Evaluation of the Usefulness of Sea Buckthorn for Planting in Various Urban Areas According to Photosynthetic Apparatus Efficiency and Antioxidant Activity

Introduction

Various anthropogenic activities including ore mining or smelting and intense routes traffic are mainly responsible for considerable amounts of toxic substances introduced into environment. Heavy metals are strongly poisonous to plants [10, 16, 27]. Negative effects of these hazardous elements are manifested in suppressed cell division, inhibited growth rate, restrained physiological processes such as transpiration, photosynthesis or electron transport [18, 22, 24]. Moreover, plants are exposed to direct influence of other stress factors such as drought and strong winds, intense insolation, extremely low content of nutrients both on areas degraded by industrial activities and close to main roads and highways [11, 26]. They also lead to numerous metabolic dysfunctions what might be lethal for sensitive organisms. Thus, it is necessary not only to develop effective methods for removal of toxic substances from the soil and counteract further contamination. Another important issue is to create an efficient monitoring system of plant status which plays an important role in the biological barrier against the spread of pollution [11]. Many of the physiological and biochemical responses of plants can be utilized for the detection of environmental pollutants. For instance, the response to the presence of heavy metal ions might be evaluated based on the changes in photosynthetic pigments content or in chlorophylls fluorescence emission [7, 11, 20]. Drought, salinity, toxic substances, temperature stress, etc. cause uncontrolled production of reactive oxygen species (ROS) that might be detected by measuring antioxidant activity of plants and their ability to free radicals’ scavenging [4, 9, 17]. The presence of environmental stressors can also be measured by enzyme activity and the production of specific cell marker proteins [8, 25].

The aim of this study was to evaluate the conditions of Hippophae rhamnoides specimens growing in various environments. Hippophae rhamnoides is an Eurasian deciduous shrub with low soil requirements and high resistance to drought, pollution and winter frost [3]. Plants status was determined using chlorophyll fluorescence techniques that provide a lot of information about the actual state of photosynthetic apparatus of the leaves. Moreover, the content of photosynthetic pigments and antioxidant response were measured in sampled specimens.

Material and methods

Leaf samples of sea buckthorn (Hippophae rhamnoides L., Elaeagnaceae) were collected in the spring (the middle of May) and autumn (the middle of September) 2013 from female specimens growing in different polluted areas: close to main Cracow road (Grzegórzecka Street), directly on the waste heap formed after zinc and lead ore flotation in Bukowno near Olkusz (southern Poland) and on the waste heap formed after mining operations in Pszów near Wodzisław Śląski (southern Poland).
Photosynthetic pigments were performed following extraction in acetone (80%). Then, the extract was centrifuged (15 min, 4800g) and the supernatant was supplemented with the acetone to 20 ml volume. Chlorophyll \textit{a} chlorophyll \textit{b} and total carotenoid content were determined by measuring spectrophotometric absorbance of the extract at 470, 646 and 663 nm using UV-Vis spectrophotometer (Hitachi U-2900). Next, the pigment content were calculated in accordance with the equations presented by Wellburn [29]. Moreover, the chlorophyll \textit{a} fluorescence induction curves analysis were provided. For this reason, 10 leaves from plants growing in various conditions were adapted to the dark for 30 minutes. The fluorescence were induced by red light: \(\lambda_{\text{max}}=650\text{nm}, 2500\text{μmol [quants] m}^{-2}\text{s}^{-1}\). Several photosynthetic functional parameters were calculated - the maximum photochemical efficiency of photosystem II (PSII)-Fv/Fm and the index of PS II vitality- PI (called Performance Index). Chlorophyll fluorescence induction kinetics were recorded using Handy-PEA (Hansatech, UK) spectrophotometer on the basis of the relevant standard procedures. Recorded curves were analysed using the fluorometer producer’s software (PEA-Plus).

Antioxidant activity was determined using the Pekkarinen et al. [21] method with free radical DPPH (2,2-diphenyl-1-pikrylohydrazyl). The ability of examined methanolic extracts to DPPH extinction, reflecting in DPPH absorbance reduction was evaluated. The study was conducted at the maximum absorption of DPPH solution (515nm) after 30 minutes from the initiation of the reaction. Percent of neutralization was calculated according to the Maharia et al. [12]:

\[
\% \text{ neutralization} = 100\left(\frac{A_0 - A_{SR}}{A_0}\right) ,
\]

where: \(A_{SR}\) – average value of the examined extract absorbance,

\(A_0\) – absorbance of DPPH solution.

One-way analysis of variance (ANOVA) test was used separately for data for each area. Statistical significance of the results and means were evaluated according to the Duncun’s test at \(\alpha = 0.05\).

**Results**

Zinc-lead waste heap in Bukowno (WH). The total content of chlorophylls, chlorophyll \textit{a} and \textit{b}, as well as carotenoids was significantly lower at the beginning of the growing season (Table 1). There were no differences in Chl\textit{a/b} ratio. Statistical analyses revealed also significantly higher values of Fm, Fv/Fm and Pl in the autumn than in the spring and no differences in Fo (Fig. 1).

<table>
<thead>
<tr>
<th>Area</th>
<th>Date</th>
<th>Chl\textit{a} [mg/ g f.w.]</th>
<th>Chl\textit{b} [mg/ g f.w.]</th>
<th>Chl\textit{a} + \textit{b} [mg/ g f.w.]</th>
<th>Chl\textit{a}/\textit{b}</th>
<th>Car [mg/ g f.w.]</th>
</tr>
</thead>
<tbody>
<tr>
<td>WH</td>
<td>Spring</td>
<td>0.41 ±0.03</td>
<td>0.103 ±0.009</td>
<td>0.51 ±0.04</td>
<td>3.93 ±0.01</td>
<td>0.116 ±0.01</td>
</tr>
<tr>
<td></td>
<td>Autumn</td>
<td>0.60 ±0.08*</td>
<td>0.158 ±0.010*</td>
<td>0.76 ±0.10*</td>
<td>3.78 ±0.25</td>
<td>0.167 ±0.006*</td>
</tr>
<tr>
<td>CR</td>
<td>Spring</td>
<td>0.56 ±0.02</td>
<td>0.136 ±0.004</td>
<td>0.69 ±0.03</td>
<td>4.12 ±0.12</td>
<td>0.139 ±0.003</td>
</tr>
<tr>
<td></td>
<td>Autumn</td>
<td>0.57 ±0.08</td>
<td>0.162 ±0.020*</td>
<td>0.73 ±0.10</td>
<td>3.51 ±0.26*</td>
<td>0.173 ±0.009*</td>
</tr>
<tr>
<td>MH</td>
<td>Spring</td>
<td>0.47 ±0.03</td>
<td>0.120 ±0.010</td>
<td>0.59 ±0.04</td>
<td>3.92 ±0.06</td>
<td>0.128 ±0.005</td>
</tr>
<tr>
<td></td>
<td>Autumn</td>
<td>0.54 ±0.02*</td>
<td>0.147 ±0.006*</td>
<td>0.69 ±0.02*</td>
<td>3.68 ±0.04*</td>
<td>0.145 ±0.006*</td>
</tr>
</tbody>
</table>
The percent of DPPH inhibition was very high (84.2%) during the spring and decreased almost half in the autumn (47.4%) (Fig. 2).

Cracow road (CR). We noted higher content of chlorophyll b and carotenoids in the autumn, but no differences in chlorophyll a and total chlorophylls content (Table 1). In turn, the Chl a/b ratio was higher in the spring (4.1) than in the autumn (3.5) (Table 1). Fluorescence parameters: Fo, Fm and PI were significantly higher at the end of growing season (Fig. 1). There were no considerably differences between Fv/Fm measured in the spring and autumn (Fig. 1). Contrary to antioxidant activity of sea buckthorn from WH, percent of DPPH inhibition was four times higher in the autumn leaves than in the spring ones (Fig. 2).

![Fluorescence parameters](image)

Fig. 1. Fluorescence parameters (mean ±SD) of sea buckthorn growing in different areas: minimal fluorescence (Fo), maximal fluorescence (Fm), maximum photochemical efficiency of photosystem II (Fv/Fm), performance index (PI) (*significant differences for each area, Duncun test, \( \alpha = 0.05 \))

Mining waste heap in Pszów (MH). Similarly to the plants from WH, the content of photosynthetic pigments (Chl a, Chl b, Chl a+b, Car) increased significantly in the autumn (Table 1). But Chl a/b ratio was higher in the spring (Table 1). Among chlorophyll fluorescence parameters, only Fv/Fm was considerably higher in the autumn (Fig. 1). Likewise in plants from Cracow road, percent of DPPH inhibition was about three times higher during the autumn than the spring (Fig. 2).
Growing human population, forcing development of industry and urban regions, causes that exposition of plants to many abiotic stress factors such as drought, soil salinity and pollution of air, soil and water with heavy metals is still increasing. Abiotic stress leads to series of morphological, physiological, biochemical and molecular changes that adversely affect plant growth and productivity [30]. From a number of stressors, especially heavy metals affected severely process of photosynthesis [19]. Even small change in the values of chlorophyll \( a \) fluorescence indicate serious problems and unfavorable situation for the plant therefore its measurement is considered as indicator of photosystem II efficiency and helps in evaluation of plant reaction to stress [5, 14]. Also content of photosynthetic pigments is widely used as an indicator of plant physiological status [28].

In the spring, development of the photosynthetic apparatus in plants from heap in Bukowno was delayed, that could be confirmed by lower pigments content and lower values of the photosynthesis efficiency parameters. This delay may result from the impact of heavy metals, cadmium and lead, what is proved by high value of minimal fluorescence (Fo) at the beginning of the growing season. In plant cells, heavy metal lead to damage of reaction centres and impaired energy transfer between them and the collecting antennae of the PSII [6, 13]. Also the high activity of small molecule compounds with antioxidant properties indicates the occurrence of oxidative stress, which may result from the presence of heavy metals [4, 17]. In turn, the results obtained in the autumn: high content of pigments as well as increased the maximum photochemical efficiency of photosystem II and declining antioxidant activity, may indicate the adaptation of plants to stressful conditions. Decreasing the value of minimal fluorescence (Fo) may indicate a reduction in the number of oxidized reaction centers compared to properly formed ones and increasing maximal fluorescence (Fm) that the electron transport chain begins to operate more efficiently, which results in improved efficiency of photosynthesis [2].

In Cracow, changes observed in functioning of the photosynthetic apparatus may largely result from the differ climatic conditions as ending the growing season. Shortened growing season and thus accelerated the aging process may be due to the location of the growth sites in the city center (higher temperature and water shortages).Increasing minimal (Fo) together with maximal fluorescence (Fm) in the absence of variation in chlorophyll \( a \) content can prove the aging process [1, 15]. Higher antioxidant activity in the autumn additionally confirms early stages of leave senescence cause plants produce small molecule antiradicals as a defence response to the formation of large amounts of free radicals (ROS) occurring in oxidative burst during aging process [23].

No changes in the efficiency of the photosynthetic apparatus and the continued maximum photochemical efficiency of PSII at the level of 0.83 in plants growing in close proximity to coal mine heap in Pszów
may indicate a good adaptation of these plants to the prevailing conditions in a given location. However, the increase in carotenoid pigments and antioxidant activity may indicate, on the one hand, about the beginning of the aging process, on the other, about the influence of abiotic stress arising from the neighbourhood of coal mine heap. Carotenoids have greater persistence in senescence leaves but also can act as photoprotectants and antioxidants in plant metabolism [28].

Conclusions

The article provides a brief update on the progress of *Hippophaërhhamnoides* evaluation as plant material proposed to be cultivated in the extremely harsh conditions. On the basis of scientific experiments we stated that sea buckthorn specimens are able to deal well upon different stress factors associated with degraded urban areas. However, some further analyses are needed to find out the exact reasons responsible for tiny physiological disturbances when sea buckthorn is grown in unfavorable conditions.

Abstract

In different urban areas, plants are exposed to direct influence of various stress factors that frequently lead to numerous metabolic dysfunctions. For this reason, the aim of this study was to evaluate the physiological conditions of *Hippophaërhhamnoides* specimens growing in: (1) main Cracow road (CR), (2) waste heap formed after zinc and lead ore flotation (WH), (3) waste heap formed after mining operation (MH). Efficiency of photosynthetic apparatus and the DPPH free radical scavenging activity were determined in the spring and autumn. Obtained results demonstrate a good adaptation of sea buckthorn for growth and development in degraded urban areas.

Keywords: sea buckthorn, photosynthetic pigments, chl a fluorescence, DPPH

References


**Acknowledgments**

Support of experimental work by the Ministry of Science and Higher Education of Republic of Poland (DS 3500/WO/2013) is gratefully acknowledged.