Ewa Muszyńska*, Ewa Hanus-Fajerska*

Evaluation of *Scabiosa ochroleuca* L. vitality after introduction on post-flotation wastes¹

Ocena efektu wprowadzenia *Scabiosa ochroleuca* L. na odpady poflotacyjne

* Dr inż. Ewa Muszyńska, dr hab. Ewa Hanus-Fajerska - Institute of Plant Biology and Biotechnology - Unit of Botany and Plant Physiology, University of Agriculture in Krakow, 31-425 Krakow, 29 Listopada 54 Ave., Poland, e-mail: e.muszynska@ogr.ur.krakow.pl; e.hanus@ogr.ur.krakow.pl

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Abstract

The aim of this study was to evaluate the *Scabiosa ochroleuca* suitability for growth on heavy metals contaminated substrate obtained as a result of Zn–Pb ores exploitation and their processing. Three variants of cultivation were tested in vegetative hall conditions: post-flotation wastes (control), wastes enriched with either mineral fertilisation or with sewage sludge. The possibility of future direct use of the studied species on post-flotation settling pond was determined on the basis of biometric measurements and analysis efficiency of photosynthetic apparatus, that is, pigments content and chlorophyll *a* fluorescence. The results showed that untreated wastes negatively influenced on plants morphological and physiological status. On the contrary, the application of sewage sludge improved growth and development of cream scabious. Thus, not only the selection of proper plant material, tolerant to toxic levels of heavy metals and other hostile conditions, but also the waste treatment are crucial when metalliferous areas are to be reclaimed.

Streszczenie

Przeprowadzono eksperyment z zamiarem określenia przydatności *Scabiosa ochroleuca* do wzrostu na materiale odpadowym powstałym w wyniku eksploatacji i przetwarzania rud cynkowo-ołowiowych. Testowano trzy warianty uprawy w doświadczeniu wazonowym, prowadzonym w hali vegetacyjnej: surowe odpady poflotacyjne (kontrola), odpady wzbogacone w nawożenie mineralne bądź nawożone osadem ściekowym. O możliwości wykorzystania badanego gatunku na osadniku poflotacyjnym wnioskowano na podstawie pomiarów biometrycznych, analizy zawartości barwników fotosyntetycznych i fluorescencji chlorofilu *a*. Uzyskane wyniki wskazują na negatywne oddziaływanie surowych odpadów poflotacyjnych na wzrost i rozwój driakwi żółtej. Natomiast u roślin uprawianych w wazonach z dodatkiem osadów ściekowych zaobserwowano najwyższe wartości wszystkich analizowanych parametrów. Zatem zarówno dobór odpowiedniego materiału roślinnego wykazującego tolerancję na metale ciężkie i inne niesprzyjające warunki siedliskowe, jak i wzbogacenie podłoża w odpowiedni rodzaj substancji jest niezbędne, aby rekultywacja przy użyciu badanego gatunku mogła przynieść zamierzone efekty.

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1. INTRODUCTION

There are particular areas of Śląsko-Krakowska Upland (southern Poland) rich in zinc and lead ores that have been mined and processed for more than 800 years. Those activities have created huge amount of waste characterised by unfavourable physical and chemical properties, such as alkaline pH, low water capacity, lack of organic matter and low concentration of mineral nutrients [Przedpełska and Wierzbicka 2007; Szarek-Łukaszewska 2009; Wójcik et al. 2013]. Moreover, deposited materials are strongly polluted with heavy metals, especially zinc, lead and cadmium. It was found that their concentration in the substratum can exceed 16,000 mg·kg⁻¹ d. m. for Zn, 6,000 mg·kg⁻¹ d. m. for Pb and 90 mg·kg⁻¹ d. m. for Cd [Cabała et al. 2009; Hanus-Fajerska and Ciarkowska 2010; Muszyńska et al. 2013]. Owing to the information mentioned earlier as well as the limited number of appropriate species that could colonise wastes obtained after mining and processing-ore operation, the development of vegetation cover on such area is extremely

difficult and time consuming [Tordoff et al. 2000; Kasowska and Koszelnik-Leszek 2014; Pandey et al. 2015]. Nevertheless, the establishment of vegetation is highly desirable because (1) it provides the necessary surface stability that protects wastes from wind and water erosion and (2) it accelerates the process of soil development. The planted species are usually trees and shrubs, such as birch, poplar and sea buckthorn [Borghi et al. 2008; Hanus-Fajerska et al. 2009; Muszyńska et al. 2014]. Methods of reclamation based on naturally colonising and economically valuable plants are used only rarely [Hanus-Fajerska and Ciarkowska 2010; Gupta et al. 2013; Pandey et al. 2015]. Consequently, experiments should be undertaken to select proper, and possible perennial, plant materials adapted to such habitat conditions and thus to overcome the limitation of plants development allowing to formulate large-scale revegetation scheme with the use of native and metal-tolerant species.

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The aim of the presented study was to examine the suitability of *Scabiosa ochroleuca*, that is, the species that spontaneously occurred on metalliferous terrains, to be grown on heavy metals contaminated wastes. The long-term objective is to find new genera to be applied in remediation of such difficult kind of substratum, almost useless to be re-grown. Several physiological parameters were used to test the vitality of plant material. Especially, because the photosynthesis is strongly influenced by stress factors, the efficiency of photosynthetic apparatus was evaluated. Therefore, we focused on photosynthetic pigments content as well as the chlorophyll *a* fluorescence tests.

2. MATERIAL AND METHODS

The pot experiment was carried out in vegetation hall condition during the whole growth season. Initial plant material were seed samples of *S. ochroleuca* (Dipsacaceae) collected from specimens growing on old waste heap in Bolesław, located in the south-eastern part of the Śląsko-Krakowska Upland (Poland). As substratum for plant growth, post-flotation wastes obtained in processing operation of Zn–Pb ores performed by ZGH Bolesław S.A in Bukowno (Southern Poland) were used. The following treatments were tested: (1) untreated post-flotation waste (control) [described as CTR], (2) post-flotation waste enriched with NPK fertilisation applied as NH_4NO_3 , KH_2PO_4 and KCl in the dose of 1.2 g N, 0.4 g P_2O_5 , 1.0 g K_2O per pot [described as NPK]; (3) post-flotation waste enriched with sewage sludge from the mechanical and biological treatment plant in the maximal permissible dose defined by the Ministry of the Environment [Dz. U. 2010 nr 137, 924] that can be used for reclamation purpose [described as SS]. In June 2013, five juvenile specimens of cream scabious were planted per pot in six replications. Plants' status was evaluated two times (4 and 12 weeks after planting) by measuring the height and diameter of plant, size of leaves and their number per specimen. Moreover, the concentration of photosynthetic pigments as well as the chlorophyll *a* fluorescence were ascertained. The content of chlorophyll *a*, chlorophyll *b* and carotenoids were determined spectrophotometrically following extraction in acetone (80%) and calculated according to Wellburn [1994]. The analysis of chlorophyll *a* fluorescence induction curves were provided using Handy-PEA (Hansatech, UK) spectrofluorometer on the basis of the relevant standard procedures. The fluorescence was induced by red light: $\lambda_{\text{max}} = 650 \text{ nm}$, $2,000 \mu\text{mol} [\text{quants}] \text{ m}^{-2} \text{ s}^{-1}$. The photosynthetic functional parameters, such as the maximum photochemical efficiency of photosystem II (Fv/Fm) and the index of PS II vitality (PI; *Performance Index*), were calculated.

One-way analysis of variance (ANOVA) test was used for analysing biometrical and physiological data, separately for the beginning and the end of growing season (4- and 12-weeks of cultivation, respectively). Statistical significance of the results and means were evaluated according to the Tukey's test at $\alpha = 0.05$.

3. RESULTS

Obtained results showed that untreated wastes obtained after Zn–Pb ore flotation negatively influenced on plants growth (Table 1).

Regardless of measurement time, the reduction of the plant height and diameter of rosette as well as leaf size and the number of leaves were observed in control treatment. In contrast, the highest values of all biometric parameters were noted in plants growing on wastes with the addition of sewage sludge. However, at the beginning of cultivation, those plants diameter, leaves width and their number were similar to the plants of NPK treatment and ranged from 14.2 to 16.2 cm in diameter, from 2.2 to 2.3 cm in case of leaves width and from 10.1 to 12.6 for pieces of leaves per plants. With the course of experiment, growth of cream scabious proceeded the best in pots supplemented with sewage sludge, whilst in pots fertilised with N, P and K, the measured parameters of tested specimens reached rather the average values. However, in NPK treatment, the visible symptoms such as leaf chlorosis or even necrosis and their premature ageing were also observed.

We noted also that all parameters of photosynthetic apparatus efficiency varied depending on the treatment (Table 2). Applied doses of either mineral or organic fertilisers influenced considerably on the content of chlorophyll *a* and *b* as well as carotenoids. The highest values were detected in plants cultivated on wastes enriched with sewage sludge, whilst the lowest in untreated plants (control). Statistical analyses also revealed significantly higher values of Fv/Fm and PI in plants treated with both NPK and SS than in CTR ones. Nevertheless, the application of mineral fertiliser contributed to plants death. Thus, at the end of growing season, the analysis of physiological parameters were impossible to perform in such treatment, so their results were not shown in Table 2.

4. DISCUSSION AND FINAL REMARKS

The activities that allow efficient establishment of vegetation cover on industrial degraded areas have been continuously searched. New, economically viable and environmentally friendly approach is to use the biological methods based on metal-tolerant plants that occur naturally on contaminated terrains. Those plants are already adapted to other stress factors of mine sites, particularly drought and nutrient deficiency [Tordoff et al. 2000; Szarek-Lukaszewska 2009; Zhang et al. 2014]. Then, we propose calamine ecotype of *S. ochroleuca*, representing local populations of Olkusz Ore Bearing Region, to initiate and/or accelerate the spontaneous colonisation of metalliferous mine wastes on which natural colonisation is extremely difficult and time consuming. Our results have shown that wastes obtained after Zn–Pb ores flotation require special treatment to improve the growth and development of cream scabious. The highest values of all morphological and physiological parameters were observed in specimens growing on soil-free grounds with sewage sludge addition. Similarly, the positive effect of sewage sludge application on plants growth and development was reported, for example, in *Dyera costulata* [Ghafoori et al. 2011] or *Helianthus annuus* [Hao et al. 2012], during the evaluation of their suitability for phytoremediation techniques. Moreover, sewage sludge amendment can improve soil properties, such as bulk density, porosity and water holding capacity, and provides organic matter

Table 1. Growth parameters of *Scabiosa ochroleuca* (mean values $\pm$ SD) depending on the treatment (CTR, untreated wastes after Zn–Pb ore flotation; NPK, wastes enriched in NPK fertilisation; SS, wastes enriched in sewage sludge) after 4 and 12 weeks of cultivation

Term	Treatment	Plants height [cm]	Diameter [cm]	Leaf length [cm]	Leaf width [cm]	Leaves number/ plant [pcs]
4 weeks	CTR	3.91 c* $\pm$ 1.26	10.97 b $\pm$ 2.37	6.49 c $\pm$ 0.74	2.03 b $\pm$ 0.22	6.75 b $\pm$ 0.87
	NPK	4.47 b $\pm$ 1.43	14.27 a $\pm$ 1.86	8.23 b $\pm$ 0.82	2.24 a $\pm$ 0.18	10.18 a $\pm$ 2.28
	SS	7.78 a $\pm$ 2.09	16.25 a $\pm$ 2.18	9.98 a $\pm$ 0.91	2.30 a $\pm$ 0.17	12.67 a $\pm$ 2.24
12 weeks	CTR	1.25 c $\pm$ 0.35	5.19 c $\pm$ 0.72	3.68 c $\pm$ 0.34	1.55 c $\pm$ 0.29	5.71 c $\pm$ 1.11
	NPK	2.63 b $\pm$ 0.52	9.47 b $\pm$ 1.54	5.77 b $\pm$ 0.99	2.12 b $\pm$ 0.31	12.83 b $\pm$ 2.71
	SS	6.35 a $\pm$ 0.95	6.35 a $\pm$ 0.95	6.35 a $\pm$ 0.95	6.35 a $\pm$ 0.95	6.35 a $\pm$ 0.95

*Significant differences for each treatment, Tukey's test, $\alpha = 0.05$

Table 2. Photosynthetic apparatus efficiency (mean $\pm$ SD) evaluated after 4 and 12 weeks of *Scabiosa ochroleuca* cultivation on different substrata (CTR, soil-free grounds; NPK, soil-free grounds enriched with NPK fertilization; SS, soil-free grounds enriched with sewage sludge)

Term	Treatment	Chlorophyll a [$\text{mg}\cdot\text{g}^{-1}\text{f. w.}$]	Chlorophyll b [$\text{mg}\cdot\text{g}^{-1}\text{f. w.}$]	Carotenoids [$\text{mg}\cdot\text{g}^{-1}\text{f. w.}$]	Fv/Fm	PI
4 weeks	CTR	0.456 c* $\pm$ 0.097	0.108 c $\pm$ 0.028	0.161 c $\pm$ 0.024	0.785 c $\pm$ 0.014	1.462 c $\pm$ 0.325
	NPK	0.856 b $\pm$ 0.024	0.229 b $\pm$ 0.012	0.243 b $\pm$ 0.007	0.804 b $\pm$ 0.005	3.399 b $\pm$ 0.467
	SS	1.403 a $\pm$ 0.041	0.378 a $\pm$ 0.007	0.411 a $\pm$ 0.100	0.821 a $\pm$ 0.008	5.409 a $\pm$ 0.632
12 weeks	CTR	0.311 b $\pm$ 0.008	0.083 b $\pm$ 0.004	0.094 b $\pm$ 0.002	0.6618 b $\pm$ 0.027	1.303 b $\pm$ 0.128
	NPK	-	-	-	-	-
	SS	0.977 a $\pm$ 0.008	0.255 a $\pm$ 0.003	0.256 a $\pm$ 0.009	0.840 a $\pm$ 0.004	4.883 a $\pm$ 0.625

*Significant differences for each treatment, Tukey's test, $\alpha = 0.05$

and plant nutrients [Singh and Agrawal 2008; Torri and Lavado 2008]. Thus, their application on degraded areas may facilitate the establishment of vegetation cover and eliminate the need for commercial NPK fertilisers, what could be crucial for survival of plant material tested in presented study. Growth and development disturbances observed in *S. ochroleuca* specimens cultivated on wastes treated with mineral fertilisation were probably caused by certain level of substratum salinity as several studies have demonstrated that soil salinity is positively related to heavy metal uptake by plants [Bartl et al. 2002; Wu et al. 2002; Zhao et al. 2003]. This issue should be verified in the near future.

It is well known that any kind of stress factors that exceed optimum threshold may affect the process of photosynthesis. Changes in both the content of photosynthetic pigments and the values of chlorophyll *a* fluorescence can indicate serious nutritional problems and unfavourable situation for the plant development [Iqbal et al. 2010; Augustynowicz et al. 2011]. Therefore, their measurements are considered as marker of plants status and thus help to evaluate the plant response to stress factor [Kalaji and Łoboda 2010]. The reduction in photosynthetic pigment content and decline in the photosynthetic function caused by elevated metal concentrations and unfavourable environmental condition

were demonstrated in several studies [Augustynowicz et al. 2011; Fernández et al. 2013; Muszyńska et al. 2014]. Similarly to our studies, decrease in chlorophylls concentration as well as disturbance in the function of photochemical apparatus were also observed in species selected for biological restoration of heavy metal contaminated terrains in the Iberian Peninsula – *Halimione portulacoides* [Andrades-Moreno et al. 2013] and *Spartina densiflora* [Mateos-Naranjo et al. 2011]. Authors suggested that changes in photosynthetic apparatus efficiency may be attributed to the adverse effect of excess metals on photosynthetic electron transport, as a consequence of either a decrease in chlorophyll synthesis or an increase in its degradation.

Our results demonstrated that native plants species such as *S. ochroleuca* are good candidates to direct planting on wastes obtained after Zn–Pb ores mining and processing. However, in order to enable the establishment of vegetation cover at field scale projects, the organic fertiliser could be applied to the substratum.

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