Mining and Metallurgy Institute Bor
West University Timisoara

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Scientific Study 1

State of waters in the mining operations zones in considered areas

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MMI Bor and WUT engaged researchers

Study Author: PhD Renata Kovacevic

Co-authors: Jelena Petrovic

PhD Radmila Markovic

Vojka Gardic

PhD Zoran Stevanovic

Ljubisa Obradovic

Vladan Marinkovic

Adina Matica

Bianca Boros

Bianca Vulpe

PhD Vasile Ostafe

PhD Adriana Isvoran

West University Timisoara	**Mining and Metallurgy Institute Bor**
	Director Advisor
RORS-337 PM	RORS-337 TDM
PhD Adriana Isvoran	PhD Zoran Stevanovic

Contents

1. INTRODUCTION

Mining plays an important role in the global economy and will continue to allow us to access the metals and the minerals that are much needed for the development of technology and life. Over the last few decades, the gap between the economy and the mining industry has widened to the point that extracting the necessary minerals and metals has become unsustainable for the wildlife and ecosystems. The mining industry brings substantial economic growth and benefits to countries, generating revenues and creating jobs. To note though, countries with significant amounts of resources for mining activities have the potential to generate a sustainable development.

Mining operations can have a huge local impact on the environment and population. They produce many types of air, water and soil pollutants that may ultimately affect human health. In Serbia and Romania, copper mineralization is mostly porphyry type of deposits containing chiefly sulphur minerals associated with pyrites that are one of the main sulphuric acid generators in contact with the atmosphere. Main mining wastes generated during the treatment of those kinds of copper deposits and which cause the major environmental pollution are: tailings generated during flotation processes containing a variety of metallic and non-metallic minerals, spent ores consisting of the material remaining in either dump or heap leach piles when leaching ceases, acid rain resulting from the combination of rain and SO_2 causing damage to crops, trees and buildings for many miles downwind. Furthermore, the disposal of an enormous volume of tailings dumps poses a serious risk to the surrounding environment through air pollution due to air-dried out tailings, erosion of the tailings with the potential of valuable land degradation, and leaching of soluble inorganic potentially toxic chemical species (Cu, Ni, Pb, Zn, Cd, and Cr) occurring in a variety of minerals present in the tailings dump. Not at last, in the area of disposal of the mining waste, the acid mine water is generated from the mine wastes containing sulphide-rich minerals. Taking into account the complexity of the pollution produced by mining operations, adverse health effects of those leaving near, downstream or downwind of mines can be substantial. Not at last, environmental effects should also be considered.

The main goal of monitoring is to provide timely response and warning to possible negative processes and accident situations, gain a more complete insight into the state of the environment and determine the need to take protection measures depending on the degree of threat and type of pollution. Land and water in urban areas, and especially those in industrial and urban areas, are exposed to significant anthropogenic influences due to higher population density, traffic intensity, proximity to industry, etc. Knowledge of water and soil quality from the point of view of the content of organic and inorganic pollutants is reflected in the possibility of risk assessment, location and remediation of polluted areas as well as urban planning in terms of identification and relocation of pollution sources.

Prolonged accumulation of pollutants in water and soil can lead to a decrease in its buffer capacity, which can result in permanent contamination of soil and groundwater. This further, in a chain, has a detrimental effect on human health. Water and soil monitoring involves testing the content of a number of inorganic elements and organic compounds (pesticides, polychlorinated biphenyls (POPs), polycyclic aromatic hydrocarbons (PAHs), etc.) by modern methods of instrumental chemical analysis (ICPAES, ICPMS, GCMS, etc.). The pollutants present are harmful to health. According to the EPA classification some of these pollutants are very harmful and belong to the group of substances that have been proven to be carcinogenic to humans.

In order to find measures and solutions for the reduction, remediation and elimination of pollutants, it is necessary to have a true picture of the consequences of more than a century of continuous mining and metallurgical activities. In this study, aiming to assess the risk of mining activities in the vicinity of the Bor copper mine from Serbia and the Moldova Noua closed copper mine from Romania, on the pollution of surface waters, wells, sediments and soil, physico-chemical analysis of the collected samples were performed. In Serbia, sampling was performed quarterly, with 14 previously defined profiles of the Bor River (W5-1-W5-14), as well as from locations upstream and downstream of the Bor River (marked W1-W4, W6-W10). In addition, samples collected from locations on the Timok River (marked W11-W15) were analyzed. In Romania, surface water, well water, sediment and soil samples were collected in four sampling campaigns. The surface water (W18-W23) and sediment (S82-S84) samples were collected from three rivers, Radimna, Bosneag and Nera, the soil samples (S85-S88) were collected from the vicinity of the tailing pond and the well water samples (WU11-WU14) were collected from localities in the area of Moldova Noua.

A total of 164 samples from Serbia and 19 from Romania of surface water and wells were analyzed during the first year of the Project RoRS 337 to the content of the following pollutants: As, Cd, Cr, Cu, Fe, Hg, Mn, Mo, Ni, Pb, Zn, Se and S using atomic emission spectrometry with inductively plasma, mass spectrometry with inductively coupled plasma and flow injection system for mercury determination by cold-vapor atomic absorption spectrometry. In addition, pH, T and dissolved oxygen have been measured in the water samples. For the Romanian samples, conductivity and redox potential were determined and toxicological analysis were also conducted.

According to Serbian legislation for surface water, in rivers from Bor city to the confluence Bor and Timok, the content for almost all of the analyzed elements and sulfate are above the Maximum Allowed Concentration (MAC). According to Romanian legislation for surface water, in considered rivers from Romania (Radimna, Bosneag and Nera), the content for almost all of the analyzed elements was below MAC.

According to the pH values most of the analyzed samples of surface water are strong acid water with pH values lower than 3.0. The situation is different with rivers that belong to the II (Danube River) and III (Timok River) category, the recorded values for all elements and sulfates are below the allowed values for the given category of surface waters. The considered rivers in Caras Severin County near Moldova Noua in Romania did not have acidic characteristics.

The results of wells analysis show that the content for almost all analyzed elements are below the Maximum Allowed Concentration according to Serbian legislation for drinking water.

Cooperation beyond borders.
Interreg-IPA Cross-border Cooperation Romania-Serbia Programme is financed by the European Union under the Instrument for Pre-accession Assistance (IPA II) and co-financed by the partner states in the Programme.
Project RoRS 337- ROmania Serbia NETwork for assessing and disseminating the impact of copper mining activities on water quality in the cross-border area (RoS-NET2)

2. IMPORTANCE OF MONITORIZATION OF THE WATER QUALITY IN MINING OPERATION AREAS

According to the scientific literature, information regarding the water quality monitoring and water pollution with heavy metals in the area of the former mining operation near Moldova Nouă is very limited. Similarly, this type of data is also missing from the reports of Romanian Environmental Agency, branch Caras-Severin for 2018 and 2019. One study regarding the assessment of water quality was performed by Anghel and co-workers (2019) using water samples collected in 2017 from the Bosneag river, a direct affluent of Danube, with the confluence point being situated near the tailing ponds in Moldova Veche. The presence of six heavy metals (Pb, Cd, Ni, Cr, Cu, Zn) was registered in both water and sediment samples. The concentration of heavy metals in the tested surface waters decreased in the following order: Zn>Cu>Ni>Pb>Cd>Cr, while for the sediment samples, the concentration distribution was as follows: Zn>Cu>Pb>Cr>Ni>Cd (Anghel et al., 2019).

The main rivers in Bor mine area are Bor, Krivelj, Ravna, Bela and Timok. There also are small river creeks and many acid mine drainages (AMD) resulting from mining operations flow into the mentioned rivers. According to Serbian legislation, most of the mentioned rivers belong to class I/II of pollution. However, after passing the mines facilities they become very polluted mostly by heavy metals (Pb, Zn, Cd, Ni, Se, As, Fe) that are mobilized (leached) from the surrounding mining wastes. The concentration of heavy metals in the most polluted rivers in the region of Bor were in 2012 between 10 and 100 times higher than MAC (Obradović et al, 2012). We must underline that, besides these pollutants, water in most rivers smells, has a pH value in the acid range and contains suspended matters.

All these data illustrate the pollution water with heavy metals (As, Cd, Cr, Cu, Fe, Hg, Mn, Mo, Ni, Pb, and Zn) due to the mining operations in the cross-border area of Romania and Serbia. This was the reason that we proposed the Project *RoRS 337- ROmania Serbia NETwork for assessing and disseminating the impact of copper mining activities on water quality in the cross-border area (RoS-NET2).* One of the objectives of this project is the improvement of the cross border services for environmental protection. In order to achieve this objectives, one of the main activities of the project is to assess the quality of water in the region of mining operations in the cross-border region of Romania and Serbia.

Arsenic (As) is a common element in the crust of the Earth, it is toxic and has not a biological role for humans (Ekeanyanwu et al., 2020). It is a metalloid revealing both metallic and non-metallic chemical characteristics, existing in nature in several states of oxidation, the arsenates As(V) prevailing in the oxidizing conditions and arsenites As(III), prevailing in the reducing conditions. The As(III) is more toxic than the As(V) as As (III) is more water-soluble (is the dominant arsenic species in groundwater), has an increased mobility and illustrates severe effects on human health (Agency for Toxic Substances and Disease Registry). The human exposure to arsenic occurs by ingestion (mainly by drinking contaminated water) but also through inhalation and skin contact, mining activity being the predominant source of arsenic pollution (Palma-Lara et al., 2020). There also is a natural presence of high levels of arsenic in drinking water due to geological characteristics (Guha Mazumder, 2003).

Arsenic is absorbed by ingestion, inhalation, contact with mucous membranes or skin penetration, is distributed through the bloodstream to various organs (liver, kidneys, lungs, bladder) and muscle and nerve tissues, the greatest accumulation of inorganic arsenic occurring in the liver (Watanabe and Hirano, 2013). Arsenic induces the formation of reactive oxygen species (ROS) and the exposure deregulates several cellular processes at the molecular level: DNA repair, epigenetic regulation, normal gene expression, proliferation and apoptosis resistance (Ebert et al, 2011). It is commonly known that arsenic exposure may cause multiple human cancers (liver, lung, skin, kidney, bladder and prostate) the most common diseases caused by chronic exposure to arsenic being lung, skin and bladder cancer (Guha Mazumder, 2003 and the references therein).

Copper (Cu) has important roles in the metabolic processes of the human body (Ekeanyanwu et al., 2020) being an essential element required for haemoglobin synthesis, absorption of iron, and cardiovascular integrity. The recommended daily intake values for copper range from 900–1700 µg for men and 900–1500 µg for women (Kukusamude et al., 2021; Freeland-Graves et al., 2020). In excess, copper may lead to acute toxicity. Copper is one of the heavy metal found abundantly on the Earth's crust and also a major pollutant occurring both naturally and anthropogenically, major anthropogenic sources including industries and mining drainage. Human exposure to copper is similar to other metal ions, copper being ingested with vegetables that extract it from soil solution via roots or by copper-contaminated water and via the inhalation of particulate matter. Copper is absorbed in the stomach and duodenum and is transported to the liver, distributed within hepatocytes and exported to the blood and is then distributed to the various organs. Concerning human health, the toxicity of copper is relatively low compared with other heavy metals, but excess copper accumulation in subjects following high-dose chronic exposure and into sensitive populations results in hepatic cirrhosis with jaundice, haemolytic anaemia, and degeneration of the basal ganglia, cardiotoxicity (hypotension, tachycardia, tachypnea) gastrointestinal disorders (ulcerations, mucosal acute haemolysis and haemoglobinuria), nephropathy (azotaemia, oliguria) and central-nervous-system manifestations (dizziness, convulsions, lethargy, headache, stupor, coma) (National Research Council, 2000).

Cadmium (Cd) is one of the most toxic and mobile elements widely distributed in the environment coming from both natural and anthropogenic sources. Important anthropogenic Cd sources include mining (usually wastewater), industry, and waste management activities. Cadmium has no biological roles in animal organisms. Cadmium pollution is observed in soil (by contamination of irrigation water by mining activities) and groundwater. The primary route of contamination with cadmium is through ingestion (through vegetables due to high mobility in the soil-plant system) but inhalation was also observed (Qin et al., 2020). Once entered into the human body, cadmium accumulates to a high level in several organs (Pan et al., 2010). Cadmium exposure is usually associated to renal tubular dysfunction, osteomalacia and osteoporosis as a result of competition with calcium, endocrine system disruption, glucose metabolism disorders, prostate, kidney, bladder, breast and lung cancer, cerebral infarction and cardiac failure (Qin et al., 2020; Buha et al., 2019; Khan et al., 2017).

Chromium is one of the most common elements in the Earth's crust and seawater. It is found in the environment in several oxidation states: metallic (II), trivalent, Cr(III), and hexavalent, Cr(VI). There are controversial results published in specific literature concerning the effects of chromium.

Some studies have indicated that chromium supplementation had improved the glucose metabolism and the serum lipid profile, and had increased muscle gain and fat loss associated with exercise, but other studies have indicated little or no benefit of chromium on the above-mentioned effects. Chromium uptake depends on its oxidation state and solubility, the Cr(III) is mainly absorbed by ingestion and Cr(VI) is readily absorbed by both inhalation and ingestion. Ingestion of chromium in all states is due to food and drinks consumption, accidental swallowing of contaminated water or contaminated soil, gastric juice reduces hexavalent chromium to trivalent chromium. Once absorbed, chromium is distributed to various tissues of the body but appears to be most concentrated in the kidney, muscle, and liver. Chromium proved to be very toxic both when ingested, inhaled and by dermal contact. When ingested or inhaled, Cr(VI) seems to be more toxic than trivalent chromium but it is unstable in the human organism being reduced to Cr(V), Cr(IV) and Cr(III) (Engwa et al., 2019). The reduction of Cr(VI) to Cr(III) conducts to the formation of reactive intermediates that produce cytotoxicity, genotoxicity and carcinogenicity (Shrivastava et al., 2002). Both *in vitro* and *in vivo* studies have illustrated that Cr(VI) is able to induce oxidative stress through enhanced production of reactive oxygen species resulting in several types of deoxyribonucleic acid (DNA) lesions, gene mutations and inhibition of macromolecular synthesis. Furthermore, chromium affects different components of the immune system and may conduct to immunostimulation or immunosuppression (Shrivastava et al., 2002). Dermal exposure may be due to swimming, bathing/showering, or contact with contaminated water and/or soil. Inhalation of hexavalent chromium (usually found as CrO3) from dust and aerosols is also an important route of exposure. Acute dermal exposure produces contact dermatitis, but chronic exposure may conduct to deep skin ulcerations (Engwa et al., 2019).

Iron is an essential component of every living organism as it participates in oxygen transport, electron transport and DNA synthesis. However, its concentration in the human organism must be regulated because iron can form free radicals and, if found in excessive amounts, it can lead to numerous disorders: anaemia to iron overload, neurodegenerative diseases and genetic and metabolic diseases (Fraga, 2005). There are two forms of dietary iron: heme (usually found in meat and sea food) and nonheme (found both in vegetables and meat). The main routes for exposure are ingestion and inhalation. Acute exposure by drinking water contaminated mainly with iron exceeding the permissible limits conducted to liver cirrhosis, liver cancer, diabetes, infertility, and diseases related to the heart (hypotension, shocks, lethargy, tachycardia), metabolic acidosis, central nervous system disorders (Engwa et al., 2019; Kumar et al., 2017), and in case of chronic exposure it may lead to death (Engwa et al., 2019). Furthermore, the oxidation of iron conducts in generating hydroxyl radicals (OH-). These free radicals can react with biological molecules (proteins, DNA) damaging them and to induce an oncogenic effect in the colon (Engwa et al., 2019). Not at last, Fisher Material Safety Data Sheet for iron illustrates that (Fisher Scientific, 2007): (i) exposure to particulates or solutions containing iron may cause eye injuries (conjunctivitis, ulceration, corneal abnormalities), skin irritation, gastrointestinal disorders (gastrointestinal irritation, nausea, vomiting, diarrhea; (ii) acute toxicity due to exposure to iron may include weakness, shock, cyanosis and acidosis; (iii) chronic exposure may lead to liver and lung damages; (iv) repeated exposure may cause diabetes, pancreatic damage, and cardiac abnormalities.

Lead (Pb) is the most toxic element that is present in the environment as a result of both natural and anthropogenic sources. It is not necessary for human health as it is not involved in biological processes and there is no level of exposure to lead that does not conduct to harmful effects. Exposure of human populations to environmental lead is due to industrial activities, lead processing, mining and smelting activities. The primary routes of lead exposure are through ingestion and inhalation. Environmental lead may be bioavailable for humans after consumption of plants and animals that have accumulated lead and through occupational exposure. Once absorbed, lead binds to erythrocytes and travels in the blood to various tissues (liver, kidneys, lungs, brain, spleen, muscles, heart) and moves further into bones and teeth, and may affect every organ or system in the body by disparaging fundamental biochemical processes (Meyer et al, 2008). Exposure to lead may conduct to numerous human health effects, the brain and kidney being most affected by lead toxicity in both children and adults (Debnath et al., 2018). Even at low concentration, it can induce neurotoxicity (Dórea, 2019), cardiac dysfunction and vascular damage (Flora et al., 2012), nephrotoxicity as lead is eliminated through the kidney (Ekong et al., 2006), male and female reproductive system effects (Vigehet al, 2011), bone toxicity by reduction of bone calcium content (Bhardwaj and Rai, 2016) and alters the major cellular functions (Gillis et al., 2012).

Manganese (Mn) is a metal naturally occurring in soil and water. In humans, it is an essential nutrient assuring bone mineralization, metabolic regulations, cellular protection from free radical species, and an enzyme activator. The main routes for exposure to manganese are from contaminated water, inhalation of dust, intake from vegetables grown in contaminated soil. There also is a dermal exposure to manganese from dust and water, but there is little evidence that dermal contact with manganese leads to significant absorption through the skin. The population living in the areas of mining activities may be exposed by inhalation to high levels of manganese in the dust. Inhalation of particulate manganese compounds can lead to the inflammatory response in the lungs, leading to increased pneumonitis and pneumonia, especially in manganese-exposed worker populations, but also in populations living and attending school near the area with dust containing manganese (Williams et al, 2012). Chronic exposure to excessive manganese levels may conduct to cardiovascular effects (Šarić and Hrustić, 1975) and psychiatric disturbances (Menezes-Filho et al, 2011). It was shown that exposure to excess levels of contaminated drinking water with manganese ($\geq$ 0.2 mg/L) may lead to neurological (Menezes-Filho et al, 2011) and increased time spent in mining operations may increase the manganese poisoning (Schuler et al, 1957).

Molybdenum (Mo) does not exist in nature in the pure metallic state but occurs in association with other elements. It presents five oxidation states (II–VI) and the predominant ones are Mo(IV) and Mo(VI). It is an essential trace element for human health being a cofactor for enzymes, but it can lead to toxicity when ingested in high doses. The main routes for exposure are ingestion from contaminated water and inhalation of dust containing molybdenum or its oxides, the major sources of metallic molybdenum including mining operations and by products of copper mining operations (Barceloux and Barceloux, 1999). Most natural waters include low levels of molybdenum (2–3 µg/L), but anthropogenic sources may contaminate the water. Molybdenum concentrations in drinking water are usually less than 0.01 mg/L, while in areas near mining sites, it may extend up to 0.20 mg/L and considerable contamination of drinking water with molybdenum may occur (Vyskočil and Viau, 1999). Little information is available on the human toxicity of molybdenum. The blood level of molybdenum is increased in persons living in areas rich in molybdenum. Workers in the copper-molybdenum mines that were exposed to a high level of dust experienced headache, weakness, fatigue, skin irritation, and impairment of the central nervous system (Walravens et al, 1979).

Mercury (Hg) is a naturally occurring element that exists in three forms: elemental (metallic) Hg (Hg°), inorganic Hg (Hg+1, Hg+2) and the organic mercury that results from methylation process. Mercury is toxic in all its forms and has no biological roles in humans. Mercury is bio-accumulative in its elemental and organic forms, monomethyl Hg being the most toxic as it acts as a neurotoxin. Mercury is considered among the most poisonous metals adversely affecting both environment and human health. Mining and industrial activities are producing mercury pollution in the atmosphere, sediments and soils. The most common route of exposure to mercury and its compounds found in the environment is the ingestion of contaminated food as it accumulates in the various aquatic animals. Exposure to mercury or its compounds conducts to serious health effects: renal, cardiovascular and pulmonary impacts, and developmental neurotoxicity (Sharma et al, 2019; Kumari et al, 2020). Mercury also produces changes in the membrane permeability, alterations in macromolecular structures and affects the endocrine system and pancreas (Obrist et al, 2019).

Nickel (Ni) is a metal broadly distributed in the environment. It is released from both natural sources and anthropogenic activity and is present in the air, water, soil and biological materials. The main routes of exposure to nickel are inhalation and dermal contact, but also ingestion may take place. In the human body, nickel may serve as a cofactor or structural component of specific metalloenzymes of various functions. Literature data illustrate that nickel at high doses and in certain forms is toxic to both humans and animals. Drinking water usually contains nickel. In the case of professional exposure, inhalation is the most significant way of uptake for Ni compounds. It has been shown that inhalation of nickel and its compounds produces the following effects (Denkhaus and Salnikow, 2002; Muñoz and Costa, 2012): irritation and/or inflammation of the respiratory tract, bronchitis, pulmonary fibrosis, asthma, and pulmonary edema, kidney and cardiovascular diseases, carcinomas. Dermal contact and oral intake of contaminated food may conduct to allergic contact dermatitis. In case of consumption of water contaminated with nickel salts, the registered health effects were: gastrointestinal disturbances and altered haematological parameters (US Institute of Medicine, Panel on Micronutrients, 2001).

Zinc (Zn) is an essential element for humans as it is involved in numerous aspects of cellular metabolism and in the catalytic activity of various enzymes maintaining immunity, antioxidant defence and protein metabolism. Excessive Zn consumption causes deficiencies in iron and copper, fever, nausea, vomiting, tiredness, and abdominal pain. The main routes of exposure to nickel are through inhalation and ingestion of polluted water, but dermal exposure is also considered. The contributions of various sources of pollution with zinc conduct to find zinc in particulate form or in larger deposits that are leached by rainfall, forming water overflow containing high zinc concentrations and polluting river water and sediments (Vareda et al, 2019). River basins supply a large part of the available fresh water on Earth and consequently, it is important to control Zn concentrations in surface water. Literature data reveal that inhalation, exposure of the skin, or ingestion of high concentrations of zinc and/or some zinc salts can produce injuries of epithelial tissue, affect levels of pancreatic enzymes and lipoproteins in serum, alter the immunological function (Walsh et al, 1994), disturb pregnancy, produce growth retardation, anorexia and disorders in energy metabolism (Wang et al., 2019).

In the waste waters generated by the Bor copper mine, besides heavy metals, the following water pollutants have been identified: Be (0.003 mg/L- 0.074 mg/L, the allowed value being 0.0002 mg/L), Mg^{2+} (>670 mg/L), sulfate (132.51 mg/L - 4145.45 mg/L), phosphate (0.05 mg/L - 1.34 mg/L) and chloride (22.14 mg/L - 184.09 mg/L) (Gorgievski et al., 2009; Korac et al, 2007). In the sterile samples from Moldova Noua sulphur, CaO and SiO_2 were identified, while in the tailing pond compounds such as Al_2O_3, Fe_2O_3, SO_3, MgO, K_2O, Ti_2O, Na_2O, P_2O_5, CuO, Cr_2O_3, As_2O_3 and MnO were also identified (Boros et al. 2021).

Beryllium (Be) is found in nature in the composition of minerals. Professionals working in industries where beryllium is present may be exposed by inhaling or contacting beryllium in the air or on surfaces. The absorption of Be is primarily via the lungs, the deposited Be moving exceedingly slow into the pulmonary blood circulation. The entrance of Be into the organism can also occur by swallowing. Once swallowed, less than 1% is absorbed into the gastrointestinal capillaries, becoming protein-bound and being deposited for a long period in the liver, spleen and skeleton (Cooper and Harrison, 2009). The elimination of the absorbed Be occurs primarily in urine, while unabsorbed Be is eliminated via faeces. Acute beryllium disease (ABD) has been observed in humans after single massive exposure, while the exposure to lower concentrations may lead to chronic beryllium disease (CBD) in 1-5% of exposed individuals. CBD affects predominantly the lungs but it may also produce skin sensitization, an immunological response to beryllium as beryllium – specific proliferation. The dermal exposure to soluble beryllium compounds leads to a cell-mediated hypersensitivity of the skin (Bruce et al, 2001). Be and its compounds are classified in the European community as known animal carcinogens suspected to be human carcinogens (Strupp, 2019).

Phosphorus (P) is essential for life since it is part of DNA and ATP, but it is also one of the most toxic substances, being used as pesticide. Human and animal studies have revealed the toxic effects of phosphate in hastening various pathologies, from vascular calcification to tumors formation and aging. Acute phosphate toxicity is relatively rare, but it can produce hypocalcaemia, tetany, hypotension and tachycardia. Chronic phosphate toxicity can lead to the deposition of calcium phosphate crystals in tissues, including cardiovascular calcification that is a fatal disease (Razzaque, 2011).

Sulfur is essential to the entire biological kingdom due to its incorporation in various molecules and has many functions: Although groundwater can naturally contain sulfates, their high levels can have acute and chronic effects on human health. Gastrointestinal effects of sulfate on human health have been reported (Backer, 2000).

Chlorine (Cl) is one of the most common substances involved in toxic inhalation. The mechanism underlying the toxicity of Cl is related to its solubility in water-based environments. When inhaled, Cl forms hydrochloric and hypochlorous acids. It also leads to ionization, the ions being able to cross the cell membrane, leading to the formation of oxygen free radicals. Prolonged exposure to high levels of chlorides may lead to diarrhea, nausea, inflammatory bowel disease (Bashir et al, 2012).

Calcium oxide (CaO), although an important cation in the organism, in excess it can impair health. The ingestion of CaO causes damage starting with the mouth, affecting even the stomach by causing ulcer. This is due to the exothermic reaction with water and its alkalinity. It is also caustic to the skin, eyes and mucous membranes of the respiratory tract (Boros et al. 2021).

The effects of silicon dioxide (SiO_2) on human health mainly include silicosis and pulmonary silicoproteinosis. Cancer, tuberculosis, sclerosis, and other similar diseases might also occur. Another affected organ is represented by the kidneys, glomerular and tubular lesions and Si being present in kidney tissue (Boros et al. 2021).

Mild skin irritation, irritation and redness in the case of eye contact, respiratory system irritation in the case of inhalation, irritation of the throat in the case of ingestion might occur after exposure to aluminum oxide (Al_2O_3) (Boros et al. 2021).

Ferric oxide (Fe_2O_3) might cause eye and/or respiratory tract irritation, even causing permanent iron staining of the eyes (Boros et al. 2021).

The exposure to magnesium oxide (MgO) may lead to hypermagnesemia. Animal studies showed that exposure via ingestion causes reduction in body weight gain, feed intake and organ weight profile (Boros et al. 2021).

The inhalation of potassium oxide (K_2O) can cause sore throat, cough, burning sensation, shortness of breath and labored breathing, while the skin contact can cause redness, pain, irritation and serious skin burns (Boros et al. 2021).

The effects of titanium oxide (Ti_2O) on human health could not be identified in specific literature (Boros et al. 2021).

Animal studies show that exposure to sodium oxide (Na_2O) causes acute depression, nasal discharge, dyspnea and gasping. Post-mortem analysis revealed gastrointestinal hemorrhage and congestion of the kidneys, liver, heart and lungs. Na_2O acted as a severe irritant to skin in animal studies, causing severe, irreversible erythema and edema at the test site (Boros et al. 2021).

Copper oxide (CuO) can cause eye irritations, respiratory tract irritation by inhalation, skin irritations and it can be toxic if ingested, according to Safety Data Sheets (Boros et al. 2021).

Chromic oxide (Cr_2O_3) causes eye and skin irritations, skin allergies, sensitization dermatitis and possible ulcerations. Repeated inhalation may cause chronic bronchitis (Boros et al. 2021).

The poisonous effects of arsenic trioxide (As_2O_3) are well known, its effects on human health involving shortness of breath, headaches, vomiting, abdominal pains, diarrhea, peripheral neuropathy, convulsions, cardiotoxicity, liver and kidneys toxicity, etc. (Boros et al. 2021).

Exposure to manganese oxide (MnO) might lead to eyes, skin and respiratory system irritations, and even pneumoconiosis (Boros et al. 2021).

There are not consistent studies regarding the impact of pollution caused by mining operations on the health of inhabitants in the cross border area of Romania and Serbia. Serbia started recently to implement a program to investigate the human health effects produced by the contaminated sites, but in Romania the impact of pollution on the human health is not investigated. The few known data emphasize that pollution caused by mining operations in investigated areas produced especially pulmonary, kidney and skin diseases (Isvoran et al., 2021). It does not mean that other human health effects that were highlighted in this study did not appear, since they have not been investigated.

Consequently, monitoring of the water content and properties in the investigated area is very important if we take into consideration the possible human health effects (for both population and professionals working in mining areas) of heavy metals and non-metals that are found as pollutants in water. It also emphasizes the necessity of serious studies meant to assess the human health impact of pollution created by mining operations in the cross border area of Romania and Serbia.

3. MATERIALS AND METHODS

Physicochemical parameters

Methods prescribed by relevant international and European standards are used to measure pollutant concentrations. The one of the deliverables of this study paper is the procedure for the chemical analysis of field samples. The procedure describes in details the chosen wavelength of analyzed pollutants, operating conditions and steps in the analysis of the following pollutants: As, Cd, Cr, Cu, Fe, Hg, Mn, Mo, Ni, Pb, Zn, Se and S from surface water, wells, soil and sediment samples by atomic emission spectrometry with inductively coupled plasma.

Water samples and wells were analysed using atomic emission spectrometry with inductively plasma (ICPAES), mass spectrometry with inductively coupled plasma (ICPMS) and flow injection system for mercury determination by cold-vapor atomic absorption spectrometry (FIMS-AAS).

ICP-AES has become probably the most widely used spectroscopic technique for measuring heavy metals in water samples, soils, plants and related materials. Analysis of the environmental samples by atomic emission spectrometry with inductively coupled plasma (ICPOES) has been widely used due to its multi-element capability, high dynamic linear range (several orders of magnitude) and sensitivity.

Analysis of the surface water samples and wells by atomic emission spectrometry with inductively coupled plasma (ICPOES) comprises the following steps:

- stabilization of plasma,
- running the samples and
- interpretation of the results.

Prior to the analysis, the plasma need to be stabilizes and optic reprofiled according to the manufacturer recommendation. The device is calibrated by using the certified calibration solution. The recommended operating conditions for water samples using ICPOES are given in Table 1.

Samples of water are measured directly without prior preparation.

The wavelength range between 165 and 770 nm can be analyzed using atomic emission spectrometer with inductively coupled plasma, model Spectro Arcos, given in Figure 1.

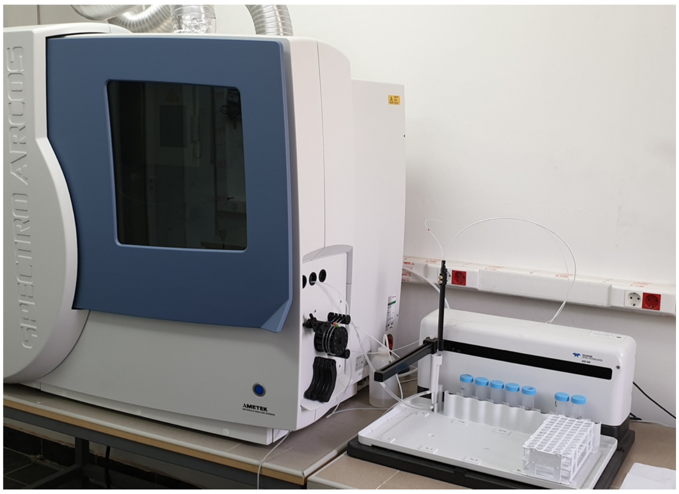

Figure 1 – Atomic emission spectrometer with inductively coupled plasma (ICPAES), model Spectro Arcos

ICP-MS is the newest atomic spectrometry technique to become widely applied in environmental analysis. ICP-MS has lower LOD comparing with ICPAES, similar to those of ETAAS (parts-per-billion or lower) but the capability to perform multi-element and isotopic analysis.

Table 1 - ICPOES operating conditions

Parameters	
Forward power (W)	1450
Coolant flow (L/min)	13
Nebulizer flow (L/min)	0.75
Auxiliary flow (L/min)	1
Plasma torch	Quartz, demountable, 2.0 mm injector tube
Spray chamber	Scott
Nebulizer	Cross-flow
Sample aspiration rate	2 mL/min

For each analyzed element with ICPOES technique it is possible to choose multiple wavelengths. Table 2 presents the recommended wavelengths for the analysis the elements of interest from water samples and wells.

Quality control of chemical analysis was performed in the laboratory by running CRM. CRMs were used to assess the accuracy of a new analytical method or instrument, to establish whether results of an established method are under control, and as an aid in training or monitoring the competence of staff while the blank samples were running to check for contamination, whilst analysis of replicate test portions will provide an indication of the repeatability and precisions of measurement.

Table 2 – Recommended wavelength for selected elements

Element	Wavelength (nm)
As	189.042
Cd	214.438, 226.502
Cr	205.618, 267.716
Cu	324.754
Hg	184.950, 194.227
Fe	259.941
Mn	257.611
Mo	202.095
Ni	231.604
Pb	220.351
S	180.731
Se	196.090
Zn	213.856, 206.191

Microbiological analysis

Enumeration of microorganisms from water samples

For the isolation and counting of microorganisms from water samples, aliquots of the water samples were inoculated on the surface of general growth media, on Petri plates. All plates were incubated at different temperature for different time intervals.

After the incubation period was over, the colonies grown on each plate were counted using the automated colony counter SCAN 300 and the CFU/mL (Colony Forming Units/milliliter) were numbered.

Dehydrogenase activity from soil samples

The principle of this method is based on a TTC solution (2,3,5 triphenyl tetrazolium chloride) that is added to the soil sample and the mixture is incubated at 25°C for 12 hours. The TPF (triphenylformazan) released is extracted with ethanol and quantified by spectrophotometry, based on a calibration curve, at a wavelength of 492 nm.

Yeast Toxicity Test to assess the toxicity of water samples from Moldova Nouă area

The Yeast Toxicity Test (YTT) is a biological method used for providing data about water toxicity.

Yeast toxicity test is a very simple, inexpensive and fast method that can provide information about the toxicity of a pollutant/contaminant, using *Saccharomyces cerevisiae* as a bioindicator.

Table 3 – Water samples from Moldova Nouă area

No. of sample	ID of the sample	Type of the sample	pH (at the time of testing)
1.	W18-M	Boşneag River (Moldova Veche)	8.02
2.	W19-M	Boşneag River (upstream Moldova Veche)	8.07
3.	W20-M	Radimna River (Pojejena)	8.15
4.	W21-M	Radimna River (upstream Pojejena)	7.48
5.	W22-M	Nera River (Socol)	7.64
6.	W23-M	Nera River (upstream Socol)	7.07
7.	WU11-M	Well from village of Coronini, near the pond Boşneag	8.05
8.	WU12-M	Well from village of Moldova Veche, near the pond Boşneag	7.37
9.	WU13-M	Well from village of Măceşti	7.34
10.	WU14-M	Well from city of Moldova Nouă	7.18
11.	WU15-M	Public well from village of Radimna	6.52
12.	WU16-M	Well from village of Măceşti	7.09
13.	WU17-M	Well from village of Măceşti	6.94
14.	WU18-M	Well from village of Măceşti	7.04
15.	WU19-M	Public water supply from Măceşti	7.27
16.	WT-M	Surface water found in tailing	5.97

The principle of this test is based on the inhibition of the fermentation process by a toxic substance. The fermentations of saccharose by Saccharomyces cerevisiae is accompanied by the production of das. In case of toxicity, the CO2 production will be suppressed in comparison to control. The toxicity of the test substance is directly proportional to the inhibition rate.

Ecotoxicity assessment

The ecotoxicity assessment involved the exposure of the aquatic macrophyte *Lemna minor* to the samples collected in Romania, from all four sampling campaigns. The assay involved the exposure for 7 days of a total number of 10 duckweed fronds, using 2 colonies of 3 fronds each and 2 colonies of 2 fronds each, for each sample.

The water samples were tested without diluting the samples, using a volume of 10 mL from each sample, while the sediment and soil samples were tested by adding 0.1 g sediment / soil to 10 mL culture media, obtaining a concentration of 1% (equal to 10 mg/mL or 10000 mg/L). The assay also included a negative control (duckweed in culture media) and a positive control (0.5% $ZnCl_2$). All samples and the two controls were tested in triplicates.

4. EXPERIMENTAL PART

Mining affects water quality through heavy use of water in processing ore, and through water pollution from discharged mine effluent and seepage from tailings and waste rock impoundments. Increasingly, human activities such as mining threaten the water sources on which we all depend.

Due to a great negative impact of mining industry on water system, the aim of cross-border collaborative project, Romania Serbia NETwork for assessing and disseminating the impact of copper mining activities on water quality in the cross-border area (ROSNET2, http://www.elearning-chemistry.ro/rosnet2/), is to perform the monitoring of surface waters close to active and abandoned mines in cross border area. Considered Romanian Serbian Cross border area is presented in Figure 2. Project includes area of mine Moldova Nouă from the Romanian side and Eastern Serbia area from the active copper mine in Bor, all the way to the confluence of the Timok into the Danube near Radujevac. This area was chosen because both mine locations have negative impact on surface water system which flow in both cases to Danube. Rivers Bosneag, Radimna and Nera from the Romanian side in Caras Severin County near Moldova Noua are also tributary of Danube River and flow in area which is affected by spreading of dust from the abandoned flotation tailing of copper mine. Bor, Krivelj, and Bela Rivers in Eastern Serbia belong to the watershed of Timok River, which is also a tributary of Danube River. All mentioned rivers flow near to the largest mining complex in Republic of Serbia where mining activities continuously exist for more than 115 years.

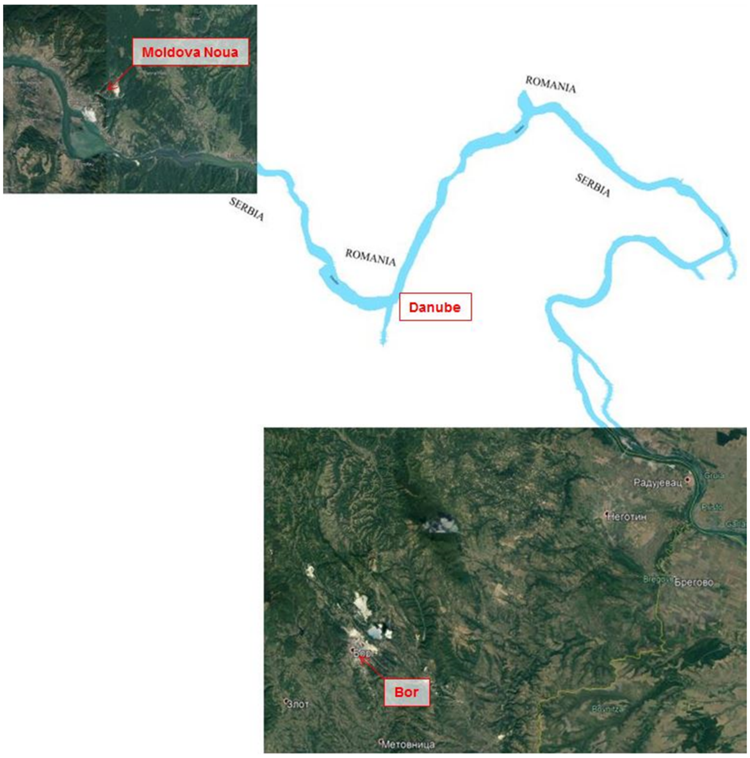

Figure 2 - Romanian Serbian Cross border ROSNET2 Project area

The influence of mining activity on the quality of regional surface water has been taken into consideration in this study. Accordingly, in Serbia sampling was done from 14 local defined profiles of the Bor River (W5-1-W5-14), as well as from locations upstream and downstream of the Bor River (marked W1-W4, W6-W10). Locations of surface water sampling in Eastern Serbia are presented in Figure 3 while the locations of surface water sampling in Romania are presented in Figure 4.

Having on mind that copper mine from Romanian side is not in operational stage, water samples were taken for monitoring purposes at first. Sampling locations were chosen to cover surface waters in surrounding area where could be expected pollution by spreading of dust from the abandoned flotation tailing. Sequence of sampling was scheduled to cover different weather condition and seasons.

Locations of surface water samples from Romanian site are: (W18, W19) Bosneag River, Moldova Veche and upstream Moldova Veche; (W20, W21) Radimna River, Pojejena and upstream Pojejena; (W22, W23) Nera River, Socol and upstream Socol. Considered rivers from Romanian side cannot be polluted by AMD because they do not have direct contact with tailing material and they are not on downstream of possible AMD leaking from tailing but they can indicate pollution by spreading of dust from the tailing. This objective was chosen because of heavy spreading of dust from the abandoned tailing due to strong winds in this area.

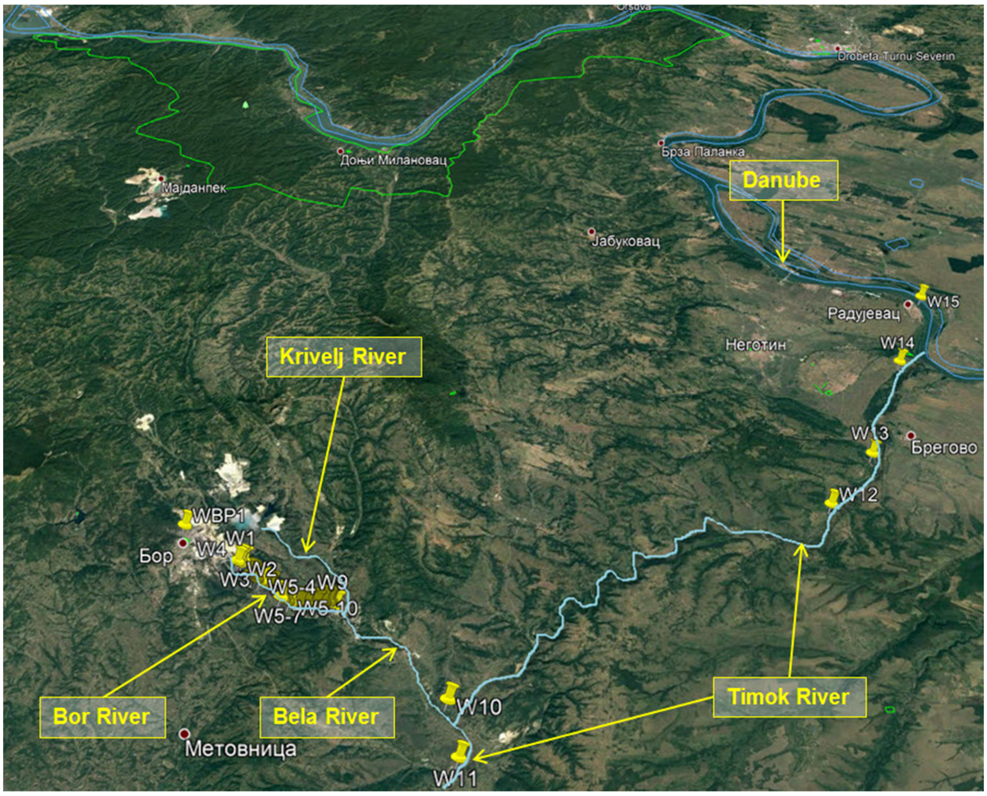

Figure 3 - Surface water sampling – Eastern Serbia side

In order to consider the effect of flow quantity on the amount of different parameters, sampling was done during the period September 2019-Jun 2021 when the local streams had the minimum and maximum flow rate respectively. The concentrations of As, Cd, Cr, Cu, Fe, Hg, Mn, Mo, Ni, Pb, Zn, Se and S were determined.

A total of **225** samples of **surface water** sampled in Serbia were analyzed during the eight quarters of the Project RoRS 337, by quarters:
1st - 28,
2nd - 29,
3rd - 30,
4th - 29,
5th - 28,
6th – 27,
7th – 27 and
8th- 27 samples.

A total of 165 samples of surface waters are of IV category, 24 of III category and 36 of II category.

A total of **24** samples of **surface water** sampled in Romania were analyzed during the eight quarters of the Project RoRS 337.

A total of **86 wells** sampled from village Slatina (Bor`s region) were analyzed during the seven quarters of the Project RoRS 337, by quarters:
1st - 14,

2nd - 10,
3rd - 11,
4th - 10,
5th - 10,
6th – 10,
7th - 11 and
8th - 10 samples.

A total of **16 wells** samples sampled in Romania were analyzed during the eight quarters of the Project RoRS 337.

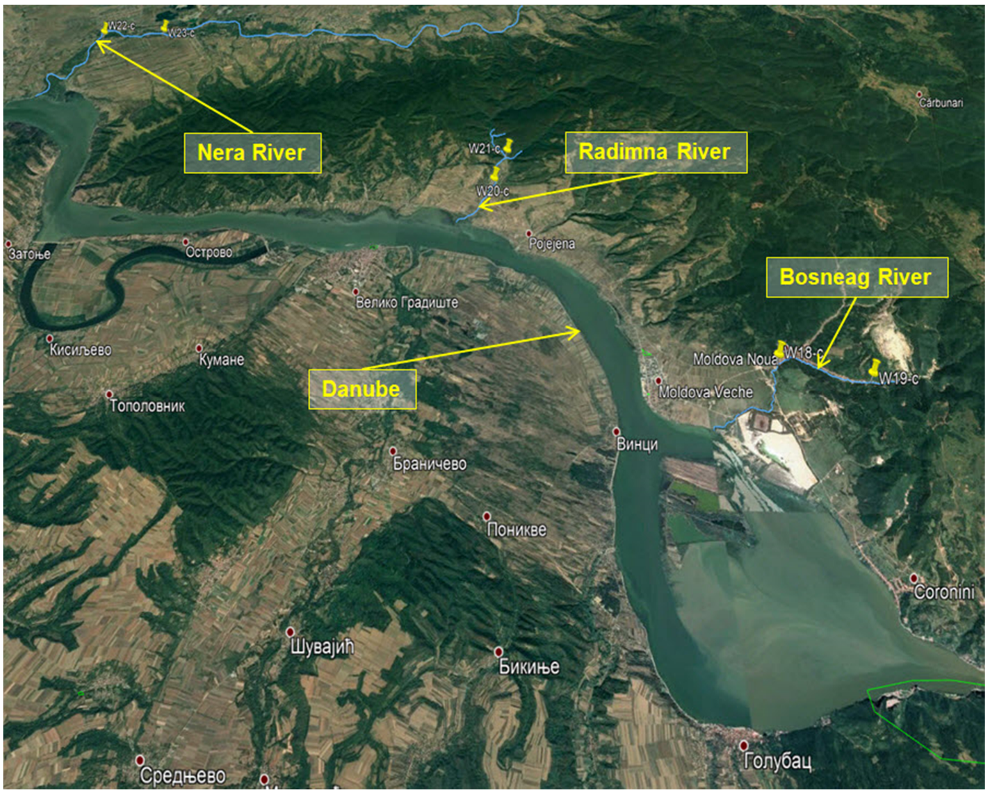

Figure 4 - Surface water sampling – Romanian side

5. RESULTS AND DISCUSSION

5.1. Results regarding the samples collected from Serbia

The present study is one of the broadest investigations of the multi-element content in wastewaters generated from copper mining activities. It may thus represent a reference point for observed concentrations in future studies conducted at Eastern Serbia or other areas of mining waste discharge.

The wastewaters from Serbia side in this study are extremely enriched with toxic elements such as arsenic and cadmium. The results showed that the concentrations of almost all metallic ions from collected surface samples during the period September 2019-Jun 2021 of study are above Maximum Allowed Concentration (MAC) according to Serbian legislation for surface water.

The summary results for the content of elements in surface water samples collected during the eight quarters of the implementation of Project RoRS 337 from Serbian side are given in Table 4.

The manganese, nickel and cadmium concentrations in IV class of surface water samples (from Bor city to the confluence Bor and Timok River) were above the Maximum Allowed Concentration (1 mg/L, 34 µg/L and 0.9 µg/L respectively) according to Serbian legislation for surface water in all analyzed samples. The sulfate content is also above the MAC value (300 mg/L) in all analyzed surface water samples of IV category. Exceedances of MAC values in these surface waters in a high percentage, above 70%, were also recorded for Cu (98.6%), Fe (95.2%), Zn (86.9%), Pb (82.1%) and As (66.2%). The only element whose content did not exceed the MAC value is chromium. The maximum recorded contents for nickel (16200.0 µg/L) and arsenic (25991.3 µg/L) are about 470 and 260 times higher than the MAC values, respectively. Of particular concern is the data for cadmium, the maximum detected value of this highly toxic metal is almost **6000** times higher than the MAC value.

Fe was the most abundant element in IV class of surface water samples with median value of 131.3 mg/L and concentrations in range from 0.069 to 1368.1 mg/L. High presence of copper in IV class of surface water is also recorded with median value of 45.7 mg/L and concentration range from 0.089 to 318.7 mg/L.

A much lower percentage of exceedances of the MAC value were recorded in the analyzed surface water samples of III category (from the confluence Bor and Timok River to the confluence of Timok and Danube River). The manganese and nickel content was exceeded in 38.1% and 42.9% of the analyzed samples respectively. The content of sulfate content for this category of surface waters was exceeded in 52.4%. The most exceedances of the MAC value for this category of water samples were obtained for cadmium (85.7%). The recorded values for the content of cadmium in surface water samples of III category are lower than those of IV category. The median value for cadmium content is 3.2 µg/L, with maximum value of 47.0 µg/L, which is times lower comparing the maximum values from surface waters of IV category. The recorded concentrations of iron, copper, zinc, arsenic, lead and chromium were below the MAC values for this category of surface water samples.

The situation is even better with II category (from border with Hungary up to border with Bulgaria) of surface waters in which increased values of manganese in 21.9%, arsenic in 12.5%, nickel in 9.4% and sulfate in 56.2% of the analyzed samples were detected. Unlike IV class of surface waters, the median values of individual elements of II class of surface waters are below the MAC values.

Table 4 – Summary results from 1st to 8th quarter for surface water samples sampled from Serbian side

Category of surface water / Location	Parameter	Range (min-max)	Median	MAC	Content > MAC (%)
IV From Bor city to the confluence Bor and Timok River Samples (W1 – W10)	Fe (mg/l)	0.069-1368.1	116.3	2	**95.8**
	Mn (mg/l)	1.5-115.8	6.1	1	**100.0**
	Cu (mg/l)	0.089-318.7	46.4	1	**98.8**
	Zn (mg/l)	0.28-43.2	7.1	5	**91.6**
	As (µg/l)	<2.1-25991.3	279.2	100	**62.4**
	Ni (µg/l)	38-16200.0	1676.9	34	**100.0**
	Pb (µg/l)	<2.1-3718	471.0	14	**84.8**
	Cd (µg/l)	8.0-5375	508.3	0.9	**100.00**
	Cr (mg/l)	<0.005-0.16	0.021	0.25	0.0
	Hg (µg/l)	N.D. (<0.5)	N.D. (<0.5)	0.07	/
	SO_4^{2-} (mg/l)	744.1-13964.3	2404.4	300	**100.0**
III From the confluence Bor and Timok River up to the confluence of Timok and Danube River Samples (W12 – W14)	Fe (mg/l)	<0.007-0.35	0.067	1	0.00
	Mn (mg/l)	<0.006-0.78	0.17	0.3	33.3
	Cu (mg/l)	<0.005-0.33	0.059	0.5	0.0
	Zn (mg/l)	<0.005-0.76	0.026	2	0.0
	As (µg/l)	<2.1-4.7	3.5	50	0.0
	Ni (µg/l)	5.3-551.9	29.3	34	37.5
	Pb (µg/l)	<2.1-5.9	5.9	14	0.0
	Cd (µg/l)	<0.14-47.0	3.0	0.6	**87.5**
	Cr (mg/l)	<0.005	-	0.1	0.0
	Hg (µg/l)	N.D. (<0.5)	N.D. (<0.5)	0.07	/
	SO_4^{2-} (mg/l)	82.2-691.6	161.8	200	**45.8**
II Danube Sample (W15)	Fe (mg/l)	<0.007-38.4	0.081	0.5	3.1
	Mn (mg/l)	<0.006-4.3	0.26	0.1	22.2
	Cu (mg/l)	<0.005-0.58	0.025	0.005(T=10) 0.112 (T=300)	**61.1** 16.7
	Zn (mg/l)	<0.005-0.40	0.031	0.3 (T=10) 2(T=500)	2.8 0.0
	As (µg/l)	<2.1-31.2	3.9	10	11.1
	Ni (µg/l)	<3.6-388.2	16.8	34	8.3
	Pb (µg/l)	<2.1-7.1	5.6	14	0.0
	Cd (µg/l)	<0.14-35.3	0.45	0.45	38.9
	Cr (mg/l)	<0.005	0.022	0.05	0.0
	Hg (µg/l)	N.D. (<0.5)	N.D. (<0.5)	0.07	/
	SO_4^{2-} (mg/l)	26.7-1950.6	73.5	100	**47.2**

As could be seen from the plot in Figure 5 pollutants show zig-zag pattern and go together, high concentrations of one species correspond to high concentration of other species. This may

Cooperation beyond borders.
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indicate the same local source of pollution. In the case of our study the mining activities is the main source.

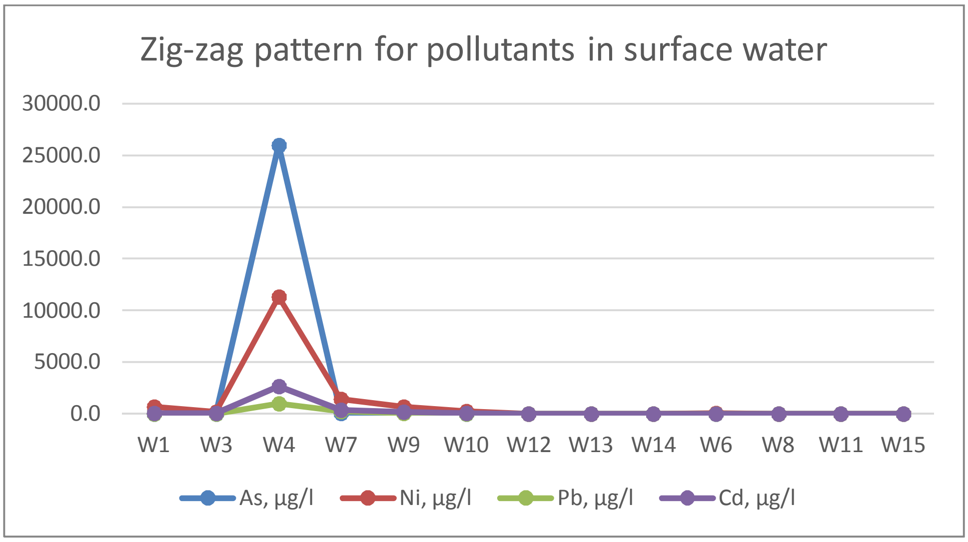
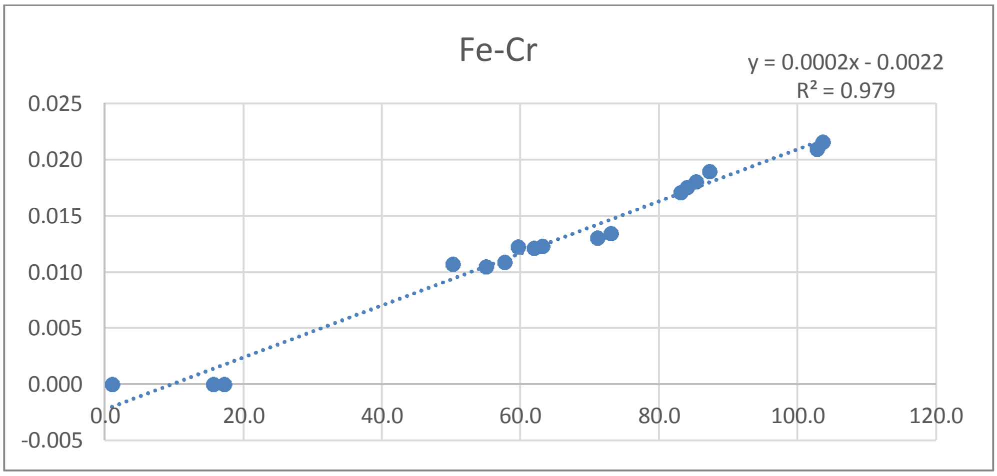
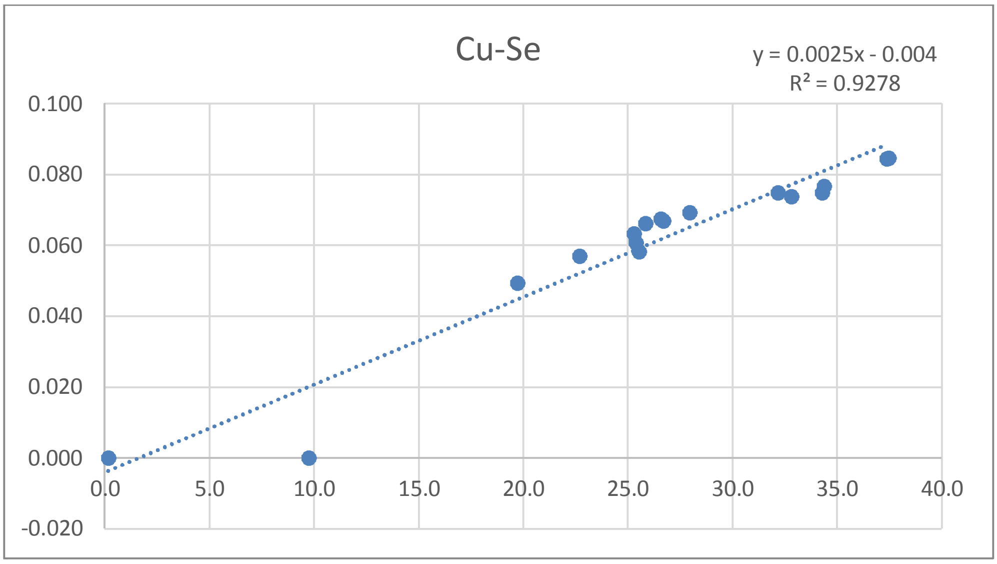


Figure 5 - Pollutants in surface water scatter plot from Serbian site

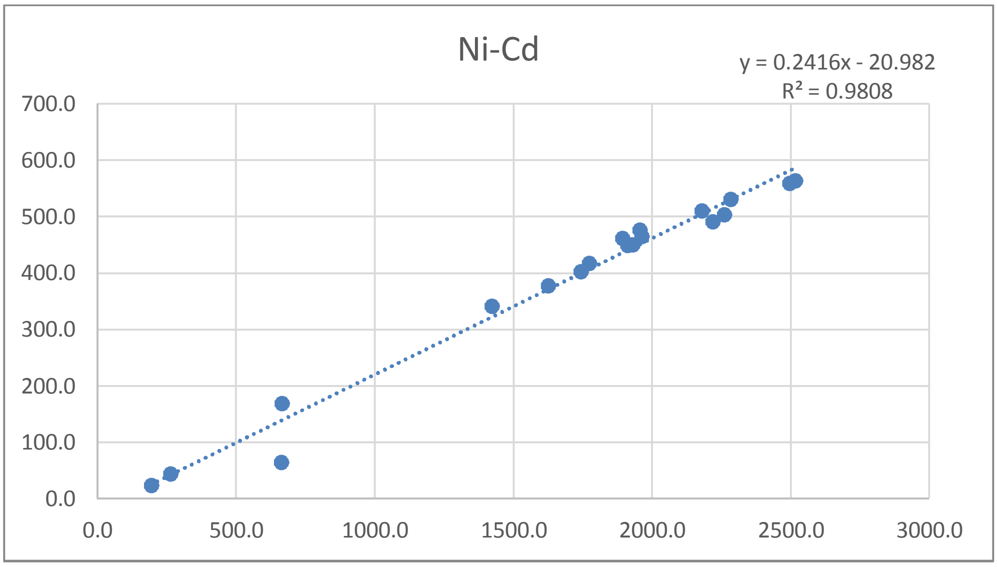
In addition, the relationships between species (Figure 6-examples for data of 7th quarter) are examined to evaluate relationships between species that may indicate a common source. According to the scatter plot results, the coefficient of determination for more than 90% of concentration of one elements are well correlated with the concentrations of other element indicating the same source of pollution for those species.



(6a)



(6b)



(6c)

Figure 6a-6c – Scatter plot for pairs Fe-Cr, Cu-Se and Ni-Cd

According to the pH values of the analyzed surface water samples (IV class) it is strong acid water with pH values lower than 3.0.

The summary results from 1st to 8th quarters for wells sampled from Serbian site are given in Table 5.

Table 5 – Summary results from 1st to 8th quarters for wells sampled from Serbian site

Element/ Parameter	Range (min-max)	Median	MAC for drinking water	Content > MAC (%)
Mn (mg/l)	<0.006-26.6	0.057	0.05	**23.3**
Cu (mg/l)	<0.005-13.6	0.040	2	3.5
Zn (mg/l)	<0.005-2.3	0.056	3	0.0
As (µg/l)	<2.1-162.6	4.8	10	**5.8**
Ni (µg/l)	<3.6-629.5	5.9	20	**3.5**
Pb (µg/l)	<2.1-29.2	2.4	10	**1.2**
Cr (mg/l)	<0.005-0.022	0.015	0.05	0.0
Mo (mg/L)	<0.007	-	0.07	-
Hg (mg/l)	<0.0005	-	0.001	-
SO_4^{2-} (mg/l)	52.7-4387.1	446.1	250	**83.7**

The exceedances for almost all of the analyzed elements from wells collected during the seven quarters of implementation of Project RoRS 337 from Serbian site are below the Maximum Allowed Concentration (MAC) according to Serbian legislation for drinking water except for the content of sulphate, manganese and arsenic. The content of sulfate is above the MAC value of 250 mg/L in 81.6% of analyzed samples while the content of manganese and arsenic are above the MAC values in 25.0% and 6.6% respectively. The median values for manganese and sulphate are above the MAC values for drinking water. The pH values for wells range from 5.76 to 7.82.

5.2. Results regarding the samples collected from Romania

Physicochemical parameters

Considered rivers in Caras Severin County near Moldova Noua in Romania do not have acidic characteristics. The main way of pollution of these rivers is by spreading of dust from nearby flotation tailing in windy conditions. According to Romanian legislation for surface water, in considered rivers, the content for almost all of the analyzed elements is below the MAC (Official Journal of Romania, No. 11/13.06.2006) for this category of surface waters. The only exceptions are Fe and Cu which were with slightly increased concentrations probably because of windy weather before and during the sampling.

Maximum recorded concentration of iron was two times higher than MAC. Median value for Fe was below MAC for Bosneag River and Radimna River while for Nera River was two times higher but still with relatively low absolute concentrations. Possible reason for increased concentration of iron could be spreading of dust from nearby flotation tailing during windy weather. In recent research it was also noted that in general there is a trend of increas of iron in river waters (Ekstrom et al., 2016). The possible causes of the increasing of Fe in the rivers are more elusive but probably also involve increased anaerobic microbial activity, considering the fact that water levels and temperature have increased during the period (Sarkkola et al., 2013). However, iron is not a strong hazardous element for human health, especially in recorded concentrations, but regarding of increased concentration it is expected that more attention will be given for clarification of noted increased transfer of Fe from soil to waters.

The summary results for the content of elements in surface water samples collected during the seven quarters of the implementation of Project RoRS 337 from Romanian sites are given in Table 6.

Table 6 – Summary results from 1st to 8th quarter for surface water samples sampled from Romanian side

Category of surface water / Location	Parameter	Range (min-max)	Median	MAC	Content > MAC (%)
II Bosneag River Samples (W18– W19)	Fe (mg/l)	0.0087-0.8903	0.4782	0.5	**50.0**
	Mn (mg/l)	<0.0016-0.0583	0.0583	0.1	0.00
	Cu (mg/l)	0.0284-0.1158	0.069	0.03	**75.0**
	Zn (mg/l)	0.0252-0.0549	0.0472	0.2	0.00
	As (µg/l)	<2.1-3.7	3.0	20	0.00
	Ni (µg/l)	<3.6-4.2	4.2	25	0.00
	Pb (µg/l)	<2.1-3.7	3.7	10	0.00
	Cd (µg/l)	<0.14-0.39	0.26	1	0.00
	Cr (mg/l)	<0.0017	/	0.05	0.00
	Hg (µg/l)	N.D.	N.D.	0.3	0.00
	SO_4^{2-} (mg/l)	78.5-295.8	133.7	120	**50.0**
II Radimna River Samples (W20 – W21)	Fe (mg/l)	0.1310-0.2652	0.2397	0.5	0.00
	Mn (mg/l)	0.0106-0.0326	0.0230	0.1	0.00
	Cu (mg/l)	0.0376-0.0546	0.0474	0.03	**100.0**
	Zn (mg/l)	0.0176-0.0333	0.0216	0.2	0.00
	As (µg/l)	<2.1	/	20	0.00
	Ni (µg/l)	<3.6	/	25	0.00
	Pb (µg/l)	<2.1	/	10	0.00
	Cd (µg/l)	<0.14	/	1	0.00
	Cr (mg/l)	<0.0017	/	0.05	0.00
	Hg (µg/l)	N.D.	N.D.	0.3	0.00
	SO_4^{2-} (mg/l)	21.9-30.3	24.8	120	0.00
II Nera River Samples (W22 – W23)	Fe (mg/l)	0.9895-1.1643	1.0292	0.5	**100.0**
	Mn (mg/l)	0.0423-0.0524	0.0497	0.1	0.00
	Cu (mg/l)	0.0136-0.0460	0.0317	0.03	**50.0**
	Zn (mg/l)	<0.0062-0.0232	0.0109	0.2	0.0
	As (µg/l)	<2.1	/	20	0.0
	Ni (µg/l)	<3.6	/	25	0.0
	Pb (µg/l)	<2.1	/	10	0.0
	Cd (µg/l)	<0.14	/	1	0.0
	Cr (mg/l)	<0.0017	/	0.05	0.0
	Hg (µg/l)	N.D.	N.D.	0.3	0.0
	SO_4^{2-} (mg/l)	21.6-29.6	24.8	120	0.0

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Increased copper concentrations were recorded for Bosneag River and Radimna River but the highest content of Cu was registered for Bosneag River, almost 5 times higher than MAC. However, median value was around 2 times higher than MAC. Having on mind that Bosneag River is closest to flotation tailing pond, probable reason for increased Cu concentration is spreading of dust from nearby flotation tailing during windy weather before and during sampling. Median value for Cu for Radimna River is 1.6 times higher than MAC. Radimna River is located farther than Bosneag River from the flotation tailing which indicate similar reason for the measured Cu concentrations.

For the considered rivers on the Romanian side, it was important that no strong pollution with heavy metals was recorded. This is a consequence of the dominant dust pollution, and not AMD which is an incomparable higher danger than dust.

Microbiological analysis

Microbiological analysis is used primarily to estimate the number of microorganisms present in a sample and subsequently, based on different biological, biochemical or chemical methods, microorganisms can be detected, listed and identified.

During the sample collection campaigns, from October 2020 until July 2021, several microbiological parameters were used in the testing of the samples collected from Moldova Nouă area: total number of microorganisms from the samples, isolation of microorganisms from samples, dehydrogenase activity in soil samples, microbial count in water samples.

The parameters listed above give information about the health of the ecosystem or the microbial contamination of well waters. The isolation of microorganisms from samples is useful for the isolation of bacteria tolerant to higher copper concentrations.

Table 7 – Water, soil and sediments sample collected through October 2020 until July 2021 and used for microbiological analysis

Sample ID	Location of the sample	Observations
W18-M	Boşneag River (Moldova Veche)	
W19-M	Boşneag River (upstream Moldova Veche)	
W20-M	Radimna River (Pojejena)	
W21-M	Radimna River (upstream Pojejena)	
W22-M	Nera River (Socol)	
W23-M	Nera River (upstream Socol)	
WU11-M	Well from village of Coronini, near the pond Boşneag	
WU12-M	Well from village of Moldova Veche, near the pond Boşneag	
WU13-M	Well from village of Macesti	
WU14-M	Well from city of Moldova Noua	
WU15-M	Public well from village of Radimna	Extra samples collected in the second sample collection campaign (February 2021) – private
WU16-M	Well from village of Măcești	
WU17-M	Well from village of Măcești	

WU18-M	Well from village of Măcești	wells from local people in Măcești
WU19-M	Public water supply from Măcești	
WT-M	Surface water found in tailing	Water found in the surface of the tailing pond
WR	Well from village of Măcești	Extra sample collected in the third sample collection campaign (May 2021) – private wells from local people in Măcești
S82-M	Sediments from W18 location (Bosneag River)	
S83-M	Sediments from W19 location (Radimna River)	
S84-M	Sediments from W20 location (Nera River)	
S85-M	Soil near Bosneag tailings pond	
S86-M	Soil at 200 m from Bosneag tailings pond (N-W direction)	
S87-M	Soil at 400 m from Bosneag tailings pond (N-W direction)	
S88-M	Soil at 600 m from Bosneag tailings pond (N-W direction)	

Enumeration of microorganisms from water samples

During October 2020 until February 2021, all samples collected from Moldova Nouă area were subjected to microbiological analysis in order to count the number of viable microorganisms. Different growth media was used in order to count as many microorganisms as possible, taken into account that in water, sediment and soil samples numerous microorganisms are present and all require different growth conditions. Different incubation temperature and time intervals were used as well in the attempt of culturing various microbial strains.

Table 8 present the CFU determination for water samples (surface water as well as wells) collected from October 2020 until July 2021 and the Figures 7 – 10 show the microbial growth on different culture media and at different temperature conditions. The surface water samples in Moldova Noua showed high microbial growth, which was expected given the fact that we collected samples from rivers close to rural and urban areas. Well water samples tested showed a very high number of microbial growth. The microbiological quality parameters of drinking water are regulated by Romanian legislation through the 458/08.07.2002 Law regarding the quality of drinking water and these microbiology parameters are given in the Table 9 below. The drinking water must not contain a number of microorganisms that could jeopardize human health.

Table 8 – Microbiology quality parameters - allowed values for drinking water

Parameter	Allowed value
Escherichia coli	0/number/100 mL
Enterococci	0/number/100 mL
Clostridium perfringens	0/100 mL
Total viable count at 22°C	100/mL
Total viable count at 37°C	20/mL

Table 9 – CFU determination for water samples collected in all four sample collection campaigns

	October, 2020	February, 2021		May, 2021	July, 2021	
Sample ID	CFU/mL	CFU/mL (PCA media)	CFU/mL (NA media)	CFU/mL	CFU/mL at 22°C	CFU/mL at 37°C
W18 M	147000	25000	20000	14425	1900	1100
W19 M	51000	662000	25800	196000	15200	10200
W20 M	1160000	7000	0	115600	2400	2800
W21 M	139000	0	0	135433	6200	3100
W22 M	45000	15000	9000		12900	20100
W23 M	70000	9000	16000		11600	20300
WU11 M	16000	2000	0		38900	800
WU12 M	823000	65000	0		51100	52300
WU13 M	798000	1000	0		73700	11600
WU14 M	321000	0	0		98800	36800
WU15-M		0	0			
Wu16-M		1000	6000			
WU17-M		6000	1000			
WU18-M		92000	155000			
WU19-M		87000	90000			
WT-M		15000	27000			
WR				6266		

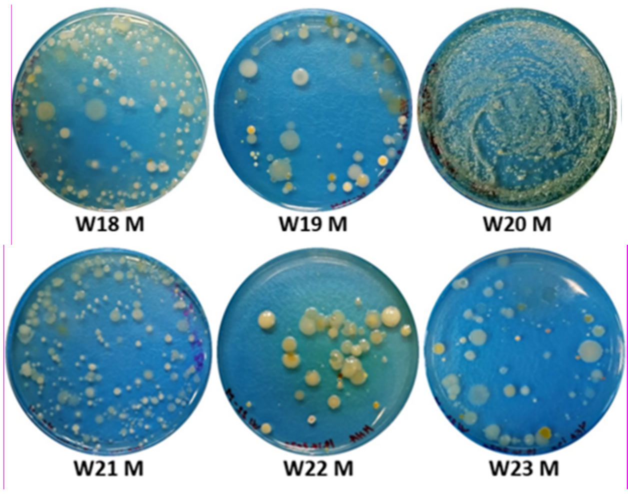

Figure 7 – CFU determination on nonselective media from surface water samples collected in October 2020

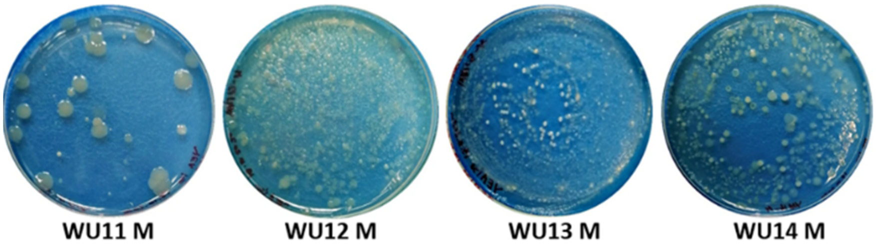

Figure 8 – CFU determination on nonselective media from well water samples collected in October 2020

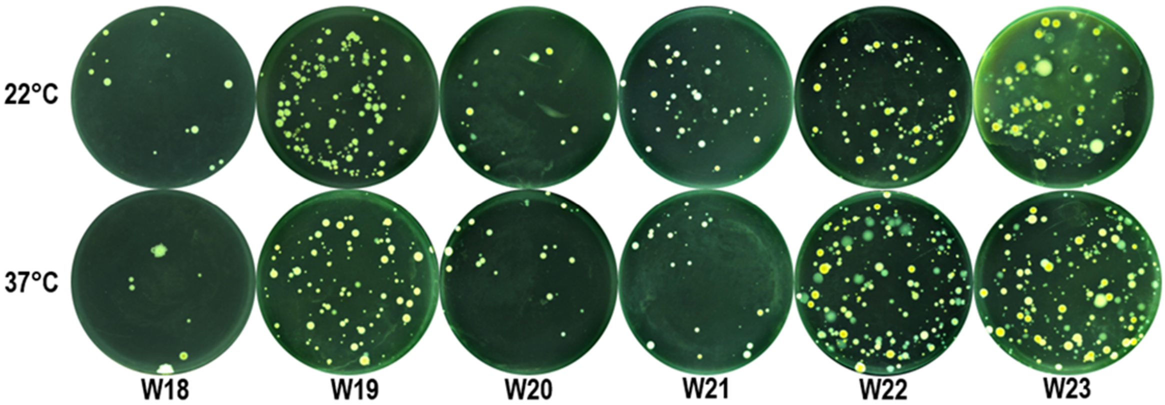

Figure 9 – SCAN 300 images of Petri plates with microbial counts from surface water samples

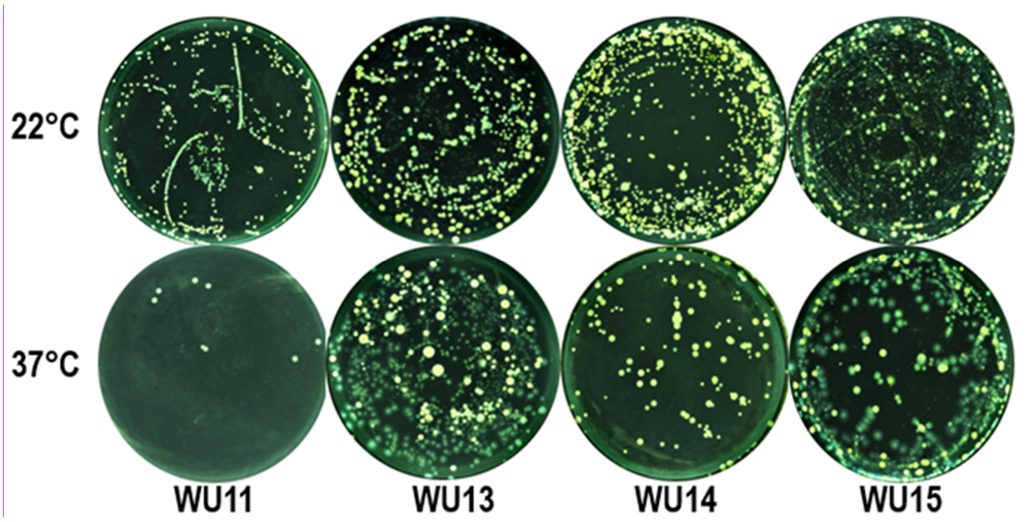

Figure 10 – SCAN 300 images of Petri plates with microbial counts from well water samples

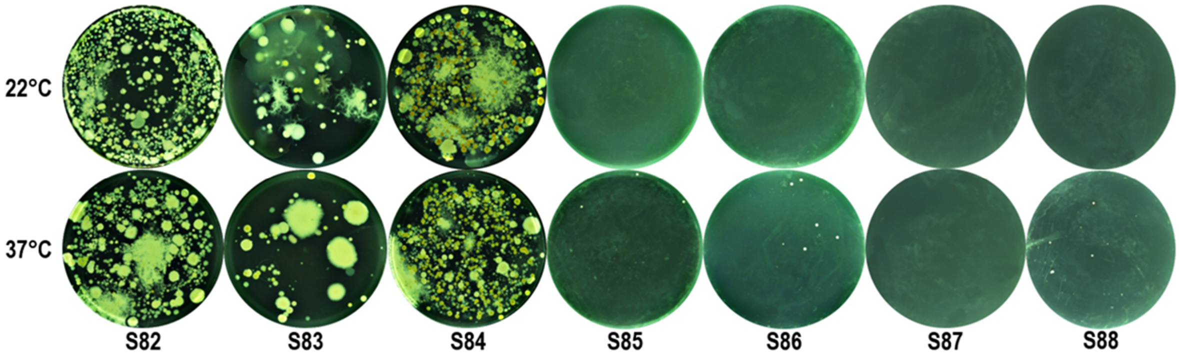

Figure 11 – SCAN 300 images of Petri plates with microbial counts, after 24 hours, from soil and sediment samples

Figure 11 and Figure 12 present the microbial growth from sediment and soil samples. The images and colony counting were made using SCAN 300 automated colony counter. The sediment samples showed a high number of microbial growth, while the soil samples from Tăușani – Boșneag tailing pond, as expected showed a very low number of microbial colonies. Moreover, for the soil samples from tailing pond, more colonies appeared after 48 hour of incubation, meaning that in those soil samples various types of microorganisms, not only bacteria, but also fungi.

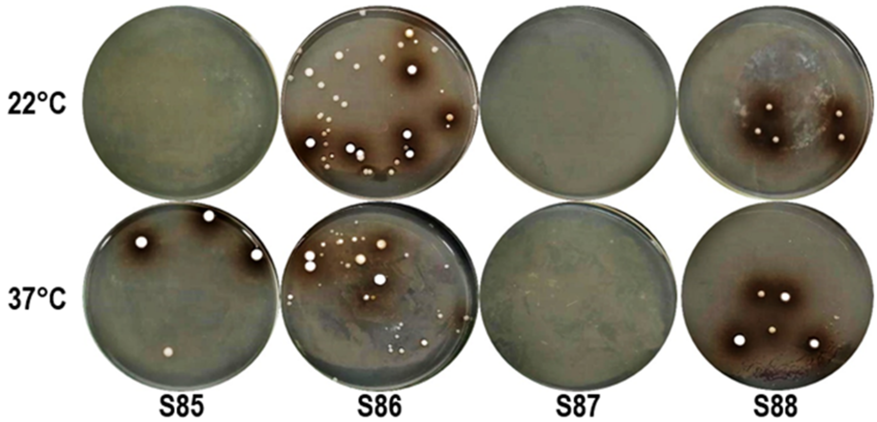

Figure 12 – SCAN 300 images of Petri plates with microbial counts, after 48 hours, from soil samples collected from Tăușani – Boșneag tailing pond

Dehydrogenase activity from soil samples

Dehydrogenase activity in soils indicates the quality and health of the soil sample investigated. The soil microflora is responsible for decomposing and converting the organic matter, which is further utilized by higher organisms.

Dehydrogenases are intracellular enzymes that components of the respiratory chain of microorganisms, therefore these enzymes can be used as indicators of biological redox systems and as a measure of viable and physiological active soil microbial communities.

The results obtained showed weak dehydrogenase activity on soil samples, meaning that bacterial growth is very low. The results obtained from TVC (Total Viable Count) analysis confirm the results obtained by monitoring the dehydrogenase activity, hence the low microbial biomass present in soil samples from Tăușani – Boșneag tailing pond.

As seen below, in Figure 13, although none of the soil samples tested show a positive dehydrogenase activity, based on visual observations, the spectrophotometric measurements showed weak dehydrogenase activity on the soil samples collected.

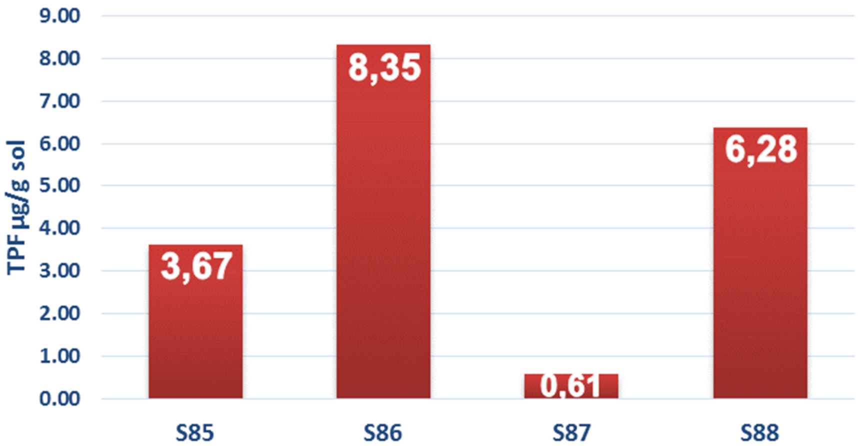

Figure 13 – Dehydrogenase activity on soil sample collected from tailing pond

Yeast Toxicity Test to assess the toxicity of water samples from Moldova Nouă area

Figure 14 shows the inhibition of the fermentation process by the water samples collected from Moldova Nouă area, during the second sample collection campaign. The majority of the samples showed low inhibition of the fermentation process (25% and under for W21, W23, WU11, WU13, WU14, WU17, WTM samples) while some samples showed no inhibition of the fermentation process (W19, WU15, WU16, WU18, WU19). Some samples inhibited the fermentation process by 37,5% (WU12) and 43,75% (W20) and up to 50% (W18, W22).

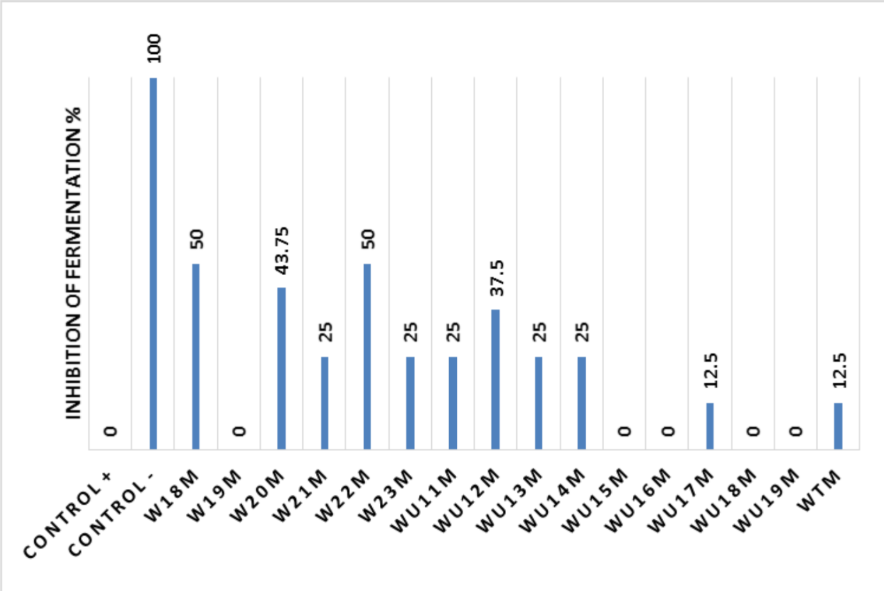

Figure 14 – Inhibition of fermentation (%) by water samples from Moldova Nouă area

Ecotoxicity assessment

None of the tested samples showed ecotoxic effects to duckweed, although some of the samples showed a reduction of green frond number, compared to the negative control.

As the water samples were tested undiluted, these samples are considered as not toxic to *Lemna minor*. Although the soil and sediment samples showed a reduction in green frond number, this is due to the high concentration in which these samples are tested. If the tested concentration would be considered to be the EC_{50} value of these samples, these would be classified according to both U.S. Environmental Protection Agency (U.S. Environmental Protection Agency, 2017) and United Nations (United Nations, 2013) as practically non-toxic. An EC_{50} value higher than 100 mg/L is considered as practically non-toxic, and the tested concentration is 10000 mg/L, which is 100 times higher than the reference concentration.

The river water sample with the lowest percentage of green fronds compared to the negative control was sample W22 and sample W19 had the highest value (Figure 15). The well water sample with the lowest percentage was sample WU14 and with the highest percentage was sample WU12 (Figure 16).

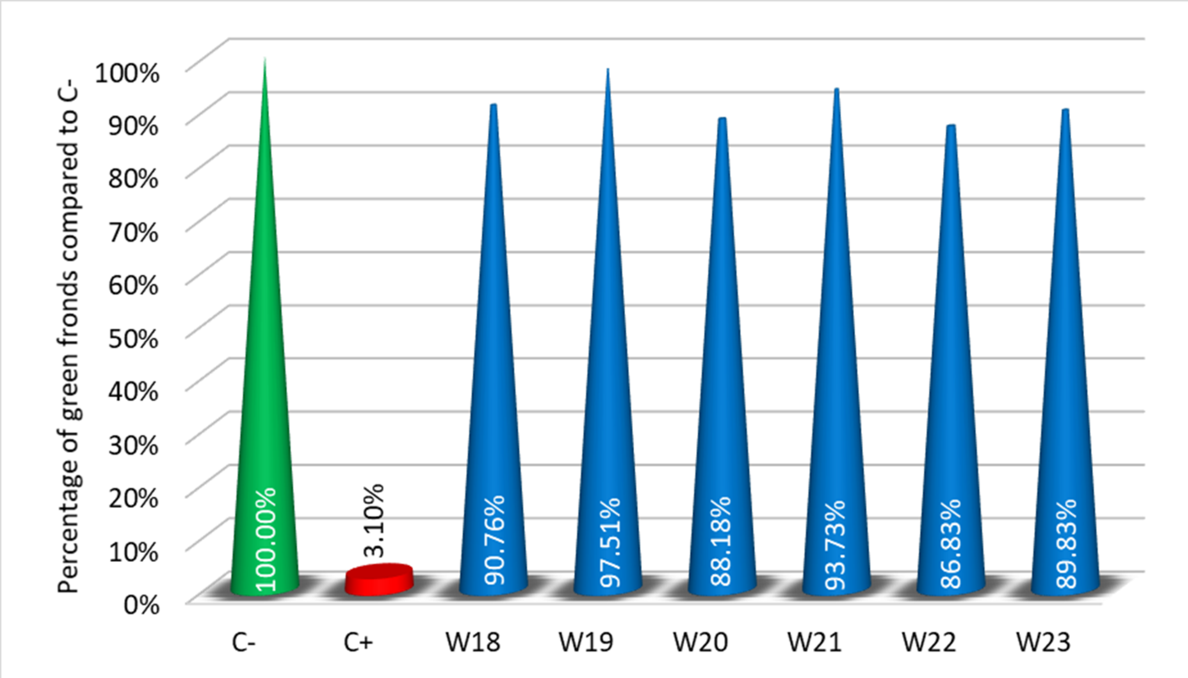

Figure 15 – Percentage of green fronds for each tested river water sample compared to the negative control

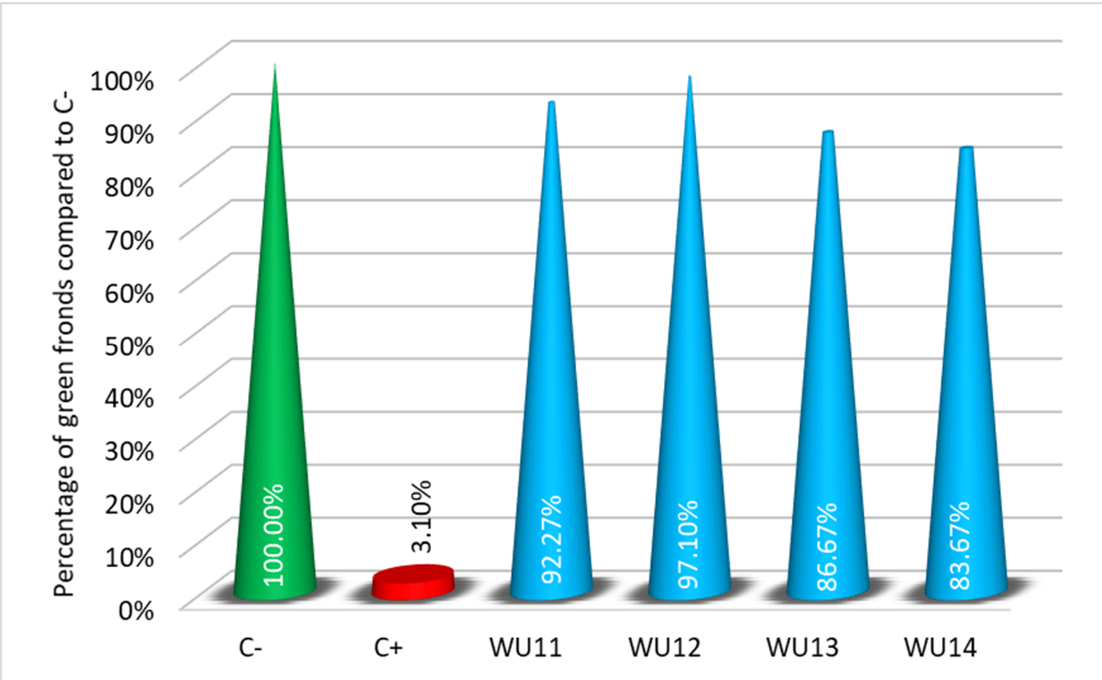

Figure 16 – Percentage of green fronds for each tested well water sample compared to the negative control

The sediment sample with the highest value of the percentage of green fronds compared to the negative control was sample S82, and sample S84 had the lowest value (Figure 17). For the soil samples, the highest value was observed for sample S85, while the lowest value was observed for sample S88 (Figure 18).

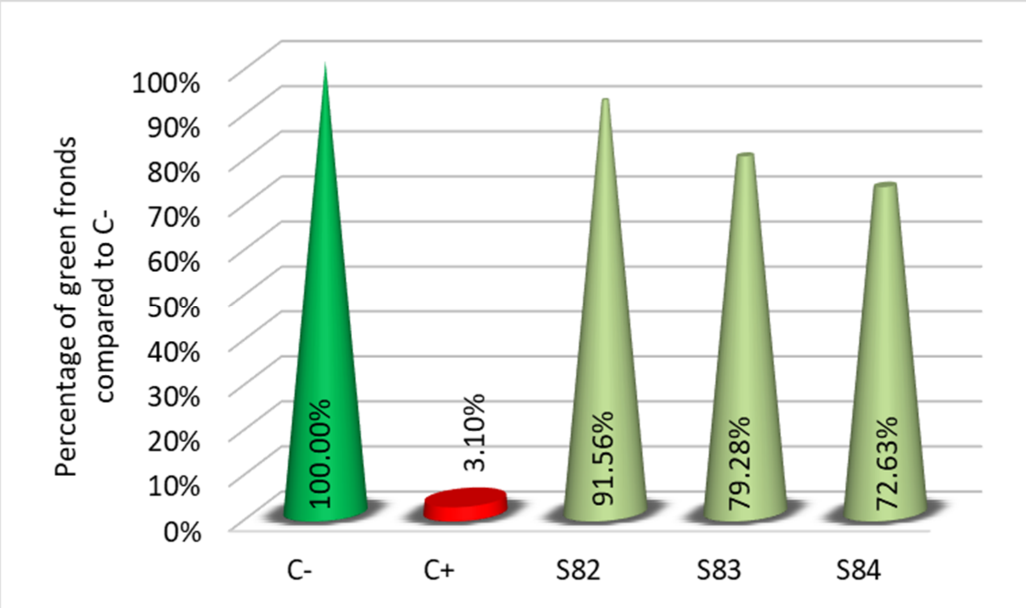

Figure 17 – Percentage of green fronds for each tested well sediment sample compared to the negative control

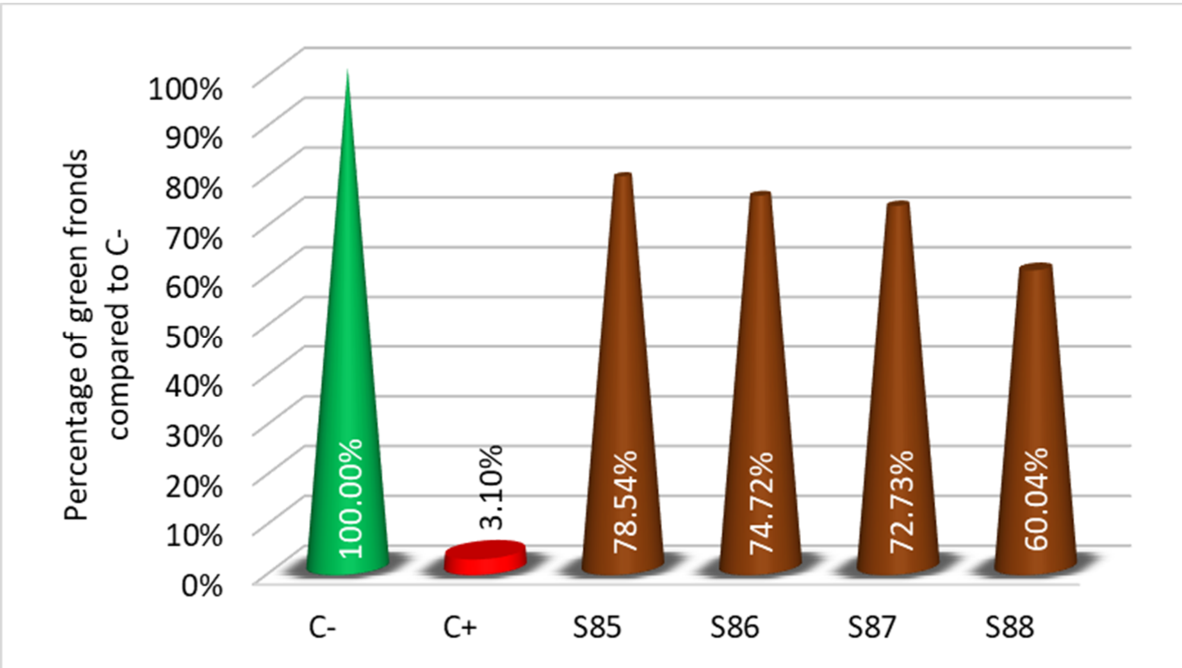

Figure 18 – Percentage of green fronds for each tested soil sample compared to the negative control

6. CONCLUSION

For Bor region, the recorded content of **all analyzed elements** in surface water samples of IV category except chromium and mercury is above the MAC values in almost all of the analyzed samples. The maximum recorded value for cadmium of 5375 μg/L is more than 5500 times higher than allowed concentration. The content of recorded metals exhibited a high degree of contamination, indicating that these surface water samples may **pose a significant to extreme risk to nearby residents.**

The **pH** values for IV category of surface water range from 1.49 to 7.72. According to the pH values most of the analyzed surface waters are **strong acid** water with pH values **lower than 3.0**.

The situation is different with rivers that belong to the **II** (**Danube River**) and **III** (**Timok River**) category, the recorded values for most of the analzyed elements are below the allowed values for the given category of surface waters.

The content for all analyzed elements from wells are under the Maximum Allowed Concentration (**MAC**) according to Serbian legislation for drinking water except for the content of arsenic, manganese and sulfate during measuring campaign.

The **pH** values for wells range from 6.76 to 7.82.

For the considered rivers on the Romanian side, it was important that no strong pollution with heavy metals was recorded. This is a consequence of the dominant dust pollution, and not AMD which is an incomparable higher danger than dust.

The quality of water can be measured through microbiological analysis in which the methods used give an estimate of the number of microorganisms present and allow the recovery, isolation and identification of microbial cells. The sediment and water samples tested showed the presence of a variety of bacteria and fungi, which are commonly present in waters. However, the microbial count for well water samples showed a high number of viable microorganisms that can affect human health if present in potable water.

The microbiological analysis of the soil samples collected from Tăușani – Boşneag tailing pond showed a low number of microorganisms present, results confirmed also by the dehydrogenase activity test, which revealed low enzymatic activity in soil samples, meaning that these soil samples from the tailing contain a very small number of bacteria, due to the lack of nutrients.

The eco-toxicological assessment of the samples collected from Romania showed that these samples did not have an eco-toxic effect towards *Lemna minor*. Although some samples did cause a reduction of the frond numbers, these samples did not prove to be eco-toxic as the tested concentration was very high for sediment and soil samples, and water samples were tested without any dilution.

All the collected data regarding the surface water state in the investigated regions are publicly available within the knowledgebase that war created in the project (Ostafe et al, 2021) following the link: http://www.elearning-chemistry.ro/rosnet2/knowledge-base/.

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