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# Laser Biotechnology for Enhanced Rooting and Shooting of *Salix Viminalis* in Hydroponic Condition for Better Adaptation in Industrially Contaminated Land

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**Abstract** Laser Photo stimulation of selected plant and microbial species has been very important in reclamation of several degraded land masses as well as effective treatment of waste and contaminated water sources. The experiment on Laser Photo-stimulation of different size of willow cuttings is carried out in laboratory condition at Department of Environment protection Engineering of AGH-UST, Krakow, Poland. Laser irradiation with selected wavelength and time of exposition of willow cuttings and establishment in hydroponic culture system resulted in enhanced development of roots and shoots compared to the non-irradiated control group. Experimental observation made every week after the irradiation has results of increased number of roots and sprouts. Root buds also increased rapidly in number after second week. Roots changed into highly dispersed secondary roots in II and IV experimental groups and sprouts also started to develop leaves after second week. Remarkable differences in control and experimental groups were seen after five weeks of hydroponic culture. The enhanced development of roots, shoots and other protective organs like root caps after laser irradiation is supposed to help willow species to grow fast and adapt in industrially contaminated land.

***Keywords:*** *laser, biostimulation, willow, root, shoot, cuttings*

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## 1. Introduction

This article highlights the use of laser biotechnology in land reclamation with a cost and labor efficient method. Laser biostimulation can be used for efficient waste water treatment, soil reclamation, increased bioremediation abilities and biomass increment in plants [1]. Experimentally selected wavelength, energy density and time of exposition to low power laser has been found as an effective tool for acceleration of cells division and physiological activity of various bacteria, fungi, protozoa, algae and higher plants [2]. Laser stimulation has also been effective in increasing the uptake of biogenic and trace elements from contaminated soil and water. Results of the experiments showed the enhanced growth of roots and shoots in willow (*Salix*) cuttings and better adaptation in industrially contaminated soil supplemented with mycorrhiza inoculums. Since Laser biotechnology has diverse area of application, it can be connected with sustainable environmental engineering and preventive biotechnology. *S. viminalis varietas Turbo* cuttings treated with proper wavelength of coherent light is effective in elimination of lead from contaminated soil and stimulation of *S. viminalis varietas Spring* has been proved to be effective in nickel removal [3]. This signifies the crucial role of willow cuttings in land reclamation as well as establishment of willow as efficient energy crop with the use of contaminated and out of use lands. Long term laboratory and field experiences of the last author of this article have proved that selected algorithm of laser stimulation can accelerate growth and increase resistance of energy crops like *Salix viminalis*, *Miscanthus giganteus*, *Sida hermaphrodit*, *Phragmites australis* as well as potent algal species for waste water treatment like *Lemna minor*, *Spirulina* etc. in the contaminated land and water [4].

Willow (*Salix*), is considered one of the best species for the reclamation of degraded land masses which was taken into account after the development of concept of watering tree plantations with sewages. These days *Salix* is highly used as energy crop. Different experiments conducted in laboratory and fields proved the possibility of using *Salix viminalis in sewage treatment at household level* [5]. Though the primary objective of the work is to analyse the difference in root and shoot development in willow cuttings of different sizes irradiated with different algorithm of coherent light and check the adaptability of growth of cutting in industrially contaminated soil, the long term objective is to promote the excessive use of laser treated willow plants for solving problems of heavy metal contamination in lands, waste water treatment and reclamation of degraded landmasses.

## 2. Materials and Methods

150 cuttings of 1 feet length of *Salix viminalis* were collected from Malopolska Region of Poland. Cuttings were divided into 5 different groups each with 30 cuttings further divided into five different sub groups according to the size of cutting namely DI, DII, DIII, DIV and K. K is the control group i.e. not treated with laser. The laser treated specimens were of following specifications:

DI: Treated with 3 times 30 seconds by medical laser (660 nm; red light) with 3 seconds of pause after each irradiation

DII: Treated with 3 times 10 seconds by medical laser (660 nm; red light) with 3 seconds of pause after each irradiation

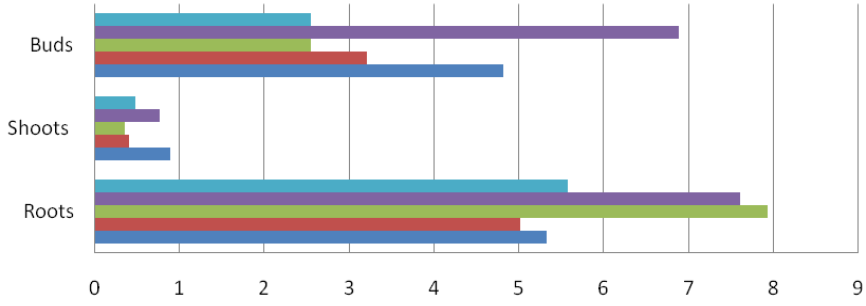
DIII: Treated with 3 times 10 seconds by medical laser (660 nm; red light) and 3 times 3 seconds with Laser diode (514 nm; blue light) with 3 seconds of pause after each irradiation

DIV: Treated with 3 times 3 seconds by laser diode (514 nm; blue light) with 3 seconds of pause after each irradiation.

The laser irradiation and culture of each cutting was done in same physical condition. All the cuttings (laser treated and not treated) were cultured in the hydroponics condition for 5 weeks. The cuttings were dipped in tap water and cultured in normal room temperature throughout the experiment. 10 homogenously selected (on the basis of size) cuttings from same group were cultured in one culture bucket therefore, 15 culture buckets were used in total. Each bucket was supplemented with 2.5 L of tap water. Every week the changes in the morphology of cuttings were noted. The number of roots, shoots and root buds were counted every week and the changes were documented. The cuttings after five weeks of hydroponic culture were transferred to industrially contaminated soil obtained from various industrial estates of Poland.

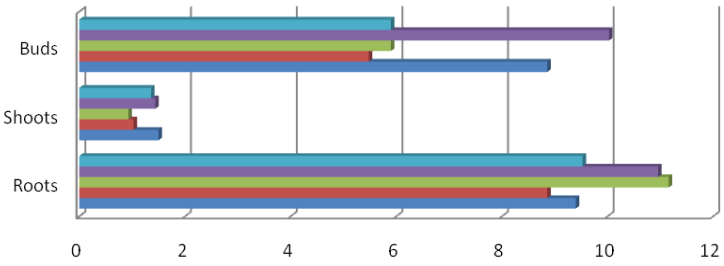
## 3. Results

The rooting and shooting both started after first week of culture. The rooting was seen in all the groups after a week including controls but the size and numbers of roots were significantly different in experimental and control groups. DII and DIV groups had the best result in terms of number of roots and shoots after 7 days of hydroponic culture. The best growth of roots and shoots in overall experiment was seen in DIV. DIV cuttings showed the enhanced growth after three weeks of cultivation of rooted cutting in industrially contaminated soils.




| | Roots | Shoots | Buds |
|---|---|---|---|
| Avg K | 5.58 | 0.48 | 2.56 |
| Avg DIV | 7.62 | 0.77 | 6.89 |
| Avg DIII | 7.94 | 0.367 | 2.56 |
| AvgDII | 5.02 | 0.413 | 3.22 |
| Avg DI | 5.34 | 0.9 | 4.82 |

**Figure 1.** Graphical Representation of growth of roots, shoots and root buds after 7 day of culture




| | Roots | Shoots | Buds |
|---|---|---|---|
| Avg K | 9.53 | 1.36 | 5.9 |
| Avg DIV | 10.96 | 1.44 | 10.03 |
| Avg DIII | 11.16 | 0.93 | 5.9 |
| Avg DII | 8.86 | 1.03 | 5.48 |
| Avg DI | 9.4 | 1.5 | 8.86 |

**Figure 2.** Graphical Representation of growth of roots, shoots and root buds after 5 weeks of culture

**Figure 3.** Representative cuttings of control and experimental samples after one week of culture

**Figure 4.** Growth of Roots, Shoots and buds after 5 weeks of culture in different groups

## 4. Discussion and Conclusion

Cellular functions could be influenced by visible light (400-700 nm). A low energy laser has been found to modulate various biological processes in the cell cultures and animal modules [6,7]. Effects of low energy lasers in mammalian cells can be cellular proliferation, collagen synthesis and release of growth factors from cell [8,9]. Photochemical processes are based on the excitation of the electronic state of the atom or molecule by visible light irradiation[10]. It has been suggested that laser biostimulation affect the cell function by modulating the production of growth factors at mitochondrial level [11,12]. Mitochondria might be a special target of He-Ne laser light since it contains most of the cellular chromophores [13]. One hypothesis explaining the mechanism of photostimulation is that the energy is absorbed by intracellular chromophores and is converted into metabolic energy in the respiratory chain [14,15]. Activation of electron transport chain due to the low energy laser irradiation leads to increase the electric potential across mitochondrial membrane which increases the ATP pool ultimately leading to activation of Nucleic Acid synthesis. This is how laser biostimulation contributes for the enhanced growth of cells [16].

On the basis of the results obtained after the regular observation for 5 weeks of hydroponic culture of experimental and control willow cuttings, it is inferred that laser biostimulation has remarkable contribution in enhanced rooting and shooting in willow which is highly important in increasing the adaptive capacity of the plant to grow in the industrially contaminated land.

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