

**REDUCTION OF POLLUTANT CONCENTRATIONS IN THE  
PLANT SYSTEMS WITH THE INERT FILTER BED.  
PART II:  
HUMIFICATION OF *EPILOBIUM PARVIFLORUM* SCHR.  
RHIZOSPHERE IN THE MICRO-CULTIVATIONS  
FERTILIZED WITH DOMESTIC WASTEWATER**

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The article details the processes accompanying transformations of organic substances in the compounds of humus rhizosphere area of *Epilobium parviflorum* Schr. The functions of the root organ together with root hair system were developed. The ion functions in the inert filter bed (made of porous clay) with phosphorus sorption were described and the dependence between C:P in the phosphorus mineralization was demonstrated. The important role of porous clay in the inert filter bed for biochemical transformations was justified, in particular, for phosphorus. In the analysis of humic transformations, it was emphasized that nitrogen compounds present in domestic wastewater and filter bed solution are important. The relation of C:N was defined describing the mineral origin of humic substances in the rhizosphere humus. These results were related to the results obtained from *Phragmites communis* Trin.

Keywords: C:N, C:P, humus, *Epilobium parviflorum* Schr., keramzite

## 1. INTRODUCTION

As a result of micro-biological processes which occur in the plant systems inert filter beds, there appears to arise humic substances. Vascular plants within the filter solution environment contact with their filter bed solution through the root organ hairs system. The area of the hairs system is several times larger than the basic root part. This elongation zone is very important for the plant, due

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to its contact with the macro-elements present in the filter bed solution. In 1903, the area, which constitutes the root hairs system, was called rhizosphere<sup>1</sup>. In the rhizosphere dying detritus tissues form humic compounds which, in turn, activate the plants growing process and influence the solution properties.

In rhizosphere there are humic compounds which react and create organic and mineral compounds. Those may be divided in four basic groups:

1. Low-molecular octanes, oxalates and other organic acids salts. They react to minerals in the filter, inter alia: calcite, magnesite or mineral acids salts, mainly, potassium, calcium and phosphorus.
2. Humic and fulvic acids whose salts are typical for rhizosphere humic compounds. In the keramzite bed solution they originate in the process of humic acids reactions on the soluble alkaline salts representing functional groups such as COOH and OH on the elements cations:  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ ,  $\text{Na}^{2+}$  and  $\text{K}^{2+}$ . Mainly, humic and fulvic compounds exist as iron or aluminum hydroxides in the filter bed solution.
3. Chelates are complex humic compounds of the ring structure, that form several coordination bonds with free electron pairs of a ligand (a group of atoms or molecules) and free metallic element valences (with keramzite). The ligands part in the filter bed solution is fulfilled by simple organic compounds, groups of metals or active humic organic compounds. These groups can be arranged according to their diminishing affinity of chelation. The example is a group of metals which originated from the keramzite ions:  $\text{Fe}^{2+} > \text{Cu}^{2+} > \text{Ni}^{2+} > \text{Co}^{2+} > \text{Zn}^{2+} > \text{Fe}^{2+} > \text{Mn}^{2+}$
4. Adsorptive clay compounds. The reaction of organic compounds and clay fractions on one another results in the filter adsorption of humic compounds. Adsorption of humic or fulvic acids in the inert filter bed occurs on its surface owing to existing cations in the solution, forming the bindings [3]. Unfortunately, existing in the keramzite cations  $\text{Ca}^{2+}$ ,  $\text{Fe}^{2+}$ ,  $\text{Al}^{2+}$ , do not form such strong bonds. Adsorption with van der Waals forces is rather weak. However, important bonds can ensure polar groups of humic compounds (COOH,  $\text{NH}_2$ , OH), which connect with  $\text{O}_2$  from the net crystal structure of the clay mineral. Paradoxically, when the filter humidity lessens, the bonding and attraction of organic compounds to the minerals in the filter becomes stronger. The hydrogen bonds free the iron and aluminum oxides, and this results in a more thorough accumulation of oxides on the filter bed ceramic particles. Thus, it is followed by surface adsorption of the humic compounds. These compounds are connected with the oxides with the

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<sup>1</sup> Rhizosphere – the term was introduced in 1903 by Hiltner and Stornel as the area of about 1 mm around the root. It constitutes a climax biocoenosis niche and correlation between the soil life and chemical substances exchange.

Coulomb forces. They can be removed by increasing the pH in the solution or adding a little amount of the salt: NaCl or NH<sub>4</sub>Cl which in turn makes the phosphorus bound free.

Other mechanisms that react in the keramzite bed solution are connections of humic compounds with the iron and aluminum oxides using the organic compound anion group. Penetrating the area of oxides layer on the grain surface of the film in the filter bed, anions are able to form the bonds in the clay material (keramzite) interlayer space. Such penetration was well documented by Schnitzer and Kodam in 1967 [8] and later, also confirmed by Thenga in 1976 [9]. This adsorption demands an environment with a reaction pH < 5.5 because humic acids contain molecules too large to penetrate the interlayer spaces.

The aim of the studies undertaken was to define the quantity of nitrogen and phosphorus absorption in the plant and designate a parameter illustrating the origin of organic matter of *Epilobium parviflorum* Schr.

## 2. MATERIALS AND METHODS

The series of the research was carried out in 2010 at the Research Station for Experimental Phytotechnologies in Młodziejowice near Kraków. It included work upon the nitrophile plants of the species *Epilobium parviflorum* Schr. or the over-ground vegetative plant organs and the root together with the rhizosphere. The experiment was conducted in the micro-cultivation with the subsurface flow (HF-CW), in the plug-flow system. The plot for micro-cultivation was a rectangular area of 1.5 meters wide and 2.0 meters in length, insulated horizontally with polyethylene film PE-HD 1 mm thick.

The construction first filter layer was 10 cm thick and consisted of dense, fat clay of 25 cm thick laid on the film. The second layer was 25 cm thick, raised on the spacer made of non-woven geotextile fabric, and filled in with an artificial inert aggregate, keramzite<sup>2</sup>, with the grain size of 8 to 16 mm. The keramzite (porous clay) of the filter bed characterized the parameters: gravity 740 kg·m<sup>-3</sup>, thermal conductivity at the temperature 23°C 0.141- 0.07 W·mK<sup>-1</sup>, absorption capacity 21.3±2.1 %, with a reaction pH 6.2. In the filter bed the drain pipes made of polyethylene PCV 70 were placed, an inlet on the shorter rectangle side and the infiltrative pipe on its opposite side. The bottom fall of the plot bottom maintained with the value  $i=1.0\%$ . The excess of fertilizing wastewater was transported back to the treatment system. The fertilization was controlled with the gardening timer, in two-hour intervals, the outflow time equal to 60 seconds and the stream flow equal to  $8 \cdot 10^{-2} \text{ dm}^3 \cdot \text{s}^{-1}$ . The fertilization

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<sup>2</sup> inert aggregates- arising from the ceramic materials: recycled or minerals, in the technological process that gives them the end -demanded properties

of the micro-cultivation was performed from the Sun rise to Sun set, also in direct insolation. The wastewater was transported through the pipe of PCV 110 from the settling tank (OS) equipped with the solid phase separator with the Čoanda effect [2]. The wastewater from the settling tank OS (for four persons household) and pollution parameters:  $BOD_5$   $280 \text{ mgO}_2 \cdot \text{dm}^{-3}$ ,  $COD$   $385 \text{ mgO}_2 \cdot \text{dm}^{-3}$ , total suspension  $216 \text{ mg} \cdot \text{dm}^{-3}$ ,  $TN$   $45.90 \text{ mgTN} \cdot \text{dm}^{-3}$ ,  $NH_4-N$   $30.74 \text{ mg NH}_4-N \cdot \text{dm}^{-3}$ ,  $TP$   $3.36 \text{ mg TP} \cdot \text{dm}^{-3}$  was entered in every two hours and for 120 seconds with the flow intensity  $0.8 \text{ dm}^3 \cdot \text{s}^{-1}$ . The fertilization was controlled with a gardening time programmer. For the purpose of chemical quantitative analysis at the beginning of September from the micro-cultivation, 5 plants were selected and measured: stem diameter = 2 cm, height = 3 cm count from the filter bed surface. After cutting the stem, from the depth area of 5 to 8 cm the root with the organic part was taken out. The dark brownish organic substance adjacent to the hairs was gently flipped. The substance collected from the area within the hairs zone was recognized to be the organic substance of the rhizosphere (R). Minor hairs and roots were removed carefully. Analogically, there was organic rhizosphere substance (N) collected from the plants growing off the experimental cultivation. In both samples a reaction pH was marked as well as the organic carbon with the Tiurin's method. Total nitrogen was measured with the Kjeldachl method. Both elements: total nitrogen and total phosphorus were tested according to the producer's procedures with the spectrophotometer HACH 2800ECO.

### 3. THE RESEARCH RESULTS AND DISCUSSION

The studies on nitrogen stabilization in the micro-cultivation filter bed were carried out in the in years 2008 -2010. The plants were watered with the separated wastewater from the well settling tank [2].

After the second year of the plants cultivation and watering with sewage from the well settling tank in their growth period there was a hypothesis set that Kjeldahl nitrogen in the plot filter bed solution should undergo the stabilization to organic nitrogen [3]. This was confirmed by the results of the chemical analyses done and the evidence was shown in Table1 and Table 2.

Table 1. Average nitrates concentration in the wastewater in the filter bed solution during two-year lasting experiment

Period	Nitrates reduction [ $\text{mg} \cdot \text{dm}^{-3}$ ]		
	Total Kjeldahl Nitrogen	Ammonium Nitrite	Organic Nitrite
2009	30.90	10.36	20.54
2010	25.85	5.23	20.62

Table 2. Kjeldahl nitrogen and phosphorus contents in the vegetative parts of the plants *Epilobium parviflorum* Schr. in  $\text{mg}\cdot\text{g}^{-1}$  s.m.

The plant species	Part of the plant	Concentration of biogenes in the plant	
		TKN	TP
<i>Epilobium parviflorum</i> Schr.	root	28.7	7.8
	rhizome	25.8	6.8
	stem	19.7	5.8
	leaves	42.5	5.5

According to Reed et al. (1995) [7], in the first two years of exploiting the filter bed, removing  $\text{N-NH}_4$  from the wastewater can be higher, and it is caused by absorption into the organic compounds present in the filter bed within the rhizosphere area. That was confirmed by the research done by Dąbek (2010) [4] that the cultivation biomass grew near the end in the second year of the experiment (Table 2). In the rhizosphere the nitrogen absorption analysis showed that the absorption level of organic nitrogen in the filter did not undergo significant changes, as seen in Table 1. During two years it maintained the levels: in 2009 it was  $20.54 \text{ mg}\cdot\text{dm}^{-3}$  and in 2010 it measured  $20.62 \text{ mg}\cdot\text{dm}^{-3}$  for the last two years. The biological elements analysis on *Epilobium parviflorum* Schr. Table 2, illustrates a high drawing of nitrogen and phosphorus from the filter bed solution particularly in the vegetative plant parts which also means high adsorption of the nitrogen in the rhizosphere. The chemical analysis of organic carbon  $\text{C}_{\text{org}}$ , (Tiurin method) in the humus organic substance gave the following results of dependencies, shown in Table 3.

Table 3. Nitrogen and phosphorus contents and parameters of humic organic substance in the rhizosphere of *Epilobium parviflorum* Schr. keramzite filter bed [ $\text{mg}\cdot\text{g}^{-1}$  s.m.]

Object	pH	Organic carbon	Organic nitrogen	Total phosphorus	C: N	C: P
R	6.8	333.4	20.5	5.0	16.2	66.7
N	6.4	19.8	2.0	1.4	9.8	14.1

Also, the biological absorption of total nitrogen and phosphorus, see Table 2 by humic organic substance in the rhizosphere is connected with a rhizosphere effect<sup>3</sup>. The results of the analysis, particularly the quotient C:N, showed that Pempkowiak et al. (1989)[5,6] for the hydrophytes treatment system gave the quotient C:N equal to 9.0. However, Baran and Bielińska (2008) [1], got the quotient C:N equal to 13.5 in their studies on the *Teraxaceum officinale* Web. rhizosphere.

<sup>3</sup> the rhizosphere effect – it is the increased intensity of bio-chemical transformations. This effect was first described in 1946 by Katznelson.

- The quotient C:P, is the characteristic of the transformations which occur in the rhizosphere, while C:N sets the organic substance origin. The quotient C:N included within the values 15 and 25 is typical for the humus forms between Moder and Mull and the soils with organic bedding not exceeding 2-3 cm.
- If  $C:P \geq 300$ , it means, the phosphorus was bound in the filter bed, and formed the organic compounds, the quotient  $C:P < 200$  proves the phosphorus mineralization to the forms of calcium, iron or aluminum phosphates which constitutes the evidence of keramzite ions influential role.

#### 4. CONCLUSIONS

The research undertaken upon the *E. parviflorum* Schr. rhizosphere confirmed the aims set out in the introduction. The analysis results allowed to draw the following conclusions:

1. The organic nitrogen in the inert filter bed, filled in with keramzite, undergoes a stabilization with the falling tendency of the ammonium nitrogen concentration.
2. The quotient C:N equal to 16.2 points to the organic origin of humic compounds in the rhizosphere as typical for the humic chelate complexes.
3. The quotient  $C:P < 200$  and the analysis result equal to 66.7 implicates that the phosphorus was bound into the mineral phosphorus compounds: calcium, iron and aluminum phosphates.
4. The filter bed with the keramzite is adequate for phosphorus precipitation and phosphorus sorption from the domestic wastewater.
5. The *Epilobium parviflorum* Schr. can be regarded as the useful alternative for the reduction of pollutant concentration in the wastewater.

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## REDUKCJA STĘŻEŃ ZANIECZYSZCZEŃ W ROŚLINNYCH SYSTEMACH ZE ZŁOŻEM INERTNYM

Część druga

### HUMIFIKACJA RYZOSFERY *Epilobium parviflorum* Ochr. W MIKROUPRAWACH NAWOŻONYCH ŚCIEKAMI BYTOWYMI

#### Streszczenie

W pracy przedstawiono procesy towarzyszące przemianom substancji organicznej w związki próchnicowe w ryzosferze *Epilobium parviflorum* Schr. Przedstawiono funkcje organu korzeniowego i systemu włósnikowego w tworzeniu ryzosfery. Opisano udział jonów w tworzeniu kompleksów organicznych z metalami, w złożu inertnym wypełnionym keramzytem. W krótkich rysie przedstawiono formy kwasów huminowych i fulwowych, oraz mechanizm tworzenia helatów w ryzosferze. Podkreślono znaczenie związków azotowych w tworzeniu organicznej substancji próchnicowej ryzosfery. Dokonano analizy zależności wynikających z ilorazu C:N oraz C:P. Opisano istotność tych ilorazów dla określenia pochodzenia związków organicznych w próchnicy ryzosfery jak i miejsca związania organicznego bądź mineralnego fosforu. Otrzymane wyniki z analizy odniesiono do innych badań tematycznych, części dotyczącej azotu, przytoczono wyniki Pempkowiaka i in. (2008, 2009), oraz w części dotyczącej fosforu, powołano się na podobieństwo wyników do badań Barana i Bielińskiej (2008). We wnioskach końcowych odniesiono się do rezultatów badań uznając azotolubną roślinę *Epilobium parviflorum* Schr. za interesującą alternatywę uzupełniającą redukcję biogenów na oczyszczalniach roślinnych typu nitrofitowego bądź makrofitowego.

