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HYDROCHEMICAL AND ECOTOXICOLOGICAL CHARACTERISATION OF WATER SAMPLES FROM MOLDOVA NOUA AREA, ROMANIA

Abstract: The abandoned copper mine in the Moldova Noua area is still producing mining pollution today due to the drying of tailings ponds and the carrying of contaminated dust by the winds, leading to potential contamination of surface waters and groundwaters. The purpose of this study is the assessment of some surface waters and groundwaters potentially contaminated by mining pollutants from Moldova Noua area regarding their hydrochemical and ecotoxicological properties. The physicochemical parameters (pH, conductivity, dissolved oxygen, and turbidity) were determined *in situ*, with the concentrations of heavy metals (As, Cd, Cr, Cu, Fe, Mn, Ni, Pb, and Zn) being determined in the laboratory. Inhibition of acid phosphatase enzymatic activity and yeast toxicity tests were the two ecotoxicological tests used for the evaluation of mining pollution effects on the quality of surface waters and groundwater. The results highlighted that most of the physicochemical parameters and heavy metal concentrations were within normal limits. With the exception of the surface water sample collected from the Bosneag River, upstream of Moldova Noua, all samples induced inhibition compared to the positive control in the ecotoxicological tests applied. Statistically significant correlations were observed, with the total concentration of iron ions presenting the most statistically significant correlations with other parameters. A grading scale was used to assess the state of the waters, highlighting the weak impact of mining pollutants on the analysed samples.

Keywords: mining pollution, physicochemical parameters, heavy metal concentration, ecotoxicity tests

Introduction

The copper mine in the Moldova Noua area has played an important role for the industrial sector throughout Europe; it has existed in Romania for more than 100 years and represents the second largest copper mine in the country. The mining activities in this area were stopped in 2008, with 130 hectares of approximately 20 m deep tailings ponds containing 30 million m^3 of tailings abandoned. After the cessation of mining activities in

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the Moldova Noua area, environmental pollution did not stop. This is due to the abandonment of the tailings pond, which dried up over time, allowing the resulting dust to be transported over long distances by wind. The mining pollutants contaminated dust has the potential to affect both the environment and the health of people living in the affected areas [1-4].

Due to this potential environmental hazard, water quality testing is imperative, along with soil and air quality monitoring, and the development of diverse remediation methods is also important. Water quality monitoring is based on the identification of some physicochemical parameters, such as pH, conductivity (*EC*), dissolved oxygen (DO), turbidity (T), etc., as well as the determination of the concentration of polluting compounds such as heavy metals, chlorides, and nitrates. There are various methods for determining these parameters, such as direct methods *in situ* or in the laboratory, but also methods that use remote monitoring systems [1, 5]. There is some consensus on using biological assays, such as ecotoxicity assays, which lead to fast results based on assessing the biological effect of the pollutants. When testing a large number of samples, it is preferable that the methods used not be time-consuming, not require sophisticated equipment or technicians with specialised skills, and also give reproducible results. The yeast toxicity test, one such method that meets the criteria mentioned above, 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. The principle of this test is based on the inhibition of the fermentation (IF) process by a toxic substance, represented by the suppression of CO_2 production [6]. Classic ecotoxicity tests use organisms or populations of organisms to test the quality of potentially contaminated waters. There are possible alternatives to the classic ecotoxicity test, one such test being the acid phosphatase enzyme activity inhibition test which represents a potential ecotoxicity test at the molecular level [7]. The enzymatic activity (EA) of acid phosphatases can be influenced by pollutants present in the environment, inducing an inhibition of the enzymatic activity, revealing possible toxic effects of the analysed pollutants [8].

The purpose of this study was the assessment of the hydrochemical and toxicological properties of some surface and groundwater samples contaminated with mining pollutants generated by the former copper mine in the Moldova Noua area. This study represents a continuation of a previous one in which the state of mining polluted surface waters in 2019-2020 was assessed for both the Moldova Noua area, Romania, and the Bor area, Serbia [1].

Materials and methods

Reagents and materials

All reagents (such as nitric acid, acid phosphatase, p-nitrophenyl phosphate, p-nitrophenol, ammonium molybdate, sodium acetate, acetic acid, peptone, dextrose, saccharose, yeast extract, agar, etc.) and materials (such as centrifuge tubes, microplates, etc.) were purchased from Carl Roth, Karlsruhe, Germany, or Merck, Darmstadt, Germany.

Study area

The study area is the Moldova Noua region in the southwest of Romania, a region where an abandoned copper mine is located. The copper mine ceased operations in 2008, leading to the abandonment of the tailing ponds and their drying out over time. This led to

a potential environmental hazard due to the heavy metal polluted dust, which is carried by wind over long distances and pollutes surface waters, as well as due to the possibility of the heavy metals' infiltration into the groundwater through contaminated rainwater [1-3].

Sampling

A total of 80 surface water and groundwater samples were collected from 10 sampling points, in the period November 2020 - July 2021, in each season (autumn, winter, summer and spring). Surface water samples were collected from six sampling points: Bosneag River from Moldova Noua (SW-1), Bosneag River upstream of Moldova Noua (SW-1'), Radimna River from Radimna (SW-2), Radimna River upstream of Radimna (SW-2'), Nera River from Socol (SW-3) and Nera River upstream of Socol (SW-3'). Groundwater samples were collected from four public wells from Coronini (GW-1), Moldova Veche (GW-2), Macesti (GW-3) and Divici (GW-4) (Fig. 1).

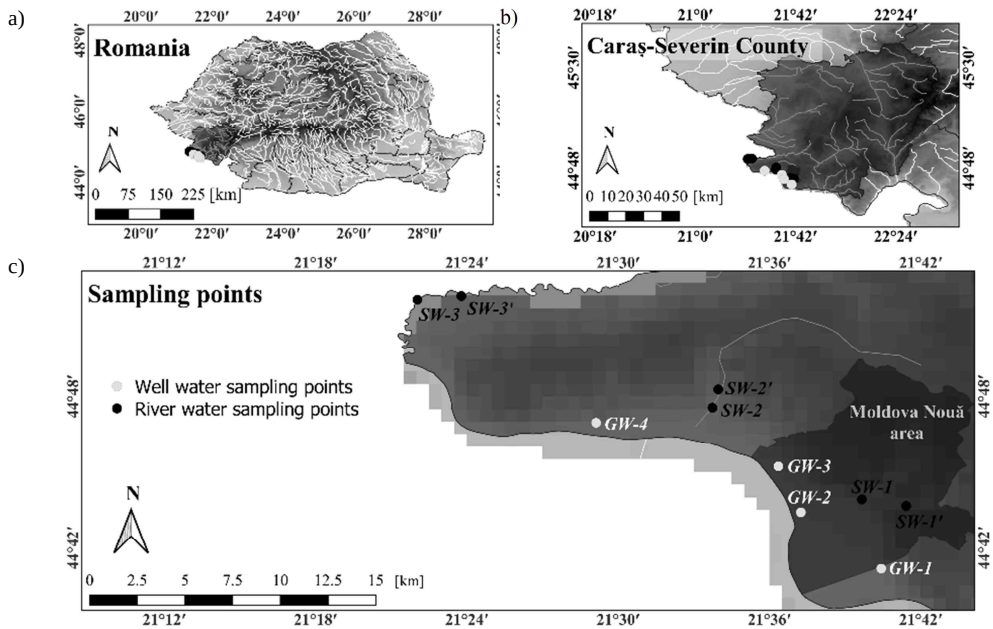


Fig. 1. Map of sampling area: a) Romania country, b) Caras-Severin county and c) sampling points in Moldova Noua area

Two types of samples were collected from each sampling point, specifically ecotoxicological and chemical samples. The surface water samples (SW) and groundwater samples (GW) were collected by specific sampling procedures according to the type of collected sample (Fig. 2). *In situ*, physicochemical measurements were carried out, as well as laboratory analyses depending on the type of collected sample.

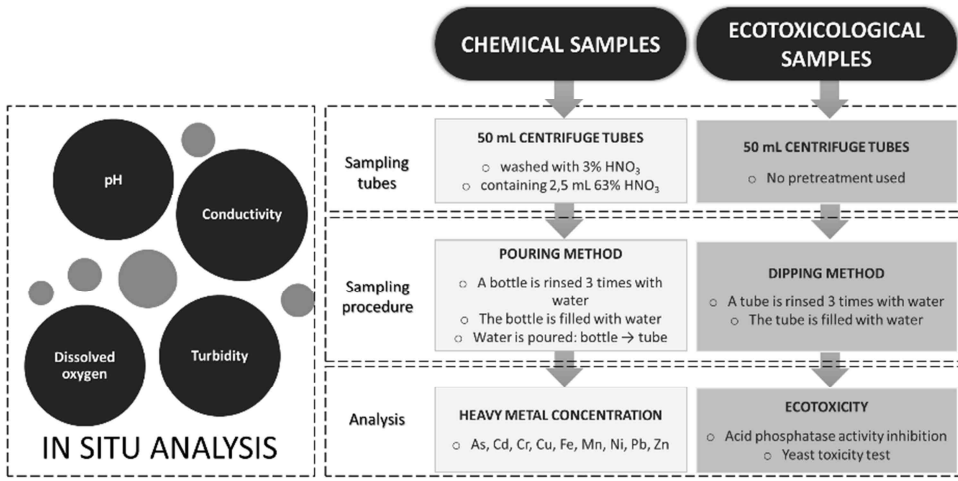


Fig. 2. Overview of sampling and analysis done *in situ* and in the laboratory, by sample type

Physicochemical parameters analysis

Parameters like pH, conductivity (*EC*), dissolved oxygen (DO) and turbidity (T) were determined *in situ* using a field multiparameter (Multi 340i, WTW) and a portable turbidimeter (2100Qis, Hach Lange). For the analysis of data, Romanian legislation (Order of Ministry of Environment and Water Management 161/2006, for the approval of the Normative on the Classification of Surface Water Quality to establish the ecological status of the water bodies [9] and Law No. 458/2002 regarding the quality of drinking water in Romania republished in 2011 [10]) was used to assess the state of the tested water samples (Table 1). Where no data was available for surface water, the groundwater limits were used for comparison. In all charts showing physicochemical values, the diamond symbols (♦) are used to represent the measured values, while the grey rectangles (▬) are used to represent the normal value range according to Table 1 for pH, conductivity, and turbidity. For dissolved oxygen data regarding surface water, the lightest grey rectangles (▬) represent the values for class I, lighter grey rectangle (▬) represents the values for class II, medium grey rectangle (▬) represents the values for class III, darker grey rectangle (▬) represents the values for class IV and darkest grey rectangle (▬) represents the values for class V according to Table 1.

Table 1

Limit values of physico-chemical parameters for surface water and groundwater samples

Parameter	**Limit values for SW samples**					**Limit values for GW samples**
pH [-]	6.5-8.5					6.5-9.5
Conductivity [S cm^{-1}] at 20 °C	No data available					2500
Turbidity [UNT]	No data available					≤ 5
Dissolved oxygen [mg/L]	**Quality classes**					5
	I	**II**	**III**	**IV**	**V**	
	7	6	5	4	< 4	

The chemical samples were used for the determination of heavy metals content. The collected samples were analysed by the Mining and Metallurgy Institute Bor using an ICP-MS (Agilent 7700 Series, Agilent Technologies) and an ICP-OES (Spectro Arcos, Spectro, Ametek) (Fig. 3).

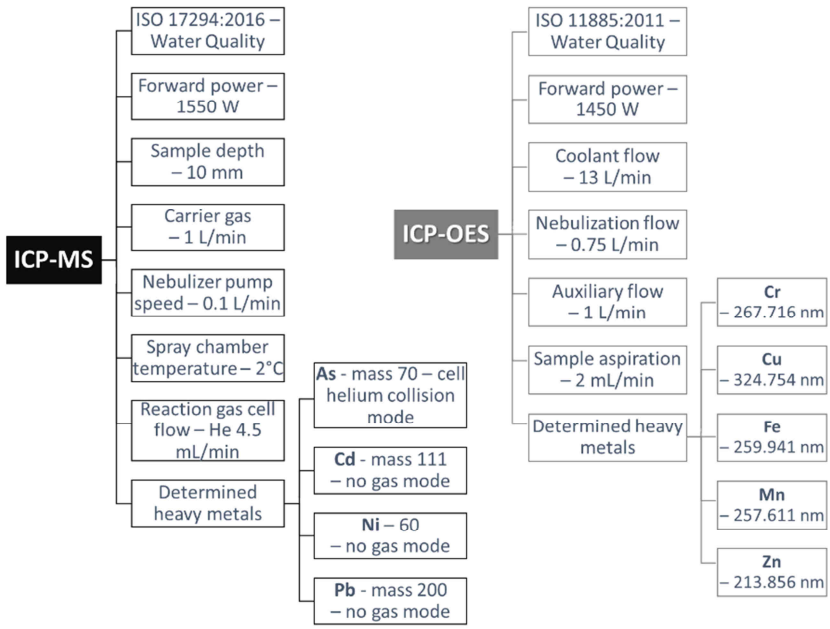


Fig. 3. Conditions used for the ICP-MS (black outline) and ICP-OES (grey outline) determination of heavy metal content

Chemical analyses were subjected to quality control using accredited reference materials (VHG Labs, QWPTM-15). For the analysis of the data, Romanian legislation [9, 10] was used to assess the state of the tested water samples (Table 2).

Table 2

Maximum acceptable concentrations (MAC) of heavy metals in surface water samples and in groundwater samples

Heavy metal [µg/L]	Quality classes for SW samples					Limit values for GW samples
	I	II	III	IV	V	
As	10	20	50	100	> 100	10
Cd	0.5	1	2	5	> 5	5
Cr	25	50	100	250	> 250	50
Cu	20	30	50	100	> 100	0.1
Fe	0.3	0.5	1	2	> 2	200
Hg	0.1	0.3	0.5	1	> 1	1
Mn	0.05	0.1	0.3	1	> 1	50
Ni	10	25	50	100	> 100	20
Pb	5	10	25	50	> 50	10
Zn	100	200	500	1000	> 1000	5000

Inhibition of acid phosphatase enzymatic activity

The ecotoxicity of the samples was assessed based on their inhibitory effect on acid phosphatase activity. The enzymatic activity of potato acid phosphatase was determined by hydrolysis of the chromogenic substrate p-nitrophenyl phosphate (p-NPP), p-nitrophenol (p-NP) being obtained as a reaction product. Due to the yellow colouration of p-NP at an alkaline pH, the formation of reaction product was assessed at 405 nm wavelength using an UV-Vis spectrophotometer (Synergy H1 Hybrid Multi-Mode Reader, BioTek, Agilent Technologies). The enzymatic activity assays were carried out in 96-well microplates with a final reaction volume of 250 µL. A volume of 10 µL of enzyme solution, obtained by dissolution in 0.1 M acetate buffer at a pH of 4.8, was incubated for 15 min with 50 µL of either the analysed samples, or with a positive (acetate buffer/C+) or a negative ($(NH_4)_6Mo_7O_{24} \cdot 4H_2O$/C-) control. A volume of 90 µL substrate, 5.6 mM p-NPP in acetate buffer, was added to the enzyme and sample mixture. The reaction was stopped after 2 min, 150 µL 1M NaOH being used. The calculation of enzymatic activity was done using the molar extinction coefficient of 18530 $M^{-1}cm^{-1}$ of p-NP.

Yeast toxicity test

The ecotoxicity of the samples was also assessed through a yeast toxicity test. Prior to performing the test method, all reagents and materials were sterilised at 121 °C for 15 minutes and all procedures took place under aseptic conditions. *S. cerevisiae* DSM 70449 strain, used in this study, kept on glycerol stock at –80°C was thawed on ice and cultivated, under aseptic conditions, on YPD agar plates (20 g/L of peptone, 10 g/L yeast extract, 20 g/L dextrose, 15 g/L agar, pH = 7), to obtain isolated colonies. Further, one isolated colony was transferred in YPD broth (20 g/L of peptone, 10 g/L yeast extract, 20 g/L dextrose, pH = 7) until reaching a log-phase culture (~5 hours at 30 °C). The yeast toxicity test (YTT) was performed in 20 mL glass bottles as follows: a volume of 4 mL of sterile liquid YTT culture media (4 g/L saccharose, 2 g/L peptone, 1.7 g/L yeast extract, pH = 7) was pipetted into each of the glass bottles. The yeast strain culture was centrifuged at 4000 rpm for 5 minutes and resuspended in sterile distilled water to obtain a cell density of 3.0 a.u. (OD 550 nm) against distilled water as blank. Each of the glass bottle was inoculated with 1 mL of yeast suspensions and then the bottles were filled up with 15 mL of the water samples to be tested. Sterile needles and syringes were placed into the center of the rubber bottle caps, into the liquid media. For the positive control sample, the surface and groundwater were replaced by sterile distilled water and for the negative control sample, a fungicidal agent was added. The bottles were incubated into the dark, at room temperature for 16 hours. After the incubation period, the volume of the liquid in the open syringe, which is equivalent to the amount of gas produced during the fermentation process, is measured and the relative inhibition of the fermentation is determined.

Data analysis

The correlation of obtained data was analysed through a Pearson linear *r* correlation test in PAST software [11]. A significant difference was considered as having a *p* values less than 0.05. To identify the samples that were most strongly affected by the mining pollutants, a grading scale from 1 to 5 points was defined. On this scale, 1 point is assigned to samples that are within the permitted limits, the increase in the number of points representing various percentages above the permitted limits (Table 3). The graded

parameters were pH, conductivity (*EC*), dissolved oxygen (DO), turbidity (T), heavy metal concentrations, enzymatic activity (EA) and the inhibition of fermentation (IF).

Table 3

Grading scale used for the comparison of analysed samples

Points	CG*	GS_{Class}**	GS_{MAC}***	GS_{C+}****
1		**I**	0-MAC	≥ C+
2		**II**	MAC - 50 % over MAC	C+ - 25 % C+
3		**III**	50 % over MAC - 100 % over MAC	25 % C+ - 50 % C+
4		**IV**	100 % over MAC - 500 % over MAC	50 % C+ - 75 % C+
5		**V**	> 500 % over MAC	75 % C+ - 100 % C+

* color coding; ** grading scale according to water quality classes; *** grading scale based on MAC (maximum acceptable concentrations); **** grading scale based on C+

Results and discussions

Physicochemical parameters

All surface water samples had pH values within the normal range (Table 1) (Fig. 4a). The SW samples from Nera River (SW-3 and SW-3') had the lowest pH values, while SW samples from Bosneag River (SW-1 and SW-1') had the highest values. All upstream SW samples had lower pH values than the downstream samples. A higher pH of downstream waters may be due to rural pollution, as these samples are collected from villages. Discharge of wastewater, pollution with agricultural fertilisers or contamination with detergents from household water are possible causes of the higher pH values in the localities compared to the pH values of the samples collected upstream. Groundwater samples also had pH values within the normal range (Table 1) (Fig. 4b). The GW sample from Coronini village had the highest pH value, while the GW sample from Divici village had the lowest pH value. The pH difference of the analysed groundwater samples can be due to both natural factors, such as geological ones, as well as anthropogenic factors. Rural pollution due to wastewater and agricultural pesticides or fertilisers is a possible anthropogenic cause. Another cause could be contamination with mining pollutants such as heavy metals carried by wind or acid carried by rainwater.

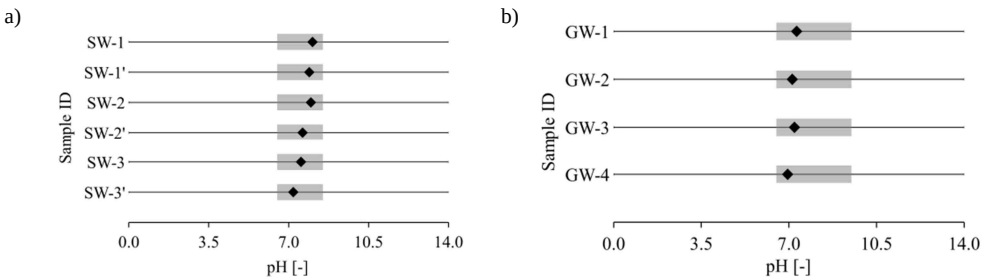


Fig. 4. pH values: a) surface water samples and b) groundwater samples in comparison with the normal range according to Table 1

The obtained results are in good agreement with the data identified in the scientific literature that highlight different pH profiles, characteristic for the studied surface waters.

Both surface water samples with pH values within normal ranges [12] and lower pH values [13] were studied in the proximity of active copper mines. In the case of some abandoned copper mines, varying values of pH of the analysed surface water samples were observed, ranging between 3-7 [14]. Regarding groundwater, the identified studies emphasised a weak effect of the active or abandoned copper mines on the pH of the groundwater samples, as the majority of the well water samples were within normal ranges [15].

All surface water samples had conductivity values within the normal range for drinkable water (as there are no available data for surface water) (Table 1) (Fig. 5a), Bosneag River samples (SW-1 and SW-1') showing the highest values. None of the groundwater samples exceeded the normal range (Table 1) (Fig. 5b).

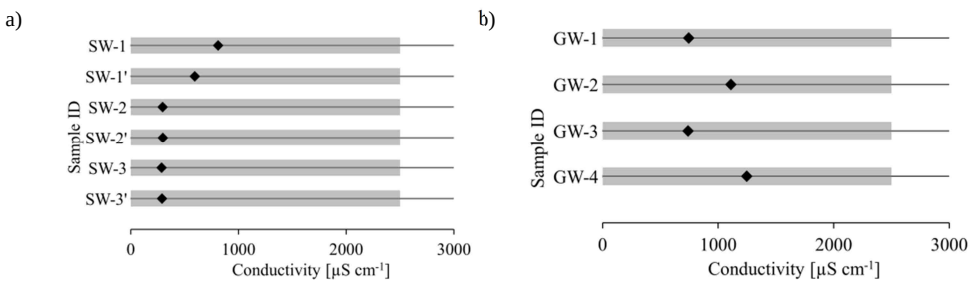


Fig. 5. Conductivity values: a) surface water samples and b) groundwater samples in comparison with the normal range according to Table 1

Literature data reveals diverse effects of surface water contamination with mining pollutants on conductivity. Studies were identified in which the conductivity was below the MAC [16], but also where the analysed samples exceeded the limit by up to approximately 2 times [17]. Regarding the groundwater, it was observed that the conductivity oscillates between the various study areas. Both normal values of conductivity in mining areas [18] and values above the permissible limits have been observed, even after the cessation of mining activities [15]. Oscillations of the electrical conductivity of water can appear due to normal physicochemical processes or due to anthropogenic processes. Evaporation, dilution, ion exchange, osmotic processes, sulphate reduction, oxidation processes and the interaction of water with rocks and soil are natural processes that can influence the electrical conductivity of water. In the case of surface waters, heavy rains can lead to the dilution of rivers, causing an increase in conductivity, while in the case of underground waters, evaporation is a source of normal variations in conductivity. Anthropogenic processes that can lead to conductivity oscillations are represented by mining, industrial and agricultural processes.

According to the dissolved oxygen normal ranges by class (Table 1), all surface water samples belong to class I, except for SW-3', which belongs to class II (Fig. 6a). The GW samples exceeded the normal range of dissolved oxygen (Table 1), the highest value being registered for GW-1, 2.4 times higher than the MAC, and the lowest being registered for GW-4, 1.7 times higher than the MAC (Fig. 6b). The concentration of dissolved oxygen in rivers can vary both in time and geographically. These variations can be caused by a multitude of factors such as photosynthesis carried out by aquatic plants, riparian vegetation, urban pollution with wastewater, etc. In the case of groundwater, the concentration of dissolved oxygen is mainly controlled by the rate of microbial respiration

in the infiltration bed and can be influenced by rainwater infiltration that is almost saturated with dissolved oxygen.

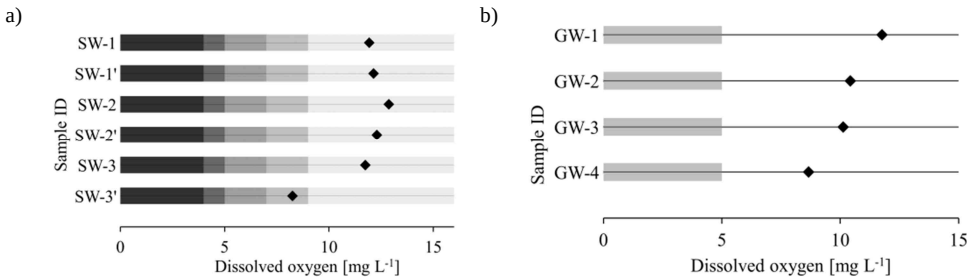


Fig. 6. Dissolved oxygen values: a) surface water samples and b) groundwater samples in comparison with the normal range according to Table 1

Various studies have been identified in which dissolved oxygen values were analysed for surface waters in copper mining areas. The rivers analysed in these studies were classified in various classes, from 2 to 5.8 [19, 20], unlike the rivers analysed in the current study which were included in class 1. In the case of groundwater, studies were identified in which dissolved oxygen had concentrations both below the lower limit allowed, but not low enough for anoxia, and with slightly increased values, up to 9.9 mg/L [21-23].

The turbidity of the surface water samples was higher than the normal range for drinkable water (as there are no available data for surface water) (Table 1 and Fig. 7a). Nera River samples had the highest values of turbidity, 7.3 times higher than the maximum acceptable value. The groundwater samples were within the normal range (Table 1), having similar turbidity values (Fig. 7b).

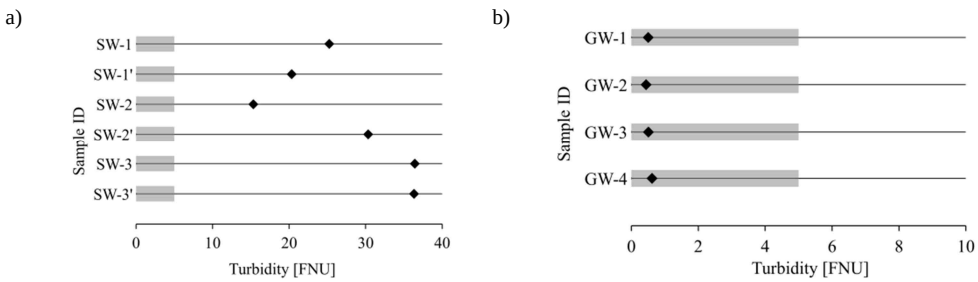


Fig. 7. Turbidity values: a) surface water samples and b) groundwater samples in comparison with the normal range according to Table 1

According to specialised literature, values above the permissible limit of surface water turbidity can be observed both due to natural phenomena, such as meteorological ones [24], and to intense mining activity. In the case of this study, mining activities are non-existent, thus the most plausible explanation is the heavy rains at the time of sampling for most of the river water samples [25], which can also be the cause of the recorded variations. In scientific studies, groundwater turbidity was within acceptable limits, with few

exceptions [24, 26]. High turbidity can be due either to contamination with potentially toxic elements, or to the concentration of water with mud or clay in the summer [27].

Both the experimental data and those from the scientific literature revealed varied values of the physicochemical parameters analysed. These parameters can be influenced by the potential mining pollution, but also by both natural factors, such as present organisms, geological, geographical, or meteorological factors, and by anthropogenic factors, mainly rural pollution represented by agricultural pesticides and fertilisers, wastewater, household water, and industrial pollution.

Heavy metal concentrations

All surface water samples belong to class I regarding the concentration of As, Cd, Cr, Ni, Pb and Zn Tables 2 and 4. According to the copper concentration, samples SW-1 and SW-1' belong to class III, while samples SW-2 and SW-2' belong to class II. The concentration of iron highlights the classification of samples SW-2', SW-3 and SW-3' in class IV, having the highest iron concentrations from all surface water samples. SW-1 and SW-1' have the lowest concentration of Fe, belonging to class II, SW-2 belonging to class III. Considering the Hg concentration, all surface water samples can be included in class III. All SW samples can be included in class II according to the concentration of manganese, except for SW-1'.

Table 4

Heavy metal concentrations of surface water samples in comparison with the corresponding MAC values according to Table 2

Heavy metal	**Sample ID**					
[µg/L]	**SW-1**	**SW-1'**	**SW-2**	**SW-2'**	**SW-3**	**SW-3'**
As	3.76	2.74	2.37	2.45	2.40	2.37
Cd	0.20	< 0.14	< 0.14	< 0.14	< 0.14	< 0.14
Cr	< 1.7	< 1.7	< 1.7	< 1.7	2.27	2.17
Cu	36.75	38.18	25.72	24.65	17.48	16.55
Fe_{tot}	385.10	421.47	671.81	1046.56	1721.27	1663.57
Mn	74.24	36.57	51.88	60.46	71.26	68.84
Ni	< 3.6	3.74	< 3.6	< 3.6	3.82	3.82
Pb	2.91	< 2.1	2.13	2.48	2.44	2.36
Zn	34.24	22.47	17.84	15.53	14.42	63.33

White background shows heavy metal concentrations that do not exceed the MAC values for class I SW samples, light grey background shows heavy metal concentrations that do not exceed the MAC values for class II SW samples, medium grey background shown heavy metal concentrations that do not exceed the MAC values for class III SW samples, dark grey background shows heavy metal concentrations that do not exceed the MAC values for class IV SW samples and black background shows heavy metal concentrations that do not exceed the MAC values for class V SW samples

The highest concentration was registered for Fe, followed by Mn, Cu and Zn. The heavy metal with lowest concentration was Cd, followed by Cr.

Two studies were identified in the literature in which the concentration of heavy metals was determined for surface water samples from the Moldova Noua area. In a previous study, by Stevanović et al. [1], the concentration of metals determined was lower than the concentration determined in the current study. In the study made by Popescu et al. [28], lower concentrations were observed for As, Cr, Fe, Hg, Mn and Zn. An increase in the

concentration of heavy metals over time may be due to the accumulation of these metals due to continuous pollution [1, 28]. Also, the concentration of heavy metals can present variations due to meteorological conditions, or even due to the exact sampling sites [29]. Data on the concentration of heavy metals in surface water from other countries were also identified, with different trends being observed. In all analysed articles, the concentrations of Cd and Ni were higher than those determined experimentally in this study, while for the rest of the metals both higher and lower values were observed [13, 16, 30-32]. The highest values identified in the literature were observed for Cu, being up to approximately 4000 times higher than the values in the current study [13]. Very high concentrations were also identified in the case of Cd, being up to approximately 1800 times higher [1]. The most similar values were observed in the case of Fe, with most values being below those determined experimentally [13, 31, 32].

None of the groundwater samples exceeded the MAC values (Table 2) for any of the identified heavy metal concentrations (Table 5). The heavy metals with the highest concentrations were Fe, Cu and Zn, while the lowest concentrations were registered for Cd and Hg. The heavy metals that were found in the well water samples are due to pollution by wind loaded with dust from the tailing pond in Moldova Noua. Moreover, the heavy metal composition of the mining waste is closely related to the heavy metal content of the rock processed during mining activities. Copper is the main product, secondary products being obtained in high concentrations such as iron and zinc, or others in much lower concentrations compared to the main product [2, 33].

Table 5

Heavy metal concentrations of groundwater samples

Heavy metal [µg/L]	Sample ID			
	GW-1	GW-2	GW-3	GW-4
As	2.10	2.10	5.24	2.10
Cd	0.14	0.14	0.14	0.14
Cr	2.41	1.70	1.70	4.89
Cu	16.49	11.95	13.09	16.36
Fe_{tot}	67.94	82.29	177.37	73.79
Mn	2.79	1.60	2.26	1.83
Ni	3.60	3.60	3.60	3.60
Pb	2.10	2.10	2.10	2.10
Zn	13.40	7.29	7.37	10.82

To the best of our knowledge, there are no other studies that determined the heavy metal concentration of groundwater samples from Moldova Noua area. Studies of groundwater from mining areas from different countries have identified concentrations of several heavy metals higher than those identified in this study. There were also exceptions, such as Cd, Cr, Cu and Ni, in these cases the concentrations were lower than those in this study, or below the detection limit for the methods used [7, 18, 34].

Inhibition of acid phosphatase activity

The enzymatic activity of acid phosphatase was slightly affected by all surface water samples (Fig. 8a). None of the SW samples lead to a higher than 26 % inhibition of the enzymatic activity.

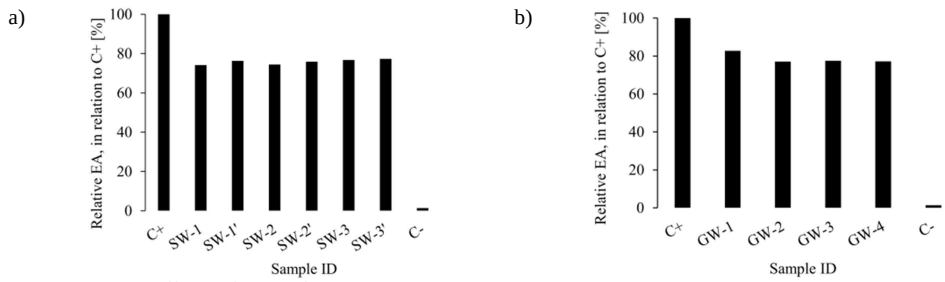


Fig. 8. The effects of: a) surface water samples and b) groundwater samples on the percentage relative enzymatic activity of acid phosphatase in relation to the negative control

The groundwater samples had a similar effect on the enzymatic activity of acid phosphatase as the surface water samples. The GW samples slightly inhibited the enzymatic activity, no more than 23 % inhibition being observed (Fig. 8b). GW-1 (well from Coronini village) had the lowest inhibition percentage from all GW samples. These data highlight the fact that the analysed water samples do not influence the enzymatic activity of acid phosphatase, used as a biomarker for the environment. A significant inhibition of more than 50 % of the enzyme activity would indicate that the analysed sample could affect the life of aquatic organisms in the sampled waters, thus highlighting the presence of a potential pollutant [35].

To the best of our knowledge, there are no other studies that address the effects of water samples potentially contaminated by mining activities on acid phosphatase in pure form. Although studies have been identified that used this enzyme in the analysis of the effects of some pollutants, the enzyme is either obtained by extraction from certain target organisms exposed to pollution [36], or the presence of the free enzyme in the analysed sample is determined [7].

Yeast toxicity test

Regarding the surface water samples, the results obtained showed a maximum inhibition of fermentation process of 50 % for samples SW-1, SW-2 and SW-3, while the other surface water samples showed no inhibition (SW-1') or no more than 25 % (SW-2' and SW-3') inhibition of fermentation process (Fig. 9a). Most groundwater samples inhibited no more than 25 % (GW1, GW2, GW3) the fermentation process, reaching to a maximum of 37.5 % for the GW4 sample (Fig. 9b).

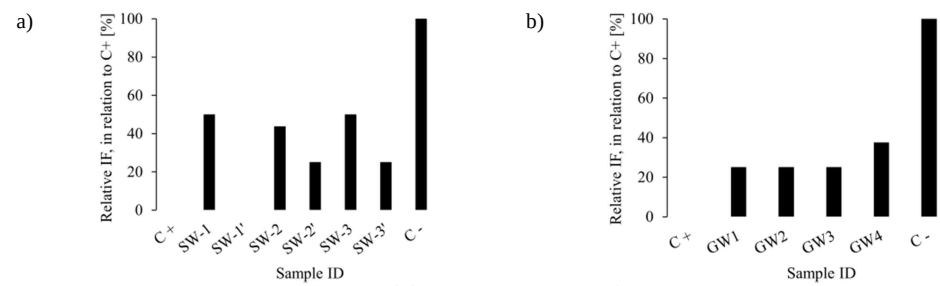


Fig. 9. Percentage relative inhibition of fermentation by: a) surface water sample and b) groundwater samples, in relation to the negative control

The inhibition of fermentation, although for most of the samples, was low, still the differences compared to the Control may be attributed to several factors, such as a pH high value (~8) of the surface and groundwater samples, considering that the fermentation process requires a more acidic environment. At pH values over 8, *S. cerevisiae* showed a decrease in growth rate of yeast cells, with a complete stop at a pH value of 9 [37].

The presence of different heavy metals in water samples can be another cause for the slight inhibition of fermentation. Metal toxicity towards yeast cell can be caused by oxidative stress, DNA alteration, inhibition of some metabolic enzymes and by disturbing the function of various proteins involved in cell proliferation [38].

Data analysis

The statistical analysis of the correlation of data indicated significant correlations between some of the analysed parameters (Table 6), either for all water samples, either for surface or for groundwater samples only. In Table 6, the probability is shown above the diagonal (statistically significant probability values, p values < 0.05, are shown with coloured background: black background for data regarding all samples, dark grey for data regarding surface water samples only and light grey regarding groundwater samples only), statistics of correlation are shown below the diagonal (for the statistically significant probability values, the statistical values of correlation are shown in bold if values are positive and in italic if values are negative). Only parameters with significant correlations are shown.

Table 6

Statistical analysis of correlation of physicochemical parameters, heavy metal concentrations, enzymatic activity (EA) and inhibition of fermentation (IF) using Pearson Linear r correlation test

		pH	***EC***	**DO**	**T**	**As**	**Cd**	**Cr**	**Cu**	**Fe_{tot}**	**Mn**	**Ni**	**Pb**	**Zn**	**EA**	**FI**
pH	**All**		0.155	0.005	0.182	0.721	0.135	0.083	0.001	0.678	0.048	0.951	0.153	0.675	0.084	0.709
	SW		0.161	0.051	0.035	0.215	0.304	0.068	0.042	0.013	0.477	0.121	0.851	0.280	0.028	0.805
EC	**All**	–0.484		0.261	0.009	0.902	0.643	0.123	0.545	0.005	0.015	0.113	0.481	0.240	0.555	0.890
	SW	0.651		0.681	0.504	0.004	0.037	0.325	0.027	0.096	0.975	0.537	0.290	0.892	0.315	0.952
DO	**All**	**0.808**	–0.393		0.652	0.970	0.576	0.093	0.082	0.930	0.397	0.551	0.562	0.336	0.535	0.819
	SW	0.810	0.216		0.160	0.727	0.827	0.172	0.284	0.196	0.449	0.159	0.914	0.010	0.152	0.772
T	**All**	0.459	*–0.773*	0.163		0.741	0.584	0.388	0.266	0.0004	0.281	0.024	0.042	0.048	0.207	0.599
	SW	*–0.843*	–0.345	–0.653		0.688	0.823	0.051	0.136	0.030	0.141	0.175	0.487	0.461	0.114	0.779
As	**SW**	0.593	**0.948**	0.184	–0.211		0.002	0.413	0.110	0.187	0.607	0.422	0.089	0.846	0.218	0.652
	GW	0.283	–0.571	–0.066	0.836		1.000	0.571	0.600	0.007	0.822	1.000	1.000	0.470	0.723	0.667
Cd	**All**	0.506	0.168	0.202	0.198	0.351		0.627	0.090	0.709	0.243	0.556	0.004	0.431	0.232	0.193
	SW	0.508	**0.840**	0.116	–0.119	**0.967**		0.544	0.267	0.321	0.347	0.393	0.035	0.757	0.179	0.378
Cr	**SW**	–0.779	–0.489	–0.639	0.809	–0.416	–0.314		0.061	0.010	0.292	0.027	0.998	0.496	0.105	0.629
	GW	–0.781	0.651	–0.685	0.135	–0.429	0.000		0.287	0.541	0.811	1.000	1.000	0.544	0.874	0.025
Cu	**All**	**0.871**	–0.218	0.575	0.389	0.093	0.563	–0.308		0.979	0.130	0.937	0.174	0.497	0.121	0.774
	SW	**0.827**	**0.863**	0.517	–0.682	0.716	0.541	–0.791		0.005	0.343	0.305	0.800	0.662	0.242	0.552
Fe_{tot}	**All**	0.150	*–0.806*	–0.032	**0.901**	–0.210	–0.135	–0.189	–0.010		0.006	0.006	0.269	0.095	0.507	0.520
	SW	*–0.907*	–0.735	–0.612	**0.854**	–0.622	–0.492	**0.915**	*–0.944*		0.037	0.115	0.923	0.589	0.087	0.705
	GW	0.240	–0.509	–0.103	0.807	**0.993**	0.000	–0.459	–0.494		0.924	1.000	1.000	0.376	0.635	0.657
Mn	**All**	**0.636**	*–0.738*	0.301	0.949	–0.061	0.407	–0.361	0.512	**0.792**		0.123	0.010	0.057	0.082	0.288
Ni	**All**	0.022	–0.532	–0.215	**0.702**	–0.197	–0.213	–0.068	0.029	**0.794**	0.521		0.754	0.089	0.968	0.791
	SW	–0.700	–0.319	–0.654	0.635	–0.408	–0.431	**0.864**	–0.507	0.709	0.105		0.602	0.444	0.022	0.693
Pb	**All**	0.487	–0.253	0.209	**0.650**	0.186	**0.819**	–0.243	0.467	0.387	**0.762**	0.114		0.208	0.170	0.124
	SW	0.099	0.520	–0.058	0.357	0.746	**0.844**	0.001	0.134	–0.051	0.790	–0.272		0.751	0.511	0.249
Zn	**All**	0.152	–0.410	–0.340	**0.635**	–0.072	0.281	–0.133	0.244	0.557	0.618	0.565	0.436		0.643	0.964
	SW	–0.530	0.072	*–0.919*	0.378	0.103	0.164	0.351	–0.229	0.281	0.339	0.391	0.168		0.496	0.809
EA	**SW**	*–0.859*	–0.498	–0.662	0.709	–0.590	–0.631	0.722	–0.565	0.748	–0.004	**0.875**	–0.339	0.350		0.357
IF	**GW**	–0.892	0.741	–0.828	0.217	–0.333	0.000	**0.975**	0.546	–0.343	–0.369	0.000	0.000	0.248	–0.342	

The concentration of total Fe presented the most statistically significant correlations with other parameters, a possible explanation being related to its highest concentration among all tested metals in all water samples. On the other hand, the inhibition of fermentation showed the least correlations with other parameters. All physicochemical parameters, as well as the concentrations of Cd, Cu, Fe, Mn, Ni, Pb and Zn, showed correlations for all water samples. All physicochemical parameters and all heavy metal concentrations, excluding Mn, showed correlations for surface water samples, while only the concentration of As, Cr, total Fe, and the fermentation inhibition showed correlations for groundwater samples. The enzymatic activity of acid phosphatase showed statistically significant correlation only for the surface water sample for pH and Ni concentration. A strong positive correlation between As and total Fe in groundwater was also observed by Singh and Singh [39], the arsenic mobilisation being caused by the reduction of iron oxide. It was observed that the concentration of chromium can affect the fermentation process at higher concentration [40].

The comparison of analysed samples using the grading scale (Table 3) emphasised that all the samples had bellow 2 points, being slightly affected by the mining pollutants (Fig. 10a). The lowest number of points were observed in the case of groundwater samples, these being the least affected by mining pollution. Regarding the surface water samples, SW-1' had the lowest value.

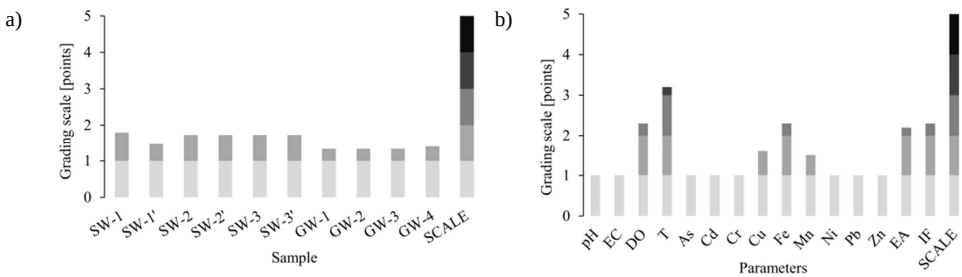


Fig. 10. Grading of: a) samples for the identification of stronger mining pollution effect according to Table 3 and b) of analysed parameters for the identification of the strongest effect of mining pollution according to Table 3

The comparison of the determined parameters using the grading scale (Table 3) highlighted that turbidity had the highest number of points, exceeding 3, thus being the parameter that had the highest values over its limit (Fig. 10b). Dissolved oxygen, total Fe concentration, enzymatic activity and inhibition of fermentation had values between 2 and 3, while Cu and Mn concentration had values between 1 and 2. The other parameters had only 1 point showing that these had no values over their limit.

Conclusion

In this study, we assessed hydrochemistry and toxicity of surface and groundwater samples contaminated with pollutants generated by the abandoned copper mine in the Moldova Noua area.

To the best of our knowledge, the current study is one of the most comprehensive studies regarding the hydrochemical and toxicological characterisation of surface and

groundwaters contaminated with mining pollutants in the Moldova Noua area. Regarding the physicochemical parameters, the pH and conductivity values were within normal limits for both surface waters and groundwaters. In the case of turbidity and the concentration of heavy metals, only the groundwater samples had values within normal limits, the parameters for surface water samples being above the maximum allowed concentrations. This is also reflected by the ecotoxicological study which revealed the fact that all the analysed water samples induced an inhibition compared to the positive control used, except for sample SW-1' (Bosneag River upstream of Moldova Noua) which did not influence the fermentation process.

Statistically significant correlations were observed in the case of some analysed parameters: (i) all physicochemical parameters and Cd, Cu, Fe, Mn, Ni, Pb and Zn concentrations for all water sample; (ii) all physicochemical parameters and all metal concentrations excluding Mn for surface water samples; (iii) and As, Cr, total Fe concentrations and inhibition of fermentation for groundwater samples. The grading scale applied highlighted the fact that all analysed samples were slightly affected by mining pollution, the parameters that had the highest values above the accepted limit being turbidity, dissolved oxygen, iron concentration and the ecotoxicological parameters.

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