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Management of environmental quality and Kostolac mine areas natural resources usage

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Abstract

Purpose – This paper aims to define and theoretically analyse the performance of modern methods investigated to speed up the recultivation process of degraded areas, and apply it into the mining basin, open pit mines, tailings and all other areas, which need environmental quality improvement.

Design/methodology/approach – This paper presents methods of conservation and substitution of natural resources for the purpose of faster land recultivation (reclamation) in degraded coal mine areas. Two types of methods have been developed: forest slope recultivation and agricultural recultivation of flat landfills. Indicators of positive changes include: changes in chemical properties of tailings, increase in biogenic properties and changes in biodiversity.

Findings – The research results show that the application of these methods, along with the holistic approach and adequate investment, can significantly contribute to the length of recovery process and accelerate it.

Research limitations/implications – Mining basin Kostolac is used as a model test. The results can be applied on all mining basin, open pits and any degraded area.

Originality/value – All results and conclusions were drawn, based on the original measurements and experiments. Stake holders, wanting to manage environmental quality after exploitation in mining basin, applying those methods, can find the value of this paper.

Keywords Resources, Management, Environmental quality, Ecology, Method

Paper type Research paper

1. Introduction

Soil fertility is important factor in vegetation growth and productivity. The relationship between vegetation and soil fertility is important for both scientific and practical reasons (Jiao *et al.*, 2011). For the realization of sustainable development, it is necessary to ensure the principle of sustainable use of natural resources and the principle of substitution. Over the past two decades, progress has been made towards the development of a balanced range of energy indicators for sustainable development (Stevović *et al.*, 2010), which include the broader themes such as economy, society and environment (Vera and Langlois, 2007). Significance of renewable resources that are being jeopardized by the exploitation of coal, bind to the preserve the value of biodiversity and geodiversity. Careful planning and continual interventions can restore the initial value and purpose of the areas severely devastated by the coal exploitation, such as ash deposits and tailings.

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A number of scientific studies have shown that these areas, tend to restore the biodiversity that existed prior to the exploitation of coal, and that this process lasted for decades and centuries. Through the proper recultivation (reclamation) planning and managing, revitalization processes can be accelerated and it can be ensured that the post-exploitation landscapes become semi-natural, but still attractive and multipurpose for the residents of nearby settlements.

Kostolac coal basin, observed in the broadest sense, is bounded by the Morava River in the west, Golubac Mountain in the east, the Danube River (Mladenović-Ranisavljević *et al.*, 2012) in the north and Mlava basin in the south. In the narrow sense, it covers an area of 100 km^2 with the city of Kostolac in its centre, which is located 80 km southeast of Belgrade. It has natural reserves of lignite, estimated to about 400 million tonnes of lignite coal with four layers of lignite. Its relief is mostly characterized as a plain type, and the highest point does not exceed 300 metres above the sea level, in the radius of 20 km while its climate is temperate continental one. In Kostolac coal basin, coal is being exploited through the open pit method, and the annual production is estimated to approximately 9 million tonnes of coal, which is accompanied by 30 million tonnes of tailing, being indiscriminately disposed around the coal mines. This coal is mostly used in thermal power plant which installed capacity is 1,000 MW, for the generation of power. Today, in Kostolac basin, mining surface, facilities, machinery and tailing dump cover the area of 3,000 ha (Plate 1).

Such challenges in open pits and tailings environments, are reflected through the range of disorders in the domain of geomorphology, pedology, hydrology and ecology (Radosavljević and Radosavljević, 2011). Studies have shown that these areas still tend to auto regenerate (Jalali, 2012). Biocenosis that existed prior to the exploitation of coal in these areas, tended to rebuild themselves, but the natural processes lasted for decades and even centuries (Griffith and Bhutto, 2008).

The exploitation of coal and tailings disposal extinct all of the biocenosis (Stevović *et al.*, 2011), and instead of agricultural and forest land, what remained is severely damaged, unproductive soil. The soil composition and biogenic is significantly altered, with the accumulation of heavy metals (Stevović *et al.*, 2010/2011), and irreversible chemical processes (Stevović *et al.*, 2010/2012).

This paper presents a modern approach to accelerate the recultivation of degraded areas, applied and presented in the example of Kostolac coal basin. Two types of

Plate 1.
A view of open pit mine

methods have been developed: forest slope recultivation and agricultural recultivation of flat landfills Indicators of positive changes include: changes in chemical properties of tailings, increase in biogenic properties and changes in biodiversity with increasing biogenic features. The results show that the application of these methods, with appropriate investment (Obradović *et al.*, 2012) can significantly contribute to the recovery process and accelerate it.

2. Materials and methods

2.1 Site of investigation

The survey was conducted in different locations of open pit coal landfills in Kostolac coal basin, in the period May-June 2012. The most active lignite coal mine was opened back in 1987. Today it is characterized by the annual production of 9 million tonnes of coal with the excavation and disposal of approximately 30 million tonnes of tailings. Immediately after its opening, the first external tailing landfill was generated, so-called southern tailing landfill. Few years later, a temporary recultivation of this landfill was performed. Since then, the tailings are being deposited in the internal landfill (inside the open pit), to the west of the progress of excavation. The extensive recultivation works of the external southern landfill as well as of the internal landfill slopes, began in 2008. Recultivation of flat landfills was performed in several phases, which were initiated after the technical preparation, i.e. grading (Plate 2). Research site (JD01) is a flat, technically prepared, tailing landfill with spontaneous vegetation consisting of *Polygonum lapathifolium* and *Calamagrostis epieios*. Next sampling site (JD02) is an empty slope of internal landfill, which is composed of loess and sand. Sites marked with JD03, JD04 and JD05 are being in various stages of recultivation, in which humat was applied and legumes and grasses sown (Table I). Their composition is characterized as sandy-clayey tailings. At the site JD06, an experimental vineyard was cultivated five years ago, and the preparations are ongoing for cultivation of big, 15-hectare vineyard. Research sites, were also at internal open pit landfill slopes with acacia plantations (JD07) and external landfill slope, recultivated 20 years ago with poplar trees. In poplar forest, the beginning of creation of humus-accumulative layer of soil was observed.

For the purpose of comparison (benchmarking), the research has been made in the agricultural areas next to the tailing landfill, and on the soils planted with corn (ZD01). About a mile from the landfill, remnants of, once widespread flooding, oak forests were

Plate 2.
Technical preparation of tailings

Table I.
Research sites in Kostolac open pit coal mine tailing landfills, type of substrate used, vegetation and recultivation

Mark	Description of substrate	Vegetation type
JD01	Tailings on the external landfill, 5 years, technical preparation, sandy-clay fraction	Spontaneous colonization *Polygonum lapathifolium*, *Calamagrostis epigios*
JD02	Slope of internal landfill, 3 years old, loess and sand	Empty under spontaneous vegetation *Sorgum halepense, Tussilago farfara, Lotus corniculatus*
JD03	The second phase of recultivation, use of humates, 3 year old, sand-clay	*Lotus corniculatus*
JD04	The second phase of recultivation, use of humates, 3 year old, sand-clay	*Trifolium repens*
JD05	The second phase of recultivation, use of humates, 5 year old, sand-clay	*Phleum pratense, Bromus arvensis, Festuca rubra*
JD06	Recultivation by planting grapevines, 5 years old, sand-clay	*Vitis vinifera*
JD07	Slope of the internal landfill, recultivation by acacia forest, 3 years old, loess and sand	*Robinia pseudoacacia*
JD08	Slope of the external landfill, 20 years old poplars forest, sand, clay, humus	*Populus tremula, Rubus cesius*
ZD01	Chernozem soil, the corn crop	*Zea mayis*
ZD02	Alluvial smonitsa, the remaining of the mixed oak-ash tree forest	*Quercus robur*

found, dominated by *Qurcus robur* on old alluvial layers of black earth and extra-zonal vegetation (ZD02). Today, the remaining of these forests are either strip forests or individual trees.

2.2 Chemical analyses

Substrate fir and soil was investigated in five samples (in the surface layer at a depth of 0-10 cm) in all sites. The usual methods of agro-chemical analysis were applied: replacement acidity of pH (in KCl), the percentage of humus by Kotzmann, the total nitrogen in the soil by micro-Kjeldahl method, the quantitatively determining $CaCO_3$, phosphorus and potassium Al-method (Conklin, 2005).

2.3 Analysis of diazotrophic bacteria diversity

The tailing from coal mines is characterized by few microorganisms, and deficit of overall and available forms of nitrogen. Hence, free, asymbiotic nitro-fixators are significant for further process of revitalization of tailings. Three kinds of diazotrophic bacteria have been observed: *Beijerinckia* sp., *Derxia* sp. and *Azotobacter* sp. At each of the above presented ten sites, five samples of tailings and soil were taken from the surface layer and stored in sterile polyethylene bags. Method of agar plates was used for identification and determining concentration of bacteria in the sample. The concentration and taxonomy of *Bijerinckia* sp. Bacteria, was determined using 0.5 ml of soil suspension, diluted 10^{-2} on the Becking (1961) medium and the incubation period of 14-21 days at 28°C. Taxonomic differentiation was based on the results of biochemical and cultural tests (Kennedy and Genus, 1994). Identification and concentration of *Derxia* sp. bacteria was determined by the Jansen medium at dilution of 10^{-2} and incubation period of five to seven days at 28°C (Jensen *et al.*, 1960;

Shankar *et al.*, 1986). Isolation and identification of *Azotobacter* sp. was performed at substrate Ashby medium, without a nitrogen medium (Lü and Huang, 2010). All results were re-calculated and presented in the form of average number of colony forming unit (CFU) of bacteria per g of absolutely dry soil.

2.4. Statistical analysis of data

The data obtained from soil and tailings' chemical analyses were standardized to the mean and standard deviation, by using SPSS (Mihajlović *et al.*, 2010). Species richness index was estimated in the plot. To quantify the diversity of the bacteria species, the Shannon index (H'), as a measure of species abundance and richness, was applied (Shannon, 1948). For each sample it was calculated as:

$$H' = -\sum_{i=1}^{s} p_i \text{In} p_i \tag{1}$$

Where, s equals the number of species and p_i is the relative number of ith species. In addition, the Simpson index (D) and the evenness index (E) are considered as a measure of species dominance and a measure for evenness of spread, respectively (Simpson, 1948). The Simpson index is defined as:

$$D = \sum p_i^2 \tag{2}$$

As biodiversity increases, the Simpson index decreases. Therefore, to get a clear picture of species dominance $D = 1 - D$ is used. Whereas, the evenness index (E) is defined as:

$$E = \frac{H'}{H_{\max}} = \frac{H'}{\text{In} s} \tag{3}$$

Where, $H_{\max}$ is the natural logarithm of the total number of species.

3. Results

Construction and exploitation of coal in Kostolac area (on the right bank of the Danube River) has been increasing from year to year, ever since 1987. This augmented the problem of tailings disposal and its recultivation. During the first decades of exploitation, tailings was dumped in landfills of so-called South-West area. In recent years, with the advancement of the open pit mine to the north and opening a wider front of excavation, the tailings has been stated to be deposited in the interior of the mine dump. This temporarily resolved the issue of tailings deposit, but not the issue of land rehabilitation and restoration of its original purpose and function. Therefore, the first and the oldest tailing landfill from Kostolac coal open pit mine, are being a subject of intensive recultivation in recent years.

From 2007, part of external landfill was technically recultivated in a planned manner. This included: grading and shaping of slopes, by alternating planes and slopes, in order to reforest such land slope with forest trees species, and to use planes for agricultural production. Restoration and recultivation of technically prepared tailings deposits in external landfill until now included biological recultivation by planting the rapeseed (*Brassica napus*) as a green manure or a bio-culture carrier.

Next step is growing the alfalfa legumes (*Medicago sativa*) and clover (*Trifolium repens* and *T. pratense*). In the final stage, a grass seed mixtures will be grown to stabilize the substrate and to accelerate the process of humification. Besides, an experimental vineyard was cultivated in 15 ha, as a part of a larger project of recultivation of the external tailings landfill south for the purpose of agricultural production. Along with the development of open pit mine, an internal landfill was formed. On the slopes of the internal landfill, a recultivation commenced through binding and substrate stabilization by planting acacia.

Due to the high heterogeneity of soil cover this area and the subsequent indiscriminate depositing a small area one can find several types of soil. The largest area deposits formed from land belonging to the class of cambic soil, forest soil type and class of undeveloped land with different texture, sandy and loess-like sandy soil. The defined area of 100 ha in the location where the chemical biological recultivation for its chemical, physical and especially mechanical properties are characterized by a large heterogeneity.

Before the commencement of recultivation, the chemical properties of the active acidity values ranged from 4.19 (acid) to 7.52 (alkaline), which was not a limiting factor for the biological recultivation performance, but was rather considered a stable complex. Tailings deposits are poor in calcium, and the humus deposits were low in some places. Tailings is not rich in nitrogen, and it is low in easily accessible forms of phosphorus and potassium, as well (Table II). During the recultivation of tailings, the irreversible changes in the chemical composition of the substrate occur on the flat deposits of tailings.

After five years and three phases of recultivation (rapeseed-legume-grass), pH changed from acidic to alkaline, the percentage of humus increased ten times, the total level of nitrogen tippled and the concentration of readily available potassium doubled (Table II). According to these parameters, tailings is still poor in NPK complex when compared to Chernozem type of soil that is present in the surrounding area, but it can be used for the agricultural production, fruit, vegetable and vineyard cultivation.

Landfill slopes are covered by forest trees. Old, external tailings landfills are being under the poplar trees since about 20 years ago, while the slopes of internal landfill have been under acacia since about three to five years ago. In poplar forest, there is a change in the level of carbonates; tailings is richer in nitrogen (of average value) and is well supplied with potassium. Compared to oak and ash trees on alluvial soils, which represent the rest of the climax community in the Danube region, the recultivated

Table II.
Results of chemical analyses of studied tailings and soil from Kostolac open pit coal mines

Mark	pH (in KCl)	$CaCO_3$	Humus %	Nitrogen %	P_2O_5 mg/100 g	K_2O mg/100 g
JD01	4.19	0.00	0.097	0.048	5.79	6.50
JD02	7.52	0.00	2.92	0.046	6.79	6.50
JD03	6.17	0.00	0.18	0.009	6.95	11.50
JD04	7.52	0.00	0.35	0.017	6.15	10.00
JD05	7.58	1.34	0.93	0.146	8.19	11.00
JD06	4.61	0.00	0.35	0.017	5.57	5.75
JD07	7.80	0.00	1.02	0.051	6.35	13.00
JD08	7.87	1.17	1.28	0.064	5.44	42.00
ZD01	5.81	0.00	3.34	0.177	5.87	23.75
ZD02	6.87	0.00	13.79	0.69	7.22	46.00

tailings underneath the forest vegetation is, by all parameters, of lower quality. Recultivation of tailings with fast-growing species, holds the potential for: raising forest plantations, timber production of all types and for various purposes, biomass production or sequential assimilation of emissions.

The tailings from coal mines is characterized by poverty in microorganisms, and low in total and affordable forms of nitrogen. Because they are free, asymbiotic fixing bacteria are important to further tailings revitalization process. The following three genea of diazotrophic have been observed: *Beijerinckia* sp., *Derxia* sp. and *Azotobacter* sp. At eight tailings sites and at the two on the ground in their vicinity, 11 species of the above diazotrophs have been identified. *Beijerinckia* sp. were identified in the tropical and subtropical regions, and were found, mostly, in acid soils, but are also present in moderate-continental areas. They are able to fix atmospheric nitrogen in the aerobic and microaerobic conditions, while in unfavourable environment, they form cysts. In Kostolac Region they have never been studied before, so their appearance is certainly significant, although unexpected. Taxonomic differentiation has been based on biochemical and cultural tests, which resulted in identification of species: *Beijerinckia indica var. alba*, *Beijerinckia indica*, *Beijerinckia fluminensis*, *Beijerinckia derxia* and *Beijerinckia mobile*. *Derxia gumosa* is the best known bacteria of the *Derxia* genus, with the ability to fixate nitrogen. Although it is mainly identified in the tropical and subtropical regions, it shows greater tolerance to pH value and can grow at event pH 5-9. On the medium without nitrogen, it showed intensive growth, the colonies were mostly white, and by lowering pH of medium, they became dark red-brown. Molybdenum encourages nitrogen fixation. A potentially new species was observed – *Derxia gumosa var new* which uses ascorbin as a source of carbon. Species of the genus *Azotobacter* sp. were studied the most in soils of moderate-continental climate areas. These include mobile Gram− bacteria (peritrichal flagella) and six species of them have been identified until now. They are fixing atmospheric nitrogen, and in unfavourable conditions they form cyst. It is actually a polysaccharide and aligate capsule which protects them from adverse impacts. They are capable of forming spores as well. Their taxonomic classification has been explored and the following species were determined: *Azotobacter chroococcum*, *Azotobacter beierinckii*, *Azotobacter vivalendi* and *Azotobacter paspali*. Table III summarizes their taxonomic diversity, abundance of species and presence in specific sites.

Name of species	JD01	JD02	JD03	JD04	JD05	JD06	JD07	JD08	ZD01	ZD02
Beijerinckia indica var. alba	6	0	0	0	0	2	0	4	6	0
Beijerinckia fluminensis	0	45	29	20	19	0	20	30	40	23
Beijerinckia derxia	24	0	20	14	11	14	40	11	8	14
Beijerinckia mobile	10	9	0	0	0	5	0	0	0	0
Beijerinckia indica	0	7	0	0	0	0	0	0	0	0
Derxia gumosa	0	40	38	18	34	15	12	10	23	46
Derxia gumosa var. new	32	28	18	0	0	0	75	15	0	0
Azotobacter chroococcum	0	19	15	0	0	0	14	0	0	0
Azotobacter beierinckii	45	47	37	52	63	74	43	58	53	46
Azotobactr vivalendi	0	0	0	0	10	0	0	0	0	0
Azotobacter paspali	12	14	0	0	0	0	10	0	0	0
Number of species (S)	6	8	6	4	5	5	7	6	5	4

Table III. The overall diversity of diazotrophs *Beijerinckia* sp., *Derxia* sp. and *Azotobacter* sp., species' presence in specific sites as the average of $n \times 10^2$ CFU/g d w soil

In tailings landfill of Kostolac open pit mine, four species of *Beijerinckia* genus were identified and one variation. The most spread specie is *Beijerinckia fluminensis* (Plate 3), while occurrence of other species depends on the change in tailings chemical characteristic, and it especially depends on the applied recultivation measures. Different variety of crops used in recultivation, causes slow but noticeable increase in diversity during recultivation. The remoteness/similarity among species *Beijerinckia* sp. After UPGMA and 13 physiological tests was 0.68, which is at the same time measure of γ – diversity of this specific and useful genus of bacteria.

Already mentioned diazotrophic have been settling the empty tailing landfills from the beginning, and measured index of diversity varied from 1.588 to 1.900 (by the Shannon-Wiener index) and from 0.235 to 0.167 (the Simpson index; Table IV). With further recultivation of tailings, changes in composition of diazotrophic species occur as well as the changes in diversity. Species that are constantly present in tailings, and soils in their vicinity are *Beijerinckia fluminensis* and *Azotobacter beierinckii*. A constant increase of the diversity of species in all communities was observed in the tailings, as well as a constant increase in diazotrophic diversity (by the Shannon-Wiener and Simpson; Figures 1 and 2). Diversity index value is affected by the presence of some species such as *Azotobacter vivalendi*, which occurs only in the community JD05 (on sandy-clayey substrate in the second phase of recultivation), thus can be considered a rare species.

Evenness shows a homogeneous representation of species with the value of index ranging from 0.630 to 0.926, i.e. approximately 1 (Table IV) with small deviation (Figure 3). Species homogeneously populate habitats, even when taking successive turns, until a stable climax community is reached, in site ZD02, i.e. in the soil under the oak tree forest.

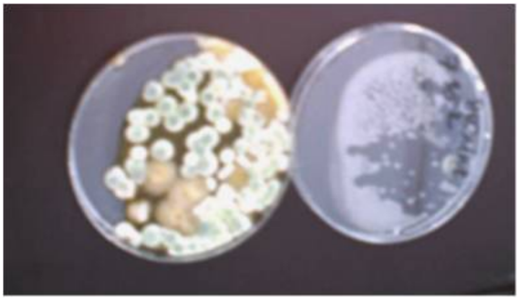

Plate 3.
Beijerinckia fluminensis, *Beijerinckia mobile i Beijerinckia derxia* from Kostolac open pit mine tailings, diluted 10^{-2} on the Becking medium without nitrogen

Table IV.
Different diversity indices for diazotrophic bacteria in the barren soil communities and forest and crop soils in the vicinity

	JD01	JD02	JD03	JD04	JD05	JD06	JD07	JD08	ZD01	ZD02
Shannon-Wiener (H)	1.588	1.900	1.731	1.237	1.370	1.014	1.708	1.468	1.348	1.283
Species richness (S)	6	8	6	4	5	5	7	6	5	4
Total abundance	129	209	157	104	137	110	204	128	130	129
Simpson diversity index (D)	0.235	0.167	0.187	0.335	0.304	0.489	0.216	0.288	0.298	0.298
Index dominance (D)	0.765	0.832	0.813	0.665	0.696	0.510	0.783	0.711	0.701	0.702
Evenness (E)	0.886	0.914	0.966	0.892	0.852	0.630	0.877	0.819	0.838	0.926

Changes in diazotrophic diversity in tailings depend on the changes in its physical-chemical conditions. During the recultivation, which was studied, application of humates, fertilizer and cultivation of certain plant species cause progressive increase in organic matter in the tailings (Table II). These changes are observed as percentage of increase of humate substances from empty, uncultivated to recultivated tailings landfills. The highest percentage of humate matters are found in the soil in their vicinity. Following this gradient, diazotrophic diversity

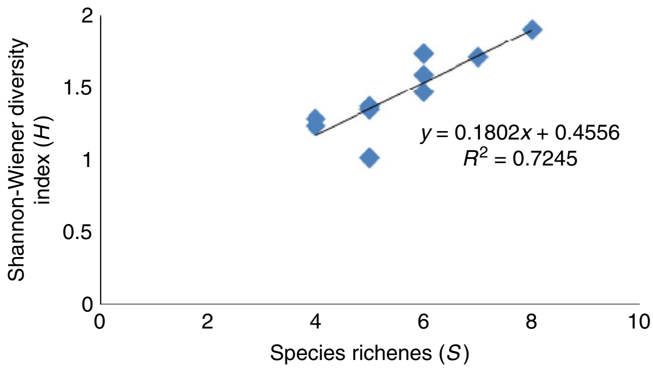

Figure 1.
Dependence between the value of Shannon-Wiener diversity index (H) and the richness of species (S)

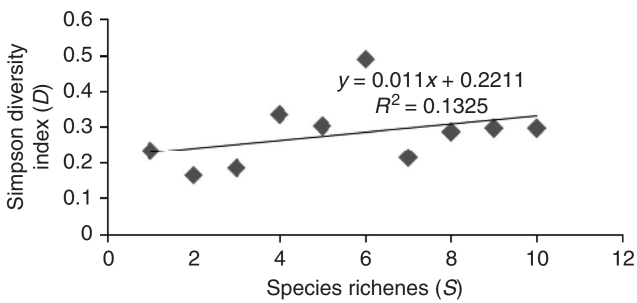

Figure 2.
Dependence between the value of Simpson diversity index (D) and the richness of species (S)

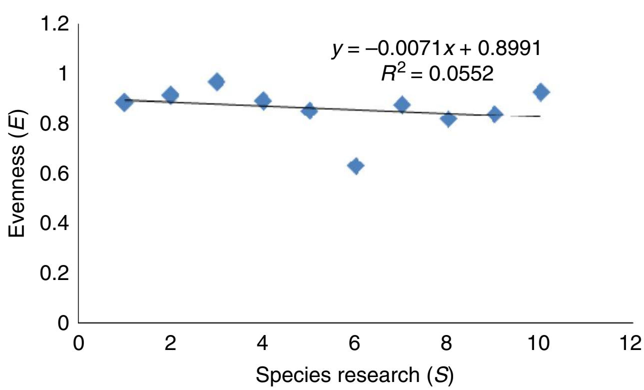

Figure 3.
Dependence between evenness (E) in communities of diazotrophs and the richness of species (S)

rapidly increases, and then has a constant value and it approaches a stable climax community (Figure 4). Humate substances stimulate the increase in diversity and concentration of diazotrophic, because they provide with matters, necessary for the respiratory metabolism, which creates special conditions for the fixation of atmospheric nitrogen.

4. Discussion

Exploitation of coal causes numerous and profound changes in ecosystems, which binds us to bring the disturbed system back to its original purpose and function, as much as possible. Other renewable resources that are being disrupt by human, bind us to preserve the value of biodiversity and geodiversity. Tailings from the exploitation of coal from open pit mines are of poor quality, which pedogenetic processes can last for centuries. Therefore, the technology of melioration and recultivation of land, degraded by the coal exploitation, was intensified in the twenty-first century, with a purpose to speed up the reversal of negative effects and to bring it back to its original purpose and function.

In our example, tailings from Kostolac mine are composed of clay or loess sand with following characteristics: active acidity vary from 4.19 (acid) to 7.52 (alkaline), low in calcium, low in humic substances, not supplied with enough nitrogen, low in easily accessible forms of phosphorus and potassium. Recultivation is being conducted in two directions: for the agriculture land in flat areas on technically prepared landfills and forest recultivation on the slopes. New recultivation technology implies a combination of organic fertilizers (humus) and seeding a mix of cultures (canola, legumes, grasses), as well as planting fast-growing trees (such as acacia and poplar).

During the recultivation of tailings on flat tailings landfills, irreversible changes in the chemical composition of the substrate occur. After five years and three phases of recultivation, pH changes from acidic to alkaline, percentage of humus increases ten times, the total nitrogen triples, while the concentration of readily available potassium doubles. Thus, the physical and chemical conditions on flat tailings landfills change in the direction of restoring the ecosystem and restoring the original purpose and function of the land, i.e. fit for agricultural production. Other researches came to similar conclusions in various land rehabilitation programmes and presented similar results (Mummey *et al.*, 2002; Munshower, 1993).

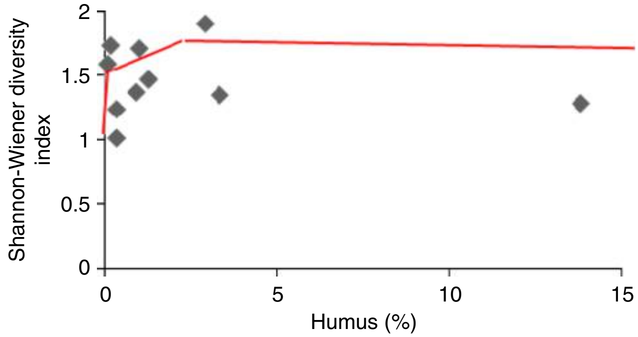

Figure 4.
Dependence between the value of Shannon-Wiener index of diversity and the content of humic substances, in all communities in tailings and soil

Land on the slopes of tailings landfills is composed of sandy loess, and it is characterized as of poor quality, and subject to water or any other type of erosion (Milosevic *et al.*, 2013). Hence, the most effective form of recultivation is by planting the fast-growing tree species (acacia and poplar). Within only five years of acacia planting, the land stabilizes and the proportion of available potassium increases. In total, 20 years after the planting of poplar, land is changing and humus layer is being created, the proportion of available nitrogen and potassium increases and stabilization and creation of forest litter is observed. These irreversible changes bring tailings landfills slopes closer to the original forest community, which existed in the area and enable planting and cultivation of plantation forests, orchards and vineyards (Boca *et al.*, 1984; Karlen *et al.*, 1997).

In the process of recultivation of tailings, the aim is to achieve the biodiversity complex that exists in the immediate surroundings, in places that are not devastated by the exploitation of coal. Diversity of species is observed through the concentration of species, the total number of species present and evenness of distribution (Trevors, 1998; Ovreas, 2000). Traditionally, research on diversity include the relative diversity of the communities in relation to the gradient of stress, impairment or other biotic and abiotic factors (Hughes *et al*,. 2001). It is difficult to study the true diversity using current techniques, so it is a common approach to study the diversity of a functional or taxonomic group. Scientists will often attempt to reduce the information obtained through the research on diversity to discrete, numerical measurements, such as the diversity index (Atlas and Bartha, 1993).

Nitrogen in tailings from the coal mines is low, which creates a big problem or stress to the plants used in the recultivation process. The annual rate of accumulation of nitrogen in the soil, through bacteria, in natural and undisturbed conditions, is about 100 kg N ha^{-1} (Killham, 1994). In fact, 80 per cent of nitrogen are being effectively assimilated by symbiotic bacteria (*Rhizobium*) in the nodules of legumes. At the tailings in Kostolac, the presence of symbiotic nitrogen-fixing bacteria is insignificant, because tailings are almost sterile after its removal and disposal. Strains of *Rhizobium* bacteria are also absent (Djordjevic-Miloradovic *et al.*, 2012). Therefore, in this paper, we focus on asymbiotic, so-called free nitrogen-fixing bacteria, diazotrophe, which, to a lesser extent, but still significantly fixate atmospheric nitrogen. Species of *Beijerinckia* sp. genus were discovered in tropical and subtropical regions, and were mostly present in acid soils, but were also found in moderate-continental areas as well. They are able to fix atmospheric nitrogen in the aerobic and microaerobic conditions, and in unfavourable environments they form cysts. In Kostolac Region they have never been studied before, so their appearance is certainly significant, although unexpected. Taxonomic differentiation has been based on biochemical and cultural tests, which resulted in identification of species: *Beijerinckia indica var. alba*, *Beijerinckia indica*, *Beijerinckia fluminensis*, *Beijerinckia derxia* (Kennedy and Genus, 1994). *Derxia gumosa* is the best known bacteria of the *Derxia* genus, with the ability to fixate nitrogen. Although it is mainly identified in the tropical and subtropical regions, it shows greater tolerance to pH value and can grow at event pH 5-9. On the medium without nitrogen, it showed intensive growth, the colonies were mostly white, and by lowering pH of medium, they became dark red-brown. Molybdenum encourages nitrogen fixation. A potentially new species was observed – *Derxia gumosa var new* which uses ascorbin as a source of carbon. Species of the genus *Azotobacter* sp. were studied the most in soils of moderate-continental climate areas. These include mobile

Gram− bacteria (peritrichal flagella) and six species of them have been identified until now. They are fixing atmospheric nitrogen, and in unfavourable conditions they form cyst. It is actually a polysaccharide and aligate capsule which protects them from adverse impacts. They are capable of forming spores as well. Their taxonomic classification has been explored and the following species were determined: *Azotobacter chroococcum*, *Azotobacter beierinckii*, *Azotobacter vivalendi* and *Azotobacter paspali* (Pietrzykowski and Krzaklewski, 2007). All researchers agree that in one area or one region, α-diversity is the best to be studied on a specific taxonomic or functional group of organisms.

Through this research, 11 species of diazotrophs were investigated, in the Kostolac Region, in the tailings from the coal mine, on one hand, and in relatively stable and undisturbed systems, on the other. Obtained values for diversity index varied from 1.588 to 1.900 (by the Shannon-Wiener index) and from 0.235 to 0.167 (the Simpson index). Similar fluctuations were notices in other considerations of diversity (Jensen *et al.*, 1960). During the process of further recultivation of tailings, changes in species composition and changes in diazotrophic diversity were observed. Species that are constantly noticed as present in tailings and soil in their vicinity are *Beijerinckia fluminensis* and *Azotobacter beierinckii.* Continual increase in species' diversity has been observed in tailings as well as constant increment in diversity of diazotrophs. Species homogeneously populate habitats, even when taking successive turns, until a stable climax community is reached, in site ZD02, i.e. in the soil under the oak tree forest. According to Whittaker (1972), an increase in diversity is always accompanied by the increase in number of species, which in the long-term share the resources. Recultivation of tailings is accelerating the processes of shifts in species richness and diversity index. It is common practice to observe diversity along a certain gradient (time, resource, elevation, humidity, area, etc.; Hughes *et al*,. 2001). In this study, it was demonstrated that the increase in the amount of humus substance (by adding organic fertilizer, humus, zeolite or growing grass and leguminosa), rapidly affected increase of diversity, and then the approximation of its value towards the value of stable communities in its surroundings.

The question is imposed, why the increase of humic substances stimulates the increase of diversity. The explanation of this phenomenon within the investigated diazotrophs, is in their physiology and genetics. Mentioned diazotrophs fix nitrogen from the atmosphere, in aerobic soil condition, through the enzyme nitrogenase. The nitrogenase enzyme's activity is conditioned by the existence of microanaerobic conditions. Mentioned bacteria achieve these conditions in the cytoplasm, thanks to the increased respiration. Hence, by consuming organic substances while breathing, they create microanaerobic conditions and activate the nitrogenase enzyme (Duc *et al*, 2009).

5. Conclusion

This paper presents methods of usage and substitution of natural resources in recultivation of areas degraded by the coal mining. In the area of Kostolac coal mine's external landfill, a planned technical recultivation was carried out, which included grading and shaping of slopes, by alternating planes and slopes, in order to reforest such land slope with forest trees species, and to use planes for agricultural production. Restoration and recultivation of technically prepared tailings deposits in external landfill until now included biological recultivation by planting the rapeseed

(*Brassica napus*) as a green manure or as a bio-culture carrier. Next step is growing the alfalfa legumes (*Medicago sativa*) and clover (*Trifolium repens* and *T. pratense*). In the final stage, a grass seed mixtures will be grown to stabilize the substrate and to accelerate the process of humification.

The landfill slopes are recultivated with forest. Old external landfills slopes are under the 20 years old poplar trees, and the inner slopes, while the internal landfill slopes were recultivated by planting acacia. In our example, tailings from the Kostolac mine are composed of clay or loess sandy of active acidity ranging from 4.19 (acid) to 7.52 (alkaline), low in calcium, low in humic substances, not supplied with enough nitrogen, low in easily accessible forms of phosphorus and potassium.

During the recultivation of tailings, the irreversible changes in the chemical composition of the substrate occur on the flat deposits of tailings and biogenic features. After five years and three phases of recultivation (rapeseed-legume-grass), pH changed from acidic to alkaline, the percentage of humus increased ten times, the total level of nitrogen tippled and the concentration of readily available potassium doubled. Thus, the physical and chemical conditions on flat tailings landfills change in the direction of restoring the ecosystem and restoring the original purpose and function of the land, i.e. fit for agricultural production. In total, 20 years after the poplars are being planted, the beginning of creation of humus-accumulative layer of soil was observed, increasing the proportion of available nitrogen and potassium. Stabilization and education of forest litter is also being observed. These irreversible changes bring tailings landfills slopes closer to the original forest community, which existed in the area and enable planting and cultivation of plantation forests, orchards and vineyards. In the process of recultivation of tailings, the aim is to achieve the biodiversity complex that exists in the immediate surroundings, in places that are not devastated by the exploitation of coal.

Through this research, 11 species of diazotrophs were investigated, in the Kostolac Region, in the tailings from the coal mine, on one hand, and in relatively stable and undisturbed systems, on the other. Obtained values for diversity index varied from 1.588 to 1.900 (by the Shannon-Wiener index) and from 0.235 to 0.167 (the Simpson index). A constant increase of the diversity of species in all communities was observed in the tailings, as well as a constant increase in diazotrophic diversity. In this study, it was demonstrated that the increase in the amount of humus substance (by adding organic fertilizer, humus, zeolite or growing grass and leguminosa), rapidly affected increase of diversity, and then the approximation of its value towards the value of stable communities in its surroundings.

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