

USE OF HIGH-ENERGY IONIZING RADIATION FOR MICROBIOLOGICAL DECONTAMINATION OF COASTAL SOIL IN THE KOLUBARA RIVER BASIN, SERBIA

by

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The Kolubara river pollutes the coastal land in the river basin and makes it unsuitable for agricultural activities in that area. Also, contaminated land poses a risk to the environment. Different methods can be used for soil decontamination. These methods include biological treatment/bioremediation, chemical oxidation, soil stabilization, physical methods, such as soil leaching, or treatment with high-energy ionizing radiation. Gamma irradiation of soil is a well-known method of inhibiting microbial activity. This paper investigated the influence of different doses and dose rates of gamma irradiation on microorganisms' decontamination of coastal soil, in the Kolubara river basin. The irradiation effects on reducing the total number of microorganisms and removing mold and pathogenic bacteria from soil samples were examined. Gamma radiation affects the soil's organic matter, causing the formation of free reactive radicals, which act as reducing and oxidizing agents, cleaving C-C bonds, and depolymerizing carbohydrates. It was found that a dose of 3 kGy of gamma radiation, neutralizes all pathogenic bacteria, a dose of 5 kGy deactivates mold in soil samples, and a dose of 10 kGy is optimal to kill all microorganisms in the samples and sterilize exposed soil. The research showed that the dose rate does not significantly affect microbiological decontamination of soil using gamma irradiation. The content of heavy metals in soil was determined, and the obtained values were compared with the remediation limit values prescribed by the regulations. It was concluded that the content of heavy metals in the analyzed soil samples is below the limit of remediation values. The only exception is the slightly increased copper content in one sample. The result of this research is the conclusion that the coastal land from the Kolubara basin can be decontaminated by gamma radiation treatment. This advanced soil treatment technology is available in Serbia because there is an industrial plant for gamma radiation treatment within the Vinča Institute.

Key words: microbiological decontamination, coastal soil, gamma irradiation, Kolubara river

INTRODUCTION

Coastal ecosystems may be exposed to anthropogenic pressures, including pollution from human activities [1]. In recent years, rivers and coastal land pollution have been a growing problem due to rapid population growth and industrial development [2].

Pollutants that flow into rivers and accumulate in coastal soil are increasingly investigated [3, 4]. The most common pollutants are organic matter and heavy metals, in various forms in coastal soil [5]. Organic matter consumes oxygen during decomposition. The resulting anaerobic conditions generate hydrogen sul-

fide and methane that negatively affect the environment [6]. Heavy metals can be adsorbed during the decomposition of organic matter and later eluted by desorption, dissolution, substitution, hydrolysis, or microbial activity. Heavy metals in soil can negatively affect the aquatic ecosystem and water quality [7]. Heavy metals can easily dissolve in water and be absorbed by aquatic organisms, such as fish and invertebrates, causing many biological effects, from essential to living organisms, to deadly ones [8]. They can cause toxic effects that interfere with organisms' growth, metabolism, or reproduction, with consequences for the entire trophic chain, including humans [9].

The Kolubara river is positioned in west Serbia. It is about 100 km long and rises from two rivers, Obnica

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and Jablanica, near the town of Valjevo. The Kolubara river basin covers an area of 3650 km². The basin is rich in lignite deposits. The main tributaries of the river Kolubara are Rabas, Kladnica, Tamnava, Gradac, Banja, Lepenica, Ribnica, Toplica, Ljig, Peštan, Turija, Ub, Lukavica, and Beljanica. Its maximum flow at the confluence with the Sava River is about 28 m³s⁻¹ [10]. A graphic representation of the river Kolubara and its main tributaries is shown in fig. 1.

Due to the developed industry in this area, the proximity of the lignite mine, and insufficient care for environmental protection, the water in the river Kolubara contains significant pollution. Previous studies have reported environmental pollution in the Kolubara river area [11, 12].

Water can be polluted from several sources, from wastewater treatment plants and factories to mining activities, paved roads, and agricultural runoff.

Contaminated water washes the necessary nutrients from the surrounding soil. Water pollution makes the soil acidic and negatively affects the solubility of nutrient ions [13]. Also, many heavy metals and microorganisms from the water may penetrate the soil and stay there, making this land unsuitable for agricultural activities [14]. Microorganisms from water often end up in coastal soil, which can be adsorbed by clay minerals or organic matter. These compounds tend to gradually degrade in the soil biochemically. Heavy metals in the soil are toxic in higher concentrations. Their solubility depends on environmental factors. The solubility of heavy metals in the soil is mostly affected by the pH of the solution, the total metal content, the total carbon content, and the oxide content in the soil.

Microorganisms can be found everywhere in our environment. They inhabit air, water, land and live in all habitats of our environment. Most microorganisms



Figure 1. Map of the Kolubara river basin
(Substrate map: software SAS.Planet.Nightly.201020.10106, Base map: Bing Maps-satellite zoom 15)

do not cause disease. Instead, they maintain the fertility of the soil, degrade wastes in landfills and compost piles. Only 1 % of total microorganisms are pathogenic or disease-causing bacteria.

Recently, high-energy ionizing radiation has been used as an innovative method for remediation of contaminated soil [15-17]. Gamma radiation can remove pathogens, molds, and total microorganisms from the treated soil [18, 19].

This paper determined the presence of pathogenic bacteria *Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*.

Escherichia coli (*E. coli*) is bacteria found in people and animals' environments, foods, and intestines. *E. coli* in the soil indicates the possible presence of disease-causing bacteria, viruses, and protozoans. The acceptable level of *E. coli* in soil is 1.26 cfug⁻¹ (colony-forming units in one

gram) [20]. *Staphylococcus aureus* (*S. aureus*) is a toxigenic pathogen [21, 22] and is a significant cause of numerous diseases [23, 24], including toxic shock syndrome, meningitis, and septicemia [25]. The presence of this bacteria in agricultural soil is not allowed [26]. *Pseudomonas aeruginosa* (*P. aeruginosa*) is a Gram-negative, rod-shaped bacteria. It can cause disease in plants, animals, and humans [27]. The presence of this bacteria in agricultural soil is forbidden [28].

This research aimed to optimize the most appropriate radiation dose and dose rate suitable for the inactivation of all pathogens from contaminated soil along the basin of the Kolubara river. The influence of different doses and dose rates of gamma radiation on reducing the total number of microorganisms in the samples of the coastal soil of the Kolubara river was determined. The radiation dose required for complete

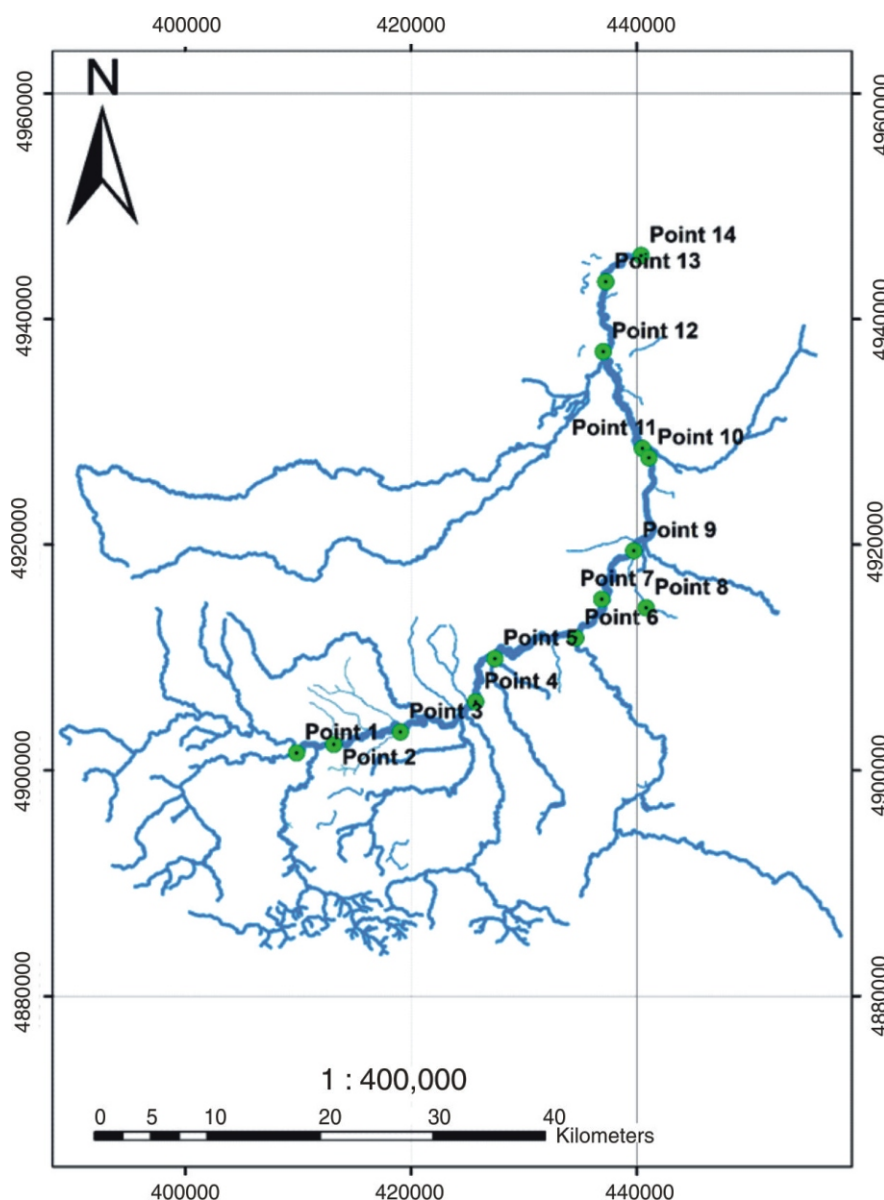


Figure 2. Coastal soil sampling points from the Kolubara river basin

sterilization of the soil was also determined. Finally, the content of heavy metals in the samples from the most crucial sampling points was determined. Based on these results, the possibilities of using soil treated with gamma irradiation in agriculture were examined, considering the directives on soil protection [29, 30].

MATERIALS AND METHODS

Sample collection

A sampling of soil was performed on an area of 3600 km² in the Kolubara river basin, for one month, with average daily temperatures of 20.2 °C, average daylight of 11.75 hours, average humidity of 69 %, and an average rainfall of 32 mm. Samples were collected at 14 key points: at the spring of the Kolubara river and its confluence with the Sava river and 12 points with the most significant pollution was expected, fig. 2. Samples were collected from depths of 20 cm and 50 cm from the surface. The weight of the sample each sample was 2 kg. After sampling, soil samples were sieved through a 2 mm diameter mesh [31] and stored in a dry and dark place.

Description of sampling points:

- Sampling point 1 (UTM coordinates: E 409870 m, N 4901541 m, altitude 193 m) – at the confluence of two constituent rivers: Obnica and Jablanica, which form the Kolubara river in the center of the city of Valjevo.
- Sampling point 2 (E 413176 m, N 4902302 m, altitude 172 m) – on at the location of the urban part of the city of Valjevo.
- Sampling point 3 (E 419073 m, N 4903407 m, altitude 150 m) – near the confluence of the Banja river and the Kolubara river.
- Sampling point 4 (E 425744 m, N 4906085 m, altitude 131 m) – at a rural location, one kilometer upstream the Ribnica river and the Lepenica river flow into the Kolubara.
- Sampling point 5 (E 427448 m, N 4909905 m, altitude 123 m) – where the Toplica river flows into the Kolubara.
- Sampling point 6 (E 434611 m, N 4911729 m, altitude 112 m) – near the confluence of the Ljig river and the Kolubara river.
- Sampling point 7 (E 436902 m, N 4915180 m, altitude 100 m) – the local road where it is possible to cross the Kolubara river. Visible traces of gravel exploitation.
- Sampling point 8 (E 440828 m, N 4914415 m, altitude 99 m) – sampled at a location downstream from the settlement of Lazarevac. The Lukavica river flows through the settlement of Lazarevac. Pollution is noticeable (biological waste, turbidity of water).
- Sampling point 9 (E 439755 m, N 4919475 m, altitude 94 m) – a sample was taken at the confluence

of the Peštan river in Kolubara. The Peštan river and the Kolubara river have been displaced from their natural flows in this area in the past, due to the need for lignite exploitation.

- Sampling point 10 (E 441087 m, N 4927716 m, altitude 86 m) – sampled at the location downstream from the Kolubara Mining Basin (open mines where lignite is exploited).
- Sampling point 11 (E 440513 m, N 4928554 m, altitude 86 m) – where the Beljanica river flows into the Kolubara river. A few kilometers upstream, the Thermal Power Plant Kolubara is located.
- Sampling point 12 (E 437052 m, N 4937122 m, altitude 76 m) – the coast is difficult to pass due to the abundant vegetation. Sampling was carried away from the coast due to an impassable approach.
- Sampling point 13 (E 437258 m, N 4943323 m, altitude 74 m) – at the location where the highway *Miloš the Great* crosses the Kolubara river, a few kilometers north of the settlement of Obrenovac.
- Sampling point 14 (E 440398 m, N 4945644 m, altitude 70 m) – at the confluence of the Kolubara river and the Sava river. There is an industrial zone and many factories (Eko-Dunav, Mei Ta Europe, MTE Automobile Parts Factory).

Gamma irradiation

Irradiation of soil samples was performed in the Radiation Unit of the Vinca Institute of Nuclear Sciences. All samples were irradiated with different doses of gamma radiation: 1 kGy, 3 kGy, 5 kGy, and 10 kGy, and with different dose rates: 300 Gy/h, 1 kGy h⁻¹, and 10 kGy h⁻¹. An ethanol-monochlorobenzene dosimeter was used for dosimetric control of the absorbed radiation dose [32, 33]. The measuring equipment was an instrument oscillotitrator type OK-302/2 of the company Radelkis (Budapest, Hungary). Since the accuracy of the oscilloscope measurement [34] is highly dependent on the measurement temperature [35], all dosimeters are thermostated to 25 °C. Measurements were performed at a constant temperature. Traceability with the primary standard was achieved via transfer dosimeters sent to the Risø High Dose Reference Laboratory from Denmark.

Microbiological analysis

The initial contamination and the total number of microorganisms, molds, and bacteria in the soil before irradiation and after gamma irradiation with different radiation doses were examined in the accredited microbiological laboratory. The method used to determine these parameters was Ph. Eur. 7.0 (2.6.12. – Microbiological examination of non-sterile products (total viable

aerobic count), and 2.6.13. – Microbiological examination of non-sterile products (test for specified microorganisms) (European Directorate for the Quality of Medicines and HealthCare, 2011).

Determination of mold

The molds count was performed by plating 1 mL of decimal dilutions on Sabouraud dextrose agar. Plates were incubated at 25 °C for 5-7 days. For qualitative examination, 10 mL of prepared sample was added to 100 mL of casein soya bean digest broth.

Determination of *E. coli*

For the determination of *E. coli*, 100 mL of broth medium A was used. The solution was homogenized and incubated at 35-37 °C for 18-48 hours. After shaking, broth medium G was added and incubated at 43-45 °C for 18-24 hours. Subculture on plates of agar medium H at 35-37 °C for 18-72 hours. The growth of red, non-mucoid colonies of gram-negative rods indicated the presence of *E. coli*. It was confirmed by appropriate biochemical tests, such as indole production.

Determination of *S. aureus*

For the determination of *S. aureus*, 100 mL of broth medium A was used. The solution was homogenized and incubated at 35-37 °C for 18-48 hours. Subculture on a plate of agar medium O and incubated at 35-37 °C for 18-72 hours. Black colonies of gram-positive cocci, surrounded by a clear zone indicated the presence of *S. aureus*. Confirmation may be affected by suitable biochemical tests such as the coagulase test and the deoxyribonuclease test.

Determination of *P. aeruginosa*

For the determination of *P. aeruginosa*, 100 mL of broth medium A was used. The solution was homogenized and incubated at 35-37 °C for 18-48 hours. Subculture on a plate of agar medium N and incubated at 35-37 °C for 18-72 h. If no growth of microorganisms was detected, the product passed the test. If the growth of gram-negative rods occurred, some material of morphologically different, isolated colonies was transferred to broth medium A and incubated at 41-43 °C for 18-24 hours. The product passed the test if no growth occurred at 41-43 °C.

Determination of the concentration of heavy metals in soil

To determine Cd, Pb, Ni, and Cu content in the soil, the soil samples were dissolved in HF, HClO₄, and HCl. After dissolution, the Ni and Cu content was determined

using AAS (PerkinElmer PinAAcle 900T). For quantitative analyses of As, Hg, and Se, soil samples were dissolved in HNO₃, HClO₄, H₂SO₄, and HCl. After dissolution, the As, Hg, and Se content was determined using the Mercury Hydride System AAS (PerkinElmer PinAAcle 900T). The applied method was chosen according to the previous research on determining the total metal concentration in the sludge [36-38].

For determination of Cd, Co, Pb, Ni, Cr, and Cu, 2 g of dry weight sludge sample was mixed with 15 mL of HF and 5 mL of HClO₄ and heated at 150 °C. The dry residue was dissolved in 10 mL of HCl (1:1) and cooled to room temperature.

To determine As, Hg, and Se, 1 g of dry sample was mixed with 5 mL of HNO₃, with gentle heating. After that, the digestate was cooled to room temperature, 0.5 mL of HClO₄ and 2 mL of H₂SO₄ were added, and dissolution was continued until the appearance of white vapors. The solution was cooled to room temperature again, and 10 mL 6M HCl was added. After cooling, the solution was transferred quantitatively to a 25 mL volumetric flask with distilled water.

RESULTS AND DISCUSSION

Determination of the total number of microorganisms in the soil at different positions of the Kolubara river flow

The total number of microorganisms was determined at 14 key sampling points of the coastal land along the Kolubara river. The results are presented in fig. 3. Similar results have been presented in previous studies [39]. Samples collected at depths of 20 cm and 50 cm are shown.

From fig. 3, one can conclude that soil pollution increases along the river's course as it approaches the confluence with the Sava river. This increasing pollu-

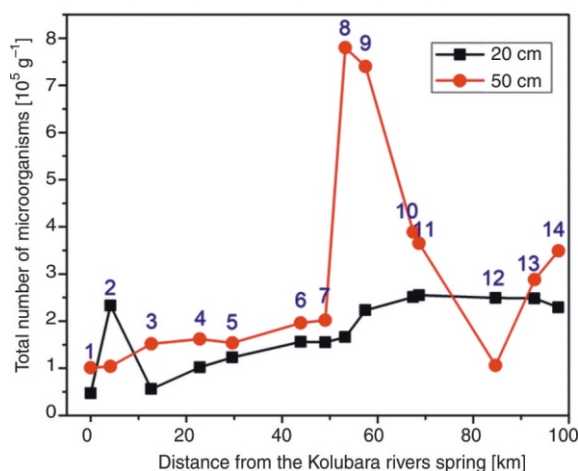


Figure 3. The total number of microorganisms in soil depending on the distance from the spring of the river Kolubara. Numbers 1 to 14 present the sampling points

tion indicates that the Kolubara river, passing through populated places and industrial zones, is becoming increasingly contaminated and pollutes the surrounding coast. Also, soil contamination is higher in the deeper layers because they are more exposed to the Kolubara river. Exceptions are Points 2 and 12. Sample 2 was taken from the urban part of Valjevo, where due to human activities, the surface layer is more polluted than the rest of the sampled points. A sampling at Point 12 was performed farther from the coast due to inaccessible access, so the Kolubara river does not soak deeper layers.

The diagram also shows the peaks in sampling Points 8 and 9, which originate from the increased pollution that the Lukavica river brings from the settlement of Lazarevac.

Microbiological decontamination using gamma radiation

The influence of different doses of gamma radiation on the inactivation of the total number of microorganisms in the key 5 sampling points (near the spring of Kolubara river, near the confluence, and three points with the highest contamination) was determined. Samples from a depth of 50 cm were analyzed. Doses of 1, 3, 5, and 10 kGy were used at an average radiation dose rate of 10 kGyh⁻¹.

The results are presented in fig. 4.

Figure 4 shows that a dose of 5 kGy neutralizes most of the microorganisms from the soil. In comparison, a dose of 10 kGy is sufficient for the complete inactivation of microorganisms from all tested samples. In this way, the soil becomes sterilized. Sterilized soils are used repeatedly to germinate seeds, propagate cuttings, or grow juvenile plants [40, 41]. Seedlings grown in sterilized soils produce higher root biomass and rhizosphere soil (RS) mass than those grown in unsterilized soil. Some researchers showed that soil

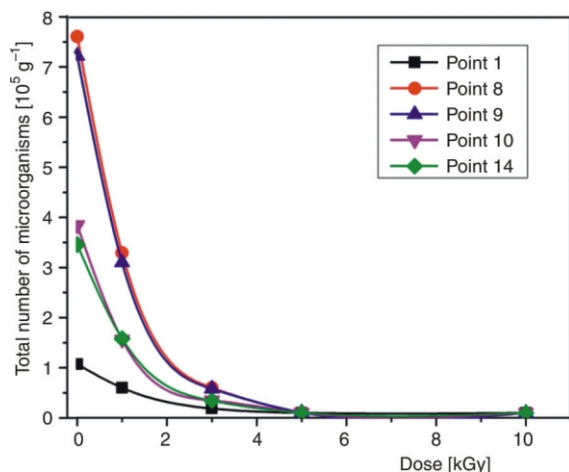


Figure 4. The effect of different radiation doses on the reduction of the total number of microorganisms

sterilization by gamma-irradiation increases wheat seedlings' root growth and RS mass [42].

Influence of different dose rates of the gamma irradiation on microbiological decontamination

In addition to the dose rate of 10 kGyh⁻¹, the effect of lower dose rates, which are often present in commercial irradiation treatment, was also examined. For this purpose, comparing the impact of dose rates of 0.3, 1, and 10 kGy was performed. The test was performed on the most contaminated sample, with initial contamination of 7.8 10⁵ g⁻¹. The obtained results are shown in fig. 5.

From fig. 5 it can be concluded that the rate of soil decontamination decreases with decreasing dose rate. However, these differences are small and practically negligible, so it can be concluded that the dose rate does not play a significant role in the inactivation of microorganisms in the soil.

Determination of the pathogen microorganisms in soil samples

The influence of gamma radiation on the complete neutralization of three pathogenic bacteria (*E. Coli*, *S. Aureus*, and *P. Aeruginosa*) was determined. Five different samples were tested: sample at the spring of river, confluence, and 3 points with the highest pollution in accordance with the results from fig. 3. The results are shown in tab. 1.

Table 1 shows that a radiation dose of 3 kGy is sufficient to remove all pathogenic bacteria from the soil. Previous research has shown that even a dose of 2 kGy is often sufficient to remove all pathogenic bacteria from the soil [43].

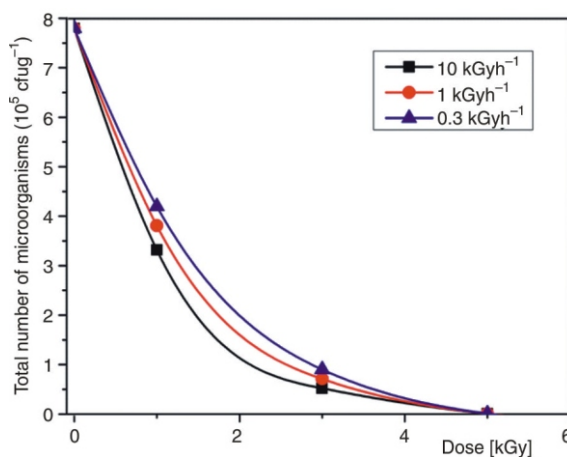


Figure 5. The effect of different gamma irradiation dose rates on microbiological decontamination

Table 1. Content of pathogen bacteria before and after gamma irradiation

Pathogen bacteria	Radiation dose [kGy]	Sampling Point 1 (50 cm)	Sampling Point 8 (50 cm)	Sampling Point 9 (50 cm)	Sampling Point 10 (50 cm)	Sampling Point 14 (50 cm)
<i>E. coli</i>	0	<1	2.56	4.11	1.68	<1
	1	<1	<1	<1	<1	<1
	3	<1	<1	<1	<1	<1
<i>S. aureus</i>	0	Not present	Present	Present	Not present	Present
	1	Not present	Present	Not present	Not present	Present
	3	Not present	Not present	Not present	Not present	Not present
<i>P. aeruginosa</i>	0	Not present	Present	Present	Present	Not present
	1	Not present	Not present	Present	Not present	Not present
	3	Not present	Not present	Not present	Not present	Not present

Content of mold in soil and influence of different doses of the gamma irradiation on molds' decontamination

Mold has been observed in soil samples. The following pathogenic microorganisms can be detected in the mold: *S. aureus*, *P. aeruginosa*, *E. coli*, *Salmonella spp.*, and *Enterobacteriaceae*. Therefore, it is necessary to inactivate mold so that the soil can be used in agriculture.

The mold content was determined at 14 key sampling points of the coastal land along the Kolubara river. The results are presented in fig. 6. Samples collected at depths of 20 cm and 50 cm are shown.

It can be seen from fig. 6 that the amount of mold in the soil does not depend on the position of the soil concerning the Kolubara river. It is assumed that the pollution of the river does not affect the molds' contamination of the coastal soil. The mold content in all the samples has values from 7000 – 25000 g⁻¹.

The effect of gamma radiation on mold inactivation was examined on a sample with the highest value of mold (25000 g⁻¹, sample 8), using radiation doses of 1, 3, and 5 kGy and a dose rate of 10 kGyh⁻¹. The results are shown in the diagram, fig. 7.

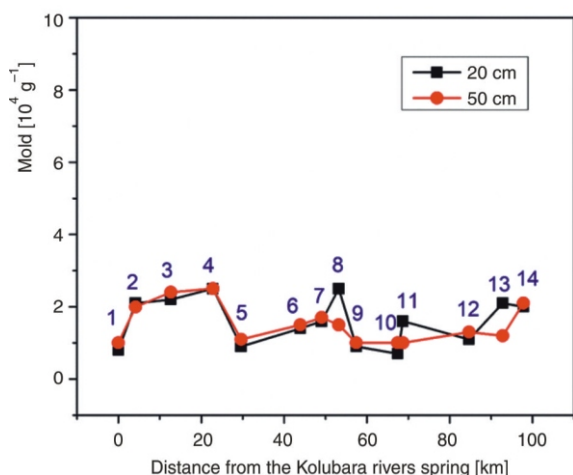


Figure 6. Mold content in soil depending on the distance from the spring of the Kolubara river. Numbers 1 to 14 present the sampling points

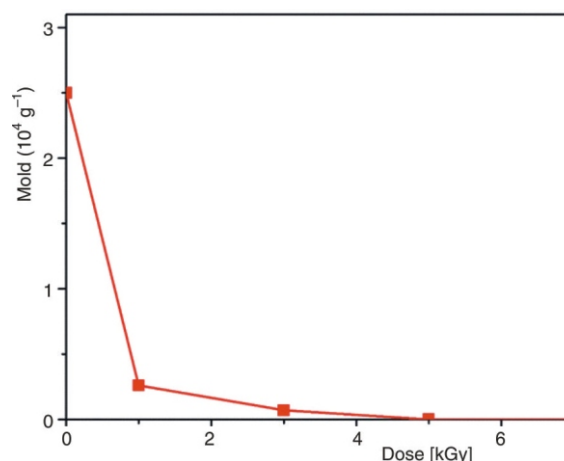


Figure 7. The effect of different radiation doses on the reduction of mold in soil samples

It can be concluded that a dose of 5 kGy is sufficient for the complete inactivation of the mold from the soil sample.

Determination of the concentration of heavy metals in soil

The content of heavy metals was determined in soil samples taken from 5 key sampling points. The effect of heavy metals on soil microbial mainly includes the influence of heavy metals on soil microbial activity, soil enzyme activity, and the composition of soil microbial community [44]. The obtained results were compared with the limit values from the Regulation on limit values of pollutants, harmful and hazardous substances in the soil [45]. This Regulation prescribes the maximum and remediation values of heavy metal content in the soil. The remediation project must be implemented when the average concentration of any polluting, hazardous and harmful soil material exceeds the remediation value. Table 2 shows the content of heavy metals in the sampled soil and remediation values.

From tab. 2, it can be concluded that the content of heavy metals in the analyzed soil samples is below the limit of remediation values. There is no need to remove heavy metals from the soil before microbiologi-

Table 2. The content of heavy metals in different soil samples

Heavy metal		Cadmium	Copper	Nickel	Lead	Zinc	Mercury	Arsenic	Selenium
Sampling point	Depth	mgkg ⁻¹ dry matter							
1	20 cm	1.9	83.5	33.6	100	150	6.2	15.3	<0.1
	50 cm	2.0	82.0	35.7	78	150	6.7	16.0	<0.1
8	20 cm	2.2	182.5	44.2	116	150	8.2	18.5	<0.1
	50 cm	2.2	178.0	45.8	110	165	9.0	18.6	<0.1
9	20 cm	1.9	185.0	46.2	98	145	9.0	16.3	<0.1
	50 cm	2.0	182.0	40.3	96	145	9.1	16.0	<0.1
10	20 cm	1.8	212.0	28.7	84	120	8.2	14.7	<0.1
	50 cm	1.6	209.0	31.3	83	95	8.3	15.1	<0.1
14	20 cm	1.6	165.0	30.2	90	110	7.5	14.1	<0.1
	50 cm	1.6	160.0	26.3	82	120	7.3	13.2	<0.1
Remediation values (mgkg ⁻¹ dry matter)		12	190	210	530	720	10	55	100

cal decontamination. The only exception is the slightly increased copper content in one sample (sampling point 10). The increased concentration of copper at one point probably originates from the mine (sampled at the location downstream from the Kolubara Mining Basin). Before gamma treatment of the soil from that sampling point, remediation should be done to reduce the copper content. Some methods for removing copper from soil include solidification/stabilization, soil washing, vitrification, and flotation [46-48]. After successfully removing copper from the soil, it can be treated with gamma radiation due to microbiological deactivation.

CONCLUSIONS

Kolubara is one of the most polluted rivers in Serbia, mainly because it passes through populated areas, industrial zones, near railways and mines. During its course, Kolubara river is in contact with the surrounding coastal land, both surface and below the surface layers. Kolubara river contaminates coastal soil, which becomes unsuitable for agriculture and the environment in general. The most significant contamination comes from pathogenic microorganisms.

This research aimed to optimize the most appropriate radiation dose and dose rate suitable for the inactivation of all pathogens from contaminated soil. It was determined that the dose of 3 kGy destroys all pathogen bacteria, 5 kGy inactivates mold, and 10 kGy completely sterilizes treated soil. Sterilized soil produces healthier plants, saves time and money, and can benefit the environment. Also, it has been shown that the dose rate does not play a significant role in microbiological decontamination of soil using gamma irradiation. Finally, the content of heavy metals was determined on in soil samples. It was shown that the content of heavy metals in the analyzed soil samples is below the limit of remediation values. The only exception is the slightly increased copper content in one

sample. In this case, remediation to reduce the copper content should be performed before gamma irradiation treatment.

The other methods used for decontamination of soil are biological treatment/bioremediation, chemical oxidation, soil stabilization, and physical methods, like soil washing. The environmental application of gamma irradiation has an advantage over other emerging processes, in that, a good understanding of the underlying chemistry is found in the radiation chemistry literature. The unique feature is that the presence of solids does not comprise the process, thus allowing the potential application of radiation processing to soils. It can also be easily engineered, as a unit process in a treatment system, because reactions destroying pollutants are rapid. Another advantage of gamma irradiation is its capability to kill bacteria and inactivate viruses. At the same time, it is destroying pollutants.

Based on all the obtained results, it can be concluded that the coastal soil from the Kolubara basin can be decontaminated by gamma irradiation treatment. The soil treated in this way has a great potential for application in agriculture.

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AUTHORS' CONTRIBUTIONS

All measurements were performed by V. A. Gajić and I. T. Vujčić. The measurement setup was conceived and prepared by S. B. Mašić. The theoretical analysis was carried out by I. T. Vujčić, G. D. Dražić, and J. M.

Milovanović. All authors analyzed and discussed the results and reviewed the manuscript.

REFERENCES

- [1] Ruiz-Fernandez, A. C., et al., Effects of Land Use Change and Sediment Mobilization on Coastal Contamination (Coatzacoalcos River, Mexico), *Continental Shelf Research*, 37 (2021), Feb., pp. 57-65
- [2] Yang, H. J., et al., Organic Matter and Heavy Metal in River Sediments of Southwestern Coastal Korea: Spatial Distributions, Pollution, and Ecological Risk Assessment, *Marine Pollution Bulletin*, 159 (2020), July
- [3] Islam, M. S., et al., Heavy Metal Pollution in Surface Water and Sediment: A Preliminary Assessment of an Urban River in a Developing Country, *Ecological Indicators*, 48 (2015), Aug., pp. 282-291
- [4] Kalnejais, et al., The Release of Dissolved Nutrients and Metals from Coastal Sediments Due to Resuspension, *Marine Chemistry*, 121 (2010), 1-4, pp. 224-235
- [5] Forstner, U., Wittmann, G. T., *Metal Pollution in the Aquatic Environment*, Springer-Verlag, 1981
- [6] Molamahmood, H. V., et al., The Role of Soil Organic Matters and Minerals on Hydrogen Peroxide Decomposition in the Soil, *Chemosphere* 249, (2020), Feb., 126146
- [7] Hakanson, L., Jansson, M., *Principles of Lake Sedimentology*, Springer-Verlag, Berlin, 1983
- [8] Gheorghe, S., et al., *Metals Toxic Effects in Aquatic Ecosystems: Modulators of Water Quality*, Water Quality, 2017
- [9] Stanković, S., et al., Biota as Toxic Metal Indicators, *Environmental Chemistry Letters*, 12 (2013), 1, pp. 63-84
- [10] Drndarski, N., et al., Stable Isotopes in Periphyton and Sediments from the Kolubara River and its Tributaries, *Environmental Pollution*, 80 (1993), 3, pp. 287-292
- [11] Dragičević, S., et al., Land Use Changes and Environmental Problems Caused by Bank Erosion: A Case Study of the Kolubara River Basin in Serbia, *Environmental Land Use Planning*, 2012
- [12] Samardžić, I., Occupation and Land Degradation Due to Mining Works: Example of the Kolubara Mining Basin, *Zbornik radova – Geografski fakultet Univerziteta u Beogradu (65-1a)*, (2017), pp. 307-318
- [13] Eugenio, N. R., et al., *Soil Pollution: A Hidden Reality*, Food and Agriculture Organization of the United Nations Rome, Italy, 2018
- [14] Hillel, D., *Soil Pollution and Remediation*, Soil in the Environment, 2008, pp. 211-222
- [15] Wen, H.-W., et al., Application of Gamma Irradiation in Ginseng for Both Photodegradation of Pesticide Pentachloronitrobenzene and Microbial Decontamination, *Journal of Hazardous Materials*, 176 (2010), 1-3, pp. 280-287
- [16] Al-Bachir, M., et al., Synergetic Effect of Gamma Irradiation and Moisture Content on Decontamination of Sewage Sludge, *Bioresource Technology*, 90 (2003), 2, pp. 139-143
- [17] Zhang, J., et al., Immediate Remediation of Heavy Metal (Cr(VI)) Contaminated Soil by High Energy Electron Beam Irradiation, *Journal of Hazardous Materials*, 285 (2015), pp. 208-211
- [18] Lessel, T., The Sewage Sludge Irradiation Plant in "Geiselbullach", In: Joint Meeting of American Society of Civil Engineers, Task Committee on Radiation Energy Treatment of Water, Wastewater and Sludges IAEA Coordination Research Group, Arlington, Tex., USA, 1990, pp. 1-21
- [19] Etzel, J. E., et al., Sewage Sludge Conditioning and Disinfection by Gamma Irradiation, *Am. J. Public Health*, 59 (1969), pp. 2067-2076
- [20] Navab-Daneshmand, T., et al., Escherichia coli Contamination across Multiple Environmental Compartments (Soil, Hands, Drinking Water, and Handwashing Water) in Urban Harare: Correlations and Risk Factors, *The American Journal of Tropical Medicine and Hygiene*, 98 (2018), 3, pp. 803-813
- [21] Haagsma, J. A., et al., Systematic Review of Foodborne Burden of Disease Studies: Quality Assessment of Data and Methodology, *Int J Food Microbiol*, 166 (2013), 1, pp. 34-47
- [22] Castro, A., et al., Staphylococcus Aureus, a Food Pathogen: Virulence Factors and Antibiotic Resistance. In *Foodborne Diseases* ed. Holban, AM & Grumezescu AM Academic Press, Massachusetts, 2018, pp. 213-238
- [23] Azara, E., et al., Antimicrobial Susceptibility and Genotyping of Staphylococcus Aureus Isolates Collected Between 1986 and 2015 from Ovine Mastitis, *Vet Microbiol* 205: 2017, June, pp. 53-56
- [24] Fursova, K. K., et al., Exotoxin Diversity of Staphylococcus Aureus Isolated from Milk of Cows with Subclinical Mastitis in Central Russia, *J Dairy Sci.*, 101 (2018), 5, pp. 4325-4331
- [25] Aguilar, J., et al., Staphylococcus Aureus Meningitis: Case Series and Literature Review, *Medicine (Baltimore)*, 89 (2010), 2, pp. 117-125
- [26] Ingham, E. R., *Soil Bacteria (2020)*, Natural Resources Conservation Service Soils – United States Department of Agriculture
- [27] Diggle, S., Whiteley, M., Microbe Profile: Pseudomonas Aeruginosa: Opportunistic Pathogen and Lab Rat, *Microbiology* 166 (2020), 1, pp. 30-33
- [28] Deredjian, A., et al., Low Occurrence of Pseudomonas Aeruginosa in Agricultural Soils with and Without Organic Amendment, *Frontiers in Cellular and Infection Microbiology*, 4, 2014, Apr.
- [29] ***, Council Directive 86/278/EEC, (1986), Official Journal OJ L 181, 6
- [30] ***, Council Directive 91/271/EEC, (1991), Official Journal L 135, pp. 40-52
- [31] Page, A. L., et al., 919820., *Methods of Soil Analysis*, part 2, 2nd edition. American Society of Agronomy, Madison, Wisconsin, USA; 148 (1985), 1, pp. 360-368
- [32] ***, ISO/ASTM 51538 – Practice for Use of the Ethanol-Chlorobenzene Dosimetry System, International Organization for Standardization, 2009
- [33] Kovacs, A., et al., Evaluation methods of the Ethanol – Monochlorobenzene Dosimeter System, *Proceedings, 6th Tihany Symposium on Radiation Chemistry*, 2 (1987), pp. 701-709
- [34] Kovacs, A., et al., Oscillometric and Conductometric Analysis of Aqueous and Organic Dosimeter Solutions, *Radiation Physics and Chemistry*, 46 (1995), 4-6, pp. 1211-1215
- [35] Vujčić, I., et al., Accuracy of Determining Absorbed Irradiation Dose at Different Temperature Measurements Using Ethanol-Chlorobenzene Oscillotitrator System, *Nucl Technol Radiat*, 33 (2018), 4, pp. 363-368
- [36] Hu, Z., Qi, L., *Sample Digestion Methods*, Treatise on Geochemistry, 2014, pp. 87-109
- [37] Liu, J., Sun, S., Total Concentrations and Different Fractions of Heavy Metals in Sewage Sludge from Guangzhou, China, *Transactions of Nonferrous Metals Society of China*, 23 (2013), 8, pp. 2397-2407
- [38] Luo, G., Determination of Total Arsenic in Wastewater and Sewage Sludge Samples by Using Hydride-Generation Atomic Fluorescence Spectrometry Under the Optimized Analytical Conditions, *Analytical Letters*, 45 (2012), 17, pp. 2493-2507

- [39] Djordjević, S., *et al.*, Mikrobiološke i Biohemijske Osobine Deposola RB "Kolubara", *Zaštita Materijala* 55 (2014), 1, pp. 91-94
- [40] Gamliel, A., *et al.*, Microbial Phenomena Related to Increased Growth Response in Solarized Soils and to Monoculture Systems, *Proceedings*, 7th Congress of the Mediterranean Phytopathological Union
- [41] Fernando, D. R., Lynch, J. P., Manganese Phytotoxicity: New Light on an Old Problem, *Annals of Botany*, 116 (2015), 3, pp. 313-319
- [42] Mahmood, T., *et al.*, Soil Sterilization Effects on Root Growth and Formation of Rhizosheaths in Wheat Seedlings, *Pedobiologia*, 57 (2014), 3, pp. 123-130
- [43] Correa, W., *et al.*, Inactivation of Bacteria by γ -Irradiation to Investigate the Interaction with Antimicrobial Peptides, *Biophysical Journal*, 2019, July
- [44] Chu, D., Effects of Heavy Metals on Soil Microbial Community, *IOP Conference Series: Earth and Environmental Science*, 113, 2018
- [45] ***, Official Gazette of the Republic of Serbia. (2019), Regulation on Limit Values of Pollutants, Harmful and Hazardous Substances in the Soil, 30/2018, 64/2019
- [46] Baskaran, V., *et al.*, Electrokinetic Remediation of Copper Polluted Soil Concatenated with an Adsorption Zone, *Environmental Nanotechnology, Monitoring & Management*, 14, 2020, June
- [47] Peng, J., *et al.*, The Remediation of Heavy Metals Contaminated Sediment, *Journal of Hazardous Materials*, 161 (2009), 2-3, pp. 633-640
- [48] Yao, Z., *et al.*, Review on Remediation Technologies of Soil Contaminated by Heavy Metals, *Procedia Environmental Sciences*, 16 (2012), Sept., pp. 722-729

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**ПРИМЕНА ВИСОКОЕНЕРГЕТСКОГ ЈОНИЗУЈУЋЕГ ЗРАЧЕЊА ЗА
МИКРОБИОЛОШКУ ДЕКОНТАМИНАЦИЈУ ПРИОБАЛНОГ ЗЕМЉИШТА
У СЛИВУ РЕКЕ КОЛУБАРА, СРБИЈА**

Река Колубара загађује приобално земљиште у свом сливу и чини га неподобним за пољопривредне активности на том подручју. Такође, контаминирано земљиште представља опасност за животну средину. Различите методе се могу користити за деконтаминацију земљишта. Ове методе укључују биолошки третман/биоремедијацију, хемијску оксидацију, стабилизацију земљишта, физичке методе, као што је испирање земљишта, или третман високоенергетским јонизујућим зрачењем. Гама зрачење земљишта је добро позната метода инхибиције микробне активности. У овом раду испитан је утицај различитих доза и јачина дозе гама зрачења, на микробиолошку деконтаминацију приобалног земљишта у сливу реке Колубаре. Испитивани су ефекти зрачења на смањење укупног броја микроорганизама и уклањање плесни и патогених бактерија из узорка земљишта. Утврђено је да доза гама зрачења од 3 kGy неутралише све патогене бактерије, доза од 5 kGy деактивира плесни у узорцима земљишта, а доза од 10 kGy је оптимална за уклањање свих микроорганизама у узорцима и стерилизацију третираног земљишта. Истраживање је показало да јачина дозе гама зрачења не утиче значајно на микробиолошку деконтаминацију земљишта. Одређен је садржај тешких метала у земљишту, а добијене вредности су упоређене са граничним вредностима санације прописане регулативом. Закључено је да је садржај тешких метала у анализираним узорцима земљишта испод границе санационих вредности. Једини изузетак је био незнатно повећан садржај бакра у једном узорку.

Резултат овог истраживања је закључак да се приобално земљиште из слива Колубаре може деконтаминисати гама зрачењем. Ова напредна технологија третмана земљишта доступна је у Србији с обзиром да у оквиру Института Винча постоји индустријско постројење за третман гама зрачењем.

Кључне речи: микробиолошка деконтаминација, приобално земљиште, гама зрачење, Колубара