Our main purpose was to study the flora, the microflora and the enzyme activity in soil of polluted areas, and to identify the factors affecting them. Besides studying the percentage distribution of species in the soil, we determined the total number of cultivable microbes as well. Enzyme activities of phosphatase, invertase, dehydrogenase, and cellulase, were also measured in the samples of the contaminated soils. Total heavy metal content in soil samples was determined by analysis of Cd, Cu, Fe, Pb and Zn using XRF technique. According to our examinations, ratio of *Actinomycetes* and *Pseudomonas* genera decreased while the *Bacillus* sp. was found to be increased in the soils. Wastes reduce the activity of soil enzymes (cellulase, phosphatase, invertase), however, the activity of dehydrogenase increase.

**Keywords:** enzyme activity, soil microorganisms, plants, contaminated soil.

**INTRODUCTION**

In soils, degradation and transformation of natural and xenobiotics depends on the activities of soil enzymes and the number and activities of soil microorganisms. Deposited waste of dumps as an ecological factor changes the chemical composition, microflora and enzyme activity of soils.

Biodynamic of soils can be inspected through the examination of soil enzymes; however it does not give complete information. Currently such experiments mainly generate contradictory results which refer to the effects of the numerous determinant factors. For this reason, comparison of results can be carried out only under the same experimental circumstances. Moller (1979) found that urease activity correlates positively with the pH of the soil, the degradation stage of organic materials and the carbon-to-nitrogen ratio (C/N), while Borie and Fuenteacba (1982) and Reddy et al. (1996) observed a negative correlation between urease activity and the pH of the soil. Despite of all contradiction, Kovács (1990) applied the values of catalase activity as indicators of the biological activity in the soil. Zhang et al. (2000) consider that urease, catalase, cellulase, dehydrogenase, and protease enzymes are important indicators, regarding the changes of organic material content of soils. There is also growing evidence that soil biological properties are mostly affected by environmental factors and may be potential indicators of ecological stress (Dick and Tabatabai 1992). For instance, it has been reported that higher rates of fertilizers suppress microbial respiration (Thirukkumara and Parkinson 2000) and dehydrogenase activity (Simek et al. 1999).

Our examinations were carried out to test correlation between the composition of microflora, the enzyme activities and the heavy metal content in soils. We also planned to determine how the wastes as ecological factors influence the biodynamic of soils.

**MATERIALS AND METHODS**

**Site description**

Our sampling sites were dumpsites which are located near to small settlements in Szabolcs-Szatmár-Bereg County along River Tisza.

**Tiszacéce**


**Tiszabecs**

Illegal dumpsite can be found on the fields of the village and a paved road leads to the end of the site. Waste is carried here by trucks and the area is severed by a ditch. Waste is randomly strewn along the road.
as well. Waste consists of a large amount of plastics, flacons, paint boxes, chemical bottles, sacks, paper and metal waste, furniture and relatively just a few organic wastes. Deposition of wastes is carried out randomly, on two larger area, where ceratin wastes such as plastic bags can be easily blown away by winds. Continuous coat of green covers 50% of the area. Height of the grass covers 1-1.5 m, its dominant species is Calamagrostis arundinacea (L.) Roth. Productivity of grass cover is high (Approximately 3.3 kg/m$^2$). Besides other grass species, we can also find here: Achillea millefolium L., Alisma plantago-aquatica L., Anthriscus sylvestris (L.) Hoffm., Arctium lappa L., Artemisia absinthium L., Artemisia vulgaris L., Capsella bursa-pastoris (L.) Medik., Cardamine impatiens L., Carex elata (L.) All., Cirsium arvense (L.) Scop., Convolvulus arvensis L., Crepis biennis L., Dactylis glomerata L., Deschampsia caespitosa L., Echium vulgare L., Elitrigia repens L., Euphorbia platyphyllos L., Galium palustre L., Glyceria maxima (L.) Hartm., Iris pseudocorus L., Juncus effusus L., Lycopus europaeus L., Medicago sativa L., Matricaria inodora L., Phleum pratense L., Plantago major L., Polygonum persicaria L., Polygonum sachalinense Fr. Schmidt. Ranunculus repens L., Rumunculus arvensis L., Rumex crispus L., Silene vulgaris (L.) Moench., Stenactis annua (L.) Nul, Symphytum officinale L., Tanacetum vulgare L., Taraxacum officinale Weber., Trifolium medium L., Tympha latifolia L., Urtica dioica L., U. urens L., Xanthium strumarium L.

**Tiszaköröd**


**Control area**


<table>
<thead>
<tr>
<th>Table 1. Physico-chemical properties of the soils of sampling sites</th>
</tr>
</thead>
<tbody>
<tr>
<td>Samples</td>
</tr>
<tr>
<td>---------</td>
</tr>
<tr>
<td>1. Tiszabecs</td>
</tr>
<tr>
<td>2. Tiszaköröd</td>
</tr>
<tr>
<td>3. Tiszacséce</td>
</tr>
<tr>
<td>4. Control</td>
</tr>
</tbody>
</table>
SAMPLING

In case of dumps, soil samples were collected from three sampling sites and from two depths (0-20 cm and 20-25 cm). Three sampling points was marked out on each sampling sites. Samples were taken from three places of a given sampling site, within a square of 50 x 50 cm, from two depth (0-20 cm and 20-25 cm). The three samples which were taken from the same depth was mixed with each other.

METHODS

After mixing the samples, we isolated the bacteria from them, and repeated isolation three times. 1g soil was mixed with 100 ml sterile distilled water by shaking and was diluted to $10^3 - 10^5$ times thinner solutions. From the dilutions, 50 μl were spread over the surfaces of Nutrient culture-media. After 48 hours-long incubation on 27 °C, the bacterium colonies were counted and morphologically different colonies were isolated. For the evaluation, we used the number of colonies in 1g soil (CFU/g). Separation of bacterial genera was carried out on the basis of colony morphology, Gram’s stain, (Hucker 1922), spore stain (Bartholomew and Mittwer 1950), oxidase-reaction (Kovács 1956), catalase-reaction and metabolism of glucose (Hugh and Leifson 1953).

Total heavy metal content as well as the content of available heavy metals in soil samples was determined by analysis of Cd, Cu, Fe, Pb and Zn after acid digestion, using inductively coupled plasma mass spectrometry (ICP-AES). Total organic carbon was analysed with TOC, using a Shimadzu TOC-5000A analyser.

We determined the enzyme activities of phosphatase (Krámér and Erdei 1959), invertase (Frankenberg and Johanson 1983), dehydrogenase (Mersi and Schinner 1991) and cellulase (Unger, 1979) from soils.

STATISTICAL ANALYSIS

For each parameter data were submitted to a two-factor analysis of variance (ANOVA). SPSS statistical package version 14.0 programs for Windows 98.

RESULTS

Examinations of microorganisms

![Figure 1. Total number of microbes (CFU/g) in the soils](image)

Microbe populations of dump soils (Fig.1) can be characterized by high diversity and large number of individuals at the same time. In case of contaminated soils, total number of microbes in the upper layer of the soil is higher (20 cm) than at the same depth of Control area ($5.48 \times 10^4$). Total number of microbes is the highest on the area of Tiszakóród ($20.6 \times 10^4$), which is followed by Tiszacsécse ($18.5 \times 10^4$) and Tiszabecs ($12.6 \times 10^4$). Differences also can be observed between the two soil depths: total number of bacteria is higher in the upper layer than in the lower layer (50 cm). Qualitative and quantitative compositions of waste are various on the dump sites. Large organic matter content of wastes and heavy metals form the two main sources of pollution, however, proportions these are different in the sampling sites. Organic matter content of wastes is an easily utilizable nutrient source for soil microorganisms. Additionally, it delays and prevents the toxic effects of heavy metals. Composition of soil microorganism communities of sampling sites is determined by both the plant cover of the areas and the microorganisms of the waste. According to the percentage of the studied bacteria
genera, Gram-positive bacteria are dominant in the dump soils. Our results show that proportions of Actinomyces and Pseudomonas genera decreased under the influence of contaminations, while percentage of Bacillus genus increased as a consequence of its more effective survival ability. Proportions of genera in lower soil layers were similar to that of surface layers. On these areas, wastes have been dumped for years and their organic matters infiltrate into the lower soil layers as well. Modifications, which are caused by wastes, also have effects on the lower soil layers.

**Examinations of enzyme activities**

**Cellulase activity**

Cellulase activity (Fig.2) is higher on the Control area (68.66%) than on dump sites. There are not significant differences among the cellulase activities of dump soils. Cellulase activity on the area of Tiszacsécse (52.4%) approximates the cellulase activity of the Control area. High cellulase activity of Control area is derived from the absence of wastes.

**Phosphatase activity**

High phosphatase activity (Fig.3) of the Control area (2.89 \( \text{P}_2\text{O}_5/\text{g}/2\text{h} \)) was approximated by only the phosphatase activity of the dump soil of Tiszacsécse (0.95 \( \text{P}_2\text{O}_5/\text{g}/2\text{h} \)). Phosphatase activity was significantly lower in the soil of Tiszakóród (0.78 \( \text{P}_2\text{O}_5/\text{g}/2\text{h} \)) and Tiszabecs (0.65 \( \text{P}_2\text{O}_5/\text{g}/2\text{h} \)). High phosphatase activity of the Control area can be explained by the absence of wastes and the domination of plants.
Invertase activity

Invertase activity (Fig. 4) of the Control area is significantly higher (10.24 mg glucose/10g/4h) than that of dump sites, with the exception of Tiszacsécse (9.73 mg glucose/10g/4h). The lowest invertase activity was measured on the dump site of Tiszabecs (4.98 mg glucose/10g/4h), while invertase activity on the dump site of Tiszakóród (7.25 mg glucose/10g/4h) is significantly higher. In agricultural practice, invertase activity is used for the determination of soil fertility. High invertase activity can be also explained by intensive growth of the surface flora. Lower invertase activity of the dump site of Tiszabecs can be explained by the compactness of soil, which resulted from the perpetual presence of heavy good vehicles and bulldozers. Besides the absence of easily mobilisable organic matters, the reduced air content of soil also inhibits the functioning of invertase enzyme.

Dehydrogenase activity

Dehydrogenase activity (Fig. 5) reveals the differences in the organic matter content of the soil. As a consequence, dehydrogenase activities show significant differences between the contaminated sites and the Control area (102.12 μg INTF/g). Highest value was measured in Tiszakóród (199.08 μg INTF/g), while the lowest activity was measured in Tiszabecs (212.29 μg INTF/g). Dehydrogenase activity of Tiszacsécse was 153.09 μg INTF/g, as this site is in intermediate position between the two previous areas. Microbial activity of soils is connected to the degradable organic material content of the soil and both of them can be characterized by the dehydrogenase activity. On the illegal dump site of Tiszakóród, organic materials are in advanced stage of degradation or these materials originally are in an easily degradable stage. On this area, load of organic matters is also increased by the communal wastewater deposition. Dehydrogenase activity of Tiszacsécse can be explained by the abandoned status of the dump site, where the area is covered by soil and plants and mineralisation of the formerly deposited wastes has already begun. On the dump site of Tiszabecs, we can primarily find synthetic wastes and a few easily degradable organic materials. Consequently, there are not significant differences in the dehydrogenase activity of this site and that of the Control area. On this dump site, dehydrogenase activities reflect the original activity of the soil.
Heavy metal content of soils

Significant differences could be measured in the heavy metal contents of soils (Fig.6). The dump sites of Tiszabecs (205.06 mg/kg), Tiszacsécse (96.4 mg/kg) and Tiszakóród (70.9 mg/kg), zinc content is significantly low on the Control area (54.86 mg/kg). Tendencies of lead content are similar to that of zinc content. Dump soils (Tiszabecs 66.41 mg/kg, Tiszacsécse 45.12 mg/kg, Tiszakóród 48.23 mg/kg) contain significantly more lead than the Control area (22.5 mg/kg). Copper content of dump sites is similar to the lead and zinc content of the Control area.

Correlation analyses

For mathematical evaluation of the results we used correlation analyses (Tabl.2). These analyses were done on the basis of the enzyme activities and metal concentrations, which were measured from the soil samples of the upper soil layer (20 cm). Analyses showed positive correlation between the cellulase and phosphatase activities ($r = 0.74$), the cellulase and invertase activities ($r = 0.26$) and the phosphatase and invertase activities ($r = 0.24$), while the connection between dehydrogenase activity and other enzyme activities can be described with negative correlation. There was also positive correlation between dehydrogenase enzyme activity and the total number of microbes.
Table 2. Correlation between different enzyme activity values and the total microbial value (TMV)

<table>
<thead>
<tr>
<th>Correlation</th>
<th>Cellulase activity</th>
<th>Invertase activity</th>
<th>Phosphatase activity</th>
<th>Dehydrogenase activity</th>
<th>TMV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cellulase activity</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td>-0.17</td>
</tr>
<tr>
<td>Invertase activity</td>
<td>0.26</td>
<td>1</td>
<td></td>
<td></td>
<td>-0.38</td>
</tr>
<tr>
<td>Phosphatase activity</td>
<td>0.74</td>
<td>0.24</td>
<td>1</td>
<td></td>
<td>0.02</td>
</tr>
<tr>
<td>Dehydrogenase activity</td>
<td>-0.04</td>
<td>-0.26</td>
<td>-0.15</td>
<td>1</td>
<td>0.72</td>
</tr>
<tr>
<td>TMV</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1</td>
</tr>
</tbody>
</table>

Lead and cadmium concentrations of the examined dump soils were significantly different from those of the Control area. Toxic effect of heavy metals influenced the cellulase, invertase and phosphatase activities, therefore these enzyme activities are significantly lower in all dump soils than on the Control area.

Table 3. Correlation between certain indices of chemical composition of the dump soils and enzymatic activity

<table>
<thead>
<tr>
<th>Correlation</th>
<th>Cellulase activity</th>
<th>Invertase activity</th>
<th>Phosphatase activity</th>
<th>Dehydrogenase activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cu</td>
<td>0.83</td>
<td>0.05</td>
<td>0.74</td>
<td>0.006</td>
</tr>
<tr>
<td>Pb</td>
<td>0.005</td>
<td>-0.49</td>
<td>-0.45</td>
<td>-0.08</td>
</tr>
<tr>
<td>Zn</td>
<td>-0.76</td>
<td>-0.33</td>
<td>-0.34</td>
<td>-0.15</td>
</tr>
<tr>
<td>Cd</td>
<td>-0.53</td>
<td>-0.55</td>
<td>-0.62</td>
<td>-0.73</td>
</tr>
<tr>
<td>Fe</td>
<td>0.34</td>
<td>-0.32</td>
<td>0.43</td>
<td>-0.21</td>
</tr>
</tbody>
</table>

Cellulase enzyme showed negative correlation with zinc \( r = -0.76 \) and cadmium \( r = -0.53 \) (Tabl.3). Invertase enzyme is inhibited by lead \( r = -0.49 \) and cadmium \( r = -0.55 \). Phosphatase enzyme is in negative correlation with cadmium \( r = -0.62 \). In case of dehydrogenase enzyme and cadmium, negative correlation \( r = -0.73 \) is not effective. According to Moreno et al. (2000), Cd inhibits the dehydrogenase enzyme activity, even when large amount of organic matters is available in the soil. According to our results, the enzyme activity of all the dump soils is higher than that of the Control area; however, distribution of organic matters is not homogenous on the dump sites. Dehydrogenase enzyme activity is correlated with the total number of chemoorganotrophic microorganisms. This explains the high dehydrogenase activity of dump soils, which is higher than on the Control area, in spite of the available organic matters and the high cadmium content.

**DISCUSSIONS**

Wastes as ecological factors significantly reduce histolysis. In our opinion, the extent of the reduction depends on the chemical composition of wastes. Through the examination of cellulose decomposition in soils, Hattori (1991) established that Cd\(^{2+}\)-ion (depending on its concentration) inhibits or stimulates the rate of cellulose decomposition. In soils which are treated with sewage-sludge and contain heavy metals, the microbial biomass content and the activity of cellulase is half as much (50%) as in not contaminated soils (Chader and Brookes 1991).

Dehydrogenase activity does not correlate with total organic material content of the soil, and as a consequence, total content of organic materials does not correlate with microbial activity of the soil (Garcia 1997). Microbial activity can correlate with concentration of degradable organic materials and both of these values can be estimated according to dehydrogenase activity.

The dumpsite of Tiszacséce has not been used for a while, which explains the outstanding activity of dump soil. Mineralization of deposited waste has started on the area which is covered by soil, plants and mainly trees. The dumpsite of Tiszaköröd also serves as a recipient of communal wastewater. Easily decomposable organic materials are in advanced stage of decomposition. Dehydrogenase activity is also high on dump of Tiszaköröd which is entirely filled with litter and has soil cover on half of the site. Consequently, nor the dehydrogenase activity of Control areas, neither that of Tiszaköröd, differs significantly in dehydrogenase activity of the standardly used dump soil. Values of
dehydrogenase activity on this site probably reflect the original state of the soil.

Invertase plays a role in transformation processes of glycopolymers which release from humous substances as well as from decomposing plant materials. In agricultural practice, invertase enzyme is used for the determination of soil fertility. Addition of farmyard manure had enhanced the amount of organic matter, total microbial biomass, and xylanase and invertase activity (Poll 2003). The highest invertase activities were found on Control areas and on the unused dumpsite of Tiszacsécse, being significantly different from other sampling sites. Among other things, a high invertase activity can be explained by the intensive growth of the aboveground flora. High invertase activity was measured in soil samples of Tiszakóród; however, these activities were significantly lower in relation to invertase activity of the previous sites. In our opinion, low invertase activities of Tiszabecs can be explained by the compaction of soil which resulted from the continuous presence of working machines on the soil surface. Plant remains or easily available and mobilizable organic matters are missing, although these materials make the activity of invertase enzyme possible.

Phosphatase activity of untreated lands is significantly lower than phosphatase activity of lands which are treated with compost or vegetable fertilizer (Pascual 2002). Additionally, this statement comes true for areas where plant cover is abundant. High phosphatase activity on Control areas is owing to the rich macrovegetation by which we mean mainly forest vegetation. We measured lower activities on the sampling site of Tiszacsécse, where vegetation started to develop again; however, these activities were higher than the phosphatase activity of constantly used sites. Waste cover prevails over plant cover on dump soils of Tiszakóród.

Wastes remarkably reduce the activity of soil enzymes (cellulase, phosphatase, invertase). Activity of dump soils depends on the chemical composition of wastes, the number of aerobic chemoorganotroph bacteria in the soil, the quantity of heavy metals and the solutions of phosphorous and nitrogenous materials. High cellulose and phosphatase activities were found on Control area, while the lowest phosphatase activities were found in the dump soils of Tiszabecs and Tiszakóród.

We determined the highest invertase activity in the dump soil of Tiszacsécse. This high activity can be explained by the fact that aboveground vegetation grows intensively on the area and plant remains serve as substrates for the given enzyme. Invertase activity is low in the dump soil samples of Tiszabecs.

According to the results of correlation, the connection between dehydrogenase activity of the soil and other ferments can be characterized by negative correlation indices. Activity of dehydrogenase enzyme considerably depends on the total quantity of chemoorganotroph micro-organisms in the soil. Pascual et al. (2006) found similar correlation, according to which dehydrogenase activity depends on the metabolic state of soil microorganism, which may be reflected by a good relationship between dehydrogenase activity and microbial biomass C ($r=0.77; p<0.001$).

Examinations of cellulase, phosphatase, invertase and dehydrogenase activities refer to the fact that cellulase activity is the most sensitive to concentration of Zn, invertase and phosphatase activities are sensitive to concentration of Pb and Cd, while phosphatase and dehydrogenase activities are the most significant to effects of Cd. Lee et al. (2002) examined enzyme activity in soils which had been contaminated with heavy metals. They found that the total concentration of heavy metals shows negative correlation with the enzyme activities (dehydrogenase, acid-phosphatase, and beta-glycosidase).

**CONCLUSIONS**

Our main purpose was to study the flora, the microflora and the enzyme activity in soil of polluted areas, and to identify the factors affecting them. On dumpsites, waste deposition as ecological factor changes the chemical composition, the microflora and the enzyme activity in soils and the composition of flora too. Total number of microorganisms in the soil correlates with the dehydrogenase activity ($r=0.72; p<0.005$) and the content of available organic matters, instead of organic matter content of the soil. Invertase activity of contaminated sites is lower than that of Control area, with the exception of these sites, where content of mobilizable organic materials has increased. Phosphatase activity on the contaminated areas is significantly lower than on the Control site. Phosphatase activity of Control site is approximated by the phosphatase activity of those sites which has significant plant cover. On unplanted areas, phosphatase activity is significantly lower than on other contaminated sampling sites.

Heavy metal content of the soil shows negative correlation with soil enzyme activities. Enzyme activities are the most sensitive to the Cd and Pb content of the soil. Contamination influences the qualitative and quantitative composition of microflora in contaminated soils. Total number of microbes in case of the examined heterotrophic bacteria (Bacillus, Enterobacter, Coryneform) is significantly higher, as compared to the Control area. There are changes in the composition of flora in the contaminated sites. Numerous adventive plants (Urtica spp., Artemisia spp., Stenactis annua (L.) Nucl, Polygonum sachalinense Fr. Schmidt, etc.) that force out autochthonous species and frequently are mono- or sub-dominants of plant groupings, are bioindicators of the polluted areas. Examinations of changes in the composition of flora and of soil enzymes, microbial researches can be useful for the experiments, which examine the effects of wastes on soils.
REFERENCES


