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PHENOLIC COMPOUNDS IN *HIPPOPHAË RHAMNOIDES* LEAVES COLLECTED FROM HEAVY METALS CONTAMINATED SITES

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We examined total phenols, phenylopropanoids, flavonols and anthocyjanins content in leaves of sea buckthorn (*Hippophaë rhamnoides* L., *Elaeagnaceae*) growing in different heavy metals contaminated areas. Leave samples were collected in spring, and in autumn from female specimens. In the spring the concentration of total phenolic compounds in samples collected from specimens growing in the city center was on the control level (material from unpolluted area). The analogous concentration in samples taken in autumn was different in particular contaminated sites, and amounted from 240 to 300 mg 100 g^{-1} f. w. Regardless of the season and site anthocyanins content reached 5 mg 100 g^{-1} f.w. In the course of time the content of phenolic compounds increased in leaves of plants growing on the waste heaps, especially concentration of phenylopropanoids and flavonols.

Keywords: contamination, heavy metals, phenols, sea buckthorn, urban areas

Introduction

In different urban areas, plants are exposed to direct influence of various stress factors that have a negative impact on living organisms and lead to numerous metabolic dysfunctions. In these sites to the most harmful and dangerous is included increasing concentration of heavy metals ions, mainly lead and cadmium, generated during the combustion of gasoline, oil and diesel oil as well as the abrasion of tires and road surfaces and other activities (Akbar et al., 2006; Gupta et al., 2010; Lu et al., 2010).

In the course of numerous experiments it has been shown that elevated level of heavy metals is toxic for living organisms (Peltarta et al., 2001; Banddh and Singh, 2011; Evangelon et al., 2012). In plants heavy metals affect physiological processes such as transpiration, photosynthesis, electron transport, and cell division which lead to inhibition of growth and development (Qufei and Fashui, 2009; Nagajyoti et al., 2010; Pourrut et al., 2012). Another, well-documented effect is uncontrolled production of reactive oxygen species (ROS) causing oxidative stress (Karuppanapandian et al., 2011; Biesiada and Tomczak 2012). Excessive amounts of ROS result in lipid peroxidation, the enzymes and other proteins inactivation and DNA damage (Karuppanapandian et al., 2011; Sharma et al., 2012). To prevent the negative effects of high ROS content, intracellular plant defense mechanisms are activated among which synthesis of phenolic compounds should be mentioned. They are known for their antioxidant properties, and ability to free radicals' scavenging (Michalak, 2006). Thus phenols counteract the effects of stress induced by balast metal ions. In addition, they may also take part in the accumulation of such ions by their coordination binding (Lavid et al., 2001a, b). For above mentioned reasons there were scheduled analyses of phenolic compounds concentration in *Hippophaë rhamnoides* leaves exposed to heavy metals from different sources.

Material and methods

Representative leave samples of sea buckthorn (*Hippophaë rhamnoides* L., Elaeagnaceae) were collected in spring, and in autumn 2013 from female specimens growing in polluted areas: (1) Grzegórzecka Street in Krakow, Poland (described as GS) where the average traffic per hour is high (about 1000 cars), (2) waste heap (described as WH) obtained after zinc and lead ore flotation in Bukowno near Olkusz (southern Poland). As a control, reference samples were taken from plants (described as CTR) growing outside contaminated areas. In plant material the concentration of phenolic compounds were analysed according to the Fukumoto and Mazza (2000) protocol, based on the method of UV-Vis spectrophotometry. Samples were homogenized in methanol. The content of total phenols, phenylopropanoids, flavonols and anthocyjanins were calculated by measuring the absorbance at 280, 320, 360 and 520 nm respectively. To identify the different classes of phenolic compounds, the following standards were used: chlorogenic acid (total phenols), coffee acid

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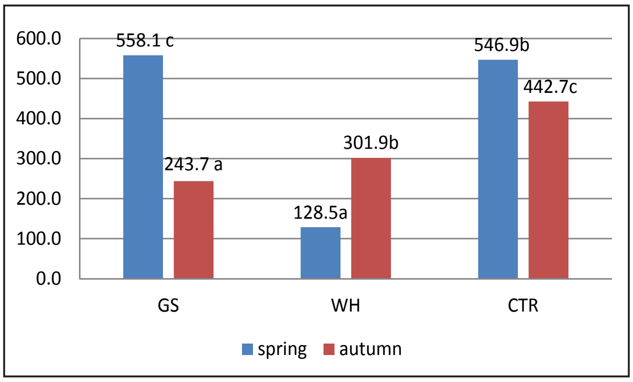
(phenylpropanoids), quercetin (flavonols) and cyanidin (anthocyanins).

One-ways analysis of variance (ANOVA) test was used separately for spring and autumn data. Statistical significance of the results and means were evaluated according to the Tukey's test at $\alpha = 0.05$.

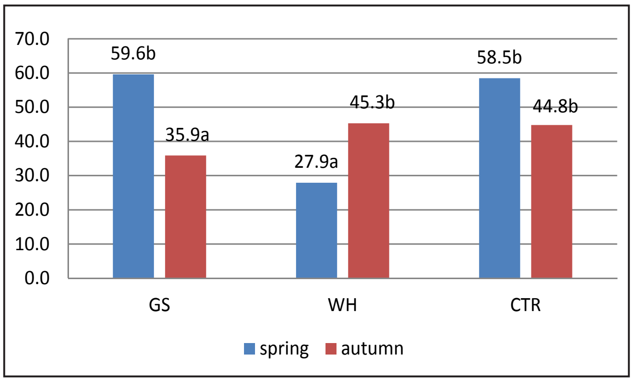
Results and discussion

In the present experiment the concentration of phenolic compounds, as the low molecular weight antioxidants protecting plants cells from negative impact of ROS, was evaluated. It was found that in samples collected in spring from specimens growing close to main Krakow road (GS) the total phenols concentration was three times higher in comparison to waste heap taken samples (Fig.1), whereas in respect to the analogous concentration concerning samples taken in autumn, the difference between the contaminated sites was proved to be statistically insignificant, and amounted to almost 240 and 300 mg 100 g^{-1} f.w. respectively. Considering phenylopropanoids, their concentration in samples collected at the beginning of the year from Grzegórzecka street (GS) was similar to concentration ascertained in leaves taken from plants growing outside the polluted areas (CTR, Fig. 2), and lower during the autumn. In both periods the concentration of phenylopropanoids in leave samples collected from waste heap (WH), has been more than three times lower in comparison to the control. In the case of flavonols, quite similar relationship was observed (Fig. 3). Their highest content (about 59 mg 100 g^{-1} f.w.) was determined in the spring in samples from Grzegórzecka street and from those from control *Hippophaë rhamnoides* plants. In autumn, flavonols concentration in waste heaps' leaves was not statistically different from the control ones (45 mg 100 g^{-1} f.w.). Regardless of the season, anthocyanins concentration in tested material was similar (Fig. 4). In the course of time, the phenolic compounds content in the leaves of examined plants decreased, with the exception of leaves from plants growing on the waste heap. In these material quite the contrary interdependence was ascertained. The concentration of total phenols as well as phenylopropanoids and flavonols increased in the autumn.

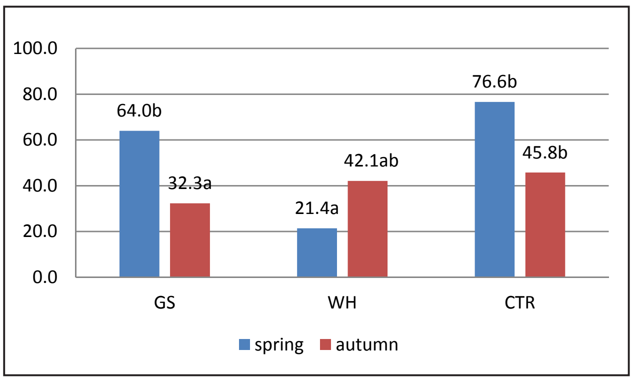
Possibilities of plants existence in the specific environment, and their ability to grow and develop there depends on the level of resistance to local stress factors. On areas degraded as a result of industrial activity or areas located close to main roads and highways plants are exposed to different level of large variety of toxic substances. Heavy metals are ranked to the most dangerous. They frequently cause the imbalance of redox processes which lead to excessive generation, and further accumulation of free radicals in particular



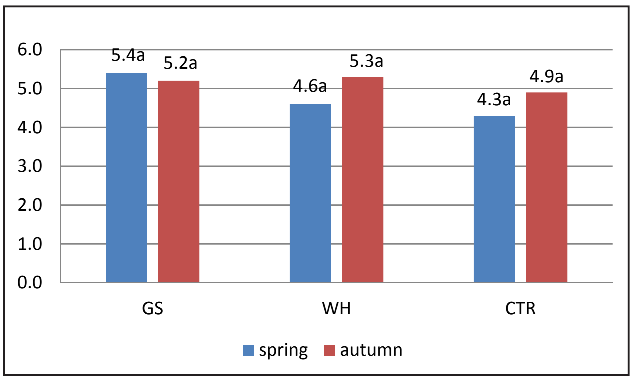
Total phenols in mg 100 g^{-1} f.w.



Flavonols in mg 100 g^{-1} f.w.



Phenylopropanoids in mg 100 g^{-1} f.w.



Anthocyanins in mg 100 g^{-1} f.w.

Figure 1–4 Phenolic compounds content in *Hippophaë rhamnoides* leaves collected in two periods (spring and autumn) from plants growing on Grzegórzecka street in Krakow (GS), waste heaps in Olkusz (WH) and polluted site (CTR)

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reactive oxygen species (ROS) (Groppa and Benavides, 2008; Biesiada and Tomczak, 2012). Plant damage occurs when the amount of ROS production is higher than the capacity of antioxidant processes or detoxification mechanisms (Michalak, 2006). In presented work the content of phenols was evaluated, because they both carry out ROS in their inactive derivatives and inhibit their formation and thus counteracting the effects of heavy metals stress. The obtained results have shown that the concentration of phenolic compounds was different in examined objects. In the spring, variation of phenols concentration in *Hippophaë rhamnoides* leaves collected from plants growing down the main road in Krakow and control site was low. Futhermore, in the autumn, differences between phenylopropanoids and flavonols content in leaves from waste heaps plants and control ones were insignificant. Similar results have been obtained using *Adhatoda vasica, Cassia fistula* and *Withania somnifera* plants grown in two contrasting environmental conditions – copper mining site and unpolluted site (Maharia et al., 2012). In this material total phenolic content and total flavonoid content in specimens from mining site and from control site did not reveal significant differences. Opposite results regarding the increased biosynthesis of phenolic compounds have been noted by Smeets et al. (2005) in *Phaseolus vulgaris* treated with Cd ions and by Pawlak-Sprada et al. (2011) in *Lupinus albus* under the influence of Cd and Pb ions. Phenolic compounds were also the first line of defense against Cu stress in experiment with red cabbage and radish (Sakihama et al., 2002; Posmyk et al., 2009). Moreover, accumulations of this antioxidants were observed not only in shoots but also in roots of maize (Shemet and Fedenko, 2005) and scot pine (Schützendübel et al., 2001) under Cd treatment. Also in halophytic plant called *Aeluropus littoralis*, the protective function of these compounds against heavy metals (Cd, Pb, Ag, Co) stress was demonstrated (Rastgoo and Alemzadeh, 2011). The increasing levels of toxic metals resulted in a general increase in levels of total phenolic contents also in the cells of other taxon, such as *Phaeodactylum tricornutum* diatom and *Amanita caesarea, Clitocybe geotropa* and *Leucoagaricus pudicus* fungi (Sarikurkcu et al., 2010; Rico et al., 2013). In examined sea buckthorn leaves collected from plants growing in industrial areas, the content of phenolic compounds increased in the course of the season and it decreased in leaves taken from other sites. Probably, it was the result of successive stress factors changes with the time. On the open spaces of waste heaps, plant species are subjected not only to heavy metals ions but also to drought, strong winds and high radiation which could influence on higher flavonoids and phenylopropanoids concentration in comparison to plants growing in the city (Przedpełska and Wierzbicka, 2007; Rakov and Chibrik, 2009; Fini et al., 2011; Agati et al., 2012). Another complexion on the matter is put by the fact that some studies have also shown that the concentration of phenols strongly depends on the type of tissue and of respective organs, the development stage, the metabolic activity of analyzed material (Michalak, 2006; Augustynowicz et al., 2011; Agati et al., 2012). Therefore, to clearly determine the role of antioxidant compounds in *Hippophaë rhamnoides* response to stress caused by heavy metals further detailed study should be conducted.

On the other hand fruits of *Hippophaë rhamnoides* are known for their medical properties mainly due to the high level of phenolic substances (Gao et al., 2000), so probably not only fruits but also leaves can be relatively rich in antioxidant compounds. For this reason, reported experiment high concentration of total phenolic compounds, phenylopropanoids and flavonols were observed in the control, and their concentration decreased in autumn. Similarly, decreased content of phenolics with increased maturity of sea buckthorn fruits was noticed by Gao et al. (2000).

Conclusion

On terrains degraded by industrial activities as well as close to main roads and highways, plants are frequently exposed to elevated concentration of heavy metals and to other stress factors that affect negatively their metabolism. Therefore monitoring of the physiological status should be the key factor during the selection of proper material to be grown in contaminated areas.

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