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Short Communication

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Comparative Phytoremediation Capacity of *In-vivo* and *In-vitro* Produced Plants of *Polyscias fruticosa* (L.) Harm.

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Key words: *In-vitro* and *In-vivo* plantlet production, Phytoaccumulation capacity, *Polyscias fruticosa* (L.) Harm.

Polyscias fruticosa (L.) Harm is plant which belonging to Araliaceae family, also known as Ming Aralia. It is dicot shrub native to India. This is shade loving and planted for its foliage purposes. It has compound leaves with seven or more than seven leaflets. Generally, the leaves are deeply lobbed and opposite arrangement is observed. The growth of the plant is seen highest from 19-29°C temperature. Its sensitive plant for any type of stress specially it cannot survive at high temperature. It bears rare flowers and mostly used as an ornamental foliage plant. It is not directly edible by any animals or humans. The leaves have so many important phytochemical constituents and that can be utilized for drug designing.

The shoot apexes (tips) of *Polyscias fruticosa* (L.) Harm were selected for the propagation of plants through *In-vitro* and *In-vivo* approaches. The Experimental work was completed at Plant Biotechnology Laboratory and Botanical Garden of Gujarat University.

In-vitro plantlet production: All the Explants were sterilized with the help of 0.1-1% $HgCl_2$ solution and inoculated in M. S. Media which is standardized media for plant tissue culture. And after the development of shoots after 20 days all the plantlets were sub cultured in rooting media through M.S. Media and after 30days the mature plantlets were harden in the net house of Botanical garden, Gujarat University.

In-vivo plantlet production: Different sized stem cuttings were selected for the plantlet production of *Polyscias fruticosa* (L.) Harm. The media was prepared for the propagation in which 70% garden soil, 20% Organic manure and 10% soil were added. All the cuttings were separately cultured in the small sized plastic pots for 30 days.

Table 1 Showing accumulation of lead in *in-vitro* produced plants

In-vitro produced *Polyscias fruticosa* (L.) Harm.			
Pb concentration in different parts / treatment	Pb in roots mg/kg	Pb in stem mg/kg	Pb in leaves mg/kg
Control	9.21±0.09	3.39±0.05	1.02±0.01
400mg/kg	1309±11.48	1149±40.02	859±19.40
800mg/kg	1420±70.98	1125±60.09	979±2.42

Table 2 Showing accumulation of lead in *in-vivo* produced plants

In-vivo produced *Polyscias fruticosa* (L.) Harm.			
Pb concentration in different parts / treatment	Pb in roots mg/kg	Pb in stem mg/kg	Pb in leaves mg/kg
Control	7.09±.20	2.98±0.09	0.92±0.92
400mg/kg	1290±17.04	1120±10.02	769±1.42
800mg/kg	1331±20.04	1113±13.26	667±11.30

Treatment of cadmium and lead: After proper Hardening process of all the plantlets produced through *In-vitro* and *In-vivo* approaches they were transplanted in the pots in which 5 kg soil was filled per pot. After proper maturation all the plants were separately treated with different metals named lead nitrate and cadmium nitrate. The concentrations were selected for lead 200mg/kg, 400mg/kg, 600mg/kg and 800mg/kg and same way for cadmium 5mg/kg, 110mg/kg, 15mg/kg and 20mg/kg. for all these sets triplicates were selected and one set was kept as

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control (without metal treatment). Here the metal treatment provided in the form of lead nitrate and cadmium nitrate. The solutions were prepared and treatment was given directly through the rootzone. The plants were incubated for 75 days.

Table 3 Showing accumulation of cadmium in *in-vitro* produced plants

In-vitro produced *Polyscias fruticosa* (L.) Harm.			
Cd concentration in different parts / treatment	Cd in roots mg/kg	Cd in stem mg/kg	Cd in leaves mg/kg
Control	0.80±0.04	0.67±0.03	0.41±0.03
10mg/kg	72.02±5.40	49.09±2.35	23.03±4.53
20mg/kg	89.09±2.31	59±3.24	33.33±2.10

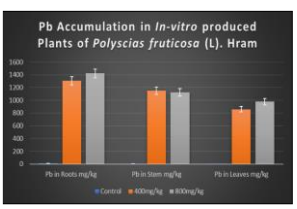
Table 4 Showing accumulation of cadmium in *in-vivo* produced plants

In-vivo produced *Polyscias fruticosa* (L.) Harm.			
Cd concentration in different parts / treatment	Cd in roots mg/kg	Cd in stem mg/kg	Cd in leaves mg/kg
Control	0.69±0.03	0.45±0.02	0.23±0.02
10mg/kg	65.60±2.41	40.30±3.45	13.03±1.04
20mg/kg	80.81±3.47	43.40±4.21	23.13±1.09

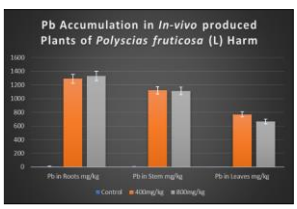
Collection, drying of the plant parts: After the incubation time of 75 days all the plants were collected individually and the parts roots, stem and leaves were segregated and dried in the oven at 80°C for 45 minutes.

Quantitative estimation of lead and cadmium in plant part: All the collected and segregated plant parts of each concentration crushed segregate. 1gm dry powder of each sample was weighed and taken in to conical flask and 10ml of concentrated HNO_3 was added. The mixture was boiled at constant temperature for 10min. After cooling 5ml of 70% $HClO_4$ was added and the mixture was further boiled until the realize the dense white fumes. After cooling, 20ml distilled water was added and heated until a clear solution was obtained. The mixture was filtered after cooling with the help of Whatman filter paper no. 44 and transferred quantitatively to a 50ml volumetric flask by adding de-ionized and double distilled water. Samples were analyzed through AAS (Atomic Absorption Spectroscopy) for quantification of Cd and Pb. Results were expressed as mg/kg metal content in dry powdered material of respective plant part. The results were collected as Mean ± S.D. (Standard Deviation).

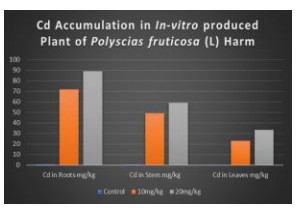
After 75 days of incubation time the plant part accumulation is seen high in roots as compare to stem and leaves. As the table showing the data of accumulation of lead and cadmium in the different plant parts that *In-vitro* produced plants has more phytoaccumulation capacity of the heavy metal as compare to *In-vivo* produced plants. as the table shows that as the metal concentration increases the accumulation rate of metal also increases. Few researchers worked on this plant for remediation activity. Yang *et al.* (2009) described the Octane indoor air pollutant remediation capacity of the *Polyscias fruticosa* (L.) Harm., here the research work focused on the remediation of soil pollutant heavy metals through *Polyscias fruticosa* (L.) Ham.



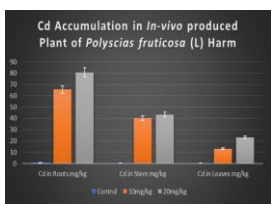
Graph 1 Pb accumulation in *In-vitro* produced plant



Graph 2 Pb acculation in *In-vivo* produced plant



Graph 3 Cd accumulation in *In-vitro* produced plant



Graph 4 Cd accumulation in *In-vivo* produced plant

In control set also the results showing the accumulation because some amount of metal was present there in the garden soil that we had used for the research work which was negligible amount per kg. So, the Soil samples were previously analyzed though AAS. As per Pendias and Pendias (1992) permissible limit for the lead is 250-500mg/kg and for Cadmium it is 3-6mg/kg. But here for this research work we tried to increase it and that shows that *Polyscias fruticosa* (L.) Harm is metal stress tolerant ornamental species.

Fig A *In-vitro* production of Aralia

Fig B *In-vivo* production of Aralia

Fig C Hardening of the plantlets

Fig D Ready plants for transplantation

Fig E Treatment to the plants in pots

Fig F Plants after metal treatment

Fig G Collection after treatment

Fig H Segregated plant parts

Fig I Acid digestion of dried powder

Fig J Filtration of acid digested material

As the table data shows that the plant has high phytoaccumulation capacity of lead and cadmium metal so, it can be used to remove the soil pollutants, especially heavy metals from the soil. In future the proteins which are responsible for the uptake or the phytochemicals produced by the plants that can be identified where the metal is binding. With the help of In-silico analysis and different software's of proteomics the proteins can be docked to check the binding capacity.

SUMMARY

In this research heavy metals accumulation capacity of *Polyscias fruticosa* (L.) Harm. was assessed. Here, two different approaches *In-vitro* and *In-vivo* were used for the production of plantlets. *In-vitro* approach involved tissue culture approach and *In-vivo* direct through media (soil, cocopeat, mosses). Shoot apexes were used for the production of plantlets. After 30 days of plantlets development all the plantlets which were produced through *in-vitro* and *in-vivo* approaches and plants were transplanted in the pots and treated with two metals lead and cadmium in the form of Pb $(NO_3)_2$ and Cd $(NO_3)_2$. Different concentrations were selected for Lead 200mg, 400mg, 600mg, 800mg/kg and for cadmium 5mg, 10mg, 15mg, and 20mg/kg. Each pot was filled with 5kg of soil. The metals were given directly through root zone of plants in solution form. After incubation time of 75 days mature and treated plants were collected and root length, shoot length, number of branches were measured scientifically. On the basis of the results of AAS (Atomic Absorption Spectroscopic Analysis) we can conclude that the accumulation of lead and cadmium is higher in *In-vitro* produced plants as compared to *In-vivo* produced plants. *Polyscias fruticosa* (L.) Harm. is high metal stress tolerant species which also shows good amount of metal remediation capacity (Lead and Cadmium). So, in future for Phytoremediation of heavy metals can be done through Tissue Cultured or *In-vitro* produced plants. In future, remediation capacity of different soil pollutants can be analyzed through this method.

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