PHENOLIC COMPOUNDS IN WETLAND MACROPHYTES*

T. Dvořáková Březinová, J. Vymazal

Czech University of Life Sciences Prague, Faculty of Environmental Sciences, Prague, Czech Republic

Phenolics are the most widely distributed class of plant secondary metabolites and higher plants are able to produce several thousand different phenolic compounds. It has been reported that phenolics are more resistant to decomposition due to the negative impact on the microorganisms involved. Therefore, it may be hypothesized that the higher content of phenolics in plant tissue may cause slower decomposition rates and potentially higher carbon sequestration in the soil. The primary goal of this study was to evaluate and compare the content of phenolics in seven common macrophytes in the Czech Republic. Aboveground biomass was sampled in June 2016 at seven different localities and phenolics concentrations determined by spectrophotometric methods according to the Folin-Ciocalteau method ranged from 9.02 to 28.39 g kg⁻¹ dry biomass weight (DW). Several plants were also harvested in August, October and December in order to follow a seasonal pattern. According to the results it seems that surveyed macrophytes vary widely in phenolics concentrations in relation to sampled site, harvesting time, plant species, and plant parts as well.

INTRODUCTION

Phenolic compounds represent a large group of substances with diverse properties (Kalá et al., 2016). They are the most widely distributed class of plant secondary metabolites and they are very important for plant biochemistry and physiology. Phenolics are involved in many interactions of plants with the biotic and abiotic environment and plants can synthesize phenolic compounds as a reaction to changing environmental conditions (Hutzler et al., 1998). It has been reported that plants are able to produce several thousand different phenolic compounds (Lattanzio, 2013). Phenolics in plant play many important roles, which can be categorized into several groups. They can (a) be involved in growth and reproduction, (b) contribute to plant morphology and sensorial properties (pigmentation, aroma, taste) (Bravo, 1998; Giada, 2013), and (c) provide passive and active resistance and protection against pathogens, predators or stress, and ultra violet radiation (Richardson et al., 1999; Vermerris, Nicholson, 2006a).

Structurally, these compounds have one or more hydroxyl group attached directly to the aromatic ring and the most basic phenolic compound is phenol. The terms polyphenols depicts compounds that have more than one phenolic hydroxyl group attached to one or more benzene rings (Vermerris, Nicholson, 2006b). A large number of these compounds can be divided into several classes and they can be classified in different ways – for example according to the number of constitutive carbon atoms or the structure of the basic skeleton (Murkovíc, 2003). In general, phenolics range from simple phenols to highly polymerized...
compounds like tannins or lignins (Bravo, 1998). In most cases, they do not occur as free compounds in plants, but they are bound to other molecules and are present as esters or glycosides (Vermerris, Nichol son, 2006b).

The levels of phenolics in plants differ among various species (Hooren et al., 2003; Wang et al., 2015; Rejman kova, 2016; Harrison et al., 2017) and even among varieties of the same species (Bravo, 1998). The concentration of phenolics is partly influenced by genetic background and partly by environmental factors (Connor et al., 2002; Howard et al., 2003), especially the nutrients availability. Harrison et al. (2017) concluded that the variability was influenced by local environmental factors and the most important predictive factors for foliar phenolic compounds in some wetland species were sampling date, soil nutrients, and herbivory. Rejman kova (2016) showed that the concentration of phenolics in Eleocharis cellulosa and Typha domingensis were negatively correlated with increased growth due to increasing nutrient levels. She confirmed the protein competition model (PCM) proposed by Jones, Hartley (1999) which assumes that protein and phenolics synthesis compete for the common limiting resource phenylalanine and, therefore, protein and phenolics allocation are inversely correlated. Under suitable nutrient conditions, most of the phenylalanine is used for the formation of proteins, while during the nutrient limiting conditions phenylalanine is more used for the formation of phenolics (Rejman kova, 2016). The concentration of phenolics can differ even in different parts of the same plant. For example, formation of flavonol and flavone glycoside in common vegetables depends on light, so these compounds are mostly concentrated in outers organs like leaves than in any other part of the same plant (Herrmann, 1988).

Polyphenols have been recognized as regulators of soil processes and it has been suggested that they inhibit nitrification, as well as plant litter decomposition and nutrient recycling (Horner et al., 1988; Xu et al., 2013). Phenolics, such as lignins, unlike proteins and carbohydrates, are more resistant to decomposition due to the negative impact on the microorganisms involved in the decomposition of plants, and, therefore, phenols can accumulate temporarily in soil. In the environment, soluble polyphenols face four different fates. They can be: (a) degraded and mineralized as a carbon source by heterotrophic microorganisms, (b) transformed into insoluble and recalcitrant humic substances by polymerization and condensation reactions (with the contribution of soil organisms), (c) adsorbed to clay minerals or chelated with Al or Fe ions, or (d) leached by percolating water, and leave the ecosystem as part of dissolved organic carbon (DOC).

The effects of polyphenols on soil microorganisms were reviewed by many researchers, for example by Ku iters (1990), Hattenschwille r, Vito sek (2000) or Wang et al. (2015). According to these studies the most resistant compounds to decompose in plants are phenolics and, therefore, it may be hypothesized that higher content of phenolics in plant tissue leads to slower decomposition rates. It can also be expected that concentrations of phenolics may play an important role in carbon sequestration in wetlands, and consequently contribute to mitigation of global warming effects.

Decomposition is very important in wetlands, because wetlands have been proved to be one of the major carbon sinks, mainly due to the incomplete decomposition of plant material. In wetlands, the term decomposition is mostly confined to the breakdown and subsequent decay of dominant macrophytes, which leads to the production of detritus (Boy d, 1970). Most annual plant production in wetlands is not consumed by herbivores due to the poor digestibility, thus it decomposes on the wetland surface and becomes a part of a particulate carbon pool (Teal, 1962; Gallagher, 1978; Polunin, 1982).

Despite this significant fact, studies focused on phenolics concentrations in wetland macrophytes are very limited. Studies by Rejman kova (2016), Wang et al. (2015), and Harrison et al. (2017) focused on wetland macrophyte species in salt, brackish, and freshwater wetlands in North and Central America, whereas information about this topic in European climate conditions is limited to the information from the Netherlands and northern Sweden (Hooren et al., 2003; Dorr e p all, 2005; Aerts et al., 2006). Hence, the primary goal of this study was to evaluate and compare the content of phenolics in seven common wetland macrophytes in the Czech Republic and thus extend the knowledge about phenolics content in macrophytes.

### MATERIAL AND METHODS

#### Study sites and plant sampling

Aboveground biomass of seven common wetland macrophytes was sampled at seven different localities

<table>
<thead>
<tr>
<th>Location</th>
<th>Type of wetland</th>
<th>Monitored plants</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breznice</td>
<td>wet meadow</td>
<td>1,2,3,4,5,6</td>
</tr>
<tr>
<td>Bělisky</td>
<td>floodplain</td>
<td>1,2,3,5,7</td>
</tr>
<tr>
<td>Chmelná</td>
<td>floodplain</td>
<td>1,2,3,5,6,7</td>
</tr>
<tr>
<td>Horní Bradlo</td>
<td>stream bank</td>
<td>2,3,5,7</td>
</tr>
<tr>
<td>Louňovák (pond)</td>
<td>pond littoral zone</td>
<td>1,2,4,6</td>
</tr>
<tr>
<td>Moravče</td>
<td>riparian wetland</td>
<td>2,5,7</td>
</tr>
<tr>
<td>Pařez (pond)</td>
<td>pond littoral zone</td>
<td>1,2,4,5,6</td>
</tr>
</tbody>
</table>

Table 1. Surveyed macrophytes and sampling sites characteristics. 1-Phragmites australis, 2-Phalaris arundinacea, 3-Typha latifolia, 4-Glyceria maxima, 5-Scirpus syriacus, 6-Carex nigra, 7-Juncus effusus.
across the Czech Republic (Table 1). The macrophytes involved in the study were *Phragmites australis* (Cav.) Trin. ex Steud., *Phalaris arundinaca* L., *Typha latifolia* L., *Glyceria maxima* (Hartman) Holmberg, *Scirpus sylvaticus* L., *Carex nigra* (L.) Reichard, and *Juncus effusus* L. In *P. australis* and *P. arundinacea*, leaves and stems were analyzed separately. In *T. latifolia*, only leaves were analyzed as flower-stems did not occur at all locations. In order to eliminate varying concentrations in different parts of the leaves and stems, the whole stems and leaves were analyzed. The study was carried out in 2016 and all surveyed plants were harvested in the last week of June. Several plants were also harvested in August, October, and December in order to follow a seasonal pattern. All samples consisted of three plants and all samples were taken in three replicates.

**Analytical methods**

Harvested biomass was separated into stems and leaves (in the case of *P. australis* and *P. arundinacea*), dried to a constant weight at 60°C, and ground in the cutting mill Pulverisette 15 (Fritsch, Idar-Oberstein, Germany) using the 0.5 mm mesh size screen. The ground plant material (approximately 0.1 g) was extracted in 5 ml of 70% acetone for 1 h at 4°C. Total content of phenolics was determined by spectrophotometric methods according to the Folin-Cioacalteau method (Barlocher, Graca, 2005). Standards solutions ranging from 0 to 250 mg l–1 were prepared from tannic acid (Sigma Aldrich, St. Louis, USA) dissolved in 70% acetone. Standards solutions and spine extracts were transferred to the test tubes, solution of 2% Na₂CO₃ in 0.1 M NaOH and Folin-Cioacalteau reagent (Sigma Aldrich) diluted 1 : 2 with deionized water were added. Absorbance at 760 nm was measured after 120 min using Cary UV-Vis 60 spectrophotometer (Agilent Technologies, Santa Clara, USA). The phenolics concentration was expressed in g per kg dry biomass weight (DW).

The statistical analysis of variance (ANOVA) followed by post-hoc Tukey’s HSD test was used to evaluate differences between phenolics contents of various plants. The significance level was set at $P < 0.05$.

**RESULTS**

**Total phenolics in macrophytes**

The average concentrations of phenolics in aboveground biomass of all monitored plants are shown in Fig. 1. The concentrations of total phenolics varied among surveyed species and in various plant parts as well. The lowest concentrations were found in stems of *P. arundinacea* ($9.02 \pm 0.14$ g kg$^{-1}$ DW) followed by stems of *P. australis* ($10.47 \pm 0.78$ g kg$^{-1}$ DW). The highest concentrations were observed for *Scirpus sylvaticus* ($27.74 \pm 0.96$ g kg$^{-1}$ DW) and *Carex nigra* ($28.39 \pm 0.54$ g kg$^{-1}$ DW).

**Seasonal pattern of total phenolics concentrations**

The results revealed several different seasonal patterns of phenolics concentrations in aboveground biomass of *Scirpus sylvaticus* sampled during the period June–December 2016; data presented are means ± SD DW = dry biomass weight

$^{a,b}$ different letters indicate a significant difference at $\alpha = 0.05$ between the means

---

**Fig. 1.** Average concentrations (± SD) of total phenolic compounds in aboveground biomass of seven macrophytes: *Phalaris arundinaca* ($n = 7$), *Phragmites australis* ($n = 5$), *Glyceria maxima* ($n = 3$), *Typha latifolia* ($n = 4$), *Juncus effusus* ($n = 4$), *Scirpus sylvaticus* ($n = 6$), *Carex nigra* ($n = 4$) harvested in June 2016

**Fig. 2.** Concentrations of total phenolic compounds in aboveground biomass of *Scirpus sylvaticus* sampled during the period June–December 2016; data presented are means ± SD DW = dry biomass weight

$^{a,b}$ different letters indicate a significant difference at $\alpha = 0.05$ between the means
The concentrations of total phenolics in aboveground biomass of *Scirpus sylvaticus* sampled during the period June–December 2016 are shown in Fig. 2. The concentration decreased from the highest value in June (27.84 ± 1.53 g kg\(^{-1}\)) to the lowest one recorded in October (7.88 ± 0.733 g kg\(^{-1}\)) and remained low until December. Similar behaviour was observed in leaves of *Phalaris arundinacea* (Fig. 3). The average concentration was the highest and steady in June (19.36 ± 0.84 g kg\(^{-1}\)) and during the summer months, then the concentration decreased until December, where the lowest value (7.03 ± 0.30 g kg\(^{-1}\)) was observed. On the other hand, the seasonal pattern of phenolics concentration in stems of the same plant exhibited a different course (Fig. 3). The average concentrations also decreased from June to December, but the decline was very slow, from 9.97 ± 0.06 to 6.07 ± 0.57 g kg\(^{-1}\).

A different seasonal pattern was observed for total phenolics concentration in *Typha latifolia* (Fig. 4.). The highest value was recorded in August (18.12 ± 2.85 g kg\(^{-1}\)) while the lowest value was observed in December (14.52 ± 2.99 g kg\(^{-1}\)). However, the differences between the average concentrations of each month are not significant.

**Concentration of total phenolics in macrophytes at different localities**

Phenolics concentrations in leaves and stems of *Phragmites australis* sampled in June at five different natural wetlands are shown in Fig. 5A. The highest average concentration in leaves (21.53 ± 1.16 g kg\(^{-1}\)) was recorded in Chmelná. The lowest value was observed in Březnice (16.38 ± 1.26 g kg\(^{-1}\)), but statistically, no significant difference between means was proved. In the case of stems, concentrations varied significantly between the highest one in Louňovák (12.44 ± 0.31 g kg\(^{-1}\)) and the lowest one in Březnice (8.92 ± 0.64 g kg\(^{-1}\)). Differences in phenolics concentrations between the highest and the lowest values for leaves and stems amounted to 25 and 28%, respectively. A similar pattern was found for *P. arundinacea* (Fig. 5B), where no significant differences were observed between the highest (22.50 g kg\(^{-1}\) in Moraveč) and the lowest (15.26 g kg\(^{-1}\) in Horní Bradlo) values in the leaves. Phenolics concentrations in stems varied between only 9.97 ± 0.06 g kg\(^{-1}\) in Březnice and 7.99 ± 0.26 g kg\(^{-1}\) in Chmelná but the significant differences were recorded.

*Carex nigra* (Fig. 5C) exhibited very similar phenolics concentrations at three locations – 28.92 ± 0.39, 28.50 ± 0.86, and 26.66 ± 0.33 g kg\(^{-1}\) at Březnice, Pařez and Louňovák, respectively. A significantly lower value was observed in Chmelná, where the concentration reached only 10.86 ± 0.92 g kg\(^{-1}\). This value was by 62% lower as compared to that at Březnice.

A similar pattern was observed for *Typha latifolia* (Fig. 5D) – the highest concentrations were observed in Běloky (23.52 ± 6.32 g kg\(^{-1}\)) and Horní Bradlo (23.49 ± 4.71 g kg\(^{-1}\)), a lower concentration was found in Březnice (16.20 ± 0.41 g kg\(^{-1}\)), and the lowest concentration was again recorded in Chmelná (12.44 ± 0.48 g kg\(^{-1}\)). However, the only significant difference was found between Březnice and Chmelná due to large concentration fluctuations in Běloky and Horní Bradlo. Phenolics concentrations in *Scirpus*...
sylvaticus (Fig. 5E) were very high and varied between 31.30 ± 2.24 g kg⁻¹ at Běloky and 21.31 ± 0.07 g kg⁻¹ at Moraveč.

The concentrations of phenolics in Glyceria maxima (Fig. 5F) were low and varied only between 16.37 ± 0.22 g kg⁻¹ at Louňovák and 12.02 ± 0.03 g kg⁻¹ at Pařez (Fig. 5F). For Juncus effusus, no significant differences between four different localities were observed and values of phenolic compounds ranged from 22.23 ± 1.33 g kg⁻¹ in Běloky to 17.76 ± 0.13 g kg⁻¹ in Moraveč (Fig. 5G).

**DISCUSSION**

There is a limited amount of information on phenolics content in wetland herbaceous macrophytes but the available data indicate that the concentrations are

---

**Fig. 5.** Average concentrations of total phenolic compounds in above-ground biomass of studied macrophytes harvested from various sites. DW = dry biomass weight; *“different letters indicate a significant difference at α = 0.05 between the means**
lower as compared to wetland shrubs. The concentration found in *C. nigra* was very similar to the concentration of 31.4 g kg⁻¹ found in the same species in the Netherlands (*Hooresens* et al., 2003). *Dorrepaal* (2005) reported phenolics concentrations between 7.3 and 19.8 g kg⁻¹ for five *Carex* species (*rotundata, vaginata, lasiocarpa, rostrate, acutiformis*) in Dutch fens indicating variability within the *Carex* genus. On the other hand, shrubs and scrubs usually exhibit higher concentrations of phenolics. For example, blueberry leaves are very rich source of phenolics. *Routray*, *Orsat* (2014) recorded phenolics concentration of 156 g kg⁻¹ in North American highbush blueberry (*Vaccinium corymbosum*) leaves. Also *Dorrepaal* (2005) reported high phenolics concentrations in shrubs such as *Arctostaphylos alpinus* (black bearberry) – 301.6 g kg⁻¹, *Vaccinium uliginosum* (bog bilberry) – 118.5 g kg⁻¹ or *Vaccinium vitis-idaea* ( lingonberry or cowberry) – 53 g kg⁻¹ sampled in Dutch fens. On the other hand, *Sphagnum* species have low phenolics concentrations (< 10 g kg⁻¹) and herbaceous wetland macrophytes seldom exceeded the concentration of 30 g kg⁻¹ (*Dorrepaal*, 2005).

The total average value for all seven species found in our study was 18.59 ± 1.20 g kg⁻¹ DW (ranging from 9.02 to 28.39 g kg⁻¹ DW). *Harrison* et al. (2017) reported a very similar range of phenolics in 19 emergent wetland plants from 0.0 to 27.6 g kg⁻¹ DW with the average value 10.4 ± 6.20 g kg⁻¹ DW. *Rojmanova* (2016) recorded a relatively higher concentration of phenolics in tropical grown *Typha domingensis* leaves (phenolics: 22–46 g kg⁻¹; lignin: 19–38 g kg⁻¹) and relatively similar results in *Eleocharis cellulosa* stems (phenolics: 7–23 g kg⁻¹; lignin: 12–28 g kg⁻¹). In the same study it has also been shown that the difference in soluble phenolics concentrations of the greenhouse grown plants could be even higher – 87–133 g kg⁻¹ for *Typha* and 16–53 g kg⁻¹ for *Eleocharis*.

Our results also revealed that phenolics concentration varies between leaves and stems (Fig. 1). *Hermann* (1988) pointed out that the distribution of phenolics in the plant can be affected by light and some groups of phenolics can be mainly concentrated in tissues in parts exposed to more light. It has also been reported that various groups of phenolics are distributed differently in plant parts. *Bujor* et al. (2016) compared the diversity of phenolic compounds in leaves, stems, and fruits of bilberry (*Vaccinium myrtillus* L.). Although the variation of the total phenolic content was not so significant, there were large differences in the qualitative composition. Analyses showed the predominance of anthocyanins in fruits, caffeic acid derivates in leaves, whereas flavonol oligomers represented more than half of the phenolic compounds in stems. In all bilberry parts, 106 phenolic compounds were evaluated. Of these 106 compounds, 62 were found in leaf (17 only in leaves), 73 in stem (32 only in stems) and 40 in fruit extracts (9 only in fruits).

A variation of phenolics concentration depending on the harvesting time was also described by several authors for different plant species. *Routray*, *Orsat* (2014) evaluated the total phenolic content in *Vaccinium corymbosum* leaves harvested in May, July, September, and October. They observed the highest concentration in October and the lowest in July. The seasonal dynamics of polyphenols in submerged macrophyte *Myriophyllum verticillatum* L. (whorled water-milfoil) was evaluated by *Bauer* et al. (2009) during the growth seasons in four successive years. Total phenolic compounds contents significantly differed in the growing season and also even between years. *Harrison* et al. (2017) observed a significant decline of the foliar phenolic content in *Lythrum salicaria* (purple loosestrife) even during a very short period of sampling (from mid-July to the beginning of August).

The results supported the information that the concentrations of total phenolics in the aboveground biomass of herbaceous wetland macrophytes differ among species and localities as well. In addition, our study revealed that the phenolics concentrations vary between stems and leaves with concentrations being higher in leaves. Our study also showed that the concentration of phenolics decreases during the growing season. This is a quite important finding in relation to decomposition and potential carbon sequestration. Our further studies will focus on the relationship between the phenolic content in the aboveground biomass of wetland macrophytes and the nutrient status of the soil or sediment at the macrophyte stand. Also the relationship between the phenolics content and the decomposition rate will be evaluated. The future research will be aimed at the selection of plants that can be used for constructed wetlands treating effectively agricultural runoff and that can simultaneously contribute to carbon sequestration under the conditions of the Czech Republic.

**CONCLUSION**

During this study, concentrations of total phenolics were evaluated in seven common macrophytes in natural wetlands of the Czech Republic. The highest concentrations of phenolics were found in *Scirpus sylvaticus* and *Carex nigra*, while the lowest concentrations were observed in stems of *Phalaris arundinacea* and *Phragmites australis*. There was also a significant difference in phenolics concentrations in stems and leaves of these plants with leaves concentrations being higher. The evaluation of a seasonal dynamics of phenolics revealed that the concentration decreases throughout the year, however, the pattern varies among monitored species. The results also indicated a differ-
ence in the phenolics content in relation to sampling sites. The evaluation of the relationship between the nutrient status of soil and the phenolics in plants is the next phase of this research.

REFERENCES


Corresponding Author:
Prof. Ing. Jan Vy m a z a l , CSc., Czech University of Life Sciences Prague, Faculty of Environmental Sciences, Kamýcká 129, 165 00 Prague 6-Suchdol, Czech Republic, phone: +420 224 383 825, e-mail: vymazal@yahoo.com