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THE POTENTIAL OF FOUR WOODY SPECIES FOR THE REVEGETATION OF FLY ASH DEPOSITS FROM THE 'NIKOLA TESLA - A' THERMOELECTRIC PLANT (OBRENOVAC, SERBIA)

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Abstract- Four woody species, *Tamarix tentandra* Pallas*, Populus alba* L. and *Robinia pseudoacacia* L. (planted) and *Amorpha fruticosa* L. (naturally colonized) were studied at two fly ash deposit lagoons, weathered 3 (L1) and 11 years (L2). All species were assessed in terms of their invasive ability, photosynthetic efficiency, photosynthetic pigments and damage symptoms, while the characteristics of the habitat were assessed in terms of trace element content and the pH and EC of the ash. A reduced vitality of all populations growing on the ash was observed, except for the naturally colonized *A. fruticosa*. High vitality on all sites, except at L2, increased chlorophyll content and absence of damage symptoms indicates a tolerance in relation to the uptake of toxic elements from the ash. Therefore, the characteristics of naturally colonized species can be used for modeling future actions of biological restoration of fly ash deposits.

Key words: Amorpha fruticosa L., *Populus alba* L., *Robinia pseudoacacia* L., *Tamarix tentandra* Pallas, fly ash deposits, multiple stress, revegetation, trace elements, Serbia

INTRODUCTION

Every year thermoelectric plants all over the world produce enormous quantities of coal combustion residues, 70-75% of which is fly ash (Haynes, 2009). There are attempts in developed countries to make use of certain amounts of the produced ash in the production of concrete and other building materials, land reclamation or fertilization in agriculture (Asokan et al., 2005; Jala and Gojal, 2006). Nevertheless, around 70% still ends up in landfills or settling ponds (Hassett et al., 2000). The largest amount of fly ash (around 3.6 Mt a year) in Serbia is produced by the 'Nikola Tesla - A' thermoelectric plant in Obrenovac, which is deposited in an open waste disposal, taking up an area of around 400 hectares.

Depositing ash in open spaces can be very damaging to the environment due to the potentially deleterious effects of ash particles. Dispersal of ash particles by wind and water erosion and the leaching of substances such as salts and heavy metals can be hazardous for the surrounding terrain and underground waters. Due to all of this, there are strict legal acts that regulate the management of ash deposits, making the procedure for disposing the ash a very costly and demanding practice. Covering fly ash deposits with a layer of soil and creating a permanent vegetative cover upon the termination of a thermoelectric plant's operation entails great costs, but it does not exclude the ecological problems arising from ash depositing during the course of its operation (Duggan and Scanlon, 1974; Hearing and Daniels, 1991). From an ecological perspective, revegetation of ash deposits is the most desirable method in eliminating the potentially hazardous effects of ash to the environment (Haynes, 2009). Planting herbaceous and woody plants prevents erosion and provides the stabilization of toxic trace elements from fly ash through the uptake by plants and the binding of the fly ash by their roots, thus creating conditions for soil formation (Gupta and Sinha, 2006; Djurdjević et al., 2006). With this in mind, at the 'Nikola Tesla - A' ash deposits research has focused during recent years on a step-by-step revegetation during the course of the thermoelectric plant's operation. Revegetation of fly ash lagoons is therefore important to control erosion and leachate generation as well as for aesthetic purposes. Establishing vegetation on fly ash lagoons is difficult due to physical and/or chemical limitations to plant growth (Mulhern et al., 1989; Carlson and Adriano, 1993; Pavlović et al., 2004). Physicochemical limitations for the successful revegetation of fly ash are the phytotoxicity of B, As, Mo, Se and other potentially toxic trace elements; toxicity caused by a high pH and/or high concentrations of soluble salts; nutrient deficiency due to the absence of nitrogen (N) and less available phosphorus (P); restriction of root growth due to the fine particle size of the ash; reduction in the number of free-living and symbiotic N_2-fixing micro-organisms (Hutnik and Davis, 1973; Hodgson and Buckley, 1975; Page et al., 1979; Bradshaw and Chadwick, 1980; Haering and Daniels, 1991; Adriano et al., 1980; Carlson and Adriano, 1991, 1993; Haynes, 2009).

A vegetative cover is a remedial technique utilized on coal fly ash deposits for soil stabilization and for the physical and chemical immobilization of contaminants (Bilski et al., 2011). During the process of vegetative cover formation, the selection of plant species that can grow on this specific substratum in multiple stress conditions is of vital importance (Carlson and Adriano, 1993; Cheung et al., 2000; Rai et al., 2004; Tripathi et al., 2004; Pavlović et al., 2004; Mitrović et al., 2008). The ability of plants to tolerate toxic substrates depends on their biochemical and physiological adaptation to the stressful conditions (Masarovicová et al., 1998). Metal tolerance is one of the most important criteria to select an appropriate plant for heavy metal contaminated sites. The accumulation of heavy metals induces biochemical and physiological responses by modifying several metabolic processes in vascular plants (MacFarlane et al., 2003). Long-term exposure to stress factors causes physiological change in plants at a much earlier stage than the appearance of the first morphological symptoms. The toxic effect of heavy metals on plants is manifested, among others, by photosynthesis inhibition (Králová et al., 1998; Šeršen et al., 1998; Masarovicová et al., 1998; Lux et al., 2000; Pavlović et al., 2004, 2007; Mitrović et al., 2008) and chlorophyll content decrease (Lanaras et al., 1993; Mascher et al., 2002; Borghi et al., 2007; Moreno-Jimenez et al., 2008), which results in a decrease in growth and productivity (Weng et al., 2005; Xu et al., 2006; Borghi et al., 2008). Most of the studies dealing with the effect of heavy metals on photosynthesis are focused on reactions related to photosystem II activity (PSII-activity), describing this complex as the most sensitive to metal toxicity in the chloroplasts (Arellano et al., 1994; Barón et al., 1995; Yruela et al., 2000).

This study presents a comparative analysis of the invasive ability of the species, photosynthetic efficiency, photosynthetic pigments, and plant damage symptoms of the planted (*Tamarix tentandra* Pallas, *Populus alba* L. and *Robinia pseudoacacia* L.) and naturally colonized (*Amorpha fruticosa* L.) species growing on the ash deposit of the 'Nikola Tesla - A' thermoelectric plant in Obrenovac (Serbia). The aim of this study was to compare the vitality level and invasive ability of the species planted and naturally colonized at the ash deposit, lagoons weathered 3 and 11 years.

MATERIALS AND METHODS

The study was carried out in May 2008, at three sites. The first site was an ash deposit lagoon which had been inactive for three years (L1); the second site was an ash deposit lagoon which had been inactive for eleven years (L2); the third site (CS – the control site) was a control site located on the banks of the River Kolubara, 3 km from the ash deposit site in Obrenovac, and the Botanical Garden 'Jevremovac' in Bel-

Table 1. Chemical properties of unweathered and weathered fly ash from ash deposit of 'Nikola Tesla – A' thermoelectric power plant

Ash from electrostatic precipitators * (%)			Unweathered ash ** (µg/g)	Weathered ash** (µg/g)
SiO_2	64.44	As	79	125
Al_2O_3	18.20	B	710	1090
Fe_2O_3	5.59	Ba	465	450
CaO	5.18	Cr	360	375
MgO	2.98	Cu	240	248
Na_2O	0.30	Cl	<10	<10
K_2O	0.72	F	<1	<1
TiO_2	0.72	Ga	50	23
P_2O_5	0.09	Hg	<0.01	<0.01
SO_3	1.70	Li	80	125
		Mn	262	208
		Mo	<5	<5
		Nb	20	15
		Ni	140	159
		Pb	34	49
		Rb	125	165
		Sc	<1	<1
		Sr	190	180
		V	120	120
		Zn	112	37
		Zr	110	110
		Y	29	29

Source: * Vinča Institute for Nuclear Sciences; ** Holding Institute of General and Physical Chemistry

Table 2. Electrical conductivity (EC) and pH values of control site (CS) and ash deposit lagoons (L0-unwethered ash, L1-wethered 3 years and L2- weathered 11 years)

	EC (dS m^{-1})	pH
CS-soil	0.184 (0.007)	7.54 (0.200)
L0-unwethered ash	0.720 (0.023)	8.03 (0.008)
L1-Fly ash-3	0.203 (0.027)	7.78 (0.130)
L2-Fly ash-11	0.153 (0.007)	7.72 (0.080)

n=3, values are mean (S.D.)

grade, where *T. tentandra* grows. The soil at the CS is classified as fluvisol (Duchaufour, 1976).

Fly+bottom ash deposit is performed by sluicing the ash into three wet lagoons, two of which are inactive at any one time. Ash deposit super structuring, carried out by erecting side dikes, is performed by hydro-cycling the ash, which is piped in the form of a hydraulically transported pulp (ash:water = 1:10). By means of hydro-cycling the coarser fractions (bottom ash) are separated out to form the side dike. The lagoons are occasionally irrigated with water from the River Sava and the drainage waters from the disposal site.

The ash produced by lignite combustion is alumo-silicate (approximately 80%) with a significant proportion of Fe, Ca, and Mg oxides (approximately 14 %) (Table 1). The ash has an alkaline reaction and the proportion of fly ash is 80-85%, bottom ash 15-20 % and unburnt coal 0.2-2.0 %.

The soil and ash samples for trace element analysis were collected from immediately beneath the individuals of the species examined, in the root zone at a depth of 0-20 cm, and a composite sample was formed for analysis. Concentrations of boron, arsenic, molybdenum, selenium, copper, manganese and zinc were measured in the soil from the control site (CS and, in the fly ash from the ash deposit lagoons (L1 and L2).

For trace element analysis, the soil and ash samples (0.5 g) were digested in a microwave (CEM MDS-2000) using 10 ml of concentrated HNO_3. Concentrations of As, Mo, Se, Cu, Mn, Zn and Fe were determined through atomic absorption spectrophotometry (Pye Unicam SP9), using a sodium atomic absorption standard solution (Sigma Co.). The analytical procedure was validated using standard reference materials: ash (coal ash CRM 252-BS1) and soil (grey soil CRM 054-2504-83), obtained by Spex CerpiPrep.Ltd. (Middlesex, UK). Boron concentrations were determined using the spectrophotometric method with the aid of curcumin [Wear, 1965]. Concentrations were expressed in μg/g of the dry weight.

The soluble salt content in the fly ash and soil was measured by assessing the electrical conductivity (EC) of an extract of ash (soil): water (distilled) =1:5, at a depth of 0-20 cm, with 3 replicates. EC was expressed in dS m^{-1}. The actual pH was measured potentiometrically with a glass membrane electrode by suspending 10 g of ash (soil) in 25 ml of H_20.

Photosynthetic efficiency was measured in May 2008, using the method of induced chlorophyll fluorescence kinetics of photosystem II (Fo – non-variable fluorescence; Fm – maximum fluorescence; Fv=Fm−Fo – variable fluorescence; $t_{1/2}$ – half the time required to reach maximum fluorescence from Fo to Fm; and photosynthetic efficiency, Fv/Fm). Measurements were taken with the aid of a portable Plant Stress Meter (BioMonitor S.C.I. AB, Sweden), as described by Krause and Weis (1991). Chlorophyll was excited for 2 s by actinic light with a photon flux density of 200 and 400 μmol m^{-2} s^{-1}. Prior to measuring, samples were adapted to the dark for approximately 30 min in order to maximize the oxidation of the primary quinone electron acceptor pool of PSII and to enable the full relaxation of any rapidly recovering fluorescence quenching.

Chlorophyll concentration in the leaf tissue was determined spectrophotometrically, based on the light absorption of a solution obtained after extraction with dimethyl sulfoxide (DMSO, Hiscox and Israelstam, 1979). One disc (1 cm in diameter) per leaf was harvested from five leaves and used to extract chlorophyll with 1 ml of DMSO (n=5). After incubation at 65^0C until fully extraction of chlorophyll was reached, the absorbance of each sample in 1.00 cm cuvettes was measured at 663 and 645 nm using a Shimadzu UV 160 spectrophotometer. The equations of Arnon (1949) were used for calculating Chl *a*, Chl *b* and Chl *a+b*. The ratio Chl *a/b* was also determined. Chlorophyll content was expressed in mg per gram related to dry leaf mass (mg/g d.w.).

A scale for abundance and cover was used in phytocoenological relevés (61 for L1 and 121 for L2) (Braun-Blanquet, 1965) with the following numerical marks: + (rare species), 1, 2, 3, 4 and 5 (the highest mark showing the greatest domination of species in regard to both traits). A frequency (degree of presence expressed in %) of the examined species was calculated based on all the sampling plots and number of plots with presence of the examined species.

One-way analyses of variance (ANOVA) were performed to test the differences in the Fv/Fm, Chl *a*, Chl *b* and Chl *a+b*, and Chl *a/b* in the examined plants, and the trace element content of the examined sites as well. A discriminant analysis (DA) of photosynthetic efficiency was used in order to establish the variability and significance of the physiological differentiation of the populations from the three examined sites.

RESULTS AND DISCUSSION

Trace elements in fly ash

At the ash deposit lagoons of the 'Nikola Tesla – A' thermoelectric plant, the concentrations of B (710 µg/g) in unweathered ash were significantly reduced to 450 µg/g after 3 years, and to 390 µg/g after 11 years, $p<0.001$ (Tables 1 and 3). The differences in B concentrations between the different-aged ashes were the result of a reduction in B which arises from the ash-weathering processes. Approximately 17-64% of B is immediately soluble in water (James et al., 1982), but a further 2 to 3 years is required for the content of B to decrease to a concentration which plants can tolerate (Adriano, 1986). Concentrations of B at the ash deposit lagoons were significantly higher than in the soil from the control site ($p>0.001$), and were approximately 10 times higher than the normal range for soils (Kabata-Pendias and Pendias, 2001) (Table 3).

The arsenic concentration in the ashes (unweathered ash, L1 and L2) were 23 and 34 times higher, respectively, than the normal range of As concentrations in the control soil (Table 3), and at the same time its concentrations in the ashes were far above the normal range for soils (Kabata-Pendias and Pendias, 2001). Namely, anionic complexes are the most mobile As forms, absorbed into soils with a pH range from 7 to 9 (Kabata-Pendias and Pendias, 2001). The extractability of As increases at high pH because the anionic forms of As have no free metal ions that could cause its precipitation (Theis and Wirth, 1977). It is possible that the pH range of the ash at the 'Nikola Tesla – A' (7.7-7.8, Table 2) is high enough to enable the bioavailability of As which may enable its accumulation in excessive or toxic concentrations.

The Mo content at the control site (CS) was within the normal range of Mo concentrations in soils, while at the ash deposit lagoons (L1 and L2) it was in excess (Table 3). The highest concentration of Mo was measured in the ash weathered 11 years which contains organic matter of plant origin (12.31 µg/g, $p<0.001$). This is consistent with Kabata Pendias and Pendias (2001) who suggested that Mo is readily mobilized in alkaline soils and possesses a great affinity to be fixed by organic matter. The high Mo content in the ash and the slight alkalinity of the ash at the 'Nikola Tesla – A' may cause greater availability for the accumulation of this element by the examined plants. The Se content at the control site (0.53 µg/g) was above the normal range for soils, while at both ash deposit lagoons the Se content was <0.1 µg/g and lower than the average Se concentrations in soils (Kabata-Pendias and Pendias, 2001) (Table 3).

The Cu content in the soil from the control site was significantly lower in relation to the ash lagoons, $p<0.001$ (Table 3). The Cu content at all three sites was higher than the normal range for soils (13-24 µg/g, Kabata-Pendias and Pendias, 2001) (Table 3). The binding of Cu by soil is related to the formation of organic complexes and is highly dependent on soil pH, and the overall solubility of both cationic and anionic forms decreases at about pH 7 to 8 (Kabata-Pendias and Pendias, 2001). This is the case with the ash deposit site at the 'Nikola Tesla – A' where the pH of the ash falls within this range, which can cause deficiency of this essential element (Table 2). The Mn content in the soil from the control site was higher than the normal range for soils and three times higher in comparison to that at the 11-year-old lagoon (L2). Reduced Mn concentrations in the ash can lead to its deficiency in plants. The problem of a Mn deficit for plants growing on ash sites has been highlighted by other authors (Townsend and Gillham, 1975; Carlson and Adriano, 1991; Pavlović et al., 2004; Mitrović et al., 2008). The Zn content in the soil from the control site and the ash deposit lagoons was within the normal range for soils (27-150 µg/g, Kabata-Pendias and Pendias, 2001) (Table 3). The reduced content of Zn was measured in the L2 lagoon. As with Mn, the amount of Zn at the weathered lagoons can be expected to decrease over time. For example, Carlson et al. (2002) found a significant decrease of Mn and Zn concentrations in soils amended with fly ash. Mitrović et al. (2008) also established Zn deficiency

Table 3. Trace element content in soil (CS) and fly ash (L1 and L2), expressed on dry weight basis (μg/g)

	B	As	Mo	Se	Cu	Mn	Zn
Soil (CS)	22.00 (0.757)	3.60 (0.360)	1.43 (0.045)	0.53 (0.013)	63.44 (0.437)	670.76 (8.103)	43.24 (0.440)
Fly ash (L1)	450.08*** (1.746)	84.46*** (1.560)	9.53*** (0.665)	<0.1	126.30*** (1.555)	273.20*** (3.928)	45.06*** (1.204)
Fly ash (L2)	390.26*** (2.602)	121.80*** (0.485)	12.31*** (0.292)	<0.1	173.23*** (2.236)	222.18*** (3.860)	40.34** (1.825)

$n = 3$, values are mean (S.D.), ANOVA, level of significance: ***$p<0.001$.

in two grasses growing on ash lagoons of the 'Nikola Tesla – A' thermoelectric plant. Therefore, a deficit of Zn is to be expected in plants growing in ash lagoons. However, damage symptoms resulting from a Zn deficit were not observed in either species, which leads to the conclusion that the physiological requirement for this element is very low.

Phytocoenological characteristics

Phytocoenological analysis of the planted (*T. tentandra* Pallas, *P. alba* L. and *Robinia pseudoacacia* L.) and naturally colonized (*Amorpha fruticosa* L.) species proves their significant frequency in the overall flora of the 'Nikola Tesla – A' ash deposits at Obrenovac. At the 3-year-old lagoon (L1), the highest frequency on analyzed trial sites was recorded in *T. tentandra* [20/61(32.8%), II$^{+-3.3}$] (Fig. 1). Its frequency ranges from rare (negligible cover) to relatively good frequency (25-50% cover). The above-mentioned species is far more present at the 11-year-old lagoon [81/121(66.9%) IV$^{+-4.4}$], and its frequency ranges from rare to significant cover (50-75%). Numerical values for number, cover and frequency on the analyzed phytocoenological relevés indicate a significant invasive ability of *T. tentandra* at the 11-year old lagoon. A slightly lower frequency at L1 is noted for *R. pseudoacacia* [17/61 (27.9%) II$^{+-4.4}$] (Fig. 1). This species is sporadically present in a number of relevés, while at one particular area it has a very significant number and cover (as high as 50-75%). The analysis of phytocoenological records at the L2 lagoon shows a somewhat invasive ability of the locust at the 11-year-old lagoon in relation to the 3-year-old lagoon where it covers from 1-10% to 50-75% of the area of relevés [30/121(24.8%) III$^{1.1-4.3}$]. The *P. alba* species was noted in only one relevé at L1, covering an area of 25-50% [1/61(1.6%) I$^{3.3}$], while at the L2 lagoon it is more frequent [39/121(32.2%) II$^{+-5.4}$] (Fig. 1). Its number ranges from rare to highly frequent (75-100%). A naturally colonized shrubby species, *A. fruticosa*, was observed in 12 phytocoenological relevés at L1 [12/61(19.7%) I$^{+-3.3}$]. Its number at L1 and L2 ranges from rare to a relatively good frequency (25-50%). This species shows greater invasive ability at the 11-year-old lagoon, which is additionally proved by its significantly higher frequency [39/121(32.2%) II$^{+-3.3}$] (Fig. 1).

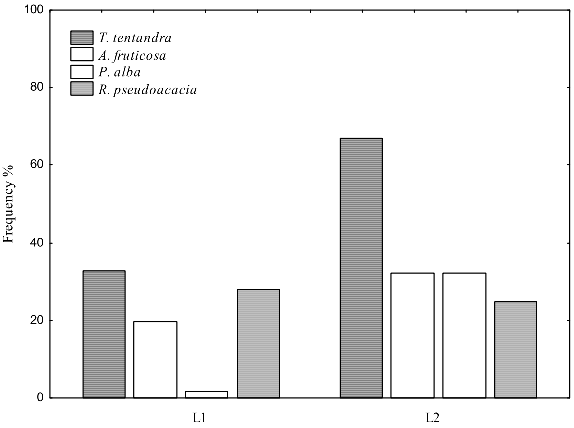

Fig. 1. Frequency of *T. tentandra, A. fruticosa, P. alba* and *R. pseudoacacia* from 3-year-old lagoon (L1) and 11-year-old lagoon (L2)

Photosynthetic efficiency of plants

The examined species differed from the control site to the ash lagoons of different age of weathering.

Table 4. Differences in photosynthetic efficiency (Fv/Fm) between species from different sites

	M (SD)	*T. tentandra*	*A. fruticosa*	*P. alba*	*R. pseudoacacia*
		Control site (CS)			
T. tentandra	0.720 (0.037)	-	ns	*	ns
A. fruticosa	0.758 (0.088)	ns	-	ns	*
P. alba	0.748 (0.040)	*	ns	-	**
R. pseudoacacia	0.689 (0.083)	ns	*	**	-
		L1-3 years old lagoon			
T. tentandra	0.619 (0.072)	-	**	ns	*
A. fruticosa	0.684 (0.072)	**	-	*	ns
P. alba	0.626 (0.078)	ns	*	-	ns
R. pseudoacacia	0.675 (0.084)	*	ns	ns	-
		L2-11 years old lagoon			
T. tentandra	0.727 (0.032)	-	**	***	***
A. fruticosa	0.650 (0.094)	**	-	***	**
P. alba	0.541 (0.085)	***	***	-	ns
R. pseudoacacia	0.563 (0.073)	***	**	ns	-

ANOVA, *n* =20, values are mean(S.D.), *p<0.05, ** p<0.01, ***p<0.001, ns= not significant

Significantly lower values of Fv/Fm were noted for *T. tentandra* at L1 lagoon in relation to the control site and L2 lagoon (p<0.001), while at the L2 lagoon the values were similar to values at the CS (ns) (Fig. 2). At the control site (CS), the mean value (Fv/Fm) was 0.720, which is lower than the general optimum range of plant photosynthetic efficiency (0.750-0.850, Bjorkman and Demmig, 1987). In *A. fruticosa* on the control site the mean value of Fv/Fm measured was 0.758, which falls within the optimum range of plant photosynthetic efficiency. At both ash deposit lagoons, however, lower values of Fm/Fv were noted in relation to the same ones at the CS (L1, p<0.01; L2, p<0.001), however, there were no differences in individual functioning on ash (ns) (Fig. 2). The *P. alba* species also exhibits considerable vitality on the control site (0.748), and falls within the lower optimum limit. At the L2 lagoon, the lowest value of Fv/Fm was noted, a mere 0.541 (in relation to the control site (p<0.001), and in relation to the L2 (p<0.01)) (Fig. 2). In *R. pseudoacacia* individuals from the L2 lagoon, a lower photosynthetic efficiency was also noted in relation to the CS (p<0.001) and in relation to the L1 lagoon (p<0.001). No differences in functioning were noted between L1 and CS (ns) (Fig. 2).

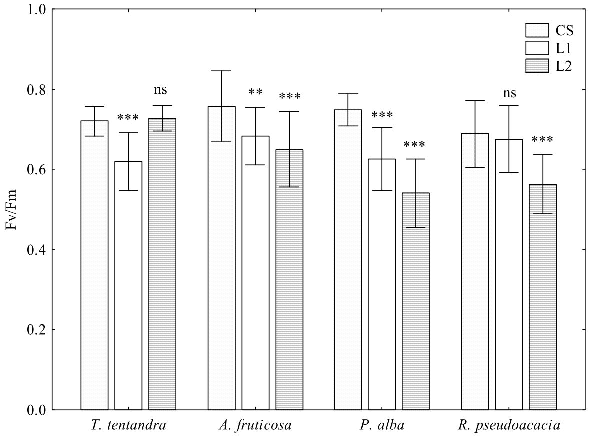

Fig. 2. Photosynthetic efficiency (Fv/Fm) of examined species from different sites

The greatest vitality at the control site was noted for naturally colonized individuals of *A. fruticosa*, and the lowest for *R. pseudoacacia* (p<0.05). *P. alba* showed a vitality similar to *A. fruticosa* (ns) and a higher one than *R. pseudoacacia* (p<0.01), while *T. tentandra* only showed a lower efficiency in relation to *P. alba* (p<0.05) (Table 4). The greatest vitality at L1 – 3-year-old lagoon, was again noted in *A. fruti-*

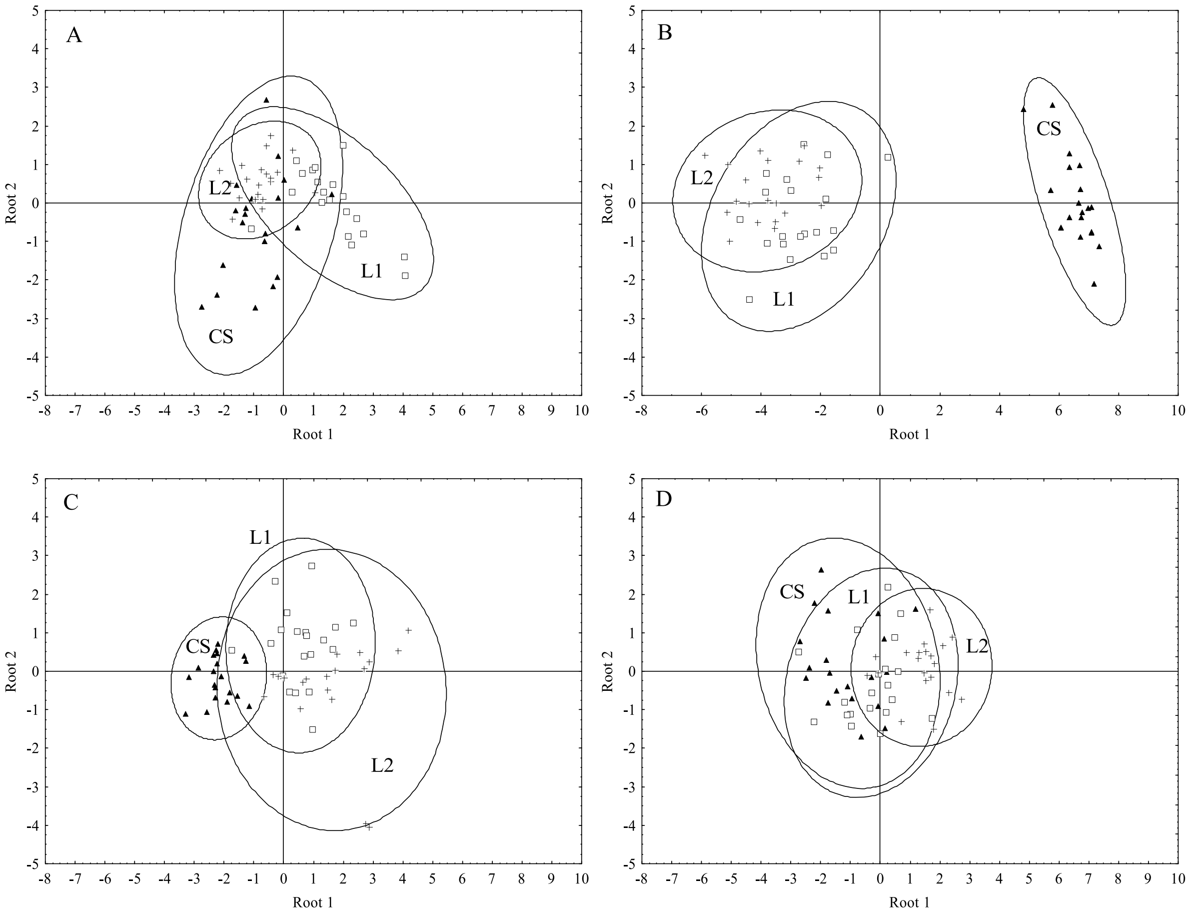

Fig. 3. Discriminant analysis (DA) for the three sampling sites based on the variations of Fv/Fm, Fo, Fm, Fv and t1/2 of *T. tentandra* (A), *A. fruticosa* (B), *P. alba* (C) and *R. pseudoacacia* (D)

cosa. R. pseudoacacia (ns) had similar vitality while *P. alba* ($p<0.05$) and *T. tentandra* ($p<0.01$) showed lower efficiency. *T. tentandra* demonstrated a lower efficiency than *A. fruticosa* ($p<0.01$) and *R. pseudoacacia* ($p<0.05$) (Table 4). At the L2 lagoon, *T. tentandra* and *A. fruticosa* had the highest efficiency ($p<0.01$), while *P. alba* and *R. pseudoacacia* a lower one (<0.001). *A. fruticosa* demonstrated a higher efficiency than *R. pseudoacacia* ($p<0.01$) and *P. alba* ($p<0.001$), while there were no significant differences in vitality between them (ns) (Table 4). In early phases of weathering, 3 years after planting, when the ash was burdened by higher salinity and a high level of toxic concentrations of B, As, Mo and Cu, the most vital were the spontaneously populated *A. fruticosa* and the planted *R. pseudoacacia,* which indicate their tolerance to the chemical toxicity of the ash. Contrary to this, lower vitality was found in planted *T. tentandra* and *P. alba*. Eleven years after planting, as weathering continued due to reduced salinity and toxicity, the ash became a substrate suitable for the colonization of species from surrounding areas, like *A. fruticosa*.

Discriminant Analysis (DA) was performed in order to detect differences between the analyzed populations of *T. tetranda, P. alba, R. pseudoacacia* and *A. fruticosa* with respect to the vitality based on the variations of Fv/Fm, Fo, Fm, Fv and $t_{1/2}$ (Fig. 3). The first principal axis clearly discriminates between populations along the pollution gradient. Therefore, we may conclude that the trace element content in

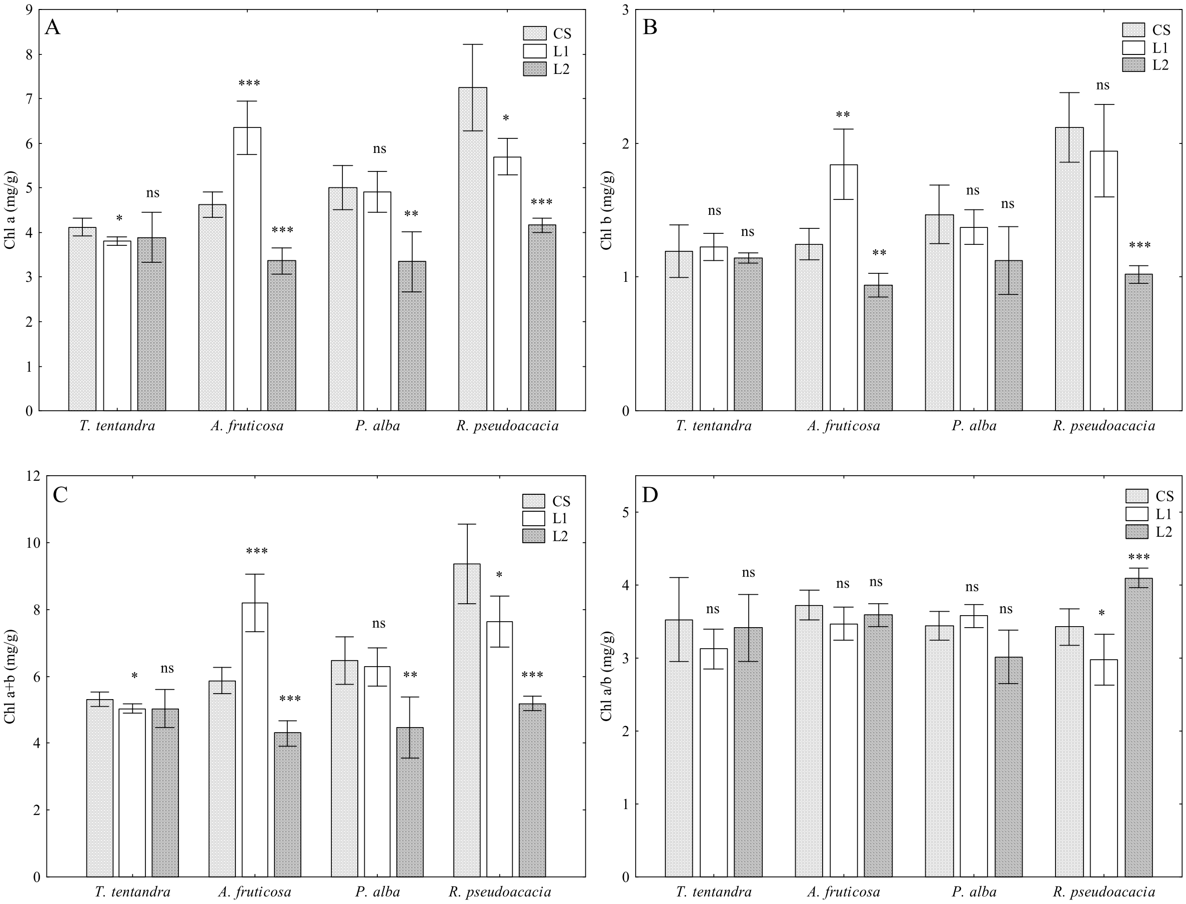

Fig. 4. Concentrations of Chl a (A), Chl b (B), Chl a+b (C) and Chl a/b (D) in examined species from different sites

the substrate is the most important for differences in vitality between populations growing in the soil from one side (unpolluted) and samples of plant material from the ash lagoons (polluted). The clear distinction of the *A. fruticosa* populations from the control site (CS) and from the ash lagoons indicates that it has merely begun to invade a new substrate burdened by pollutants.

A reduced vitality in all the populations growing on the ash was noticed in relation to the general optimum range (0.750-0.850), and in relation to the average value for deciduous trees (0.843±0.012) (Bjorkman and Demmig, 1987), except for the spontaneously colonized *A. fruticosa.* A decrease in this ratio indicates photoinhibition damage in the plants used for this study. The main reason for photoinhibition may lie in the excessive concentrations of B, As and Mo in the ash and possible deficiency of Mn. The findings of Matoh (1997) showed that toxicity symptoms at the physiological level develop in conditions of a high supply of B when the element accumulates in cell walls and the cytoplasm and can cause metabolic disturbances. At the 11-year-old lagoon, the lowest photosynthetic efficiency was measured in all the examined species except *T. tentandra,* which showed a recovery of photosynthesis. The naturally recolonized *A. fruticosa* showed highest vitality on all sites, except at L2, and had no damage symptoms, which indicates its tolerance in relation to the uptake of toxic elements from the ash.

Chlorophyll content in plants

Analysis of chlorophylls (Chl *a*, Chl *b*, Chl *a+b* and Chl *a/b*) in *T. tentandra* revealed a decrease in the content of Chl *a* at the 3-year-old lagoon, L1 ($p<0.05$), which caused a decrease in the Chl *a/b* ratio ($p<0.05$). At the 11-year-old lagoon L2, however, there occurred a complete recovery of chlorophylls (ns) (Fig 4). Contrary to this, in *A. fruticosa* at L1 there occurred a considerable increase in the content of Chl *a* ($p<0.001$), Chl *b* ($p<0.01$) and Chl *a+b* ($p<0.001$), but this increase had no effect on the Chl *a/b* ratio (ns). At L2 there was a decrease in the chlorophyll content (Chl *a*, $p<0.001$; Chl *b*, $p<0.01$), and Chl *a+b* ($p<0.001$), where the Chl *a/b* ratio remained unchanged (Fig. 4). In *P. alba* leaves at L1 there were no differences in the chlorophyll content (ns), while at the older L2 lagoon there occurred a decrease in the content of Chl *a* ($p<0.01$) and Chl *a+b* ($p<0.01$) (Fig. 4). Temporal variations of the leaf chlorophyll content (Chl *b* and Chl *a/b*) in *P. alba* were not found (ns) (Fig. 4). A decrease in the content of Chl *a* and Chl *a+b* in *R. pseudoacacia* was measured in L1 ($p<0.05$) and L2 ($p<0.001$). The content of Chl *b*, however, drastically decreased in L2 ($p<0.001$), which was reflected on the Chl *a/b* ratio ($p<0.001$) (Fig. 4).

The highest content of chlorophyll (except for Chl *a/b*) was measured on the control site in *R. pseudoacacia*, followed by *P. alba* ($p<0.01$), *A. fruticosa* ($p<0.001$) and *T. tentandra* ($p<0.001$). The lowest content of chlorophyll Chl *a* was measured in *T. tentandra* in relation to all the other species, and of Chl *b* only in relation to *R. pseudoacacia* (Table 5). At L1 a significant increase of Chl *a* was measured in *A. fruticosa* in relation to *T. tentandra* ($p<0.001$) and *P. alba* ($p<0.01$) (Table 5). This affected the quantity of the overall chlorophyll Chl *a+b* and the Chla *a/b* ratio. In this species an increased content of Chl *b* was measured in relation to *T. tentandra* and *P. alba* ($p<0.01$). The highest content of overall chlorophyll was measured at L1 in *A. fruticosa* and *R. pseudoacacia* (ns), then in *P. alba* ($p<0.01$), and the lowest was in *T. tentandra* ($p<0.001$) (Table 5). At the L2 lagoon, in all the examined species except for *T. tentandra*, there occurred a decrease in the content of all the chlorophyll parameters in relation to the CS, except for Chl *b* in *P. alba*. The highest chlorophyll ratio Chl *a/b* at this site was measured in *R. pseudoacacia*, then in *T. tentandra* ($p<0.05$), *P. alba* and *A. fruticosa* ($p<0.001$) (Tab 5).

Chlorophylls are very sensitive to the oxidative stress induced by environmental changes (Fargašova, 2001; Sharma and Shanker Dubay, 2005; Gajić et al., 2009). Chlorophyll measurement is an important tool to evaluate the effects of pollutants on plants as it plays an important role in plant metabolism, and any reduction in chlorophyll content corresponds directly to plant growth (Joshi and Swami, 2009). Therefore, photosynthetic pigments can be used as sensitive markers of environmental stress (Dietz et al., 1999; Gajić et al., 2009). One of the most common impacts of pollution is the gradual disappearance of chlorophyll and concomitant chlorosis of leaves, which may be associated with a consequent decrease in the capacity for photosynthesis (Joshi and Swami, 2007). The response of plants to stress is, however, an individual characteristic of each plant species, and they can, therefore, react differently and develop different mechanisms of defense as a response to the stress. Chlorophyll analysis in the examined species that grow at the ash lagoons of the 'Nikola Tesla – A' thermoelectric plant proved the temporal regularity of the reduction of chlorophyll concentrations and the relationship between all chlorophyll parameters (Chl *a*, Chl *b*, Chl *a+b* and Chl *a/b*) with the unfavorable aspects measured at the 11-year-old lagoon.

The only exception to this is the spontaneously colonized *A. fruticosa* species which responded to the stress of pollutants during the initial phases of colonization with a rapid increase of Chl *a* concentrations and a minor increase of Chl *b*, resulting in an increase of the overall chlorophyll Chla *a+b*. It is in these initial stages of vegetation cover formation that the least favorable conditions for plants exist in view of the high salinity and excessive content of some chemical elements. In this study, despite the

Table 5. Differences in leaf Chl *a*, Chl *b*, Chl *a+b* and Chl *a/b* of examined species from different sites

	Control site (CS)					L1 – 3 years old lagoon					L2 – 11 years old lagoon				
Species	M (SD)	T. tentandra	A. fruticosa	P. alba	R. pseudoacacia	M (SD)	T. tentandra	A. fruticosa	P. alba	R. pseudoacacia	M (SD)	T. tentandra	A. fruticosa	P. alba	R. pseudoacacia
								Chl *a*							
T. tentandra	4.120 (0.208)	-	*	**	***	3.806 (0.092)	-	***	***	***	3.890 (0.559)	-	ns	ns	ns
A. fruticosa	4.621 (0.290)	*	-	ns	***	6.349 (0.596)	***	-	**	ns	3.363 (0.299)	ns	-	ns	***
P. alba	5.011 (0.494)	**	ns	-	**	4.908 (0.453)	***	**	-	*	3.342 (0.673)	ns	ns	-	*
R. pseudoacacia	7.250 (0.919)	***	***	**	-	5.692 (0.409)	***	ns	*	-	4.164 (0.160)	ns	***	*	-
								Chl *b*							
T. tentandra	1.192 (0.197)	-	ns	ns	***	1.224 (0.102)	-	**	ns	**	1.139 (0.038)	-	**	ns	**
A. fruticosa	1.245 (0.118)	ns	-	ns	***	1.842 (0.263)	**	-	**	ns	0.938 (0.089)	**	-	ns	ns
P. alba	1.466 (0.219)	ns	ns	-	**	1.372 (0.128)	ns	**	-	**	1.124 (0.255)	ns	ns	-	ns
R. pseudoacacia	2.119 (0.260)	***	***	**	-	1.944 (0.346)	**	ns	**	-	1.018 (0.064)	**	ns	ns	-
								Chl *a+b*							
T. tentandra	5.312 (0.219)	-	*	**	***	5.030 (0.139)	-	***	**	***	5.030 (0.573)	-	*	ns	ns
A. fruticosa	5.866 (0.392)	*	-	ns	***	8.191 (0.856)	***	-	**	ns	4.302 (0.381)	*	-	ns	**
P. alba	6.477 (0.711)	**	ns	-	**	6.280 (0.570)	**	**	-	*	4.465 (0.907)	ns	ns	-	ns
R. pseudoacacia	9.370 (1.196)	***	***	**	-	7.636 (0.752)	***	ns	*	-	5.183 (0.219)	ns	**	ns	-
								Chl *a/b*							
T. tentandra	3.528 (0.578)	-	ns	ns	ns	3.126 (0.275)	-	ns	*	ns	3.414 (0.464)	-	ns	ns	*
A. fruticosa	3.725 (0.203)	ns	-	ns	ns	3.470 (0.222)	ns	-	ns	*	3.589 (0.160)	ns	-	*	***
P. alba	3.440 (0.194)	ns	ns	-	ns	3.581 (0.157)	*	ns	-	**	3.018 (0.368)	ns	*	-	***
R. pseudoacacia	3.425 (0.247)	ns	ns	ns	-	2.977 (0.348)	ns	*	**	-	4.096 (0.133)	*	***	***	-

ANOVA, n = 5, values are mean(S.D.), $^*p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$, ns = not significant

decrease of chlorophyll content, the Chl *a/b* ratio remained unchanged in *A. fruticosa*, indicating a lower sensitivity to pollution and salinity and the presence of some protection mechanisms which maintain the overall stability of photosystem II activity. Similar to our results, Zhou et al. (2011) found increased contents of Chl *a*, Chl *b*, and total chlorophyll Chl *a+b* along with the soil salt concentrations with no significant changes in the ratio of Chl *a/b*. Bearing in mind the fact that lagoon surfaces are seldom watered, water deficit in ash can certainly be one of the causes of chlorophyll increase. Namely, Yan et al. (2011) found that the decrease of soil moisture causes the increase of chlorophyll content Chl *a*, Chl *b* and Chl *a+b***.** Likewise, Paivoke and Simola (2001) assessed that an increased content of As can increase chlorophyll levels in leaves.

Symptoms of injury

Symptoms of damage in the form of leaf tip chlorosis and necrosis were noted in *T. tentandra* at the 11-year-old ash lagoon (L2). Marginal necroses, dry necrotic areas and the drying of older leaves were detected in *P. alba* at the 11-year-old lagoon. Excessive concentrations of B in the ash of both lagoons obviously resulted in the appearance of toxicity symptoms (leaf tip chlorosis and necroses). This is the result of B mobility, which generally translocates through xylem, mainly accumulating in the tips and margins of older leaves (Szabó, 1988; Kabata-Pendias and Pendias, 2001). No damage symptoms were found in *A. fruticosa* and *R. pseudoacacia*.

Selection of plant species is an important factor in determining the success of restoration on fly ash lagoons. The species selected should be capable of growing in the presence of increased amounts of trace elements and in a highly alkaline environment. The use of native plants is desirable to achieve successful restoration. In our opinion, the shrub species *A. fruticosa* meets most of the criteria for planting on a specific substrate such as fly ash. It is, actually, a N2-fixer, tolerant to high salinity and B, As and Mo, as wll as to the high temperatures and drought, which poses great potential to be a successful early colonizer on ash sites. It can, therefore, improve the short-time revegetation process together with other woody species and thus allow for the formation of an effective short-term vegetative cover at ash lagoons during the interim period before new ash deposits settle.

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