
pH-meter	Metter Gmbh-Teledo / England
Refrigerator	Ishtar
Sensitive balance	Delta Range / Switzerland
Atomic Absorption Spectrophotometer 5000	PERKIN-ELMER USA
Water bath	Gallenkamp / England
Oven	Gallenkamp / England

2.1.2-Chemicals

Chemicals	**Company**
Ethanol	BDH
Cadmium chloride	BDH
Chromium chloride	BDH
Ammonium nitrate	Mall
Potassium nitrate	BDH
Calcium chloride anhydrate	Fluka
Magnesium sulphate anhydrate	Fluka
Potassium phosphate monobasic	Fluka
Boric acid	Merk
Potassium iodide	Tetanal
Manganese sulphate.$4H_2O$	BDH
Zinc sulphate.$7H_2O$	BDH
Molybdic acid (sodium salt).$2H_2O$	BDH
Cupric sulphate.$5H_2O$	BDH
Cobalt chloride.$6H_2O$	BDH
Sodium ethylene diamine tetraacetate	Fluka
Ferrous sulfate.$7\ H_2O$	BDH
Thiamine.HCl	BDH
Nicotinic acid (free acid)	Kochligh
Pyrodoxine.HCl	BDH
Sodium hypochloride	BDH
Benzyl adinine	BDH

2,4-diclorophenoxyacetic acid	BDH
Glycine	BDH
Agar-Agar	Sleeze

2.2-Methods

This study was carried out in the plant tissue culture laboratory, Biotechnology Department, College of Science, Al-Nahrain University. The field experiment was conducted in pots in the gardens of college of Science during the period 1/10/2005 to 1/3/2007.

2.2.1-Plant Material

Sesbania grandiflora seeds were collected from one of the fields located in Sayafia village 20 Km south of Baghdad. The seeds and plants were classified by prof. Dr K.M. Ibrahim, Al-Nahrain university.

2.2.2- Seeds Sterilization

S. grandiflora seeds were surface sterilized according to Purohit, (2003). Briefly, they were rinsed with continuous shaking in 1.75% NaOCl for 5 min., then rinsed three times with sterilized DDH_2O. Seeds then submerged in DDH_2O for 24 hrs, to imbibit seeds. Embryos were dissected and discarded then cotyledons were cultured on MS medium for callus induction

2.2.3-Preparation of Culture Medium

MS (Murashige and Skoog, 1962) medium was prepared and used (Table 1). 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 was adjusted to 5.8 using NaOH or HCl (1N), 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 vesseles. 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 (Cappuecino and Sherman, 1987). The medium was left at room temperature to cool and become ready to culture explants.

2.2.4-Plant Growth Regulators

Different concentrations of the auxin 2, 4-D (0.0, 0.5, 1.0, 2.0 or 2.5) mg/l and the cytokinin BA (0.0, 0.1, 0.5, 1.0 or 1.5) mg/l were prepared and added to the culture media before autoclaving.

2.2.5-Incubation of Cultures

Surface sterilized explants (cotyledons) were inoculated into the culture vessels under aseptic conditions, placed in the incubator (Sanyo Electric Co., Ltd.) at 25°C for 16/8 hrs. light/dark photoperiod using day light inflorescent under light intensity of 1000 lux.

2.2.6-Initiation of Callus Cultures

Combinations of plant growth regulators were prepared and added to the cultural medium to determine the most effective one for callus induction. Cotyledons excised from seeds were soaked in DDH_2O for (24 hr), placed onto MS medium containing different concentrations of 2,4-D and BA as in 2.2.4. Each concentration included nine replicates. The response of cotyledons to auxin and cytokinin combinations was evaluated after 25 days in culture to determine the proper combination for callus induction.

2.2.7-Maintenance of Callus Cultures

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 as in 2.2.4. 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.

Table (2-1). MS (Murashige and Skoog, 1962) culture medium components

Components	Chemical formula	Weight (mg/l)
Macronutrients		
Ammonium nitrate	NH_4NO_3	1650
Potassium nitrate	KNO_3	1900
Calcium chloride anhydrate	$CaCl_2.2H_2O$	440
Magnesium sulphate anhydrate	$MgSO_4.7H_2O$	370
Potassium phosphate monobasic	KH_2PO_4	170
Micronutrients		
Boric acid	H_3BO_3	6.20
Potassium iodide	KI	0.83
Manganese sulphate.$4H_2O$	$MnSO_4.4H_2O$	22.30
Zinc sulphate.$7H_2O$	$ZnSO_4.7H_2O$	8.60
Molybdic acid (sodium salt).$2H_2O$	$Na_2MoO_4.2H_2O$	0.25
Cupric sulphate.$5H_2O$	$CuSO_4.5H_2O$	0.025
Cobalt chloride.$6H_2O$	$CoCl_2.6H_2O$	0.025
Chelated Iron		
Sodium ethylene diamine tetraacetate	Na2-EDTA	33.6
Ferrous sulfate.7 H_2O	$FeSO_4.7H_2O$	27.8
Vitamins		
Thiamine.HCl	$Cl_2H_{17}C_1N_4OS.HCl$	0.1
Nicotinic acid(free acid)	$C_8H_{11}NO_3.HCl$	0.5
Pyrodoxine.HCl	$C_6H_5NO_2$	0.5
Glycine(free base)	$C_2H_5NO_2$	2.0

2.2.8- Exposure of Callus Cultures to Heavy Metals

Stock solutions of heavy metals were prepared by weighting 1 mg of heavy metal salts ($CdCl_2$, $CoCl_2$, $CrCl_2$, $CuSO_4.5H_2O$, $ZnSO_4.7H_2O$) and dissolved in 1 L of DDH_2O, then added to the culture medium before autoclaving.

Callus pieces (35 mg each) were placed on the surface of culture medium supplemented with different concentrations of heavy metals salt 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 (Walstad, 2003). Each concentration was containing 9 replicates. The results of this experiment were evaluated after 25 days.

2.2.9-Field Experiment

S. grandiflora seeds were germinated on different concentrations of heavy metals at four concentrations with three replicates. Each replicate included 100 seeds for each 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 (140) g, clay (640) g, silt (220) g. Each pot contains 2 Kg of soil that contaminated with heavy metals. Heavy metal mixed with small amount of soil. Then the contaminated soil mixed with another amount of soil until whole 2 Kg of soil contaminated. These pots were put under field conditions.

Seeds started germinating after 5 days. Germination percentage and seedling height were recorded daily for 20 days.

2.3-Preparation of The Samples

Samples harvested as a whole plants(roots, stem, leaves, flower), resulted from seedlings grown on the contaminated soils and subjected for analysis. Callus pieces grown in cultures supplemented with heavy metals were also harvested then dried at 60°C for 24 hrs. and subjected for analysis. A quantity of 0.25g of the plant sample or callus sample were weighed and placed in the digestion apparatus, then 10 ml of concentrated H_2SO_4 was added and heated till boiling for 2 hours until the color of the sample turned black. The solution was cooled, and 1.5 ml of $HClO_4$ was added, reheated until the solution became clear. The volume was completed to 50 ml using a volumetric flask. The concentrations of heavy metals in this solution were measured using Atomic Absorption Spectrophotometer (Anonymous, 1986).

Conclusions

1. Callus cultures of *S. grandiflora* can be induced and maintained on MS medium supplemented with 0.5 mg/l BA and 2 mg/l 2,4-D using cotyledon explants.
2. Seed germination is severely affected with increasing metal concentrations.
3. Growth of *S. grandiflora* callus is reduced by increasing of heavy metals concentrations in cultural media.
4. Both *S. grandiflora* whole plants and callus cultures are accumulating heavy metals in their tissues as proved using atomic absorption spectrophotometer.

Recommendations

1. Investigation of other plants as a source for phytoremediation using tissue culture techniques.

2. Investigating other heavy metals such as : arsenic (As), nickel (Ni), selenium (Se).

3. Investigating the physiological basis of tolerance in selected material.

4. Regeneration of *S. grandiflora* plants result from screening and selection of callus cultures, then examination of the resulted regenerates for their tolerance to heavy metals.

6. Examination of the inheritance of heavy metal tolerance in seeds progeny in plants derived from the *in vitro* selection.

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3.1-Initiation of Callus Cultures

The cotyledons were cultured on MS medium supplemented with different concentrations of BA and 2,4-D to determine the best combination for callus induction. Table (3-1) shows the effect of different concentrations of 2,4-D and BA on the response (%) of callus induction on cotyledons.

Table (3-1): Percentage of explants showed callus of *S. grandiflora* 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

Maximum percentage of callus induction occurred at a combination of 0.5 mg/l BA and 2 mg/l 2,4-D reaching 100%. This percentage decreased with increasing of BA concentrations reaching 66.6% and 22.2% in the combinations of (2 mg/l 2,4-D and 1.0 mg/l BA), (2 mg/l 2,4-D and 1.5 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 0.1 mg/l BA), (2.5mg/l 2,4-D and 0.5 mg/l BA), (2.5mg/l 2,4-D and 1.0 mg/l BA), (2.5mg/l 2,4-D and 1.5 mg/l BA) respectively.

While no callus induction was reported on untreated cotyledon explants, and in the interaction between low concentrations of 2,4-D and BA.

This result is in agreement with Sinha and Mallick (1991) who reported that callus of *S. bispinosa* was established from both cotyledons and mature leaflets on (MS) basal medium supplemented with 0.5 mg/l BA and 2 mg/l 2,4-D. There is side effect when increase BA concentration over 0.5 mg/l and 2,4-D over 2 mg/l, while the lower concentration of BA and 2,4-D aren't adequate to callus induction.

Callus induction requires a balance between auxin(s) and cytokinin(s) as reported by Skooge and Miller (1957). In a number of plant species callus induction favours higher auxins than cytokinins (Ramawat, 2004).

Establishment of a callus from the explants divided into three developmental stages: induction, cell division and differentiation. The length of these phases depends mainly on the physiological status of the explant's cells as well as the cultural conditions including the appropriate combination of plant growth regulators (Dodds and Roberts, 1995).

3.2-Maintenance of Callus Cultures:

Callus cultures induced on cotyledon explants from the best combination of BA and 2,4-D (0.5 and 2 mg/l) respectively, were inoculated into the same combination of plant growth regulators used for callus induction to determine the appropriate concentration for callus maintenance (Table 3-2). A combination of 2.0 mg/l 2,4-D and 0.5 mg/l BA produced more callus fresh weight (615.57 mg) than any other combination (picture 3-1). Increasing both 2,4-D and BA levels reduced callus fresh weight and recorded as 522.05 and 200.95 for combinations (1.0 mg/l BA and 2 mg/l 2,4-D), (1.5 mg/l BA and 2 mg/l 2,4-D) respectively, and reaching to 211.91 and 197.21 for (1.0 mg/l BA and 2.5 mg/l 2,4-D), (1.5 mg/l BA and 2.5 mg/l 2,4-D) respectively. Thus, the combination of 2 mg/l of 2,4-D and 0.5 mg/l of BA was chosen to maintain callus culture in all subsequent experiments.

Table (3-2):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

Picture (3-1): Callus grown on MS medium supplemented with 2.0 mg/l 2,4-D and 0.5 mg/l BA, after 25 days in culture

Dry weight of callus cultures initiated from cotyledon explants on MS medium is shown in (Table 3-3).

Table (3-3): Callus dry weight (mg) grown on MS medium supplemented with different concentrations after 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

Maximum dry weight occurred in the combination of 2.0 mg/l 2,4-D and 0.5 mg/l BA (190.10 mg). While reaching to (145.52 and 89.05) mg in the high concentration (1.0 mg/l BA and 2mg/l 2,4-D), (1.5 mg/l BA and 2mg/l 2,4-D) respectively. Lower dry weight recorded as (100.01, 111.01, 95.81 and 60.10) mg in combinations of (0.1 mg/l BA and 2.5 mg/l 2,4-D), (0.5 mg/l BA and 2.5 mg/l 2,4-D), (1.0 mg/l BA and 2.5 2,4-D) and (1.5 mg/l BA and 2.5 mg/l 2,4-D) respectively.

According to the results stated above, callus was induced on cotyledon explants then maintained for many subcultures on MS medium containing 0.5 mg/l BA and 2 mg/l 2,4-D for subsequent experiments. Induction and maintenance of callus cultures in *S. grandiflora* seems to favor high levels of 2,4-D and lower level of BA.

Callus induced on cotyledon explants were friable with active meristematic cells and containing endogenous growth regulators (Kartusch and Mittendorfer, 1990).

Auxin is generally included in a culture medium to stimulate callus production, cell growth, induction of somatic embryogenesis and stimulate growth from shoot apices and shoot tip cultures and cotyledons (Stiff, 2006).

Cytokinin is very important for cell division in the presence of auxin. So, the interaction between cytokinin and auxin led to cell division and callus induction. Cytokinin is considered as a source for prevention of the oxidation of the natural auxin (IAA) (Abdol, 1987).

Callus induction and differentiation is influenced by many factors: medium components, type and concentration of plant growth regulators, plant physiology, source of plant explant and environmental conditions (Torbert *et al*., 1998). Smith (2000) considered genetic content and nutrient components as main factors affect responses of callus induction.

3.3-Callus Cultures With Heavy Metals

The *S. grandiflora* callus inoculated in MS medium containing different concentrations of heavy metals to observe the effect on callus fresh weight.

3.3.1- Effect of cadmium on callus fresh weight

The effect of different concentrations of Cd on callus fresh weight were determined as shown in (fig. 3-1). There was a progressive decline in callus fresh weight with increasing Cd concentration. Maximum callus fresh weight occurred at (0.0) mg/l Cd reached (59.5) mg. While a reduction in callus fresh weight occurred at concentration (0.05) mg/l Cd and higher. They were recorded (41.2, 38.6 and 35.9) mg for (0.05, 0.075 and 0.1) mg/l Cd respectively.

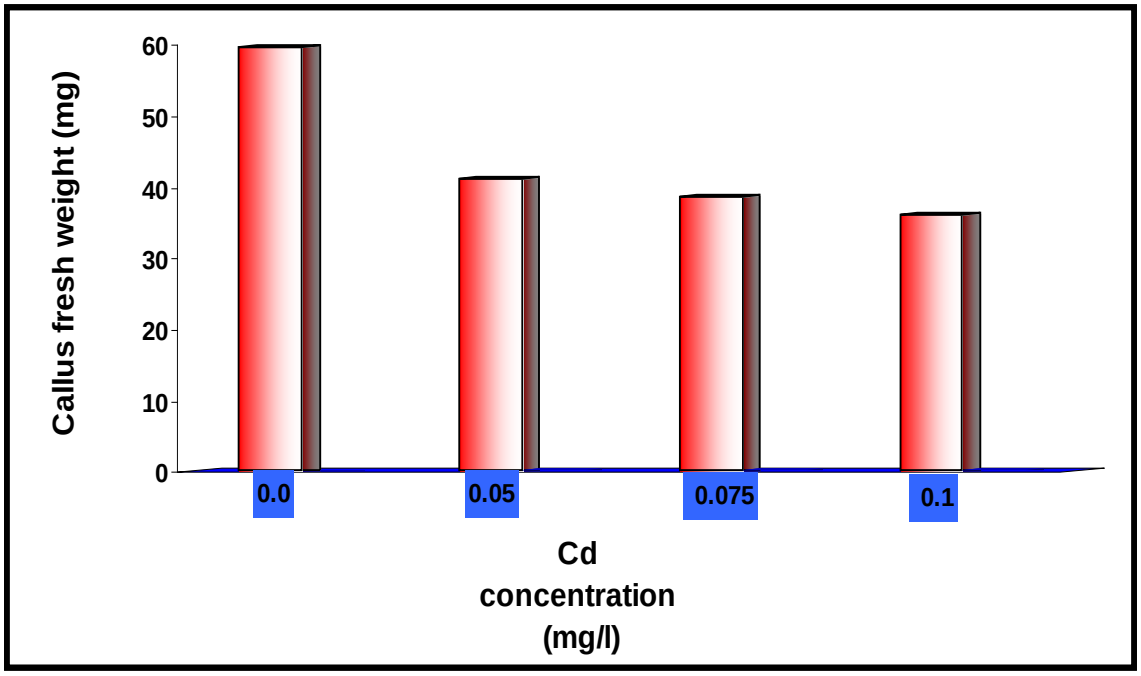

Figure (3-1):Effect of different concentrations of Cadmium on callus fresh weight grown on MS medium supplemented with 0.5 mg/l BA and 2 mg/l 2,4-D, after 25 days, the initial callus fresh weight is 35 mg (n=9)

Cadmium is a non essential element that negatively affects plant growth and development. It is relatively mobile in plants where it influences mineral nutrition (Siedlecka *et al*., 1997).

Symptoms of toxicity are leaf chlorosis, leaf and root necrosis, a decrease in growth and tissue size, causing impairment of cell respiration, inhibition of enzyme activities, protein denaturation or distruption of cell transport processes and damaging the photosynthetic apparatus (Becerril *et al*., 2001).

This result is in agreement with Bennetzen and Adams (2004) who reported that increased Cd concentration led to a decrease in callus fresh weight. They isolated cell line from suspension cultures of *Lycopersicon pernvianum* which were tolerant to Cd after stepwise exposure to increasing Cd concentrations.

3.3.2- Effect of cobalt on callus fresh weight

Fig. (3-2) represents the effect of different concentrations of Co on callus fresh weight. The highest callus fresh weight obtained in control treatment reaching up to (58.6) mg. Then begun 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.

Cobalt is considered as transition element, play an important role in plants as cofactor absorbed from soil or media by plants (Maser *et al*., 2001).

Excess Co causes phytotoxicty to plants, so the concentration less than 0.1 mg/l is the best for plant growth (Chatterjee and Chatterjee, 2003).

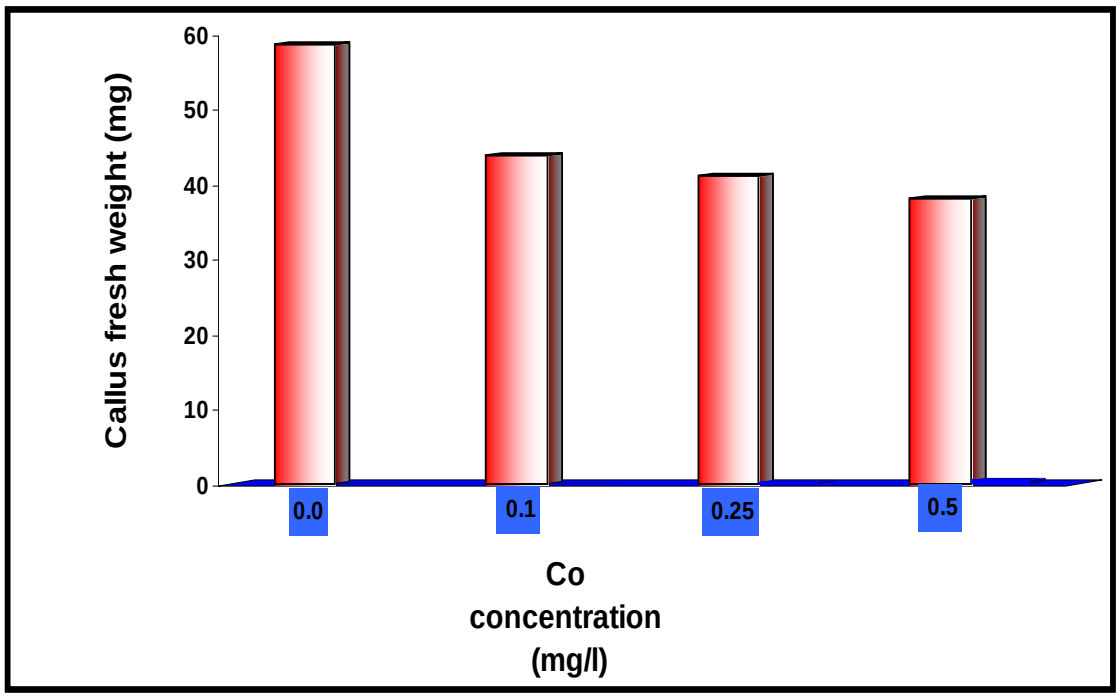

Figure (3-2):Effect of different concentrations of Cobalt on callus fresh weight grown on MS medium supplemented with 0.5 mg/l BA and 2 mg/l 2,4-D, after 25 days, the initial callus fresh weight is 35 mg (n=9)

3.3.3- Effect of copper on callus fresh weight

Callus fresh weight decreased with increasing of Cu concentration (fig. 3-3). Maximum callus fresh weight reported in (0.0) mg/l Cu reached (62.4) mg then begun to decrease at (0.075 and 0.2) mg/l recording (56.8 and 51.1) mg respectively. While the lowest callus fresh weight recorded in (0.5) mg/l Cu reaching (40) mg.

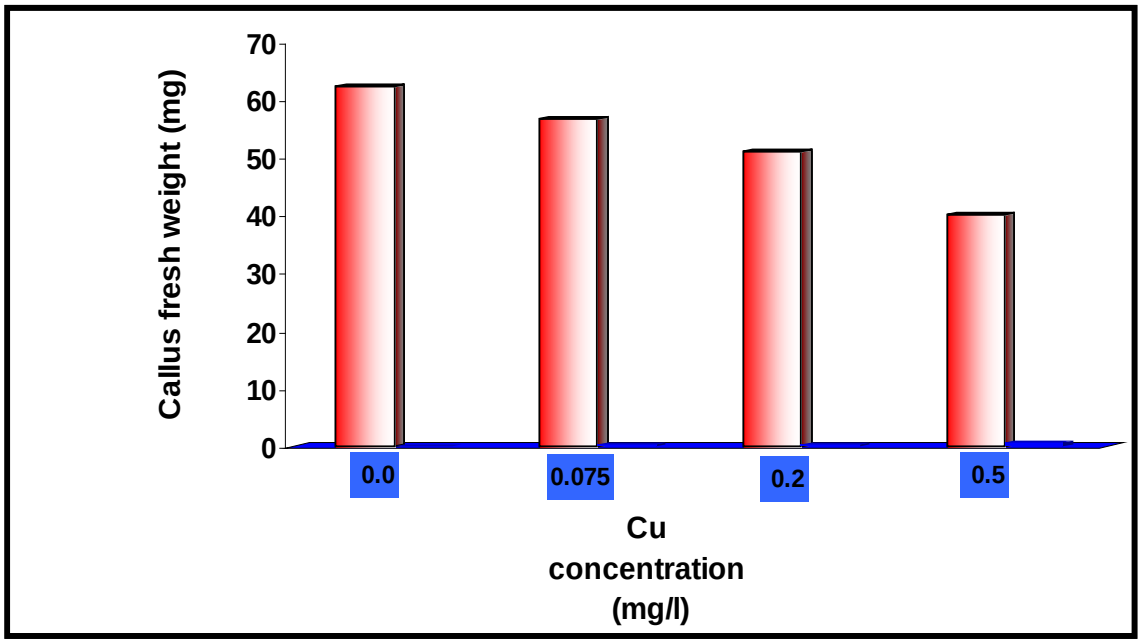

Figure (3-3):Effect of different concentrations of Copper on callus fresh weight grown on MS medium supplemented with 0.5 mg/l BA and 2 mg/l 2,4-D, after 25 days, the initial callus fresh weight is 35 mg (n=9)

Copper is essential for plant growth and development, since it interacts with many enzymes and proteins. While elevated concentrations of Cu can lead to toxicity symptoms and inhibition of plant growth (Hall, 2002). Toxicity may result from the binding of Cu to sulphydryl groups in proteins, leading to an inhibition of activity or disruption of structure (VanAssche and Clijsters, 1990).

This result is in agreements with Stefano and Edoardo (2003) who cultured the apical and single node of *Ailanthus altissima* on MS medium

containing different concentrations of Cu and different plant growth regulators in order to investigate the suitable combination for proliferation. They found that media containing Cu led to a reduction in shoot growth as compared with untreated ones.

3.3.4- Effect of chromium on callus fresh weight

The highest callus fresh weight recorded was (62) mg in callus cultures grown on Cr free medium. While callus fresh weight decreased at (0.25, 0.40 and 0.5) mg/l reaching (57.1, 49.2 and 42.5) respectively (fig. 3-4).

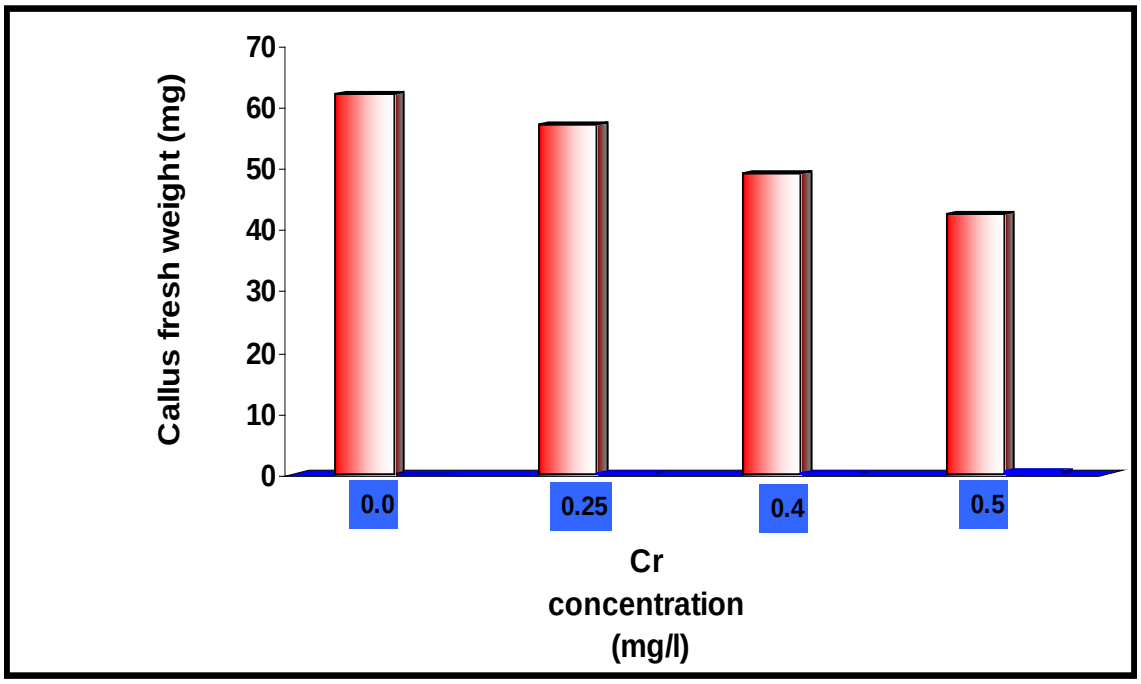

Figure (3-4):Effect of different concentrations of Chromium on callus fresh weight grown on MS medium supplemented with 0.5 mg/l BA and 2 mg/l 2,4-D, after 25 days, the initial callus fresh weight is 35 mg (n=9)

3.3.5- Effect of zinc on callus fresh weight

Fig.(3-5) 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.

Zinc among the essential metals, at low concentrations, plays critical role in a wide variety of biochemical processes as enzyme cofactors. Therefore, intracellular zinc concentration must be maintained at adequate levels to support cell growth (Blaudez, *et al*., 2003). High levels of Zn lead to toxicity symptoms and inhibition of plant growth. Toxicity lead to an inhibition of cell activity or disruption of structure. In addition, excess Zn may stimulate the formation of free radicals, perhaps resulting in oxidative stress (Dietz *et al*., 1999).

This result is in accordance with Kishinami and Widholm (1986) who reported that increased level of Zn led to a decrease in callus fresh weight. They were able to isolate Zn resistant cells of *N. plumbaginifolia* from unmutagenized cell suspensions in medium containing normally lethal concentration of Zn

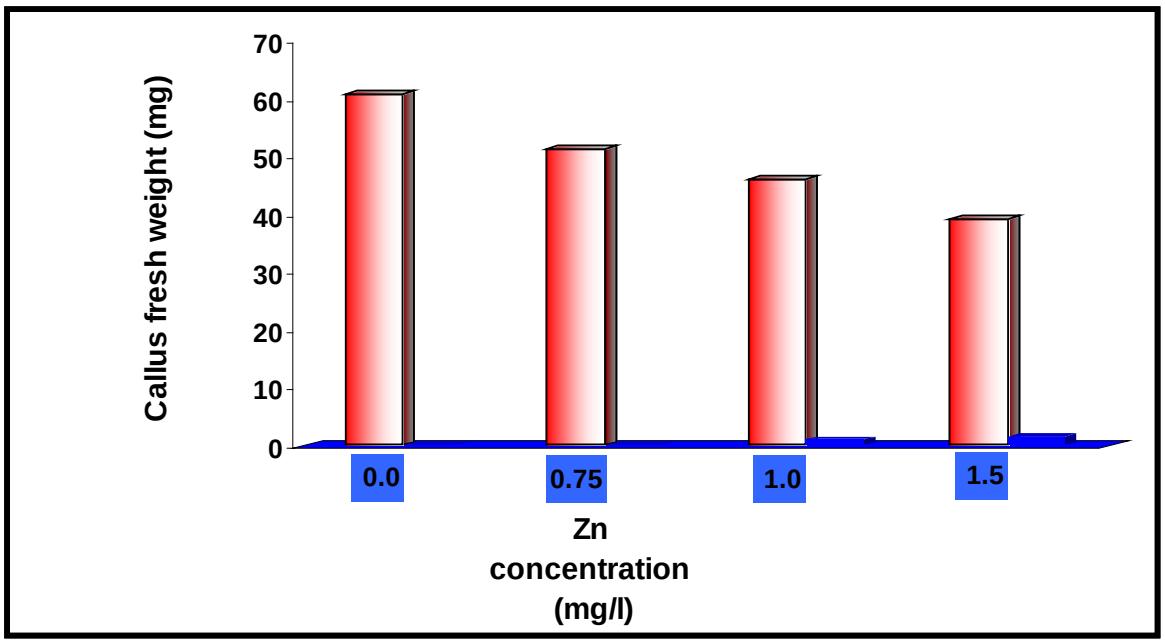

Figure (3-5):Effect of different concentrations of Zinc on callus fresh weight grown on MS medium supplemented with 0.5 mg/l BA and 2 mg/l 2,4-D, after 25 days, the initial callus fresh weight is 35 mg (n=9)

3.3.6-Analysis of heavy metals absorbed by callus culture

The callus of *S. grandiflora* cultured on MS medium supplemented with 2 mg/l 2,4-D and 0.5 mg/l BA. These medium containing different concentrations of heavy metals in order to determine the amount of heavy metals absorbed by callus.

Atomic absorption spectrophotometer was used for quantification analysis of heavy metals absorbed by callus tissues .

The concentration of Cd accumulated in callus culture increased with increasing of Cd concentration (Table 3-4).

Callus tissue may not contain enough Cd. Therefore it absorbs more Cd from medium, and intermediate concentrations of Cd are not enough to make it toxic to callus culture.

This is in agreement with Duxbury (1985) 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 until reach to (0.25, 0.2, 0.25 and 1.0) mg/l of (Co, Cu, Cr and Zn) respectively. Then this amount begun to decreased in (0.5, 0.5, 0.4 and 1.5) mg/l of by increasing of (Co, Cu, Cr and Zn) concentrations respectively. This result shown in table (3-4).

This result is in agreement with Benavides, *et al*. (2005) who found that Cobalt, Copper, Chromium, Zinc, and Nickel are toxic elements to plant tissue at high concentrations.

Table (3-4): Concentration of heavy metals absorbed by callus culture of *S. grandiflora* grown on MS medium supplemented with 2 mg/l 2,4-D and 0.5 mg/l BA, after 25 days

Heavy metal	Heavy metal concentration (mg/l)	Absorbed concentration (ppm)
Cd	٠,٠٥	١١٠
	٠,٠٧٥	١٢٠
	٠,١	١٣٠
Control	0.0	٢
Co	٠,١	80
	٠,٢٥	١٦٠
	٠,٥	٦٠
Control	0.0	١٠
Cu	٠,٠٧٥	٢٠٠
	٠,٢	٣٤٠
	٠,٥	١٥٠
Control	0.0	100
Cr	0.25	130
	٠,٤	١٢٠
	٠,٥	١٠٠
Control	0.0	١.5
Zn	0.75	450
	١,٠	٥٦٠
	١,٥	٣٤٠
Control	0.0	150

3.4-Field Experiment

In order to calculate the germination percentage of germinated seeds subjected to heavy metals under investigation under field conditions.

3.4.1- Effect of different concentration of cadmium on germination percentage and plant height

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 percentage 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. 3-6).

Also, Maximum plant height recorded in control at the end of day (20) reached (32.6) cm (fig. 3-7). 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 fertilizer material (Tucker *et al*., 2005). High amount of Cd in plants leads 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 carotenoid are reduced (Krupa *et al*., 1993).

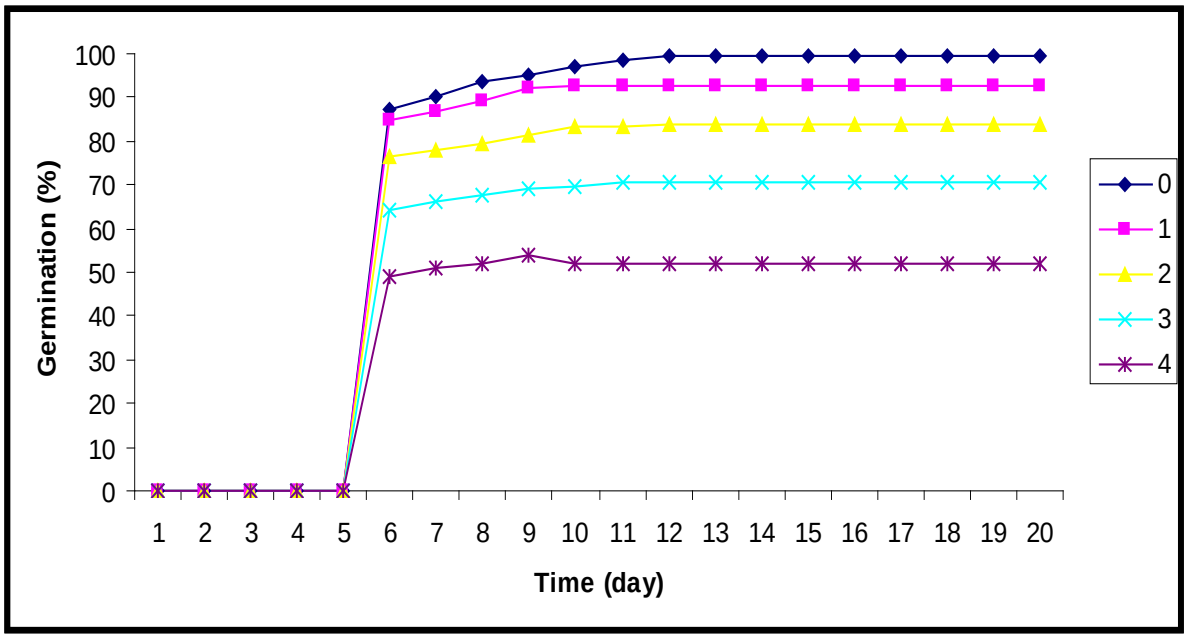

Figure (3-6):Germination percentages of *S. grandiflora* seeds germinated for 20 days on different concentrations of Cd (mg/Kg soil)

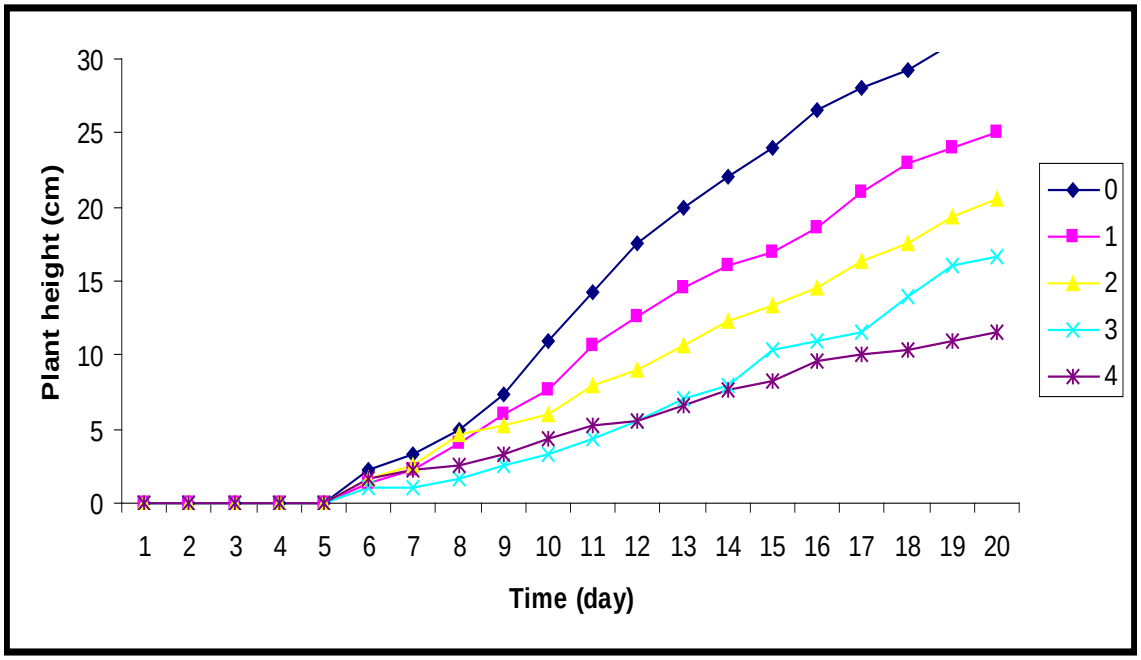

Figure (3-7):Effect of different concentrations of Cd (mg/Kg soil) on plant height after 20 days growth in pots

3.4.2- Effect of different concentration of cobalt on germination percentage and plant height

Fig. (3-8) shows that the highest germination percentage of germinated seeds 99.7% recorded in control at day (20). Germination percentage decreased with increasing Co concentration reached (95.4, 89, 75.6 and 67%) at Co concentrations (15, 30, 45 and 60) mg/Kg soil respectively.

At day (20), maximum plant height (32.6) cm was recorded in control (fig. 3-9). Plant height decreased with increasing of Co concentration over time reaching (25, 24.6, 20.3 and 19) cm for (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 assimilate production in leaves, and even inhibits the export of photoassimilates to roots and causes toxicity to plants (Li *et al.*, 2005).

This result is in agreement with Liu *et al*. (2000) who found that high concentrations of Co lead to inhibition of seedling growth and chlorosis of younger leaves in mung beans.

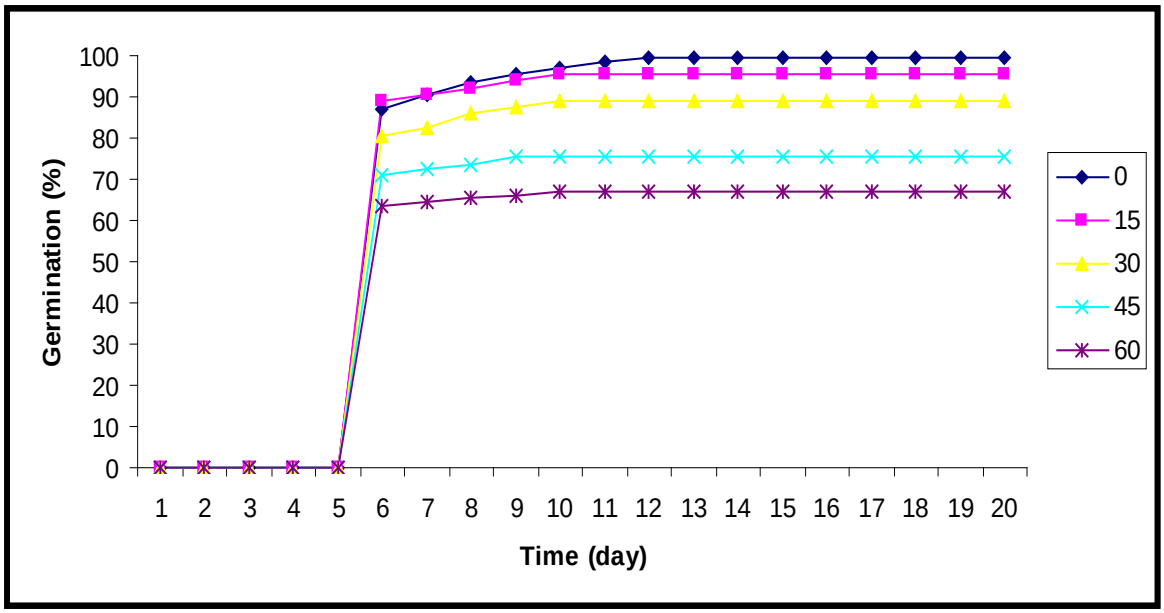

Figure (3-8):Germination percentages of *S. grandiflora* seeds germinated for 20 days on different concentrations of Co (mg/Kg soil)

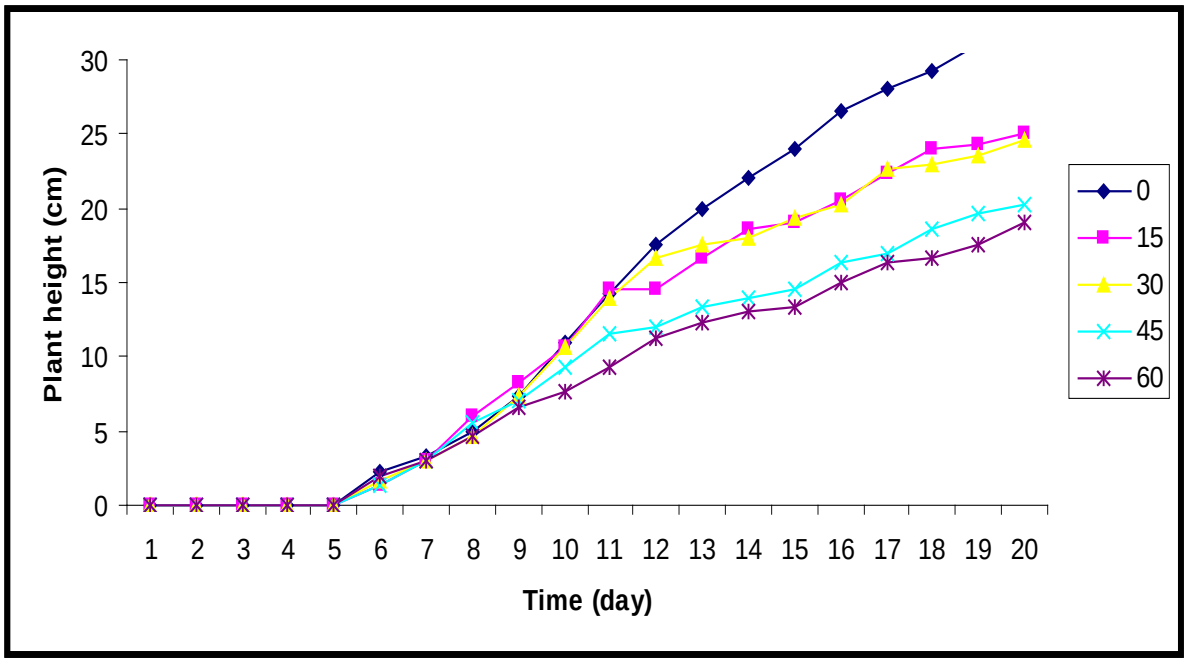

Figure (3-9):Effect of different concentrations of Co (mg/Kg soil) on plant height after 20 days growth in pots

3.4.3- Effect of different concentration of copper on germination percentage and plant height

Germination percentage decreased with increasing Cu concentrations (fig. 3-10). The highest germination percentage recorded in control (99.7%). Compared with the control, the germination percentage decreased to (25, 50, 75 and 100) mg/Kg soil reaching (89, 84.6, 77.6 and 71.6%) respectively.

Fig. (3-11) 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 (Tucker *et al*., 2005).

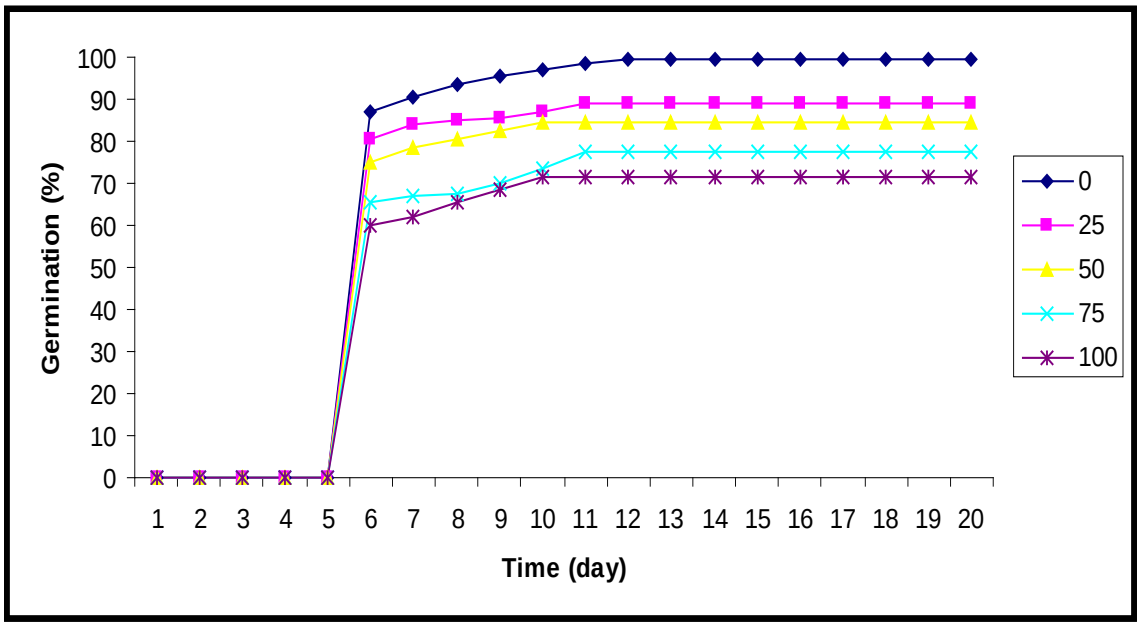

Figure (3-10):Germination percentages of *S. grandiflora* seeds germinated for 20 days on different concentrations of Cu (mg/Kg soil)

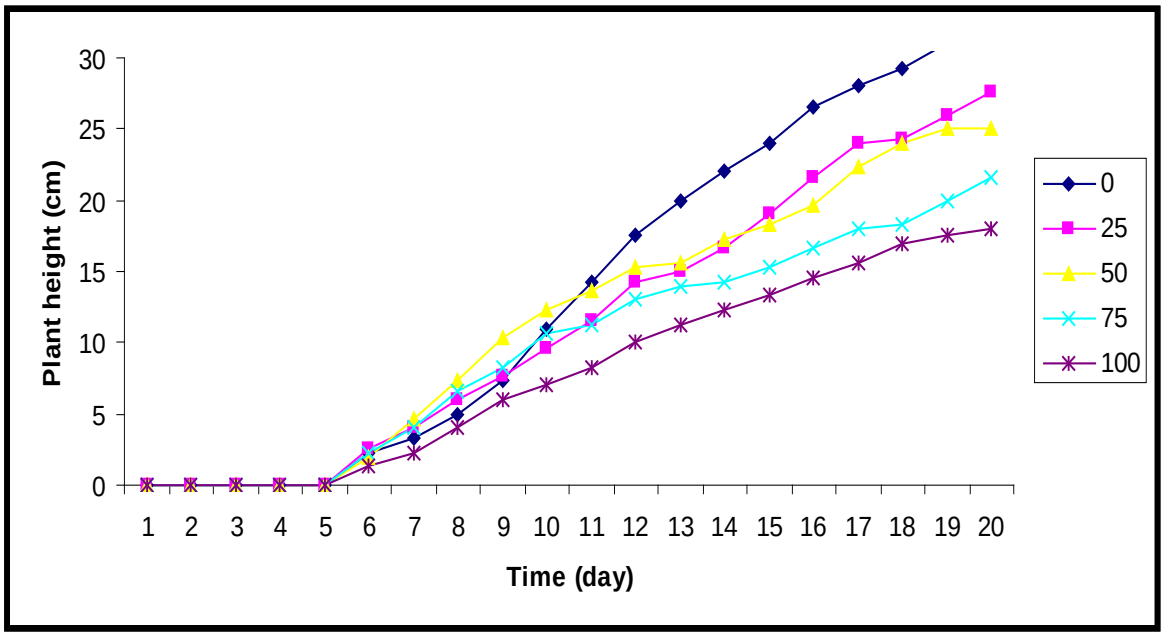

Figure (3-11):Effect of different concentrations of Cu (mg/Kg soil) on plant height after 20 days growth in pots

3.4.4- Effect of different concentration of chromium on germination percentage and plant height

Germination percentages of seeds exposed to different Cr concentration are shown in (fig. 3-12). Germination started at day (6). Maximum germination percentage was recorded in control treatment reaching (99.7%), then begun to decrease at (25, 50, 75 and 100) mg/Kg soil reaching (80, 78.5, 74.1 and 63.5%) respectively at day (20).

Fig. (3-13) 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. It is strongly attach to soil and only

small amount can dissolve in water and move deeper in the soil to underground water. Cr compounds can increase the risk of lung cancer of human (Atlanta, 2000).

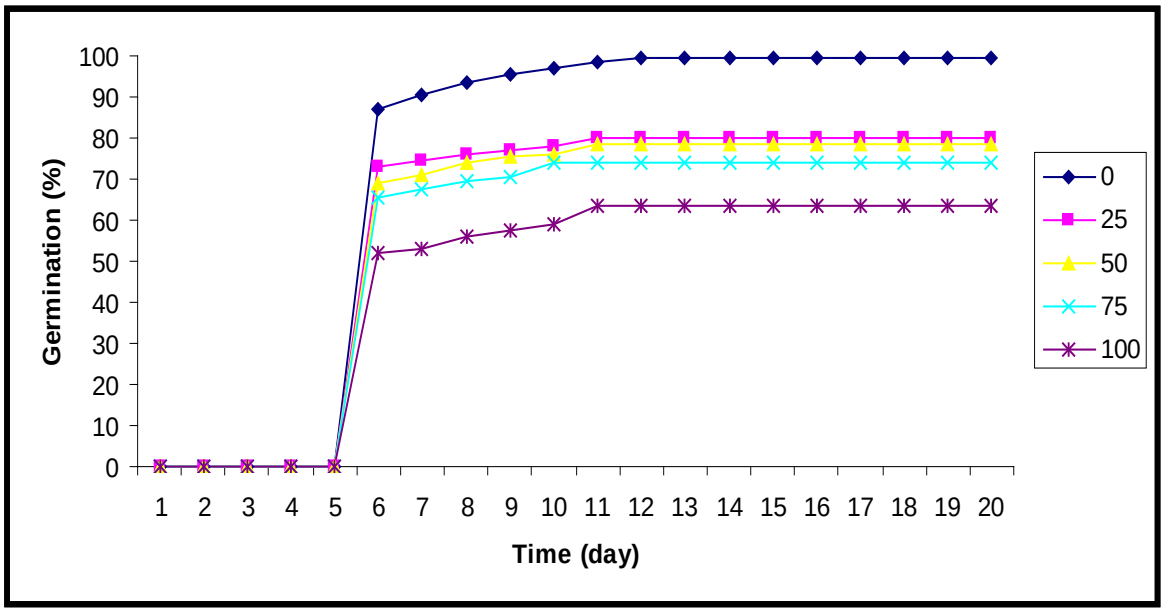

Figure (3-12):Germination percentages of *S. grandiflora* seeds germinated for 20 days on different concentrations of Cr (mg/Kg soil)

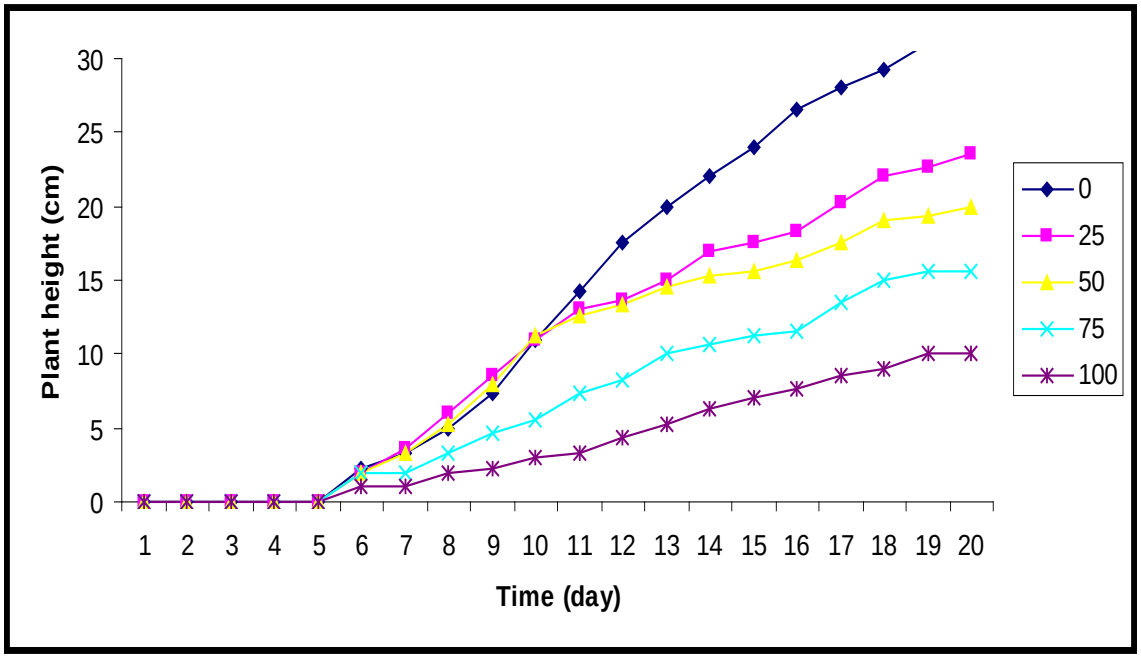

Figure (3-13):Effect of different concentrations of Cr (mg/Kg soil) on plant height after 20 days growth in pots

3.4.5- Effect of different concentration of zinc on germination percentage and plant height

Maximum germination percentage was recorded in control treatment reaching (99.7%) (fig. 3-14). While reaching to a minimum (38.4%) at (400) mg Zn/Kg soil. This percentage 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. 3-15). 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 and carbohydrate, nucleic acid and lipid metabolism (Stoyanova and Doncheva, 2002). Zn is accumulated in excess in plant tissues, it causes alterations in vital growth processes such as photosynthesis and chlorophyll biosynthesis (Doncheva *et al*., 2001).

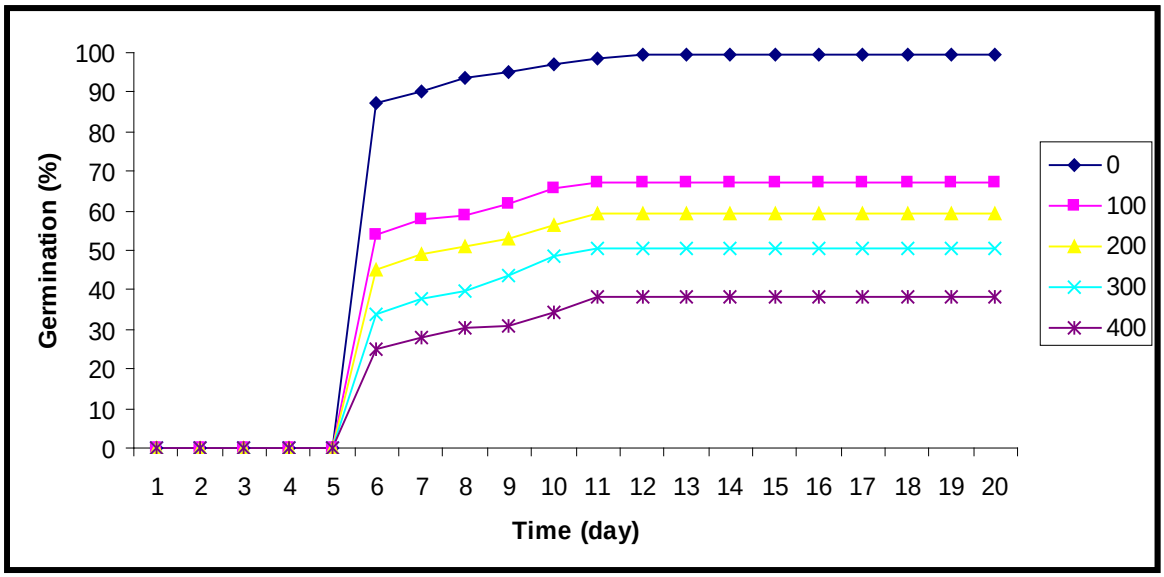

Figure (3-14):Germination percentages of *S. grandiflora* seeds germinated for 20 days on different concentrations of Zn (mg/Kg soil)

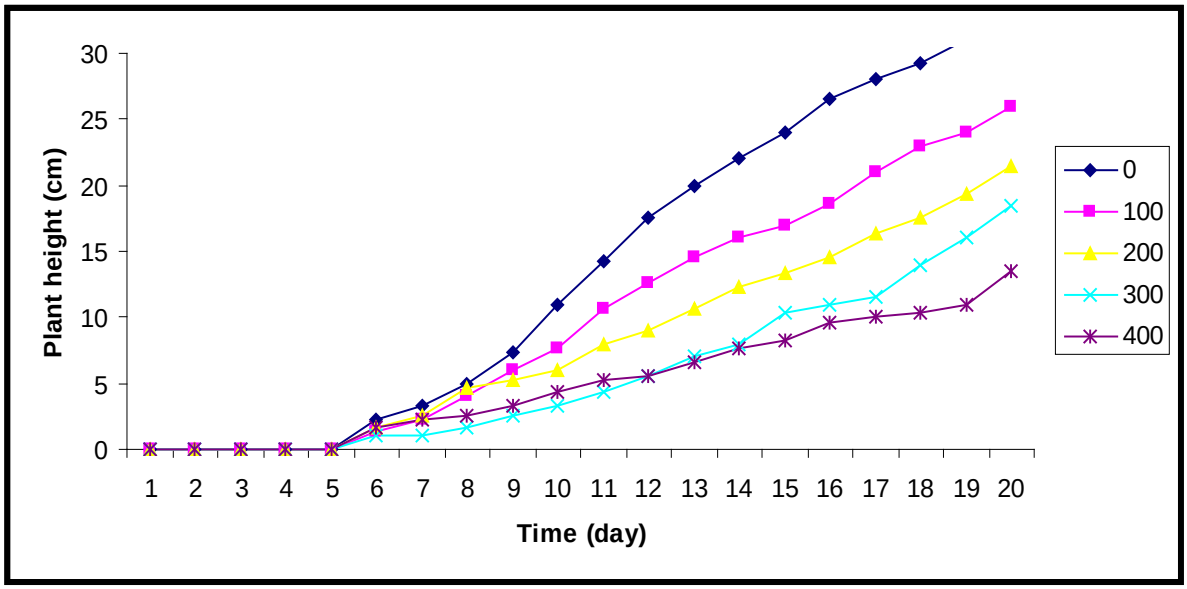

Figure (3-15):Effect of different concentrations of Zn (mg/Kg soil) on plant height after 20 days growth in pots

This decrease in plant growth may be due to the high levels of these heavy metals accumulated in plant cells that suppressed plant growth. This is in agreement with Corey *et al.*, (1981) and Barber, (1984) who reported that two mechanisms are 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.

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 extracellular binding domain to which the ion attach just before the transport, and a transmembrane structure which connects extracellular 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 extracellular space through the hydrophobic environment of the membrane into the cell (Lasat, 2001). The

heavy metal must mobile into the soil solution, for the plants to accumulate them. The bioavailability of metals is increased in soil through several means in which plants achieve it by screating phytosidophores into the rhizosphere to chelate and solublise metals that are soil bound (Ghosh and Singh, 2005). Both acidification of the rhizosphere and exudation of carboxylates are considered potential targets for enhancing metal accumulation. 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 (Ghosh and Singh, 2005).

3.4.6-Analysis of heavy metals absorbed by whole plant

Atomic absorption spectrophotometer was used for quantification of heavy metals absorbed by *S. grandiflora* whole plant.

As shown in table (3-5) 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 (80) ppm.

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 the 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 3-5).

The maximum amount of Cr absorbed by whole plant occurred at (75) mg/Kg soil reaching (1310) ppm. A decline in concentration of Cr

accumulated in plant tissue at (100) mg/Kg soil and higher reaching to (880) ppm. Level of 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 to the plant, so can't absorbed by plant at high concentration. These results are in agreement with Niess, 1999 who founded that Ni, Co, Va, Cr, Cd are toxic elements with high level in the 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 elements for plant.

Maximum amount values of Zn absorbed by whole plant tissue were recorded at (400) mg/Kg soil reaching (2220) ppm. While the lowest amount 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 increase with increasing Zn in soil because its necessary elements for plant growth and development.

Because of 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 increase with increasing of heavy metals concentrations. Zn and Cu among essential metals and play a critical roles in a wide variety of biochemical processes like cell growth and this in agreement with above results (Blaudez *et al.*, 2003). Some heavy metals are essential for normal plant growth, although elevated concentrations can result in growth inhibition and toxicity symptoms. Plant possess 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 (Hall, 2002).

Table (3-5): Concentration of heavy metals absorbed by *S. grandiflora* whole plant

Heavy metal	Heavy metal concentration (mg/Kg)	Absorbed concentration (ppm)
Cd	1	٥٥٠
	2	١١١٠
	3	٨٣٠
	4	٤٤٠
Control	0.0	٨٠
Co	١٥	٢٢٠
	٣٠	٥٥٠
	٤٥	٤٧٠
	٦٠	٤٤٠
Control	0.0	١١٠
Cu	٢٥	٤٦٠
	٥٠	٦٢٠
	٧٥	٧٦٠
	١٠٠	٨٩٠
Control	0.0	٢٥٠
Cr	٢٥	٨٠٠
	٥٠	١٢٨٠
	٧٥	١٣١٠
	١٠٠	٨٨٠
Control	0.0	١٠٠
Zn	١٠٠	١١١٠
	٢٠٠	١٣٥٠
	٣٠٠	١٨٣٠
	٤٠٠	٢٢٢٠
Control	0.0	٧٢٠

الإهداء

إلى واهب الحياة ديمومتها..... **ربي**

إلى من علمني الأخلاق والثبات على الحق..... **رسولي**

إلى الذي أهدى لي سنين عمره وعاند الهم بصبره.....**أبي**

إلى من وهبتني أغلى ما يملكه البشر نور عينها فأصبحت نور عيني.....**أمي**

إلى من هي عنوان فخري ويوم وجعي تكون راحتي.....

أمي وبنت عمي سندس

إلى من كان لي خير عون لينير لي دربي بعلمه..... **أستاذي**

إلى القلوب التي أضاءت لي درب المستقبل....**إخوتي وأخواتي**

اهدي لهم جميعا ثمرة جهدي هذا

شيماء

[قُل لَّو كَانَ البَحرُ مِدَاداً لِّكَلِمَٰتِ رَبِّي لَنَفِدَ البَحرُ قَبلَ أَن تَنفَدَ كَلِمَٰتُ رَبِّي وَلَو جِئنَا بِمِثلِهِ مَدَداً* قُل إِنَّمَا أَنَا۠ بَشَرٌ مِّثلُكُم يُوحَىٰٓ إِلَيَّ أَنَّمَا إِلَٰهُكُم إِلَٰهٌ وَٰحِدٌ فَمَن كَانَ يَرجُوا۟ لِقَاءَ رَبِّهِ فَليَعمَل عَمَلاً صَٰلِحاً وَلَا يُشرِك بِعِبَادَةِ رَبِّهِ أَحَداً*]

صدق الله العظيم

سورة الكهف

الآية (١٠٩، ١١٠)

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Shaimaa

Supervisor Certification

I certify that this thesis was prepared under my supervision in the College of Science, Al-Nahrain University as a partial requirement for the degree of Master of Science in Biotechnology.

Signature:

Supervisor: **Dr. Kadhim M. Ibrahim**

Scientific Degree: Professor

Date: / /2007

In review of the available recommendations, I forward this thesis for debate by the examining committee.

Signature:

Name: **Dr. Waleed Hameed Yousif**

Scientific Degree: professor.

Title: Head of Biotechnology Department.

Date: / /2007

Committee Certification

We, the examining committee, certify that we have read this thesis and examined the student in its contents and that, according to our opinion, is accepted as a thesis for the degree of Master of Science in Biotechnology.

Signature:

Name:

Scientific Degree:

Date: / /2007

(Chairman)

Signature:

Name:

Scientific Degree:

Date: / /2007

(Member)

Signature:

Name:

Scientific Degree:

Date: / /2007

(Member)

I hereby certify upon the decision of the examining committee

Signature:

Name: **Dr. Laith A. Z. Al- Ani**

Scientific Degree: Assistant Professor

Title: Dean of College of Science

Date: / /2007

الملخص

في محاولة لدراسة تحمل نبات السيسبان *Sesbania grandiflora* للعناصر الثقيلة على مستوى النبات الكامل ومزارعه النسيجية، تم تنفيذ عدد من التجارب لهذا الغرض.

استحث الكالس وأديم على وسط MS المجهز ب (٠,٥) ملغم/لتر من BA و (٢) ملغم/لتر من 2,4-D باستعمال الفلق كمصدر لنشوء الكالس.

أضيفت تراكيز مختلفة من العناصر الثقيلة (Cd, Co, Cu, Cr, Zn) إلى الوسط الغذائي كعوامل تلوث.

أوضحت النتائج نقصان وزن الكالس بزيادة تراكيز العناصر الثقيلة في الوسط ألزرعي.

لغرض دراسة تأثير هذه الملوثات من العناصر الثقيلة في الترب، زرعت بذور نبات السيسبان في تراكيز مختلفة من هذه العناصر لمدة ٣ أسابيع.

أوضحت النتائج زيادة نسبة الإنبات وارتفاع النبات بزيادة الوقت (الأيام) لكنها انخفضت بزيادة تراكيز العناصر الثقيلة قيد الدراسة.

استخدم جهاز الامتصاص الذري في معرفة كمية العناصر الممتصة من قبل النبات الكامل والكالس.

كمية العناصر الممتصة والمتجمعة في الكالس كانت (٠,٠٠١، ٠,٠١١، ٠,٠١٢و ٠,٠١٣%) ل (٠,٠، ٠,٠٥، ٠,٠٧٥و ٠,١) ملغم/لتر على التوالي من الكادميوم، (٠,٠٠١، ٠,٠٠٨، ٠,٠١٦و ٠,٠٠٦%) ل (٠,٠، ٠,١، ٠,٢٥و ٠,٥) ملغم/لتر على التوالي من الكوبالت، (٠,٠٠١، ٠,٠٢٠، ٠,٠٣٤و ٠,٠١٥%) ل (٠,٠، ٠,٠٧٥، ٠,٢و ٠,٥) ملغم/لتر على التوالي من النحاس، (٠,٠٠١، ٠,٠١٣، ٠,٠١٢و ٠,٠١٠%) ل (٠,٠، ٠,٢٥، ٠,٤و ٠,٥) ملغم/لتر على التوالي من الكروم، (٠,٠٢٧، ٠,٠٥١، ٠,٠٥٩و٠,٠٥٦%) ل (٠,٠، ٠,٧٥، ١,٠و ١,٥) ملغم/لتر على التوالي من الخارصين.

كمية العناصر الممتصة والمتجمعة في النبات الكامل كانت (٠,٠٨، ٠,٥٥، ١,١١، ٠,٨٣و ٠,٤٤%) ل (٠,٠، ١,٠، ، ٣,٠٢,٠و ٤,٠) ملغم/كغم تربة على التوالي من الكادميوم، (٠,١١، ٠,٢٢، ٠,٥٥، ٠,٤٧و ٠,٤٤%) ل (٠,٠، ١٥,٠، ، ٣٠,٠،٤٥,٠و ٦٠,٠) ملغم/كغم تربة على التوالي من الكوبالت، (٠,٠١، ٠,١٠، ٠,٥٧، ٠,٥٨و ٠,٧٢%) ل (٠,٠، ٢٥,٠، ، ٥٠,٠،٧٥,٠و ١٠٠,٠) ملغم/كغم تربة على التوالي من النحاس ، (٠,٠٨، ٠,٨٠، ١,٢٨، ١,٣١و ٠,٨٨%) ل (٠,٠، ٢٥,٠، ،

٧٥,٠،٥٠,٠و ١٠٠,٠) ملغم/كغم تربة على التوالي من الكروم، (٠,٠٦، ١,١١، ١,٢٠، ١,٨٣و ٢,٢٢%) ل (٠,٠، ١٠٠,٠، ٢٠٠,٠،٣٠٠,٠و ٤٠٠,٠) ملغم/كغم تربة على التوالي من الخارصين.

Ministry of Higher Education and Scientific Research
Al-Nahrain University
College of Science

In vivo and *in vitro* Studies on Heavy Metals Tolerance in *Sesbania grandiflora* L.

A thesis
Submitted to the College of Science, Al-Nahrain University
in Partial Fulfillment of Requirements for the Degree of
Master of Science in Biotechnology

By
Shaimaa Abdul Wahab Yousir
B.Sc. Biotechnology, College of Science, 2004
Al-Nahrain University

Supervised by

Dr. Kadhim M. Ibrahim

2007 **June**

1428 **Rabi' Al-Thane**

وزارة التعليم العالي والبحث العلمي
جامعة النهرين
كلية العلوم

دراسة تحمل نبات السيسبان *Sesbania grandiflora* L. للعناصر الثقيلة خارج وداخل الجسم الحي

رسالة

مقدمة إلى كلية العلوم في جامعة النهرين
وهي جزء من متطلبات نيل درجة الماجستير
في علوم التقانة الإحيائية

من قبل

شيماء عبد الوهاب يسر
بكالوريوس تقانة إحيائية- كلية العلوم- ٢٠٠٤
جامعة النهرين

بإشراف

الاستاذ الدكتور كاظم محمد إبراهيم

حزيران ٢٠٠٧
ربيع الثاني ١٤٢٨