*Polish Journal of Environmental Studies Vol. 10, No. 5 (2001), 307-311*

# Dehydrogenase and Catalase Activity of Soil Irrigated with Municipal Wastewater

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*Received: 12 March2001*
*Accepted: 18 May 2001*

## Abstract

Dehydrogenase and catalase activities were measured in a Eutric Histosol irrigated with municipal wastewaters (Lublin, Poland) purified by a two-step (mechanical and biological) treatment. Soil enzyme activity was used to test the biochemical status of the soil-plant system, the last step of wastewater purification. Three experimental fields, each of 1 ha area, were planted with *Populus nigra, Salix americana,* and grasses (with *Alopecuruspratensis, Phalaris arundinacea, Festuca pratensis* as dominating species). The fields were divided into three parts: not flooded control (A), flood-irrigated 10 times per year with 60-75 mm (B), and flood-irrigated 10 times per year with 120-150 mm (C) of wastewater per irrigation. The enzyme activity was measured several times during the first 2 years of wastewater application in soil sampled from control and flooded plots (0-10, 10-30, 30-50, 50-70 cm depth). Simultaneously, redox potential at the same depths was measured with permanently installed Pt electrodes. Irrigation with municipal wastewater elevated soil dehydrogenase activity on average by 44% and 27% for the low and high irrigation doses, respectively, but reduced catalase activity at the high irrigation dose on average by 12.4% (significant at $P < 0.001$). Plant cover significantly influenced soil dehydrogenase and catalase activities.

**Keywords:** wastewater, soil, dehydrogenase activity, catalase activity, redox potential

## Introduction

Municipal wastewaters can be disposed by controlled irrigation onto the soil. This practice may have substantial benefits for soils and their productivity [1] because wastewaters contain appreciable amounts of nitrogen, phosphorus and micronutrients (such as Fe, Cu and Zn). However, organic toxins and heavy metals may create environmental problems and the possibility of soil pollution should be taken into consideration. Soil acts as a kind of natural filter when it is irrigated with wastewater, as it removes biogenic and toxic elements from the liquid, thus protecting the ground water. The efficiency of this purification increases in the presence of plants [2].

In this research we determined dehydrogenase and catalase activities (assumed as a measure of soil biological activity), as well as redox potential (an index of soil aeration status useful especially under flood conditions) in the Eutric Histosol profiles irrigated with municipal wastewaters obtained after two-step (mechanical and biological) treatment. The soil was planted with *Populus nigra, Salix americana* and grasses. Our aim was to establish the extent to which the periodic irrigation with wastewater affects the activities of the test enzymes and modifies soil redox transformations.

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## Materials and Methods

The experimental site was located near the wastewater treatment plant of the city of Lublin, Poland. The wastewaters were obtained after two-step (mechanical and biological) treatment [1]. Three fields, each of 1 ha area, were planted in 1997 with *Populus nigra, Salix americana,* and grasses (with *Alopecurus pratensis, Phalaris arundinacea, Festuca pratensis* as dominating species). Each field was divided into three subplots A, B and C, separated by a dike (Fig. 1). Subplots A were not irrigated controls. Subplots B were irrigated with municipal wastewaters at a low dose, and subplots C - at a high dose. The low single dose (B) was based on the nitrogen and water requirements for the plants (60 mm for grasses, and 75 mm for *Populus nigra* and *Salix americana).* High single dose (C) contained twice that amount (120 mm for grasses, and 150 mm for *Populus nigra* and *Salix americana).* Irrigation was performed 10 times a year over two years (1997 and 1998). This means that subplots (B) and (C) received yearly 600 - 750 mm and 1200 - 1500 mm of wastewater, respectively. The average rainfall in the experimental area is 550 mm per year.

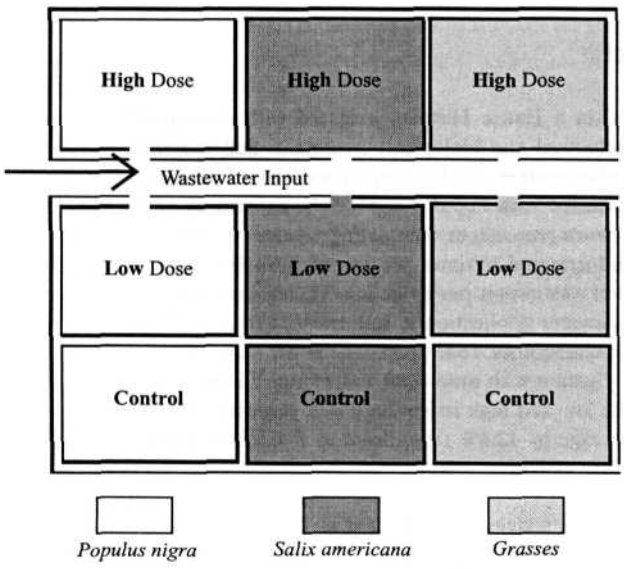


Fig. 1. The scheme of the experiment. Control - unirrigated soil; Low Dose - irrigated 10 times per year with 60-75 mm wastewater per treatment; High Dose - irrigated 10 times per year with 120-150 mm wastewater per treatment.

Concentrations of heavy metals in waters leaving the wastewater treatment plant in Lublin did not exceed 40 mg $m^{-3}$ of Pb and 20 mg $m^{-3}$ of Cu and Cd (week average values). Irrigation with the lower dose of wastewaters used in the experiment resulted in metal loading rates of about 0.9 kg Zn $ha^{-1}$, 0.12 kg Pb $ha^{-1}$, 0.09 kg Cu $ha^{-1}$ and 0.09 kg Cd $ha^{-1}$ per year [3]. Therefore, the amount of heavy metals as well as the possibility of soil pollution with wastewaters cannot be compared directly with the toxicity of sewage sludge reported by other authors. The pretreated wastewaters provided nutrients available for soil microflora and plants at concentrations comparable with intensive soil fertilization (about 180 kg N $ha^{-1}$,30 kg P $ha^{-1}$, 110 kg K $ha^{-1}$ for the lower irrigation dose [3]).

The soil under investigation was Eutric Histosol with average organic carbon 32.6% and pH in KC1 7.2. It was sampled 15 times per year at 0-10,10-30, 30-50 and 50-70 cm depths. The soil samples were placed in polyethylene bags, transported to the laboratory and immediately analysed for dehydrogenase and catalase activities. Simultaneously with soil sampling, redox potential (Eh) was measured in the field using Pt electrodes [4], permanently installed in soil profiles at the same depths (10, 30, 50 and 70 cm).

Dehydrogenase activity was determined with triphenyl tetrazolium chloride (TTC) according to Casida et al. [5] and catalase activity was determined according to Johnson and Temple [6]. The enzyme activities were expressed as nmol triphenyl tetrazolium formazan (TPF) $g^{-1}$ oven dry soil $min^{-1}$ in the case of dehydrogenase and as µmol $KMnO_4$ $g^{-1}$ oven dry soil $min^{-1}$ for catalase.

## Results and Discussion

The enzyme activities and redox potential at different depths of the soil profiles underneath *Salix americana, Populus nigra* and grasses are shown in Tables 1-3. The values are presented as two-year averages (all the consecutive determinations are considered repetitions). The results show a variability of the indexes tested among the control profiles as well as indicate that periodic application of wastewater caused different distribution of the biochemical activity under the three types of plant cover.

The dehydrogenase activity decreased with soil depth but the catalase activity remained at a similar level through the profiles. Many fine roots were observed below 50 cm, suggesting the presence of the biologically active pockets down to 70 cm. Among the control subplots, the soil planted with *Populus nigra* exhibited the highest dehydrogenase and catalase activities, followed by the soil planted with *Salix americana* and grasses.

Dehydrogenase activity decreased due to wastewater treatment in all the horizons of *Populus nigra* soil, but increased underneath *Salix americana* and grasses (Tab. 1).

Changes in the catalase activity were similar for soils of the three plantations. In surface horizons catalase activity increased after low irrigation but decreased after high irrigation. In most deeper layers catalase activity diminished in result of both treatments (Tab. 2).

The values of redox potential decreased with depth in the soil planted with *Populus nigra* and *Salix americana* - in controls down to 205 mV, and in the irrigated soils down to -36 mV. Surface horizons of soil under grasses showed lower Eh values than other profiles (by about 140-200 mV). Deeper layers of meadow profiles maintained relatively high redox potential level close to 400 mV at 50 cm and 200 mV at 70 cm - both in the control and in the treated soils (Tab. 3).

Information concerning the changes in biochemical indexes, resulting from irrigation of soil with wastewater is summarized in Figs. 2-4. Two-year irrigation of the soil

Table 1. Dehydrogenase activity in the Eutric Histosol (from a surface down to 70 cm depth) planted with *Populus nigra, Salix americana* and grasses. Averaged values of two consecutive years with standard deviations. Control - unirrigated; Low Dose - irrigated 10 times per year with 60-75 mm wastewater per treatment; High Dose - irrigated 10 times per year with 120-150 mm wastewater per irrigation.

| Dehydrogenase activity [nmol TPF $g^{-1}$ $min^{-1}$] | | | | |
|---|---|---|---|---|
| Plant cover | Soil horizon [cm] | Control | Low Dose | High Dose |
| *Populus nigra* | 0-10 | 0.266 ± 0.09 | 0.201 ± 0.05 | 0.188 ± 0.02 |
| | 10-30 | 0.092 ± 0.06 | 0.030 ± 0.01 | 0.025 ± 0.01 |
| | 30-50 | 0.054 ± 0.03 | 0.030 ± 0.02 | 0.016 ± 0.01 |
| | 50-70 | 0.038 ± 0.01 | 0.032 ± 0.01 | 0.029 ± 0.01 |
| *Salix americana* | 0-10 | 0.209 ± 0.05 | 0.346 ± 0.08 | 0.301 ± 0.06 |
| | 10-30 | 0.068 ± 0.02 | 0.194 ± 0.05 | 0.172 ± 0.04 |
| | 30-50 | 0.046 ± 0.03 | 0.153 ± 0.04 | 0.173 ± 0.09 |
| | 50-70 | 0.039 ± 0.01 | 0.161 ± 0.05 | 0.185 ± 0.04 |
| *Grasses* | 0-10 | 0.121 ± 0.08 | 0.124 ± 0.04 | 0.098 ± 0.03 |
| | 10-30 | 0.044 ± 0.01 | 0.077 ± 0.02 | 0.066 ± 0.02 |
| | 30-50 | 0.045 ± 0.02 | 0.050 ± 0.01 | 0.043 ± 0.01 |
| | 50-70 | 0.030 ± 0.01 | 0.052 ± 0.02 | 0.024 ± 0.01 |

Table 2. Catalase activity in the Eutric Histosol (from a surface down to 70 cm depth) planted with *Populus nigra, Salix americana* and grasses. Explanation as in Tab. 1.

| Dehydrogenase activity [µmol $KMnO_4$ $g^{-1}$ $min^{-1}$] | | | | |
|---|---|---|---|---|
| Plant cover | Soil horizon [cm] | Control | Low Dose | High Dose |
| *Populus nigra* | 0-10 | 8.59 ± 1.14 | 9.08 ± 0.57 | 7.21 ± 1.90 |
| | 10-30 | 8.17 ± 1.37 | 6.32 ± 0.73 | 5.59 ± 0.81 |
| | 30-50 | 6.91 ± 1.58 | 5.99 ± 1.64 | 4.54 ± 0.55 |
| | 50-70 | 7.69 ± 0.93 | 7.08 ± 0.68 | 5.71 ± 1.55 |
| *Salix americana* | 0-10 | 8.09 ± 1.06 | 8.29 ± 1.13 | 7.67 ± 1.01 |
| | 10-30 | 8.72 ± 1.06 | 7.99 ± 0.78 | 7.97 ± 1.04 |
| | 30-50 | 6.78 ± 1.55 | 8.50 ± 0.40 | 6.89 ± 0.88 |
| | 50-70 | 8.28 ± 0.89 | 8.19 ± 1.47 | 7.72 ± 1.47 |
| *Grasses* | 0-10 | 6.12 ± 0.52 | 6.27 ± 0.70 | 6.07 ± 0.60 |
| | 10-30 | 6.36 ± 1.26 | 6.60 ± 1.16 | 6.31 ± 0.72 |
| | 30-50 | 7.26 ± 1.67 | 6.75 ± 1.12 | 5.07 ± 0.88 |
| | 50-70 | 7.61 ± 1.53 | 5.94 ± 1.24 | 3.18 ± 0.77 |

Table 3. Redox potential in the Eutric Histosol (from a surface down to 70 cm depth) planted with *Populus nigra, Salix americana* and grasses. Explanation as in Tab. 1.

| Redox potential [mV] | | | | |
|---|---|---|---|---|
| Plant cover | Soil horizon [cm] | Control | Low Dose | High Dose |
| *Populus nigra* | 0-10 | 502 ± 98 | 389 ± 131 | 367 ± 132 |
| | 10-30 | 402 ± 144 | 315 ± 122 | 305 ± 144 |
| | 30-50 | 332 ± 180 | 310 ± 121 | 309 ± 115 |
| | 50-70 | 208 ± 140 | 42 ± 88 | - 9 ± 67 |
| *Salix americana* | 0-10 | 438 ± 100 | 501 ± 106 | 412 ± 177 |
| | 10-30 | 481 ± 131 | 467 ± 95 | 373 ± 176 |
| | 30-50 | 461 ± 196 | 293 ± 82 | 120 ± 84 |
| | 50-70 | 205 ± 81 | 147 ± 73 | - 36 ± 91 |
| *Grasses* | 0-10 | 304 ± 87 | 349 ± 95 | 240 ± 99 |
| | 10-30 | 495 ± 111 | 419 ± 105 | 371 ± 141 |
| | 30-50 | 470 ± 65 | 333 ± 129 | 402 ± 148 |
| | 50-70 | 272 ± 87 | 215 ± 107 | 254 ± 118 |

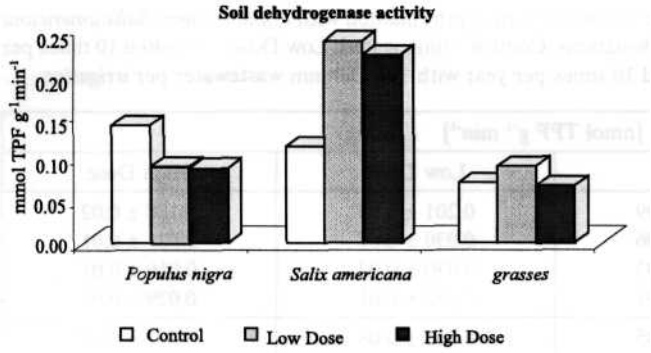


Fig. 2. Dehydrogenase activity of the Eutric Histosol planted with *Populus nigra, Salix americana* and grasses (average values of two-year experiment for the entire soil profiles - all soil layers included). Explanation as in Fig. 1.

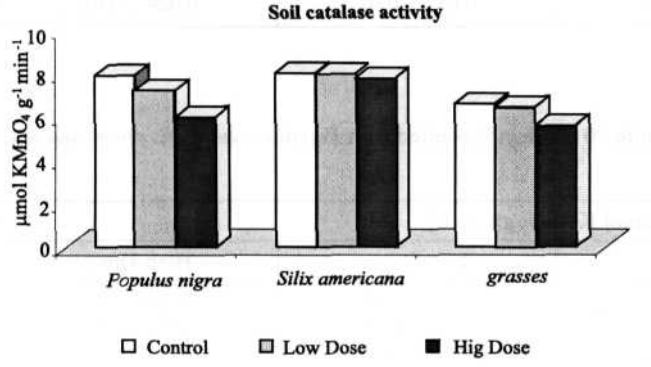


Fig. 3. Catalase activity of the Eutric Histosol planted with *Populus nigra, Salix americana* and grasses (average values of two-year experiment for the entire soil profiles - all soil layers included). Explanation as in Fig. 1.

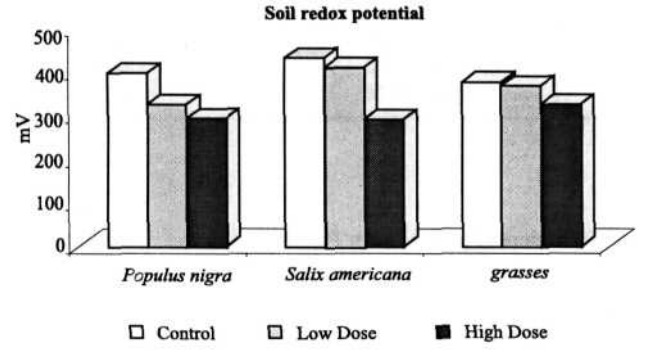


Fig. 4. Redox potential of the Eutric Histosol planted with *Populus nigra, Salix americana* and grasses (average values of two-year experiment for the entire soil profiles - all soil layers included). Explanation as in Fig. 1.

with wastewater caused a significant ($P < 0.001$) increase in dehydrogenase activity under *Salix americana* whereas no significant changes were observed underneath grasses. In *Populus nigra* planted soil, however, a decrease of the dehydrogenase activity was found ($P< 0.05$). Catalase activity decreased significantly (at $P < 0.001$) for *Populus nigra* and meadow soils and was unchanged for the *Salix americana* soil. Lowering of the redox potential was most evident in soil planted with *Salix americana* ($P < 0.001$). A relatively small decrease of Eh was observed in soil under grasses ($P < 0.05$).

Most papers concerning the results of wastewater treatment plant related studies deal with the influence of sewage sludge (but not of the wastewater) on soil enzyme activity. Brookes et al. [7], Reddy et al. [8] observed a decrease of soil biological activity due to sewage sludge application. Conversely, Eiland [9], Aichberger and Ohlinger [10], Wielgosz [11] found that the sewage sludge amendment increased bacterial population, ATP content, dehydrogenase and protease activities as well as soil respiration. According to Obbard et al. [12], dehydrogenase activity is enhanced but only if the rate of the sludge addition is limited. Baran et al. [13] found that the addition of sewage sludge into the soil stimulated the activities of dehydrogenases and protease but inhibited phosphatase activity. Kucharski et al. [14] showed that the effect of sludge on soil dehydrogenase, urease and phosphatase activities (stimulation or inhibition) was related to sludge origin and the level of sludge contamination with organic and inorganic pollutants as well as the kind of enzyme under investigation. Leszczyriska [15] observed that, despite their low concentrations, Zn, Ni and Cu inhibited nitrification activity of activated sludge probably due to permanent contact of microorganisms with heavy metals. However, the number of papers concerning the study of the influence of wastewater application on soil enzyme activity is limited. Goyal at al. [16] reported an increase in soil microbial biomass and dehydrogenase activity in soil irrigated with distillery wastewater. Filip et al. [17] found higher activities of b-glucosidase, β-acetylglucosaminidase and proteinase in the wastewater treated soil as compared to the untreated soil.

The analysis of variance of the results obtained in our experiment on the two-year periodic irrigation with wastewater showed that both factors (wastewater treatment and plantation type) significantly influenced soil enzyme activity. Average dehydrogenase activity was the highest in soil under *Salix americana* ($P < 0.001$) - about twice that of underneath grasses. Similarly, average catalase activity was also higher in soil under *Salix americana* than in the other profiles ($P < 0.001$), exceeding by about 25% the lowest catalase activity observed under grasses. Plant cover did not significantly influence soil redox potential. Wastewater treatment was a factor influencing significantly ($P < 0.001$) both enzyme activities and Eh (Fig. 5). The dehydrogenase activity increased on average by 44% and 27% for the low and high application rates, respectively. Despite the relatively low toxicity of the wastewaters, average catalase activity was reduced on average by 3.2% and 12.4% for the low and high irrigation rates, respectively. Soil irrigation with the wastewater resulted in a decrease of redox potential on average by 30 mV and 90 mV for the low and high wastewater application rates, respectively.

## Conclusions

Partly purified municipal wastewaters (after mechanical and biological treatment) influence soil biochemical

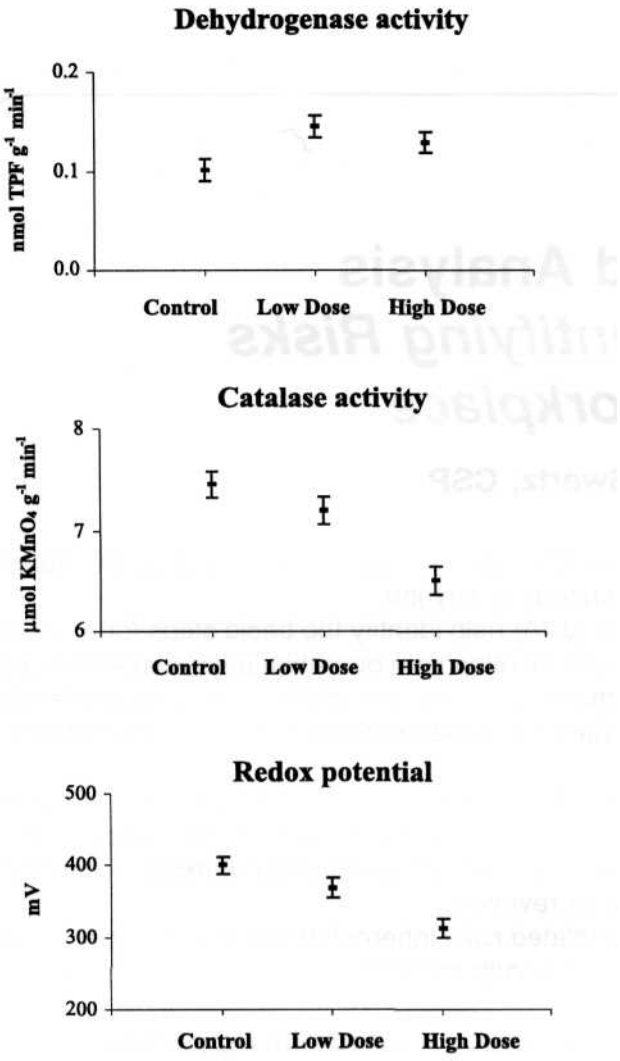


Fig. 5. The influence of wastewater irrigation on dehydrogenase activity, catalase activity and redox potential in the Eutric Histosol (analysis of variance: mean values for treatments over 2-year experiment with 95% LSD intervals). Explanation as in Fig. 1.

activity. Average dehydrogenase activity increased, while catalase activity decreased as a result of repeated application of the wastewater. However, plant cover evidently affected the response of soil microorganisms to wastewater application. It seems that the presence of *Salix americana* promoted soil enzyme activity.

## Acknowledgement

The paper was partly financed by the Scientific Investigation Committee, KBN (a grant No 5 P06H 035 16).

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