Original Research

# Photosynthetic Efficiency of Four Woody Species Growing on Fly Ash Deposits of a Serbian 'Nikola Tesla - A' Thermoelectric Plant

Miroslava Mitrović\*, Snežana Jarić, Olga Kostić, Gordana Gajić, Branko Karadžić, Lola Djurdjević, L., Ljiljana Oberan, Dragana Pavlović, Marija Pavlović, Pavle Pavlović

Department of Ecology, Institute for Biological Research "Siniša Stanković," University of Belgrade, Bulevar Despota Stefana 142, Belgrade 11060, Serbia

> Received: 23 June 2011 Accepted: 3 January 2012

#### **Abstract**

Photosynthetic efficiency and damage symptoms of *Tamarix tetrandra* Pallas, *Populus alba* L., *Robinia pseudoacacia* L. (planted), and *Amorpha fruticosa* L. (naturally colonized) were studied at two fly ash deposit lagoons of the 'Nikola Tesla – A' power plant (Obrenovac, Serbia), weathered 5 (L1) and 13 years (L2). In early phases of weathering, after 5 years, a reduced vitality of populations growing on the ash was noticed in planted *R. pseudoacacia* and spontaneously populated *A. fruticosa* (P<0.001) in comparison to *T. tentandra* and *P. alba*, due to higher salinity and elevated concentrations of As, Mo, Cu, and Mn in the ash. Thirteen years after planting, as weathering proceeded due to reduced salinity and toxicity, *A. fruticosa* species showed photosynthesis recovery and had the highest photosynthetic efficiency (P<0.001), suggesting that it poses adaptive capacity to survive and develop tolerance to stress in such habitats that strongly recommend this species for planting at fly ash lagoons.

Keywords: fly ash deposits, trace elements, photosynthetic efficiency, biological restoration

## Introduction

Increased use of coal for electric power generation has resulted in large quantities of fly ash and other residues, that require proper handling and disposal. Two methods for the disposal of the fly ash created by the burning of coal at thermoelectric plants were used: settling ponds and landfills. During the 1980s, landfills were replaced by wet settling ponds and since then, approximately 70% of thermoelectric plants dispose of their combustion products on-site (USEPA, [1]). Bare fly ash is visually unaesthetic and a source of dust and water pollution [2]. Fly ash disposal has adverse impacts on terrestrial and aquatic ecosystems due to leaching of toxic substances from the ash into soil and

groundwater followed by reduction in plant establishment and growth [3-10]. Due to unfavorable or even phytotoxic properties (lack of N and available P, extreme acidity or alkalinity, trace elements and/or B toxicity), recently abandoned deposits or landfills of coal combustion residues resemble vast desert-like areas with no or only poor vegetation cover [11]. If the ash deposit is colonized naturally, plant invasion and succession occur slowly. For example, it took about 30 to 50 years for dry ash spoils in England to resemble a normal soil and to develop birch/willow scrub woodland [12]. Therefore, the restoration of fly ash deposits in terms of their stabilization and revegetation is vital for environmental protection.

In Serbia, 6 thermoelectric plants (with total capacity of 3936 MW) use low-caloric lignite coal and produce around 5.5 Mt of ash annually, which is disposed of in the imme-

\*e-mail: mmit@ibiss.bg.ac.rs

diate vicinity of the power plants. Approximately 250-300 Mt of ash have been discarded to date. These deposits have created varied kinds of environmental hazards due to wind erosion and leachate generation and health hazards to the nearby inhabitants. In Belgrade (area of 3227 km²), up to 2 million citizens are endangered by power plant activities because two city municipalities include plants: Obrenovac (two electric power plants; over 800 ha of deposits, settling ponds technology), and Lazarevac (one electric power plant; landfill technology). The 'Nikola Tesla – A' thermoelectric plant in Obrenovac, with a total installed generator capacity of 1502 MW, burns 12 Mt of low-caloric lignite coal and disposes of approximately 2.4 Mt of ash per year. It is the largest individual thermoelectric plant on the Balkan peninsula. The ash deposit was formed on fertile cultivated land, covered an area of 400 ha, and is located in the immediate vicinity of the power plant on the right bank of the Sava River. The ash deposit is composed of three adjoining lagoons, which were performed by sluicing the ash into three wet lagoons, two of which are inactive at any one time. Biological restoration was undertaken at the inactive lagoons through the sowing of a grass-legume mixture, and the planting of tamarisk, poplar, and black locust in order to stabilize and protect the fly ash deposit sites from wind erosion. Namely, fly ash deposit do not serve as biologically productive habitat, but it is surrounded by agricultural fields, where different agricultural crops are produced, and which are permanently covered with fly ash, thus causing a number of adverse consequences to the safety of produced crops. Stabilization of ash would provide safer production of food in these areas. In addition, Obrenovac itself would be less exposed to pollution originating from a fly ash site. However, with the activation of an inactive lagoon, the established plant canopy is being covered again by ash, and recolonization of plants at this site has to start again from the beginning. This process happens in cycles from 5 to 13 years. Having this in mind, at 'Nikola Tesla-A' during recent years, research on ash deposits was focused on shortterm revegetation during the course of a thermoelectric plant's operation with special attention paid to the selection of adequate species for use in revegetation. The long-term biological recultivation of ash deposit sites is possible only after the closure of a thermal plant.

This study presents a comparative analysis of photosynthetic efficiency and leaf damage symptoms between examined species, planted tamarisk (Tamarix tetrandra Pallas), white poplar (Populus alba L.), and black locust (Robinia pseudoacacia L.), and naturally colonized false indigo (Amorpha fruticosa L.), growing at ash deposit lagoons of the 'Nikola Tesla-A' thermoelectric plant in Obrenovac (Serbia), weathered five (L1) and thirtheen years (L2) [13]. The ecophysiological characteristics of the plants at the deposit site were assessed in relation to their natural habitat (CS – the control site). The aim of this study was to evaluate the potential of these species for revegetation of ash deposits. Our hypothesis is that species that naturally colonize thermal power plant ash deposits have greater adaptive potential than species used in revegetation.

## **Experimental Procedures**

The research was carried out during June 2009 at three sites. The first site was ash deposit lagoon with an area of approximately 100 ha that has been inactive for five years (L1); the second site was an ash deposit lagoon with an area of approximately 150 ha that had been inactive for thirteen years (L2); the third site (CS - the control site) is their natural habitat, on the banks of the Kolubara River, 3 km from the ash deposit site in Obrenovac, and Botanical garden "Jevremovac" in Belgrade where T. tetrandra grows. Experimental sites have similar climatic conditions: Obrenovac (lat. 44°30' N long. 19°58' E), average altitude of 80 m, the mean annual temperature is 11.0°C (mean temperature minimum is in January of -2.1°C, and mean temperature maximum in July of 22°C), ), mean annual precipitation is 647 mm, a semi-arid period occurs during August and October; Belgrade (lat. 44°48' N long. 20°28' E), average altitude of 132 m, the mean annual temperature is 12.0°C (mean temperature minimum is in January of 1.2°C, and mean temperature maximum in July of 22.1°C), mean annual precipitation is 688 mm, a semi-arid period occurs during July and August. From the point of spreading pollutants in Obrenovac, the most significant impact are northwestern and western winds that bring ash to central zones of the city and also in agriculture fields, from ash deposits located in the western and northwestern parts of Obrenovac Municipality.

The ash disposal site consists of three adjoining lagoons that sluice the ash into three wet lagoons (ash:water=1:10), two of which are inactive at any one time. The ash produced by lignite combustion is alumo-silicate (approximately 80%), with a significant proportion of Fe, Ca, Mg, K, and Ti oxides (approximately 18%). 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%. Ash contains very low concentrations of total N (<0.05 %; [13]) and available P<sub>2</sub>O<sub>5</sub> and K<sub>2</sub>O (0.05-0.2% and 0.5-0.7%; [14]). Organic matter, if present in the ash, includes only unburnt coal particles. The concentration of overall soluble salts in the ash from the 'Nikola Tesla – A' thermal electric plant is low (at L1, 0.720 and at L2 0.110 dS·m<sup>-1</sup>, [10]). Concentrations of trace elements in bare fly ash are as follows: B (410 µg/g), As  $(172 \mu g/g)$ , Mo  $(16 \mu g/g)$ , Se  $(>2 \mu g/g)$ , Cu  $(225 \mu g/g)$ , Mn (812  $\mu$ g/g), and Zn (125  $\mu$ g/g), [10, 14]. The lagoons are occasionally irrigated with water from the Sava (EC<sub>w</sub>=0.43 dS·m<sup>-1</sup>) and the drainage waters from the disposal site (EC<sub>w</sub>=0.72-0.81 dS·m<sup>-1</sup>; [14]).

The samples of ash for trace element analysis were collected along a transect from the lagoon embankment toward the overflow channel situated in the centre of the lagoon, at 100 m intervals. The soil and ash samples 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 in three replicates.

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

Table 1. Differences in photosynthetic efficiency $(Fv/Fm)$ between species at control sites, 5-year-old lagoon (L1). and	13-year-old
lagoon (L2).	

Site	M (SD)	T. tentandra	A. fruticosa	P. alba	R. pseudoacacia
Control site (CS)					
T. tentandra	0.637 (0.043)	-	ns	ns	ns
A. fruticosa	0.649 (0.073)	ns	-	ns	ns
P. alba	0.635 (0.068)	ns	ns	-	ns
R. pseudoacacia	0.671 (0.064)	ns	ns	ns	-
L1 5-year-old lagoon					
T. tentandra	0.610 (0.059)	-	*	ns	***
A. fruticosa	0.543 (0.035)	*	-	**	ns
P. alba	0.632 (0.035)	ns	**	-	***
R. pseudoacacia	0.515 (0.044)	***	ns	***	-
L2 13-year-old lagoo	n			1	
T. tentandra	0.628 (0.022)	-	**	ns	ns
A. fruticosa	0.669 (0.035)	**	-	*	ns
P. alba	0.611 (0.055)	ns	*	-	ns
R. pseudoacacia	0.636 (0.041)	ns	ns	ns	-

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

deposit lagoons (L1 and L2). For trace elements analysis, soil and ash samples (0.5 g) were digested in a microwave (CEM MDS-2000) using 10 ml of concentrated HNO<sub>3</sub>. Plant samples (0.4 g) were digested in a microwave (CEM MDS-2000) using a mixture of concentrated HNO<sub>3</sub> (concentrated, 12 ml) and  $\rm H_2O_2$  (30%, 4 ml). 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.). Reference materials were obtained by Spex CerpiPrep.Ltd. (Middleseks, UK). Boron concentrations were determined using the spectrophotometric method with the aid of curcumin [15]. Concentrations were expressed in  $\mu g/g$  of dry weight.

Photosynthetic efficiency was measured 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 Oquist and Wass [16]. Chlorophyll was excited for 2 s by actinic light with a photon flux density of 200 and 400  $\mu$ mol m<sup>-2</sup>·s<sup>-1</sup>. 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.

One-way analyses of variance (ANOVA), was performed to test the differences in the photosynthetic efficiency (Fv/Fm) of the examined species and trace elements content in the examined sites. A discriminant analysis (CDA) was used to establish the variability and significance of the physiological differentiation of the examined species from different sites.

#### **Results**

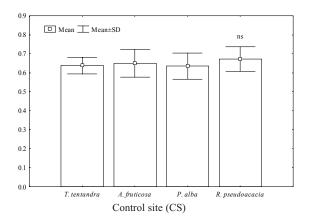
## Photosynthetic Efficiency of Plants

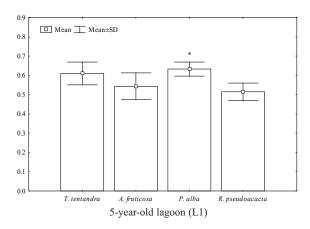
Photosynthetic plant responses to stressful conditions at fly ash lagoons of different weathering stages were analyzed with the aid of induced chlorophyll fluorescence. For the purposes of general comparison, the optimum ranges of photosynthetic efficiency parameters (Fv/Fm) of different types of vascular plants from different taxonomy groups, living forms, and site types, empirically obtained by Bjorkman and Demmig [17], were used. According to the above authors, the general optimum range of plant photosynthetic efficiency is 0.750-0.850, while 0.843 $\pm$ 0.012 is the average value for deciduous trees.

Analyses showed lower photosynthetic efficiency (Fv/Fm) than the optimum in all the species examined at control sites (Table 1). The Fv/Fm in all the species ranged from 0.635 to 0.671, with the gradient obtained: R. pseudoacacia (0.671), followed by A. fruticosa (0.649), T. tentandra (0.637), and P. alba (0.635). There were no dif-

ferences in photosynthetic efficiency (Fv/Fm) between examined species, ns (Fig. 1). Likewise, there were no differences between Fv/Fm of individual species from different sites (Table 1).

At the 5-year-old lagoon (L1), a photosynthetic efficiency was lower in all the species examined than at control sites (Table 1). Fv/Fm in all the species ranged from 0.515 to 0.632, with Fv/Fm gradient obtained: P. alba (0.632), then T. tentandra (0.610), A. fruticosa (0.543), and R. pseudoacacia (0.515), the least efficient. R. pseudoacacia and A. fruticosa varied the most in comparison to the other





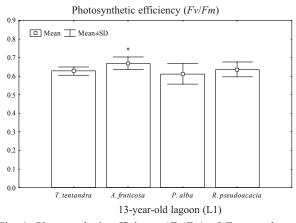


Fig. 1. Photosynthetic efficiency (Fv/Fm) of T. tetrandra, A. fruticosa, P. alba, and R. pseudoacacia at control sites (CS); 5-year-old lagoon (L1) and 13-year-old lagoon (L2).

two, and expressed photo-inhibitory damage, which occurs in conditions of exposure to stress factors at a site (Table 1, Fig. 1).

In all examined species, 13 years after planting and naturally colonizing, at fly ash lagoon (L2) a photosynthetic efficiency recovered to the values similar to those from the control sites (Table 1). Photosynthetic efficiency in all the species ranged from 0.515 to 0.632, with the gradient obtained: *A. fruticosa* (0.669), *R. pseudoacacia* (0.636), *T. tentandra* (0.628), and *P. alba* (0.611). In this period the order has been changed, *T. tentandra* and *P. alba* were the least efficient (Table 1, Fig. 1).

A discriminant analysis (CDA) based on parameters of photosynthetic efficiency showed a distinction between the populations of all examined species from the control site (CS), which are grouped to the left Axis I (unpolluted), and populations from the 5-year-old ash deposit lagoon (L1), which are to the right of Axis I (polluted) (Fig. 2). Such distinction is pronounced in *R. pseudoacacia* and *A. fruticosa*.

### Damage Symptoms

Symptoms of damage in the form of leaf tip chlorosis and necrosis were noted in *T. tentandra* and chloroses in form of black spots in *A. fruticosa* leaves at the 5-year-old ash lagoon (L1). Marginal necroses, dry necrotic areas and the drying of older leaves were detected in *P. alba*. No injury symptoms were found in *R. pseudoacacia* individuals.

# Trace Elements Concentration in the Examined Sites

The concentrations of B (410 µg/g) in unweathered ash were reduced to 25.843 µg/g after 5 years, and to 19.406 μg/g after 13 years, to values similar to those in control soil, p<0.05 (Table 2), as a result of ash weathering and lagoon processes. As content in the soil from the control site was within the normal range for soils  $(4.4-9.3 \mu g/g)$ [18]). However, As concentrations at the ash lagoons (at 5year-old L1 110.80 μg/g; at 13-year-old L2 84.460 μg/g, Table 2) were significantly higher (p<0.001). 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) there was an excess, p<0.001, (Table 2). High levels of Mo in the ash were measured (11.36 µg/g at L1, and 8.50 µg/g at L2). The Se content at the control site  $(0.53 \mu g/g)$  was above the normal range for soils, while at both lagoons the Se content was <0.1 μg/g and lower than the average Se concentrations in soils [18], (Table 2). Although lower in soil from CS (63.443 µg/g) than in fly ash lagoons, the Cu content at all three sites was higher than the normal range for soils [18]. The Mn content in the soil from the control site and from the ash deposit lagoons was similar or higher than average content in world soils (270-525 µg/g, [18]), with the highest level measured in the 5-year-old lagoon (L1), p<0.001, (Table 2). At both lagoons, as well as in control soil, the Zn concentrations

	В	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)	25.84* (0.603)	110.80*** (1.560)	11.36*** (0.085)	<0.1	96.74*** (0.475)	722.30*** (5.665)	50.18*** (1.086)
Fly ash (L2)	19.40* (1.112)	84.46*** (0.485)	8.52*** (0.446)	<0.1	73.70*** (1.503)	422.85*** (3.192)	37.38** (1.428)

Table 2. Trace element content in soil (CS) and fly ash (L1 and L2), expressed in a dry weight basis (µg/g).

were within the normal range in soils (17-125  $\mu$ g/g, [18]). The Zn average concentrations in the fly ash lagoons (L1) were higher (L1, p<0.001; L2, p<0.01) in comparison to the soil (CS) (Table 2).

#### **Discussion**

Disposal of fly ash in open ash ponds causes serious adverse environmental impact owing to its elevated trace element contents [19]. Fly ash is used in the building material industry, in civil engineering, in road construction, for

construction work in underground mining, as well as for restoration purposes in open cast mining. It can also be used as a potential nutrient supplement for degraded soils, thereby solving the solid waste disposal problem to some extent [20]. Unless utilized for the above purposes, ash is treated as waste and has to be disposed of. One of the best and most eco-friendly alternatives for fly-ash management is to vegetate the landfill or disposal area, which will serve the purpose of both stabilization and provide a pleasant landscape [3, 6]. Revegetation of fly ash lagoons is also important to control erosion and leachate generation. Establishing vegetation on fly ash basins is difficult due to physical and/or

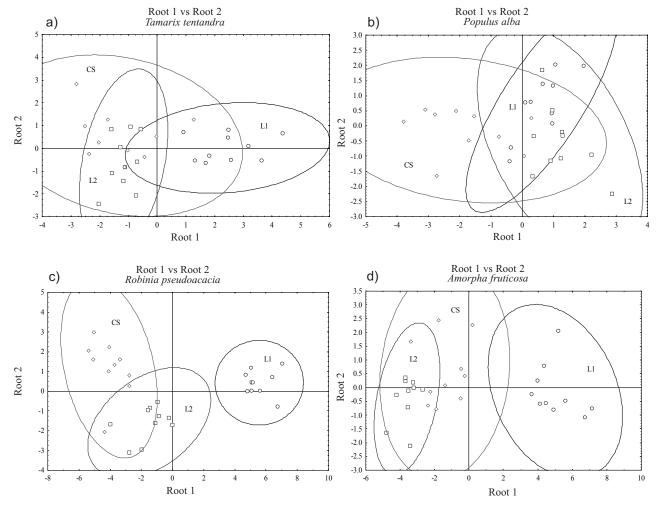


Fig. 2. Discriminant analysis (CDA) for the three sampling sites based on the variations of Fv/Fm, Fo, Fm, Fv, and  $t_{1/2}$  of a) T. ten-tandra b) P. alba c) R. pseudoacacia, and d) A. fruticosa

n = 3, values are mean (S.D.), ANOVA, \*P<0.05, \*\* P<0.01, \*\*\*P<0.001

Table 3. Comparison	of $Fv/Fm$	of T.	tentandra,	Р.	alba,	R.
pseudoacacia, and A.	fruticosa fr	om ex	amined site	s.		

Photosynthetic efficiency Fv/Fm						
Species	Species CS vs L1 CS vs L2 L1 vs L2					
T. tentandra	ns	ns	ns			
P. alba	ns	ns	ns			
R. pseudoacacia	***	ns	***			
A. fruticosa	**	ns	***			

ANOVA, n = 10, values are mean (S.D.), \*\*P < 0.01, \*\*\*P < 0.001, ns – not significant.

chemical limitations to plant growth [6, 8-10, 21, 22]. Among the possible chemical limitations for the successful revegetation of alkaline fly ash is phytotoxicity due to arsenic, boron, molybdenium, selenium and other potentially toxic trace elements; toxicity caused by high pH and/or high concentrations of soluble salts; nutrient deficiency due to the absence of nitrogen (N) and less available phosphorous (P); restriction of root growth due to the fine particle size of the ash; and reduction in the number of free-living and symbiotic N<sub>2</sub>-fixing micro-organisms [3, 5, 6, 19, 23-27].

Boron is one of the most important contaminants in most coal-ash materials [28] and is the most mobile element when coal-ash is buried [29]. In the 'Nikola Tesla – A' ash deposit in Obrenovac, concentrations of B in unweathered ash lagoons were reduced to values similar to those in control soil as a result of ash weathering and lagoon processes. James et al. [30] found that 17-64% of B is immediately soluble in water, but a further 2 to 3 years is required for B to decrease to a concentration that plants can tolerate. At ash deposit lagoon L1, a certain reduction occurs after 5 years of weathering. Concentrations of B at both ash deposit lagoons were within the normal range for soils [18]. In contrast to the soil from the control site, As content in the ash lagoons showed extremely high levels. Arsenic availability is strongly influenced by pH, with sorption of arsenate generally decreasing with increasing pH [18, 31, 32]. Low sorption of As under alkaline reaction implies restricted absorption by plants. When it is not bonded to organic matter, easily mobile As is leached down to groundwaters and creates a great health risk when used as a source of drinking water.

The Mo content at the control site was within the normal range for soils, whereas in the ash deposit lagoons there was an excess. Plants accumulate it in the form of Mo ions and its absorption is proportional to the content in the soil and depends on the pool of soluble Mo, which is linked to the pH of the soil [18]. High mobilization capacity of Mo can cause toxicity problems for vegetation at the ash deposits, especially those that accumulate it for a prolonged period, like perennial plants. The Se content at the control site was above the normal range for soils, while at both lagoons the Se content was lower than the average Se concentrations in soils [18]. In our opinion, Se is not a problem

for plants because the alkaline reaction reduced its availability to plants [33].

The Cu content at all three sites was higher than the normal range for soils [18]. The binding of Cu by soils 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 [18]. This is the case with the ash deposit site at the 'Nikola Tesla – A' thermoelectric plant, where the pH of the ash falls within this range. The Mn content in the soil from the control site and from the ash deposit lagoons were within the normal range for soils [18]. In unweathered ash and at the 5-year-old lagoon (L1) Mn concentrations were almost double in comparison to that at the 13-year-old lagoon (p<0.001).

At both the lagoons, as well as in control soil, the Zn concentrations were within the normal range of soils. As with Mn, the amount of Zn at the weathered lagoons can be expected to reduce over time. Carlson et al. [34] found a significant decrease of Mn and Zn in soils amended with ash. The Zn average concentration in the 5-year-old lagoon (L1) was higher in comparison to that at the 13-year-old lagoon (L2), p<0.001, and in comparison to the soil (CS), p<0.01 (Table 3). Zn level reduction over time can cause a Zn deficiency in plants because this element becomes less available to plants due to alkalinity of the ash [35].

Temporal reduction occurred in B (p<0.05), As (p<0.001), Mo (p<0.001), Cu (p<0.001), Mn (p<0.001), and Zn (p<0.001) concentrations in 5-year-old L1 and 13-year-old L2, due to leaching from the weathered ash. Popović et al. [36] analyzed pollution caused by leaching of elements during ash transport through the pipeline in the 'Nikola Tesla – A' power plant in Obrenovac. They found that portions of Zn, Ni, and Cr are released during the ash transport, where As and Mn are released continuously, whereas Cu and Fe do not present an environmental threat due to element leaching during and immediately after coal ash suspension and transport.

All the examined woody species showed lower overall vitality than general optimum range of photosynthetic efficiency parameters [17]. A decrease in this ratio indicates a photoinhibition in plants used for the study. At the 5-year-old lagoon, all species except of *P. alba* were less efficient than at the control site and at the 13-year-old lagoon. Reduced photosynthetic efficiency at the 5-year-old lagoon occurred due to stressful conditions at fly ash deposit. Earlier ecological studies on vegetation at 'Nikola Tesla – A' fly ash deposits indicated a number of deficiencies that ash has with regard to plant survival and growth: lack of essential nutrients N, P, Cu, Mn, and Zn, and toxicity of of B, As, and Mo in the ash, and the extreme microclimatic conditions at the deposit sites [8-10].

As expected, examined species differed from the control ones and from ash lagoons of different age of weathering. Namely, in early phases of weathering, 5 years after planting, when ash was burdened by higher salinity and high levels of toxic concentration of As, Mo, Cu, and Mn, the most vital were *T. tentandra* and *P. alba*, which indicate their tolerance to chemical toxicity of the ash and justifies their planting. In contrast, lower vitality was found in planted *R. pseudoacacia* 

and spontaneously populated *A. fruticosa* (Table 3). Thirteen years after planting, as weathering proceed due to reduced salinity and toxicity, the ash became suitable substrate for colonization of species from surrounding areas like *A. fruticosa*. Together with naturally colonized *A. fruticosa*, planted *R. pseudoacacia* showed photosynthetic recovery in the second phase, after 13 years of ash weathering.

Fly ash management is becoming more challenging due to strict environmental regulations of its utilization and disposal, which may affect soil fertility, soil quality, and soil biota, and eventually groundwater quality. To overcome this environmental problem, the revegetation of fly ash dykes/landfills with tolerant plants is one of the cheap alternatives [37]. Nutrient deficiency due to absence of nitrogen and phosphorus in fly ash can be improved by planting legume plants and by using symbiotic N2-fixing bacteria. The efficiency of different bio-fertilizers (nitrogenfixing bacteria, arbuscular mycorrhizal fungi, or mycorrhiza-associated bacterial strain) in promotion of plant growth on fly ash was demonstrated for several plant species [38, 39]. Rau et al. [40] found a considerable improvement in the seedling establishment, plant weight, and shoot length in rhizobacterial inoculated plants of Saccharum ravennae L. in fly ash environment indicated the significance of rhizobacteria in its colonization and spread to the deposits. Likewise, Jambhulkar and Juwarkar [41] used organic amendment and selection of suitable plant species along with specialized nitrogen fixing strains of biofertilizer in order to improve the revegetation process in fly ash deposits. They found that farm yard manure amendment improved ash water holding capacity and porosity together with improved nitrogen content due to the addition of nitrogen fixing strains of Bradyrhizobium and Azotobacter species, and phosphate content due to the addition of vesicular arbuscular mycorrhizal fungi that help in phosphate immobilization.

Selection of plant species is an important factor in determining the success of revegetation of fly ash lagoons. Species selected should grow in the enriched level of trace elements and a highly alkaline or acidic environment. A range of surface revegetation treatments for fly ash lagoons was applied to investigate their ability to overcome the chemical and physical limitations of fly ash for plant growth [6, 21, 24, 27, 42-44]. There are reports of trees surviving on fly ash basins [6, 21, 27]. Tree species such as Liquidambar styraciflua L., Platanus occidentalis L. [27], Cassia siamea Lamk. [41] and shrubs such as Halosarcia halocnemoides (Nees) Paul G. Wilson, and H. pergranulata (J.M.Black) Paul G.Wilson were proved for use in revegetation [45]. Tree species Acacia sp. and Leucaena leucocephala (Lam.) de Wit were found to have a high tolerance and survival in arid, infertile, and metal-contaminated areas [21, 45]. Based on analysis of natural vegetation growing on fly ash lagoons in India, Maiti and Jaiswal [46] recommended Typha latifolia L. and Saccharum spontaenum L. species for bioremediation of fly ash lagoons. Due to the large differences in species response to fly ash, more plant species should be tested when selecting species for fly ash lagoon restoration.

 $T.\ tentandra$  is the only shrub species planted at the 'Nikola Tesla – A' ash deposit in order to prevent wind erosion because of its tolerance to dry and hot habitats, which is a good precondition for the colonization of multi-contaminated habitats, such as ash deposit. Similar values of Fv/Fm (ns) at all three habitats, indicate its adaptive potential to survive in different types of habitats exposed to stressful conditions and justifies its planting on the ash.

Similarly, white poplar is tolerant to different textured soils, medium salinity, and pH reaction. It is an especially strong competitor because it can grow in a variety of soils, produce large seed crops, and resprouts easily in response to damage. As with tamarisk, there were no differences in Fv/Fm (ns) at all the habitats, which also indicates high tolerance to a variety of habitat conditions. However, air and soil humidity can be a problem considering that poplars are a hygromesophyllous species, growing in areas such as ravines and along the river valleys. Pavlović et al. [8] found functional difficulties in poplar growing at dry ash deposit.

Black locust occupies habitats like forests, regenerating old fields, naturally revegetating reclamation sites, mined sites, etc., and shows dominance in early successional stands [47]. It is a nitrogen-fixing species. For example, Montagnini et al. [48] found high net nitrogen mineralization potential, net nitrification potential, and phosphorus concentrations in the soil under dense stands of black locust occurring in a regenerating clearcut, and in a 17-year-old grass-to-forest successional stand. Although sensitive in the first stages of revegetation, planting of such a nitrogen-fixing tree is of great importance for providing a stable and functional vegetative cover on the fly ash in later stages of revegetation.

False indigo showed difficulties in *Fv/Fm* during the first 5 years after colonization of fly ash lagoons, which confirms earlier findings [8]. However, it recovered vitality between 5 and 13 years. *A. fruticosa* is an extremely efficient colonizer, especially in secondary successions, when human activity diminishes. In these cases, if the soil moisture is suitable (it is a hygrophilous species), it enters early and becomes the most important pioneer shrub species, quickly forming a close covering. For example, Kahl et al. [49] showed that *A. fruticosa* is able to grow under the poor conditions of the heap, in the potash mine Sondershausen/Harz (Thuringia).

Planted and colonized trees and shrubs could provide a good green belt and valuable vegetative cover on fly ash deposits. Plants that have native character, a perennial nature, extensive root system, vegetative multiplication ability, nitrogen-fixing ability, tolerance to high pH, salinity and toxic metals, and tolerance to high temperatures and drought are the most successful colonizers on ash sites [8, 50]. Our research has shown that all examined species have high potential for revegetation of fly ash deposits. However, *A. fruticosa*, which naturally colonizes the deposit, showed a lower viability in the first phase of revegetation, while in the later stage its vitality was the highest. The high photosynthetic efficiency, nitrogen-fixing and ability, invasive character of this species (which requires no

habitat management), strongly recommend this plant for planting at fly ash lagoons of the 'Nikola Tesla -A' power plant. Therefore, using naturally recolonized species such as A. fruticosa species can improve the short-time revegetation process and thus allow the creation of effective vegetative cover at ash lagoons for periods of five to thirteen years, as well as successful revegetation of ash deposits after the closure of the thermal plant.

## Acknowledgements

This work was supported by the Ministry for Education, Science and Technological Development of Serbia, grant No. 173018.

#### References

- U.S. ENVIRONMENTAL PROTECTION AGENCY. USEPA Rep. 530-SW-88-002. Wastes from the combustion of coal by electric utility power plants, U.S. Government Print Office, Washington, DC, 1988.
- CHU L. M. Natural revegetation of coal fly ash in a highly saline disposal lagoon in Hong Kong. Appl. Veg. Sci. 11, 297, 2008.
- ADRIANO D. C., PAGE A. L., ELSEEWI A. A., CHANG A., C., STRAUGHAN I. Utilization and disposal of fly ash and other coal residues in terrestrial ecosystems. A Review. J. Environ. Qual. 9, 333, 1980.
- EL-MOGAZI D., LISK D. J., WEINSTEIN L. H. A review of physical, chemical and biological properties of fly ash and effects on agricultural ecosystems. Sci. Total. Environ. 74, 1, 1988
- HAERING K. C., DANIELS L. W. Fly Ash: Characteristics and Use in Mined Land Reclamation-A Literature Review. Virginia Coal Energy Res. J. 3, 33, 1991.
- CARLSON C. L., ADRIANO D. C. Environmental impacts of coal combustion residues. J. Environ. Qual. 22, 227, 1993
- KUKIER U., SUMNER M. E. Boron availability to plants from coal combustion by-products. Water Air Soil Pollut. 87, 93, 1996.
- PAVLOVIĆ P., MITROVIĆ M. DJURDJEVIĆ L. An ecophysiological study of plants growing on the fly ash deposits from the 'Nikola Tesla – A' thermal power station in Serbia. Environ. Manage. 33, 654, 2004.
- PAVLOVIĆ P., MITROVIĆ M., DJURDJEVIĆ L., GAJIĆ G., KOSTIĆ O. BOJOVIĆ S. Ecological potential of Spiraea van-hauttei (Briot) Zabel for urban (Belgrade city) and fly ash deposit (Obrenovac) landscaping in Serbia. Pol. J. Environ. Stud. 16, 427, 2007.
- MITROVIĆ M., PAVLOVIĆ P., LAKUŠIĆ D., STEVANOVIĆ B., DJURDJEVIĆ L., GAJIĆ G. KOSTIĆ O. The potential of *Festuca rubra* and *Calamagrostis epigejos* for the revegetation on fly ash deposits. Sci. Total Environ. 407, 338, 2008.
- HAYNES R. J. Reclamation and revegetation of fly ash disposal sites Challenges and research needs. J. Environ. Manage. 90, 43, 2009.
- SHAW P. J. A. A Preliminary Study of Successional Changes in Vegetation and Soil Development on Unamended Fly Ash (PFA) in Southern England. J. Appl. Ecol. 29, (3), 728, 1992.

 DŽELETOVIĆ Ž., FILIPOVIĆ R. Grain characteristics of crops grown on power plant ash and bottom slag deposit. Resour. Conserv. Recy. 13, 105, 1995.

- SIMONOVIĆ B. Report on waste, surface and ground water monitoring in the 'Nikola Tesla – A' thermal power station at Obrenovac, Holding Institute of General and Physical Chemistry, Belgrade, pp. 1-33, 2003 [In Serbian].
- WEAR I. J. Method for total boron, In: Methods of Soil Analysis (Ed. A. C. Black), 1060-1062, American Society of Agronomy, Madison, Wisconsin, 1965.
- OQUIST G., WASS R. A portable, microprocessor operated instrument for measuring chlorophyll fluorescence kinetics in stress physiology. Physiol. Plant. 73, 211, 1988.
- BJORKMAN O., DEMMIG B. Photon yield of O<sub>2</sub> evolution and chlorophyll fluorescence characteristics at 77 K among vascular plants of diverse origins. Planta 17, 489, 1987
- KABATA-PENDIAS A., PENDIAS H. Trace Elements in Soils and Plants. 3<sup>rd</sup> edn., CRC Press, Boca Raton, Florida, 2001
- PANDEY V. C., SINGH J. S., SINGH R. P., SINGH N., YUNUS M. Arsenic hazards in coal fly ash and its fate in Indian scenario. Resour. Conserv. Recy. 55, 819, 2011.
- PANDEY V. C., SINGH N. Impact of fly ash Incorporation in Soil Systems. Agric. Ecosyst. Environ. 136, 16, 2010.
- MULHERN D. W., ROBEL R. J., FURNESS J. C., HENS-LEY D. L. Vegetation on waste disposal areas at a coal-fired power plant in Kansas. J. Environ. Qual. 18, 285, 1989.
- DJURDJEVIĆ L., MITROVIĆ M., PAVLOVIĆ P., GAJIĆ G., KOSTIĆ O. Phenolic acids as bioindicators of fly ash deposit revegetation. Arch. Environ. Contam. Toxicol. 50, 488, 2006.
- TOWNSEND W. N., HODGSON D. R. Edaphological problems associated with deposits of pulverized fuel ash, In: Ecology and reclamation of devastated land, I (Eds. R. J. Hutnik and G. Davis), Gordon and Breach, New York, pp. 45-56, 1973.
- HODGSON D. R., BUCKLEY G. P. A practical approach towards the establishment of trees and shrubs on pulverized fuel ash, In: The Ecology of resource degradation and renewal (Eds. M. J. Chadwick and G. T. Goodman), Blackwell Scientific Publications Ltd., Oxford, pp. 305-329, 1975
- PAGE A. L., ELSEEWI A. A., STRAUGHAN I. R. Physical and Chemical Properties of Fly Ash from Coal-Fired Power Plants with Reference to Environmental Impacts. Residue Rev. 71, 83, 1979.
- BRADSHAW A. D., CHADWICK M. J. The restoration of land. University of California Press, Berkley, California, 1980.
- CARLSON C. L., ADRIANO D. C. Growth and elemental content of two tree species growing on abandoned coal fly ash basins. J. Environ. Qual. 20, 581, 1991.
- 28. ADRIANO D.C. Trace Elements in Terrestrial Environments. Biogeochemistry, Bioavailability, and Risks of Metals, second ed. Springer, New York, **2001**.
- DOAK R. Pilot project-coal ash burial in saturated conditions. Solid Waste News and Notes 6, 1, 2003.
- JAMES, W. D., GRAHAM C. C, GLASCOCK M. D., HANNA A. S. G. Water-leachable boron from coal ashes. Environ. Sci. Technol. 16, 195, 1982.
- BECH J., POSCHENRIEDER C., LLUGANY J., BARCE-LO P.T., TOLOIAS F. J. Arsenic and heavy metal contamination of soil and vegetation around a copper mine in Northern Peru. Sci. Total Environ. 203, 83, 1997.

- SMITH E., NAIDU R., ALSTON A. M. Chemistry of arsenic in soils: I. Sorption of arsenate and arsenite by four Australian soils. J. Environ. Qual. 28, 1719, 1999.
- BOROWSKA K., LYSZCZARZ R. The selenium concentration in some grass species from mineral and peaty soils. 16th General Meeting of the European Grassland federation, Grado, Italy, 1996.
- CARLSON C. L., WEBER J., BOLAN N. S., PARAMASI-VAM S., KOO B-J., SAJWAN, K. S. Effects of high rates of coal fly ash on soil, turfgrass, and groundwater quality. Water Air Soil Pollut. 139, 365, 2002.
- SCHNAPPINGER M. G, MARTENS D. C., PLANK C. O. Zinc availability as influenced by application of fly ash to soil. Environ. Sci. Technol. 9, 258, 1975.
- POPOVIĆ A., DJORDJEVIĆ D., POLIĆ P. Trace and major element pollution originating from coal ash suspension and transport processes. Environ. International 26, 251, 2001.
- PANDEY V. C., ABHILASH P. C., SINGH N. The Indian perspective of utilizing fly ash in phytoremediation, phytomanagement and biomass production. J. Environ. Manage. 90, 2943, 2009.
- JUWARKAR A. A., JAMBHULKAR H. P. Restoration of fly ash dumps through biological interventions. Environ. Monit. Assess. 139, 355, 2008.
- HRYNKIEWIEZ K., BAUM C., NIEDOJADLO J., DAHM H. Promotion of Mycorrhiza Formation and Growth of Willows by the Bacterial Strain Sphingomonas sp. 23L on fly ash. Biol. Fertil. Soils 45, 385, 2009.
- RAU N., MISHRA V., SHARMA M., DAS M.K., AHALUWALIA K., SHARMA R.S. Evaluation of functional diversity in rhizobacterial taxa of a wild grass (Saccharum ravennae) colonizing abandoned fly ash dumps in Delhi urban ecosystem. Soil Biol. Biochem. 41, 813, 2009.

- JAMBHULKAR H. P., JUWARKAR A. A. Assessment of bioaccumulation of heavy metals by different plant species grown on fly ash dump. Ecotox. Environ. Safe. 72, 1122, 2009.
- DUGGAN J. C., SCANLON D. H. Evaluation of municipal refuse compost for ash pond stabilization. Compost Sci. 15, 26, 1974.
- HODGSON D. R., TOWNSEND W. N. The amelioration and revegetation of pulverized fuel ash. In: Hutnik, R. J., Davis, G. (Eds.), Ecology and reclamation of devastated land, Vol. 2, Gordon and Breach, London, pp. 247-270, 1973
- GUPTA A. K., SINHA S. Decontamination and/or revegetation of fly ash dykes through naturally growing plants. J. Hazard. Mater. 153, 1078, 2008.
- BARNET Y. M., CATT P. C., HEARNE D.H. Biological nitrogen fixation and root-nodule bacteria (*Rhizobium* sp. and Brady *Rhizobium* sp.) in two rehabilitating sand dune areas planted with Acacia sp. Aust. J. Bot. 33, 593, 1985.
- MAITI S. K., JAISWAL S. Bioaccumulation and translocation of metals in the natural vegetation growing on fly ash lagoons: a field study from Santaldih thermal power plant, West Bengal, India. Environ. Monit. Asses. 136, 355, 2008.
- STRAHLER A. H. Forests of the Fairfax Line. A. Assoc. Am. Geog. 62, 664, 1972.
- MONTAGNINI F., HAINES B. L., SWANK W. T., WAIDE J. B. Nitrification in undisturbed mixed hardwoods and manipulated forests in the southern Appalachian Mountains of North Carolina, U.S.A. Can. J. For. Res. 19, 1226, 1989.
- KAHL L., KLUGE R., THOMAS S. Evaluation of an attempt to cultivate shrubs and trees on a heap of a potash mine. Landscape Urban Plann. 51, (2), 109, 2000.
- 50. PANDEY V. C., SINGH K. Is *Vigna radiata* suitable for the revegetation of fly ash landfills? Ecol. Eng. **37**, 2105, **2011**.