Scientific Papers. Series E. Land Reclamation, Earth Observation & Surveying, Environmental Engineering. Vol. XIV, 2025
Print ISSN 2285-6064, CD-ROM ISSN 2285-6072, Online ISSN 2393-5138, ISSN-L 2285-6064

SOLUTIONS FOR THE RESTORATION IN THE NATURAL CIRCUIT OF CONTAMINATED SITES FROM THE PETROLEUM INDUSTRY

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Abstract

The restoration of sites contaminated by the petroleum industry aims to mitigate negative effects on the environment, and human health, and facilitate the reuse of significant land areas. In addition to reducing soil contaminant concentrations, another objective of restoration is the rehabilitation of the vegetation layer. The use of sewage sludge in decontamination processes can provide nutrients and microorganisms, potentially forming the basis for vegetation layer regeneration. However, the application of sewage sludge can also have negative environmental impacts due to high concentrations of heavy metals and the presence of various pathogenic microbiota. This study presents an experimental investigation into the potential use of sewage sludge for remediating petroleum-contaminated sites and regenerating the vegetation layer. Lawn grass was sown in the remediated soil treated with sewage sludge to study its development. Using combined SEM-EDX and electrochemical methods, we analysed the plant microstructure and the distribution of chemical elements to evaluate turf growth. The results obtained are promising and open new research perspectives for the use of sewage sludge in the remediation of sites contaminated by the oil industry.

Key words: *bioremediation, environment, petroleum, pollution, sewage sludge.*

INTRODUCTION

Contaminated sites from the petroleum industry represent a mosaic in the world's geographical landscape, occupying large areas of land. Restoring the natural environment with the aim of reusing land has become a global concern of humanity, but with the optimization of restoration activities and minimizing costs. A current approach to the remediation of contaminated sites is based on the assessment of associated risks, so that the return to the natural cycle does not pose risks to the environment and human health.

The present study is a continuation of the research on the restoration of contaminated sites from the petroleum industry using sewage sludge. Previous studies have shown the bioremediation potential of sewage sludge for lands polluted with petroleum hydrocarbons. In addition to the nutrient and microorganism content of sewage sludge that helps in the bioremediation process, it also comes with a contribution of mineral matter that, when mixed with the soil it has bioremediated, can to a certain extent complete the need for filling material in excavated areas, thus minimizing the use of natural resources. By using sewage sludge in the bioremediation processes of soils contaminated with petroleum hydrocarbons, it was found that there is a double recovery of two materials with harmful effects on the environment: sewage sludge and contaminated soil from the petroleum industry, thus reducing the disposal of hazardous waste (Iorga et al., 2025). Previous research has shown that petroleum hydrocarbons contaminated soil and bioremediated with sewage sludge can be the basis for the development of a fertile layer for plant growth (Iorga et al., 2024). Both the high content of heterotrophic bacteria and filamentous fungi identified by microbiological analyses the soil microstructure and the distribution of chemical elements with a role in plant growth were identified in petroleum hydrocarbon contaminated soils and bioremediated with sewage sludge.

In the present study, the microstructure and elemental analysis of the soil-sewage sludge/plant systems were studied using a scanning electron microscope coupled with an energy dispersive X-ray analyzer (SEM-EDX) method. The morphological and elemental characteristics of the control soil were presented before (Iorga et al., 2024). Therefore, the results of these two research papers will be correlated to validate them.

Nitrite monitoring in soil is essential as these compounds have a significant impact on agriculture and human health. Nitrite is part of the nitrogen cycle and eutrophication, and its accumulation in soil can lead to high toxic effects on plants and animals. Furthermore, nitrites and nitrates are used as additives in food processing and as inhibitors of the growth of microorganisms, and their overexpression in the body can affect the transport of oxygen in the blood and generate methemoglobinemia, which is known to cause death (Beeckman et al., 2018; Zdolec et al., 2022). In this regard, electrochemical detection methods were used because they have high sensitivity and rapidity in detection. Electrochemical sensors based on carbon nanomaterials were developed for the sensitive and rapid determination of nitrite from soil solution samples extracted with low-volume suction lysimeters. The techniques used in this study were cyclic voltammetry (CV) and amperometry.

MATERIALS AND METHODS

The analyzed samples come from three mixtures made from soil contaminated with petroleum products bioremedied with sewage sludge (S: N v/v), in three different proportions S2:N1 (2:1 v/v), S1:N1 (1:1 v/v), and S1:N2 (1:2 v/v). The soil mixtures were introduced separately into pots and sown with turf. The analysis of the samples was carried out both from each bioremedied soil mixture and from each pot, two months after the development of the plants. The turf was sown on the last day of January 2024, during a period of the year when seed germination cannot occur under natural conditions. The pots were kept in the free atmosphere of the laboratory, at a temperature of approximately 22-24°C (Iorga et al., 2024).

SEM images were captured using TESCAN VEGA microscope (Brno, Czech Republic), from CC-ITI laboratory "Dunărea de Jos" University of Galati. Prior to imagistic evaluation, the organic samples were thin metal layered by means of pulverization method (SPI Supplies, West Chester, USA) (Iorga et al., 2024). The powdered and grass samples were dried, then fixed onto aluminium stub using double adhesive carbon tape, as described by Caprita et al. (2021). The low-vacuum pressure (60 Pa) and electron accelerating voltage of 10 kV allowed to identify many chemical elements as possible, at two different magnifications (260X and 1400X). SEM-EDX results were obtained on randomly selected areas from the surface, for major and trace elements quantification and spatially mapping. Electrochemical measurements were performed with a portable Potentiostat/Galvanostat from Metrohm Dropsens, and data recording was performed using the DropView8400 software. The sensors used in this study were commercial, screen-printed, carbon paste electrochemical sensors DRP-110 (SPE) from Metrohm Dropsense, which consisted of three electrodes, the working electrode made of carbon paste, the reference electrode made of Ag, and the auxiliary electrode made of graphite. Cyclic voltammetry measurements were performed in stationary solution, by sweeping the potential between -0.2 and 1.0 V, with a scan rate of 0.05 V/sec. Amperometric measurements were performed in stirred solution, all working potentials being referenced to the reference electrode made of Ag/AgCl. Amperometric detection of NO_2^- concentration in soil solutions was performed with the portable bipotentiostat, at different applied potentials, depending on the pH of the soil solution (Gurban et al., 2023).

Preparation of nanomaterial-modified sensors

To determine the NO_2^- concentration, the sensors were functionalized by depositing on the active surface of the SPE electrodes a volume ranging from 5 to 20 μL of the multi-walledcarbon nanotubes (MWCNTs) suspension made in a 0.5% low molecular weight Chitosan solution (CS, molecular weight =

50-190 kDa) in 2% acetic acid. The sensors obtained and labelled with MC/SPE were stored at room temperature, protected from light.

Measurements on real soil samples

Small volume suction lysimeters from Hanna Instruments, 30 cm, were used to collect the soil solutions that were analyzed. Nitrite concentrations were determined by amperometric measurements using the nanomaterial-modified screen-printed sensors.

Electrochemical characterization of the sensors

Cyclic Voltammetry (CV) Studies were performed using the sensors obtained by MC/SPE (MWCNT-CS nanomaterial-based sensors) in a stationary solution of phosphate buffer electrolyte (PBS) 0.1 M, pH=7, in the absence and presence of 5 mM $NaNO_2$ (Figure 1). The potential was swept in a range between -0.2 V and 1.0 V vs. Ag/AgCl, with a scan rate of 0.05 V/sec, for a single scan cycle.

Sample metallization

SEM EDX analysis

Electrochemical determination

Lysimeter extraction of uncultivated bioremedied soil solution

Lysimeter extraction of bioremedied soil solution cultivated with grass

Data recording

Figure 1. Soil sample analysis steps

The voltammograms were recorded using the MC/SPE sensor, and it was observed that the shape of the cyclic voltammograms for both the simple electrolyte and the 5 mM nitrite solution underwent changes (Figure 2).

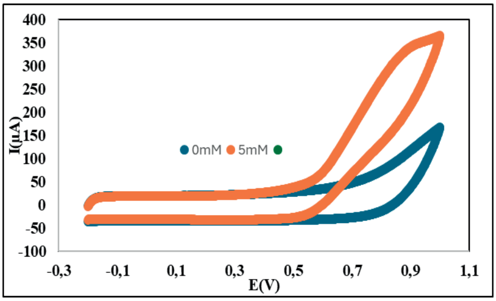


Figure 2. Influence of nitrite concentration on the oxidizing character

Cyclic voltammograms recorded at the surface of the sensor modified with MWCNTs, MC/SPE (PBS, 0.1 M, pH = 7): in the absence and in the presence of the analyte at different nitrite concentrations.

In the case of voltammograms recorded using the MC/SPE sensor, an increase in the intensity of the anodic current peak can be observed with the increase in nitrite concentration from 1 to 5 mM (Table 1).

Table 1. Influence of nitrite concentration on oxidation current and potential

[NO_2^-] (mM)	I (µA)	E (V)
0	-	-
1	145.682	0.832
2	198.581	0.904
5	292.962	0.890

The increase in the intensity of the anodic peak current with increasing nitrite concentration demonstrates the synergistic effect of multi-walled carbon nanotubes and chitosan. The sensors based on MWCNTs-CS showed high electrocatalytic behavior for nitrite oxidation, the stability of the nanomaterial on the sensor surface being considerably improved by using the chitosan solution.

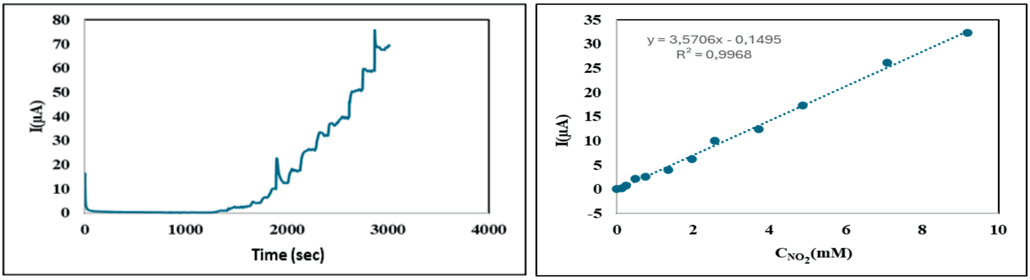
The potential value of 0.65 V was selected for performing amperometric studies of nitrite detection in order to ensure increased sensitivity and selectivity.

Amperometric determination of nitrite using MC/SPE-based sensors. Considering the previous CV studies, the working conditions for amperometric detection of nitrite were optimized by using MWCNT-CS/SPE sensors at an applied potential of 0.65 V, using PBS (pH of 7-7.5) (Figure 3A and Figure 4A). Sensor calibration was performed at room temperature in stirred 0.1 M phosphate buffer saline (PBS) electrolyte solution, pH 7 (Figure 3A) and pH 7.5 (Figure 4A), respectively, by applying a potential of 0.65 V vs Ag/AgCl and successively injecting increasing volumes of 0.1 M nitrite standard solution.

The analytical performances of the MWCNTs-CS sensors at different pH values were obtained from the calibration curves using linear regressions shown in Figure 3B (pH 7) and Figure 4B (pH 7.5), respectively. Table 2 shows the analytical parameters obtained from the calibration curves for nitrite detection.

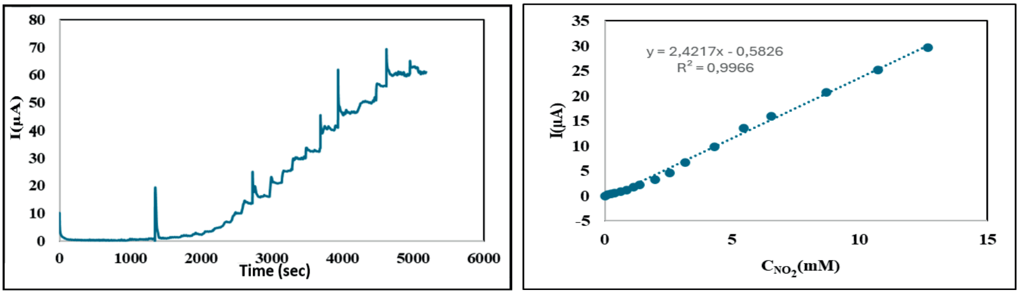
After the development and electrochemical characterization of the sensors modified with MWCNTs, using the analytical parameters obtained from the calibration curves, they were further used to determine the nitrite content in solutions of bioremedied soils.

To collect the soil solutions, lysimeters were used that were introduced into the bioremedied soil before and after turf cultivation. The sensors were introduced into the soil solution in these lysimeters. The determination of the nitrite content was performed for each sample volume by interpolating the values of the currents recorded on the calibration curves for the used sensors, using the provided portable potentiostat



A B

Figure 3. Amperogram (A) and calibration curve (B) obtained in PBS support electrolyte solution, 0.1 M, pH 7, for nitrite detection using the MC/SPE sensor



A B

Figure 4. Amperogram (A) and calibration curve (B) obtained in PBS support electrolyte solution, 0.1 M, pH 7.5, for nitrite detection using the MC/SPE sensor

Table 2. Analytical performances of MWCNTs - Chitosan based sensors for nitrite determination

pH	Linear Domain (mM)	R^2	Sensitivity (μA/mM)	Specific sensitivity (μA/mM·cm^2)
7	0-9.194	0.996	3.570	28.42
7.5	0-12.664	0.996	2.421	19.275

RESULTS AND DISCUSSIONS

Morphological and chemical characterization

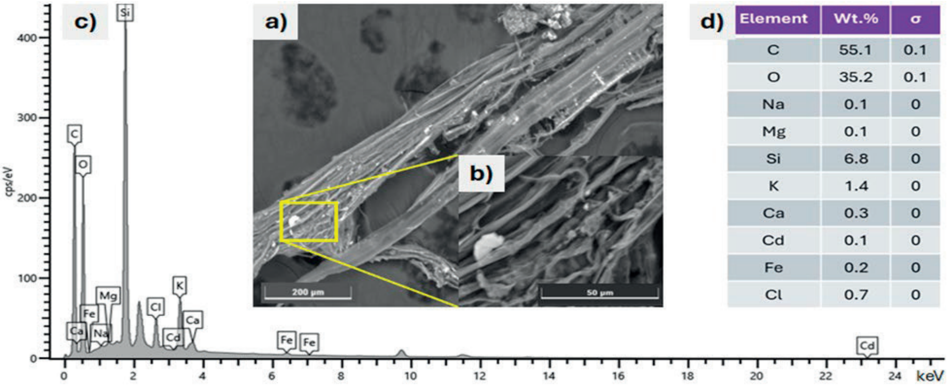
Microstructure, variability and distribution of chemical elements in plants which are developed under un-treated and treated soil systems were investigated by means of SEM-EDX combined method (Figures 5-7).

Morphological and elemental characteristics of control soil were presented by (Iorga, et al.2024). Therefore, the results from these two research papers will be corelated to validate them. The microstructure of root's lawn grass grew up from uncontaminated soil was imagistic explored at two scale bars – 200 μm (total view) and 50 μm (detail) (Figure 5a-b). Mineral compounds can be distinguished by varied shapes and gray shades, attached on the plant cellular tissue. The presence of natural elements was marked out by semi-quantitative and qualitative X-ray analysis (Figure 5c-d). Higher carbon content (55.1 wt.%) has a component derived from carbon tape, as fixation tool. The macro / micro-elements and nutrients identified from roots have very low concentrations compared to that from soil, as presented by Iorga et al. in 2024. This could be explained by the soil fertilization deficiency during these experiments, which can slow down the vegetal growing process and affect root evolution (the case of phosphorous). Figure 6a shows the mapping of macro / micro and trace of elements in the sewage sludge powder.

It is known that sewage sludge has high content in organic compounds and lower in nutrients. Usually, 2.39 wt.% of carbon in soil is comparable with the European average for the soil upper horizon (2.48 wt.%) (Salminen et al., 2005). However, higher C value of 11.03 wt.% in sludge is due to the presence of carbonates. Heavy metals were identified as minor elements in sludge, around 1 wt.%. The bioremediation effect of sewage sludge on vegetal layer is here discussed in terms of macro and micro-elements, and nutrients, respectively. SEM micrographs of underside plants developed in three soil-sludge systems are shown in Figure 7 a-c. After experimental soil remediation, a lot of necessary elements were identified in roots, with increasing concentration. So, we can remark the stimulating effect of sewage sludge, attributed to large amounts of nutrients (Mg, P, S, Na, Ca), as showed (Kominko et al., 2022). For example, P content increased from 0 wt.% (S1:N1 v/v) to 1.7 wt.% (S2:N1 v/v); Ca varied from 5.5 wt.% (S1:N1 v/v) to 9.9 wt.% (S2:N1 v/v); Fe is almost constant (2.16 wt.%). At the same time, some heavy elements (Al, Ni, Mn, Cu, Zr, Cr, Cd) from sludge (Figure 7b) are accumulated in roots. Aluminium is one of the toxic elements which has been found decreasing from 2.6 wt.% (S1:N1 v/v) to 1.9 wt.% (S2:N1 v/v) and the others are trace of element (< 0.5 wt.%). Thus, we can conclude that S2:N1 v/v mixture of soil and sewage sludge could be a promising solution for natural fertilizer of contaminated sites.

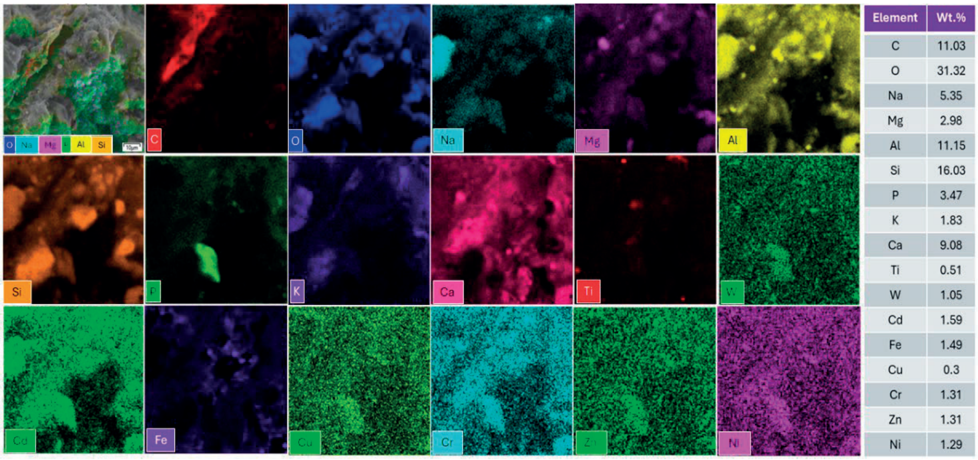
Amperometric detection of nitrite in real soil samples using MWCNTs/SPE sensors

MWCNTs/SPE sensors were used to detect nitrite content in different soil samples. Using lysimeters, a sufficient amount of soil solutions was extracted and transferred to new containers. The electrode was placed in a horizontal position and for amperometric detection, 200 μL of sample was taken and injected directly onto the sensor surface. The actual detection took place and the nitrite amounts in the samples were determined by interpolating the values of the currents recorded on the calibration curves obtained for the pH values 7, respectively 7.5 corresponding to the soil samples. Table 3 shows the results obtained from amperometric studies on soil samples. From the soil samples without plants analyzed, it is observed that at pH 7 in the S2:N1(v/v) mixture, the lowest nitrite concentration value was recorded (0.023 g/L).



Element	Wt.%	σ
C	55.1	0.1
O	35.2	0.1
Na	0.1	0
Mg	0.1	0
Si	6.8	0
K	1.4	0
Ca	0.3	0
Cd	0.1	0
Fe	0.2	0
Cl	0.7	0

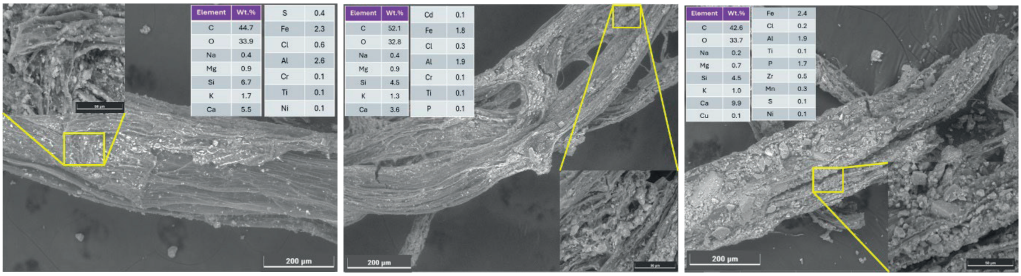
Figure 5. SEM images (a-b), X-ray spectrum (c) and semi-quantitative EDX results (d) of root's lawn grass, developed under uncontaminated soil conditions



Element	Wt.%
C	11.03
O	31.32
Na	5.35
Mg	2.98
Al	11.15
Si	16.03
P	3.47
K	1.83
Ca	9.08
Ti	0.51
W	1.05
Cd	1.59
Fe	1.49
Cu	0.3
Cr	1.31
Zn	1.31
Ni	1.29

a b

Figure 6. Elemental distribution map (a) and concentration (wt.%) (b) of sewage sludge sample, before soil mixing process



Element	Wt.%
C	44.7
O	33.9
Na	0.4
Mg	0.9
Si	6.7
K	1.7
Ca	5.5
S	0.4
Fe	2.3
Cl	0.6
Al	2.6
Cr	0.1
Ti	0.1
Ni	0.1

Element	Wt.%
C	52.1
O	32.8
Na	0.4
Mg	0.9
Si	4.5
K	1.3
Ca	3.6
Cd	0.1
Fe	1.8
Cl	0.3
Al	1.9
Cr	0.1
Ti	0.1
P	0.1

Element	Wt.%
C	42.6
O	33.7
Na	0.2
Mg	0.7
Si	4.5
K	1.0
Ca	9.9
Cu	0.1
Fe	2.4
Cl	0.2
Al	1.9
Ti	0.1
P	1.7
Zr	0.5
Mn	0.3
S	0.1
Ni	0.1

a) b) c)

Figure 7. Imagistic and chemical composition results of root's lawn grass tested in three soil-sewage sludge/plant systems: a) S1:N1; b) S1:N2; c) S2:N1

Table 3. Nitrite concentration determined from soil samples

Sample	pH	Soil without plants		pH	Soil with plants	
		mM	g/L		mM	g/L
S2-N1	7	0.491	0.023	7.5	0.471	0.022
S1-N1	7.5	0.532	0.024	7.5	0.491	0.023
S1-N2	7.5	0.880	0.040	7.5	0.749	0.034

In the mixtures where a slight increase in pH 7.5 was observed, an increase in nitrite concentration values was also observed as the amount of sludge increased from 0.024 g/L in S1:N1 (v/v) to 0.040 g/L in S1:N2 (v/v). At the time of determination, from the analysis of the results of the soil samples with plants, it is observed that at pH 7.5 of the S2:N1 mixture, the determined nitrite concentration is lower than in the initial S2:N1 mixture. For the other mixtures, the pH values did not change, remaining at 7.5, but the nitrite concentration values decreased from 0.024 g/L in S1:N1 without plants to 0.023 g/L in the S1:N1 sample with plants, respectively from 0.040 g/L (S1:N2 without plants) to 0.034 g/L (S1:N2 with plants). The results are predictable, as it is known that some nitrites are absorbed by plant roots. Considering the pH variation in the analyzed samples, a determination over time was carried out on samples of mixtures cultivated with turf, for a period of 32 days, the results being presented in the following Table 4. Sample S1-N1 had a relatively constant pH of 7.5, except for a decrease to pH 7 after 7 days. Sample S1-N2 showed a small decrease in pH from 7.5 to 7 after one week and then increased to 7.5 after 19 days, remained at 7.5 after 29 days and increased slightly to pH 8 on day 32. Sample S2-N1 showed pH variations between 7 and 7.5 over the course of 29 days, then increased to pH 8 after 32 days. Electrochemical determinations were performed as a function of pH.

Table 4. pH values recorded over time for the analysed soil samples

Time period between tests (days)	pH		
	S1-N1	S1-N2	S2-N1
0	7.5	7.5	7
7	7	7	7.5
19	7.5	7.5	7
29	7.5	7.5	7.5
32	7.5	8	8

CONCLUSIONS

The use of sewage sludge for remediating petroleum-contaminated sites and regeneration of vegetation was studied. The stimulating effect of the sewage sludge on plant growth is attributed to the presence of a large amounts of nutrients in the sludge which is confirmed by SEM-EDX analysis. Electrochemical sensors modified with MWCNTs-CS nanomaterials were used for the amperometric detection of nitrite in real soil samples collected using lysimeters, showing a decrease of nitrite concentrations due to plant growth and thus, a bioremediation effect.

ACKNOWLEDGEMENTS

The support was provided by the Ministry of Research, Innovation, and Digitization through Core Program PN 23.06.01.01.

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