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Scientific paper UDC:504.75.054 :614.3-078(497.15)

Plant and microbial diversity in coal mine-affected soil in "Kakanj" (Bosnia and Herzegovina)

Ecoremediation is one of reclamation methods, using plant-microbes-based technologies, and its application is suitable for mining activities-degraded ecosystems. The aim of this work was to estimate plant and microbial diversity on coal mine field "Kakanj" (Bosnia and Herzegovina). The results show the domination of Asteraceae species. In most of samples, bacterial number was higher in rhizosphere comparing to the surrounding bulk soil, while the abundance of fungi was higher comparing to the number of actinomycetes. Lowest microbial activity was detected in waste material. Determination of plant and microbial diversity provide valuable information for restoration of mining activities-degraded environments.

Key words: biodiversity, coal mine, microorganisms, plants

INTRODUCTION

Coal mine "Kakanj" is one of the most ancient coal fields in Bosnia and Herzegovina. The annual productivity of brown coal, over a millions of tons, has enabled the development and prosperity of the region. However, coal exploitation lead to negative consequences of terrestrial and aquatic environments [1, 2], i.e. soil, air and water pollution [3] and biodiversity loss [4]. In the same time, mining activities generate a big amount of waste, which is deposited at the soil surface [3]. Because of harsh condition, these wastelands require reclamation [5].

Different physical, chemical and biological remediation technologies have been developed during previous decades and technology selection in remediation process is site specific [6]. However, because physical and chemical technologies are unsuitable for large areas such as a mining sites are [7], plant-microbe-based technologies are more suitable and efficient for improving the quality of degraded landscapes. Plant-microbe interactions have big importance in these rigid substrates [5] and control characteristics of soil [8]. As previously showed, during soil development on coal mine field, increase of enzyme activity, soil bacteria activity and biomass is recorded [9], which directly affects the soil quality [10]. Fertility, as a parameter of soil quality, is one of major factors for plant growth [11]. Some plants have adaptation mechanisms for growth on polluted sites and can be used for restoration of degraded soils [12].

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Received for Publication: 21. 08. 2013. Accepted for Publication: 27. 10. 2013.

On the other side, these plants are important for mineralization enhance and stimulation of microbial activity [13]. Thus, these plant-microbe interactions are capable of providing site reclamation of soils degraded by mining activities [14].

The aim of this paper was to examine plant and microbial diversity at chosen location in coal mine field "Kakanj" (Bosnia and Herzegovina).

MATERIALS AND METHODS

The studying of plant and microbial diversity in coal mine filed "Kakanj" was performed during the summer 2012. at two locations: "Vrtlište" and "Stara jama i jama Haljinići". Determination of plant species was conducted on these locations, using the identification key and based on morphological properties [15].

Microbial characterisation of rhizosphere (R) and surrounding bulk soil (SBS) was performed using the dillution-plating method [16]. In order to have representative choice of plants, most dominant plants in these location were chosen for characterisation. Total number of bacteria in rhizosphere and surrounding bulk soil samples was determined using the 0.1x tryptic soy agar (TSA). Ammonification bacteria were determined on meat peptone agar (MPA), *Azotobacter* sp. and oligonitrophiles on Fyodorov agar, total number of fungi on rose bengal streptomycin agar [17] and actinomycetes on starch-ammonia agar. The total number of microorganisms (CFU x g⁻¹) was calculated after drying of samples at 105°C for 2 h.

Dehidrogenase activity was measured using the ancient method [18], while phosphomonoesterase activity was determined according to Tabatabai [19].

Appart from these samples, waste material originated from coal wash (depths 0-20, and 20-40 cm) was also included in plant and microbial characterization.

RESULTS AND DISCUSSION

At two location of coal mine field "Kakanj". twenty nine plant species, belonging to 27 genera and 18 families, were recorded in this research (Table 1). From the Asteraceae family, five plant species from five genera were registered and constituted 17.2 and 18.5% of total species and genera, respectively. Similar observation was made in previous studies [11]. Seven families including Amaranthaceae (2 species, 1 genera), Betulaceae (2 species, 2 genera), Caryophyllaceae (2 species, 2 genera), Poaceae (2 species, 2 genera), Polygonaceae (2 species, 1 genera), Rosaceae (2 species, 2 genera) and Salicaceae (2 species, 2 genera) constituted 12 plant species from 12 genera, accounting for 48.3 and 44.4% of total species and genera, respectively. These plant species had the most important effect on restoration of natural plant association. Similar trend was also observed previously [2]. In addition, the rest 10 families (Chenopodiaceae, Dipsacaceae, Equisetaceae, Phytolaccaceae, Plantaginaceae, Polypodiaceae, Portulacaceae, Ranunculaceae, Resedaceae and Scrophulariaceae) were recorded as a single species and genus only. The waste material collected near the mine spoils, was characterized only by the presence of Amaranthus albus L. Other authors have also observed same families in their reserach, such as Betulaceae, Salicaceae, Poaceae [2], Polygonaceae [12], Caryophyllaceae [20] etc. A few arbor species were detected as well at the examined locations (Betula pendula Roth., Clematis vitalba L., Prunus avium L., Salix alba L., and Populus nigra L.). Richness of herb species at these location can be explained by low crown density and low absorption rate of heat, rainfall and light by arbor plants comparing to herb

Table 1 - Plant species at locations of coal mine fields "Kakanj"

Family	Plant species	Family	Plant species		
Amaranthaceae	Amaranthus albus L.	Plantaginaceae	Plantago lanceolata L.		
Amaraninaceae	Amaranthus retroflexus L.	Plantaginaceae Poaceae Poaceae Polygonaceae Polygonaceae Polygonum av. Polypodiaceae Portulacaceae Ranunculaceae Resedaceae Rosaceae Prunus avium Potentilla rept Salicaceae Plantago lance Polygonum av. Polygonum av. (L.) Kuhn Portulaca oler Reseda luteola Prunus avium Potentilla rept Salix alba L. Populus nigra	Digitaria sanguinalis (L.) Scop		
	Artemisia absinthium L.	Poaceae	Setaria viridis (L.) P. B.		
	Doronicum sp. L.	D = l	Polygonum aviculare L.		
Asteraceae	Matricaria inodora L.	Potygonaceae	Polygonum lapathifolium L.		
Asieraceae	Turnilar a faufaur I	Dalama di mana	Pteridium aquilinum		
	Tussilago farfara L.	Рогуровівсеве	(L.) Kuhn		
	Xanthium italicum Mor.	Portulacaceae	Portulaca oleracea L.		
Betulaceae	Betula pendula Roth.	Ranunculaceae	Clematis vitalba L.		
Беннасеае	Carduus acanthoides L.	Resedaceae	Reseda luteola L.		
Camonhullaceae	Saponaria officinalis L.	Doggoogo	Prunus avium L.		
Caryophyllaceae	Silene vulgaris	Kosaceae	Potentilla reptans L.		
Chenopodiaceae	Chenopodium botrys L.	Salinganga	Salix alba L.		
Dipsacaceae	Dipsacus laciniatus L.	Sancaceae	Populus nigra L.		
Equisetaceae	Equisetum talmateia Ehrh.	Cananhulaniaa	Verbassum phlemeides I		
Phytolaccaceae	Phytolacca americana L.	Scropnulariaceae	Verbascum phlomoides L.		

Soil microorganisms play an important role in transformation of organic matter [21]. Abundance and activity of microorganisms reflects the degree of soil development [22]. Their activity is essential for nutrient cycling, formation of available forms of nutrients and development of soil [20].

As can be seen from table 2, the highest bacterial number in surrounding soil was recorded in *Polygonum aviculare* L., and in rhizosphere samples of *Tussilago farfara* L. In rhizosphere samples large diversity of bacteria was detected, which is in accordance with other authors [23]. Total number of bacteria ranged from 26.84x10⁵ g⁻¹ in *Amaranthus albus* L. and 227.42x10⁵ g⁻¹ in *Polygonum aviculare* L. These results are in accordance with previous research [24], but differs from recent data [21], which report significantly higher number of bacteria. The lowest number of bacteria was registered in samples of waste material.

In most of the samples higher abundance of ammonification bacteria in rhizosphere was noticed compared to surrounding soil (Table 3). Positive effect of plants on the abundance of ammonification bacteria was also previously confirmed [25]. Its highest number was detected in *Equisetum talmateia* Ehrh. surrounding soil, while the lowest number was determined in *Artemisia absinthium* L. On the other side, abundance of sporogenous ammonification bacteria in examined samples was considerably lower comparing to total ammonification bacteria. In waste material abundance of ammonification bacteria was lower comparing to other samples, while sporogenous bacteria were not detected.

Oligonitrophiles use small amount of organic N and partially are capable of using the atmospheric nitrogen for biosynthetical processes and they also play important role in nitrogen cycle in soil [26]. Similar to the previous results, abundance of oligo-

nitrophiles was lowest in waste material samples, while *Azotobacter* sp. was not detected in the same samples. In most of samples, oligonitrophiles number in rhizosphere is higher than in surrounding soil. The highest number in surrounding soil was obtained in *Potentilla reptans* L. and the lowest in *Plantago lanceolata* L. The abundance of this group of micro-

organisms in rhizosphere was lowest in *Polygonum lapathifolium* L. and highest in *Artemisia absinthium* L. The values of oligonitrophiles abundance in our research are higher comparing to the results obtained from previous researches [26]. *Azotobacter* sp. Abundance in all the examined samples was relatively low (Table 4).

Table 2 - Total and sporogenous bacteria in samples of coal mine fields "Kakanj"

Samples	Total number (CFUx1)		Total sporogenous bacteria (CFUx10 ⁵ g ⁻¹)		
	SBS	R	SBS	R	
Waste material (0-20 cm)	23.84	-	0.12	-	
Waste material (20-40 cm)	13.50	-	0.10	-	
Amaranthus retroflexus L.	77.45	96.26	0.48	4.09	
Matricaria inodora L.	72.67	100.40	0.42	0.52	
Saponaria officinalis L.	43.15	121.90	9.88	6.00	
Equisetum talmateia Ehrh.	161.67	159.14	3.37	1.83	
Plantago lanceolata L.	54.42	186.82	0.21	3.79	
Polygonum aviculare L.	227.42	173.06	10.95	4.22	
Polygonum lapathifolium L.	93.52	98.60	0.17	3.77	
Tussilago farfara L.	110.35	299.60	0.19	7.03	
Potentilla reptans L.	75.44	104.95	8.25	6.90	
Doronicum sp. L.	51.21	64.64	1.89	3.20	
Artemisia absinthium L.	56.47	165.00	4.82	2.64	
Xanthium italicum Mor.	53.17	69.24	1.48	1.21	
Populus nigra L.	50.77	57.09	0.38	3.09	
Amaranthus albus L.	26.84	65.44	0.33	2.75	

Table 3 - Ammonification bacteria in samples of coal mine fields "Kakanj"

Samples	Total number of amon (CFUx10		Sporogenous amonification bacteria (CFUx10 ⁵ g ⁻¹)		
	SBS	R	SBS	R	
Waste material (0-20 cm)	2.80	-	0.00	-	
Waste material (20-40 cm)	2.21	-	0.00	-	
Amaranthus retroflexus L.	23.97	28.16	2.01	1.24	
Matricaria inodora L.	28.78	31.04	0.84	0.17	
Saponaria officinalis L.	25.03	40.90	1.25	0.80	
Equisetum talmateia Ehrh.	112.08	46.34	1.84	1.29	
Plantago lanceolata L.	24.11	17.24	1.47	2.08	
Polygonum aviculare L.	20.71	16.76	3.13	2.91	
Polygonum lapathifolium L.	8.91	13.97	0.44	1.38	
Tussilago farfara L.	51.69	40.92	0.10	2.28	
Potentilla reptans L.	36.25	86.72	2.50	2.21	
Doronicum sp. L.	30.96	47.83	2.16	0.73	
Artemisia absinthium L.	4.20	139.70	1.11	2.42	
Xanthium italicum Mor.	24.80	73.98	0.52	0.09	
Populus nigra L.	29.80	39.91	0.56	0.27	
Amaranthus albus L.	15.66	15.99	1.08	1.72	

Fungi play an important role in water dynamics, nutrient cycling and organic matter conversion into available forms [27]. The highest number of fungi, in surrounding soil, was obtained in *Matricaria inodora* L. and *Saponaria officinalis* L., as well as in rhizosphere of *Artemisia absinthium* L. The abundance of

actinomycetes, which play important role in degradation of organic matter [28], was notably lower than the number of fungi in most of the tested samples (Tab. 4). Uniformly with fungi abundance, highest number of actinomycetes in surrounding soil and rhizosphere was noticed in *Matricaria inodora* L. and

Artemisia absinthium L., respectively. The obtained amount of fungi and actinomycetes was notably lower than in previous research [24], but higher comparing to the results of their abundance in "Kolubara" coal mine fields [26].

Table 4 - Oligonitrophiles and Azotobacter sp. in samples of coal mine fields "Kakanj"

Comples	Oligonitrophiles (CFUx10 ⁵ g ⁻¹)		Azotobacter sp. (CFUx10 ³ g ⁻¹)		
Samples	SBS	R	SBS	R	
Waste material (0-20 cm)	9.85	-	0.00	-	
Waste material (20-40 cm)	8.22	-	0.00	-	
Amaranthus retroflexus L.	25.49	45.85	55.15	37.81	
Matricaria inodora L.	17.30	40.35	13.29	75.65	
Saponaria officinalis L.	27.52	34.80	159.90	67.50	
Equisetum talmateia Ehrh.	25.57	25.16	58.03	50.27	
Plantago lanceolata L.	11.26	33.35	62.37	74.13	
Polygonum aviculare L.	26.14	36.24	69.49	79.05	
Polygonum lapathifolium L.	22.53	17.83	93.65	53.53	
Tussilago farfara L.	27.39	35.96	53.00	70.34	
Potentilla reptans L.	43.02	36.04	9.23	11.39	
Doronicum sp. L.	38.43	34.72	9.90	4.00	
Artemisia absinthium L.	20.14	143.00	12.67	3.85	
Xanthium italicum Mor.	25.24	41.56	0.22	2.16	
Populus nigra L.	25.95	28.73	9.40	7.73	
Amaranthus albus L.	14.75	55.98	5.39	3.22	

Table 5 - Fungi and actinomycetes in samples of coal mine fields "Kakanj"

Comples	Fungi (CFI	$Jx10^3 g^{-1}$	Actinomycetes (CFUx10 ³ g ⁻¹)		
Samples	SBS	R	SBS	R	
Waste material (0-20 cm)	5,87	-	2,28	-	
Waste material (20-40 cm)	11,76	-	3,32	-	
Amaranthus retroflexus L.	41,06	3,80	37,24	8,56	
Matricaria inodora L.	169,65	145,22	38,83	11,30	
Saponaria officinalis L.	186,03	409,00	6,71	12,00	
Equisetum talmateia Ehrh.	15,34	16,13	12,27	8,60	
Plantago lanceolata L.	115,79	69,16	37,89	26,53	
Polygonum aviculare L.	75,47	39,15	23,93	39,15	
Polygonum lapathifolium L.	31,43	43,19	14,84	14,70	
Tussilago farfara L.	69,69	161,49	30,97	11,89	
Potentilla reptans L.	20,62	16,57	24,97	27,62	
Doronicum sp. L.	8,10	16,02	33,30	42,22	
Artemisia absinthium L.	34,60	464,20	19,77	52,80	
Xanthium italicum Mor.	18,27	13,80	17,40	26,73	
Populus nigra L.	30,09	21,82	32,91	34,55	
Amaranthus albus L.	35,62	154,78	9,94	11,18	

Studies on the dehydrogenase and phosphatases activity are very important parameters of biochemical processes and fertility of soils [29]. The amount of these enzymes is usually correlated with soil conditions [30] and activity of plant roots [31]. The lowest enzyme activity was noticed in waste material samples. Highest dehydrogenase and phosphatase activity (in surrounding soil) was observed in

Saponaria officinalis L. Regarding rhizosphere, the highest phosphatase activity was observed in *Polygonum aviculare* L. Similar values of dehidrogenase activity were obtained previously [32], while the amount of phosphatase activity was lower comparing to the recent observation [33]. Our results differ from ancient research [34], which cited the positive correlation between dehydrogenase activity and bacterial number.

Table 6 - Enzyme	activity	in sampl	les of coal	mine	fields	"Kakani"
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	Dehydrogenase activity (x10 ⁻⁵ μg TPF/g/h)		phosphatase activity (μg pNP/g/h)			
Samples	SBS	R	SBS		R	
			acid	alkaline	acid	alkaline
Waste material (0-20 cm)	0,32	-	0,9	1,1	-	-
Waste material (20-40 cm)	0,22	-	0,8	0,7	-	-
Amaranthus retroflexus L.	1,73	1,68	2,2	1,4	1,2	1,5
Matricaria inodora L.	1,08	4,10	3,2	2,5	2,2	2,1
Saponaria officinalis L.	3,22	3,22	5,3	4,2	1,8	3,2
Equisetum talmateia Ehrh.	1,74	2,08	1,3	1,5	2,5	1,8
Plantago lanceolata L.	1,94	1,22	2,1	1,4	1,9	1,5
Polygonum aviculare L.	1,07	2,49	1,2	2,1	4,2	3,5
Polygonum lapathifolium L.	1,73	1,71	1,8	1,4	1,4	1,4
Tussilago farfara L.	1,65	2,50	1,6	2,2	1,0	1,3
Potentilla reptans L.	0,62	0,47	2,1	1,7	2,1	1,0
Doronicum sp. L.	0,43	0,31	3,1	2,4	1,2	1,1
Artemisia absinthium L.	1,14	0,21	1,4	1,9	0,9	0,7
Xanthium italicum Mor.	0,36	0,80	2,4	2,1	1,3	1,1
Populus nigra L.	1,15	0,57	1,1	1,2	1,0	0,8
Amaranthus albus L.	0,67	0,57	2,3	2,5	1,3	1,5

CONCLUSION

The mining industry has caused environmental devastation in Kakanj. Activities in coal mine exploitation have a notable effect on development of spontaneous vegetation, with absolute dominance of Asteraceae species. It is evident that this type of vegetation has a significant influence on abundance of microorganisms in surrounding bulk soil and rhizosphere. Also, the characterisation of microbial communities could be used as parameter of rehabilitation status of areas associated with coal mine exploitation activities. Thus, plant and microbial communities on these sites have a significant effect on soil fertility enhance and ecological reclamation. Further research will be performed on analyses of plant and microbial species in similar environmental conditions and their role in reclamation process.

Acknowledgement

This research was partially supported by the Ministry of Science and Technologal Development of Serbia, grant number TR 31080, and AREA (Advancing research in agricultural and food sciences at Faculty of Agriculture, University of Belgrade).

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IZVOD

BILJNI I MIKROBNI DIVERZITET ZEMLJIŠTA POD EKSPLOATACIJOM UGLJA U RUDNIKU "KAKANJ" (BOSNA I HERCEGOVINA)

Ekoremedijacija je jedna od metoda za obnavljanje ekosistema i efikasna je za područja pod eksploatacijom uglja. Cilj ovog rada je ispitivanje biljno-mikrobnog diverziteta na području rudnika mrkog uglja "Kakanj" (Bosna i Hercegovina). Determinacija biljnih vrsta obavljena je pomoću ključa za identifikaciju, dok su mikrobiološke osobine zemljišta i otpadnog materijala nastalog pranjem uglja ispitane standardnim metodama. Rezultati ukazuju na dominaciju biljaka iz familije Asteraceae. U najvećem broju uzoraka brojnost bakterija bila je veća u zoni rizosfere u odnosu na okolno zemljište. Zastupljenost gljiva bila je veća u poređenju sa brojem aktinomiceta. Najslabija mikrobiološka aktivnost zabeležena je u uzorcima otpadnog materijala. Determinacija biljnog i mikrobnog diverziteta obezbeđuje informacije značajne za obnavljanje ekosistema narušenog eksploatacijom uglja.

Ključne reči: biodiverzitet, rudnik uglja, mikroorganizmi, biljke

Originalni naučni rad

Primljeno za publikovanje: 21. 08. 2013. Prihvaćeno za publikovanje: 27. 10. 2013.