Experiments on the influence of laser light on some biological elements of the natural environment

J. W. Dobrowolski and T. Wachalewski
Institute of Management and Protection of the Environment, Kraków, Poland
B. Smyk, E. Różycki and W. Barabasz
Department of Microbiology, Kraków, Poland

Studies the effects of exposure to light of the laser diode Melles Griot (λ = 670nm), He-Ne laser (λ = 632.8nm) and argon laser (λ = 514nm) on selected soil micro-organisms, fungi that destroy old manuscripts, pictures, stone, etc. and on humification and mineralization of soil samples. Also studies exposure effects on seed growth and biomass production of a few species of cultivated plants and on Chlorella cells and animal spermatozoa. Finds significant changes in comparison to control material (including results of the preliminary measurement of bio-photon emission). Suggests a fruitful direction for studies on the synergistic effects of Se, laser and white light, as well as on the optimal level of exposure of living material to laser light. The data obtained seem to be useful both for land reclamation and for the protection of the indoor environment against toxicogenic moulds and bacteria.

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Materials and methods

The following sources of coherent light were used in the experimental study: diode laser produced by Melles Griot of wavelength 670nm, power 5mW; argon laser ILA 120, produced by C. Zeiss, ena, of 514nm, power 100, 421, 5mW; and He-Ne laser produced by C. Zeiss, ena of wavelength 632.8nm, power without telescope 30mW and with telescope 1.6mW. The exposure time was different (from 15 seconds to 375 seconds). Selected soil micro-organisms, soil samples, algae Chlorella sp., yeast, sper matatoza of rams and bullocks, as well as seeds of flax, watercress, radishes, different strains of tomato and potato bulbs were investigated.

Cultures of the following micro-organisms: Arthrobacter globiformis Conn, Azotobacter chroococcum Beijernick, Bacillus macerans Scardinger, Rhototurula glutinis Harrison and Saccharomyces cerevisiae Hansen were irradiated by different lasers. Measurements
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Results and discussion

The effects of laser light on selected microorganisms were investigated and showed the following outcomes.

Irradiation with a diode laser of selected species of soil bacteria (Arthrobacter globiformis, Azotobacter chlorooococcus and Bacillus macerans) in vitro under the conditions described caused morphological changes in culture and the colony incubated on solid media (agar). In the above-mentioned soil bacteria, inhibition of growth was observed. Irradiation with the diode laser (exposure time 120 seconds) on the bacteria investigated in vitro did not destroy them. But irradiation with the diode laser of the yeast Rhodotorula glutinis and Saccharomyces cerevisiae in vitro clearly showed an inhibition of growth and morphological changes in mould cultures and colonies. Laser-treated yeast cells’ vitality was reduced by about 50 per cent. Irradiation with the diode laser (exposure time 120 seconds) on the bacteria culture of Arthrobacter globiformis and Bacillus macerans (inoculated on sandstone surface) significantly destroyed the vitality of the bacteria.

An argon laser (power 42mW) was used to irradiate the following cultures of microorganisms: Arthrobacter globiformis, Bacillus macerans, Azotobacter chlorooococcus, as well as Rhodotorula glutinis and Saccharomyces cerevisiae in vitro (exposure time 40, 60 and 120 seconds respectively). Morphological changes were observed in the investigated micro-organisms. Deformations were related to exposure time. When the exposure period was extended the number of dead yeast cells also increased (e.g. after an exposure time of 120 seconds). The number of dead cells exceeded 50 per cent of the population treated. After applications of the argon laser (at 100mW power) to the yeast culture, the

were made of the effects of various powers, exposure times and wavelength of laser light on growth or inhibition and morphological changes, and some biochemical properties, such as nitrogen fixation by Azotobacter chroococcum, were also investigated. Standard media used in diagnostics and cultivation of soil micro-organisms were provided from a collection of pure cultures of the Department of Microbiology, A
gicultural University in Kraków.

Laser irradiation of micro-organisms was applied according to methods used by Gadd and Cooney (1991). The effects of laser light were measured after 24 hours and 48 hours of incubation in the control and experimental micro-organisms at a temperature of 20°C. Humus soil samples were homogenized. Mixed samples, after fortification with a water solution of CuCl₂, NH₄Cl, KCl, were irradiated with the diode laser, He-Ne laser and argon laser for 60 seconds once or twice. Soil samples were kept at constant humidity and at a temperature of 20°C. After 46 days fractional composition was analysed. Colorimetric methods were applied. The percentage of humus fraction dissolved in an alkaline solution was also measured (fulvic acid, hymatome humic brown acid, humus grey acids). The ratio of fulvic and humus acid was calculated as well as the colour quotient of humus (Q₄₃). Humification and mineralization processes were measured in laser-treated soil samples. The second sample was supplemented in humus and sand soil with cations influencing the dynamics of the soil processes. The simultaneous influence of selection cations and laser light was also investigated under laboratory conditions in relation to the trends and rate of transformation or organic substances in the soil. Germination, as a percentage of a root plant after some weeks’ cultivation, was measured. Fresh and dry biomass production and other parameters were compared between control and laser-treated seeds.

The effect of laser light was measured on the germination success of five varieties of greenhouse tomato seeds infected by pathogenic bacteria Corynebacterium michiganense var. michiganense] (Gadd and Cooney, 1991). The tomato seeds were obtained from the State Farm in Krzeszowice. Prior to the experiment the seeds were disinfected with 1 per cent solution of formaldehyde to destroy bacteria. The ability of tomato seed germination was estimated. Isolation and cultivation of the bacteria Corynebacterium michiganense var. michiganense was carried out with the addition of amino acids and tomato extract. Cultivation temperature was 24°C, as recommended (Gadd and Cooney, 1991). Differences in the kinetics of spermatozoa cells and survival ratios were taken into consideration in measurements of photon emission from control sperm and algae in relation to those treated with laser light and with alternating white light. Preliminary observations were also made of the influence of the laser beam and sodium selenate solution on a new mutant of the yeast, Pichia parish, from the Institute of Surface-Physical Chemistry and Catalysis, Polish Academy of Sciences in Kraków. Additional experiments were also conducted to assess the applying diode laser in protecting old manuscripts and paintings against destructive fungi such as Aspergillus ochraceus, Chaetomium fungicolum, Penicilium lividum and Trichoderma pysisporum (Llewellyn and O’Rhear, 1990; Smik, 1964; 1991).
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Natural elements of the environment

Experiments on the influence of CuCl₂, NH₄Cl, KCl on the retardation of microbial activity of some tomato seed strains was observed. Irradiation with the argon laser (power 42mW) and the argon laser with exposure times 120 and 180 seconds) and with the argon laser (power 42mW, exposure time 120 and 180 seconds) on a culture of fungi, Aspergillus ochraceus, Chaetomium fumicola, Penicillium lividum and Trichoderma polysporum, caused clearly morphological deformations of the moulds investigated. These deformations were associated with enzymatic changes. The laser treatment was found to be an effective method to decrease cellular activity of the mould species which destroy old manuscripts and paintings. The experimental mould material isolated from seventeenth century documents from the World Culture Heritage in the Wieliczka Salt Mine near Kraków was investigated. After the cultivation of laser-treated moulds some changes in pigment synthesis were observed. Synthesis of new atypical pigments was also observed in the fungi investigated. The influence of CuCl₂, NH₄Cl, KCl on the retardation or acceleration of humification of organic matters and mineralization was observed in the soil samples. Copper cation accelerated the process of the mineralization of fulvic acid, decreasing the quantity in soil humus. The opposite tendency was observed after fortification of soil with potassium cation. Fulvic acid concentration increased, improving soil humification. After the treatment of soil samples with the diode laser beams which were not supplemented with cations, the amount of fulvic acids and humic acids changed. The charge was related to the light dosage applied. Elevated levels of fulvic acid were observed after treatment with laser light. This situation was found to be typical for early stages of humification and for higher quality humus. The observed differences were found to be significant with a statistical error of less than 5 per cent.

Preliminary experimental results related to irradiation of the diode laser, argon laser and He-Ne laser were documented. The germination ability of some tomato seed strains was measured. Tests were also carried out to investigate the possibility of the destruction of pathogenic bacteria Corynebacterium michiganense and Enzyme. The results showed that:

1. Irradiation with a diode laser, argon laser and He-Ne laser influenced the germination ability and the energy of the greenhouse tomato variety investigated (especially strains 5, 4 and 3). Some stimulation of root system development (rhizogenesis) was observed. Strains 1 and 2 of the greenhouse tomato did not react to laser irradiation.

2. Laser treatment of infected tomato seeds did not destroy phytopathogenic bacteria Corynebacterium michiganense ensen. However, a significant diversity of biological effects of He-Ne laser treatment has been reported in relation to various biological materials (Dobrowolski et al., 1987; Gregoraszczuk and Dobrowolski, 1983; Inyushin et al., 1976; 1981). Preliminary observation indicated the possibility that the low-intensity He-Ne laser treatment of seeds of some varieties of greenhouse tomatoes leads to a protective effect in relation to virus infection (Dobrowolski et al., 1987; Gregoraszczuk and Dobrowolski, 1983; Inyushin et al., 1976; 1981).

Watercress, radish and flax seeds were irradiated with the diode laser for different periods. Dose-dependent effects were observed related to the germination process, plant survival rates and biomass production. Each exposure was 15 seconds. After one laser treatment the percentage of seeds germinating was found to have increased when compared to the control. For example, the percentage on the second day was 86.3 per cent and 72.2 per cent on watercress, 80.5 per cent and 60.0 per cent in radish, 65.1 per cent and 40.3 per cent in flax, respectively.

Laser stimulation of germination was even more significant after two treatments. In this case, on the second day of experimental study the relative percentage was 93.3 per cent in the watercress, 83.0 per cent in the radish and 81.5 per cent in the flax. The final percentage of germinating seeds after 14 days was higher in laser-treated plants (95.7 per cent and 98.1 per cent) in comparison with 83.3 per cent in control watercress, and 100 per cent in comparison with 63 per cent in the control flax. However, side effects of the overdosage of laser treatment were also observed. After 25 irradiations of radish seeds with the diode laser, the final percentage of germinating seeds was only 66.3 per cent in comparison with 86.7 per cent in the control group. Under laboratory conditions the following differences were found among three species of plants treated once or twice when compared to the untreated control: dry weight per 1 pot 0.120g, 0.150g and 0.140g of watercress, 0.410g, 0.460g and 0.020g of radish, and 0.370g, 0.090g of flax (Table 1). Typically, these data can be correlated with the percentage of plants rooting after six weeks of cultivation: e.g. 53.8 per cent in watercress treated once with a laser; 86.7 per cent in the twice-treated group; and 81.2 per cent in the control. In some cases, slight exceptions were observed in experimental radish plants in which the following percentage of rooting plants
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Results:

Table I

<table>
<thead>
<tr>
<th>Material</th>
<th>Time of cultivation</th>
<th>Control</th>
<th>One irradiation</th>
<th>Two irradiations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Watercress</td>
<td>After two weeks</td>
<td>0.111</td>
<td>±0.003</td>
<td>±0.003</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.111</td>
<td>±0.004</td>
<td>±0.003</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.017</td>
<td>±0.003</td>
<td>±0.003</td>
</tr>
<tr>
<td>Radish</td>
<td>After two weeks</td>
<td>0.120</td>
<td>±0.028</td>
<td>±0.031</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.150</td>
<td>±0.045</td>
<td>±0.031</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.140</td>
<td>±0.031</td>
<td>±0.031</td>
</tr>
<tr>
<td>Radish</td>
<td>After six weeks</td>
<td>-0.007</td>
<td>±0.002</td>
<td>±0.013</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.042</td>
<td>±0.011</td>
<td>±0.013</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.037</td>
<td>±0.013</td>
<td>±0.013</td>
</tr>
<tr>
<td>Flax</td>
<td>After two weeks</td>
<td>0.015</td>
<td>±0.004</td>
<td>±0.007</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.022</td>
<td>±0.006</td>
<td>±0.007</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.020</td>
<td>±0.007</td>
<td>±0.130</td>
</tr>
<tr>
<td>Flax</td>
<td>After six weeks</td>
<td>0.090</td>
<td>±0.028</td>
<td>±0.098</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.370</td>
<td>±0.109</td>
<td>±0.130</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.300</td>
<td>±0.095</td>
<td>±0.098</td>
</tr>
</tbody>
</table>

Table II

Progress of germination and rhizogenesis as well as mean dry biomass production after 47 days of cultivation under field conditions

<table>
<thead>
<tr>
<th>Material</th>
<th>Control</th>
<th>DL 3 + AL</th>
<th>DL 1</th>
<th>DL 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Taking root</td>
<td>25.0</td>
<td>100</td>
<td>100</td>
<td>91.7</td>
</tr>
<tr>
<td></td>
<td>±12.3</td>
<td>±21.5</td>
<td>±26.7</td>
<td>±30.4</td>
</tr>
<tr>
<td>Germination</td>
<td>86.7</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>±29.5</td>
<td>±34.6</td>
<td>±38.2</td>
<td>±42.3</td>
</tr>
<tr>
<td>Dry biomass (per one plant)</td>
<td>10.66</td>
<td>13.76</td>
<td>7.31</td>
<td>9.45</td>
</tr>
<tr>
<td></td>
<td>±3.92</td>
<td>±3.25</td>
<td>±5.13</td>
<td>±1.08</td>
</tr>
</tbody>
</table>

Notes:

- DDL1 = 1 x irradiated with the diode laser (15 seconds);
- DDL2 = 2 x irradiated with the diode laser (30 seconds);
- DL3 + AL = 3 x irradiated with the diode laser (45 seconds) and 1 x irradiated with He-Ne laser (15 seconds);
There are some differences in photon emissions of the algae Chlorella sp. after irradiation with He-Ne laser, the first five involving differing exposures to laser light (15 seconds). Repeated exposures are related to the increasing intensity of photon emissions from the algae. Longer exposure is associated with decreasing luminosity (Ezzahir et al., unpublished). Differences in photon emission were observed to be associated with different doses of laser light applied to various biological materials. Measuring luminosity appears to be a useful indicator for optimizing effectiveness of laser biostimulation, which is related to the reactivity of the material under investigation. There is a feedback relationship.

Another field deserving further study is that of synergistic effects of two or more photoreactive factors. Similarly, several short exposures of Chlorella sp. to white light are not followed by any changes in photon emission, but alternating application of white light and He-Ne laser light (during similar periods) intensifies photon emission in algae, as well as the bacteria Bacillus macerans, the yeast Saccharomyces cerevisiae and spermatozoa of the bull and the ram (Ezzahir et al., unpublished).

Experimental studies of the role of single molecules of oxygen and antioxidants in biological effects of low dosages of laser light are in progress. A topic of special interest is the survival time of irradiated cells. Preliminary observations have shown a higher survival rate of bulbck spermatozoa after 15 seconds of treatment in vitro with the diode laser (Dobrowolski et al., unpublished). Biochemical aspects of laser stimulation and energetic status of spermatozoa will be the subject of future investigations. Laser light may stimulate the mobility of frozen spermatozoa of rams and the activity of dehydrogenase, as well as an increase in oxygen consumption by these cells (Nikolov, 1989).

Some authors are convinced that low-intensity laser light could interfere with ultra-weak photon emissions from DNA and mitochondria and so with the transfer of intercellular information (Inyushin, 1976; 1981; Klima et al., 1987; Popp et al., 1979). Popp suggested that the possible role of photon storage and bioemission relates to the control of gene activity, cell metabolism and communication (Popp et al., 1979). Quantitative and qualitative differences in photon emission between normal and neoplastic cells offer indirect support for this working hypothesis (Dobrowolski, 1980; 1986; Dobrowolski et al., 1987). Gulliya et al. (1988) found increased survival in normal cells during laser photodynamic therapy, as well as in tumour cells exposed to laser light with a photoactive dye, Mercocyanine 540 (Gulliya et al., 1990). Successful direct and indirect laser treatment of neoplastic changes could be interpreted as the elimination of disturbance in the informative spectrum of a cancer cell (Dobrowolski and Tadeusiewicz, 1986). New opportunities for theoretical analyses are needed on the influence of external agents on perturbation of photon emission from living organisms, particularly in the context of applying a cyberneting approach (Kochel, 1990).

Laser microbeams are very useful for subcellular micromanipulation and for inducing cell fusion (Berns et al., 1991). There are also new opportunities for applications in photorefraction (Pepper et al., 1990) studies of biological effects of laser light related to microsemiconductors in neutrophils and other cells. Such experiments are especially promising with regard to recently discovered essential ultratrace elements such as As, Li, Ti. A review of photochemical, photothermal and photomechanical effects of lasers in tissue has recently been published (Thomsen, 1991). At lower wavelengths laser light is primarily absorbed by proteins and DNA, but at a wavelength above 1,300nm, water is primarily an absorber (Welch et al., 1991). Friedmann et al. have introduced a possible explanation of laser-induced biological effects on cellular level (Friedmann et al., 1991). According to these authors, a small dosage of visible and infrared laser stimulates cells by the intensification of a transmembrane electrochemical proton gradient in mitochondria. This concept is consistent with Inyushin et al.’s (1976) finding that mitochondrial fraction is especially sensitive to He-Ne laser light. Explosive damage to cells after the application of high laser doses is probably a result of excess Ca²⁺ – ATPase calcium pumps and the use of the ATP reserves. This process cuts down the active transport, causing hypertension of irradiated cells (Friedmann et al., 1991). Studies on the effect of visible femtosecond laser pulses on the

Table III

<table>
<thead>
<tr>
<th>Subject</th>
<th>Control (g)</th>
<th>Experimental DL + AL (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean dry biomass</td>
<td>0.319 ± 0.083</td>
<td>0.442 ± 0.107</td>
</tr>
<tr>
<td>Mean concentration of Pb (ppm)</td>
<td>1.25 ± 0.39</td>
<td>1.17 ± 0.28</td>
</tr>
<tr>
<td>Mean concentration of Fe (ppm)</td>
<td>27.3 ± 6.2</td>
<td>58.8 ± 12.3</td>
</tr>
</tbody>
</table>

Crops of control and experimental potato bulbs (per one bush) cultivated in area contaminated by metallurgical plant and mean amount of Pb and Fe in both groups.
clonogenicity of Escherichia coli has been completed recently (Karut, 1991). A rise of a new subpopulation of mitochondria could be caused by He-Ne laser irradiation (Greco and Marra, 1991). Progress in knowledge about the biological effects of the laser could improve the quality of the natural environment, stimulate production of pollutant-free food, create better living conditions and protect human health. Ecologically-oriented and interdisciplinary investigation is needed to clarify the synergistic and antagonistic relations of white light, different laser light and trace elements in water, soil plants and animals.

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