THE INHIBITORY EFFECT OF SURFACE WATER, GROUNDWATER, SEDIMENT, AND SOIL SAMPLES POTENTIALLY POLLUTED FROM THE MOLDOVA NOUA AREA ON *ZEA MAYS* L. SEED GERMINATION

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ABSTRACT

The Moldova-Noua area in Romania has long been renowned for its copper mines, which have resulted in increased concentrations of heavy metals in the surrounding environment. Maize farming accounts for 24% of the total land area in this region. This study assesses the ecotoxicological effects of polluted surface water, sediment, and soil samples on Zea mays L. seeds. We analysed the samples' impact on germination parameters, including mean germination time (MGT), germination percentage (GP), germination rate (GR), and germination value (GV). Results revealed MGT ranging from 2.4 to 3.6 days, with GP between 70% and 100%, GR ranging from 19% to 31% per day, and GV between 11% and 20%. The most affected sample was surface water from the Bosneag River downstream (SW1). This study highlights the impact of heavy metal pollution on maize crops, which have been utilized for decades by local communities. Overall, this research contributes to our understanding of the real-world consequences of heavy metal pollution on agricultural systems and ecosystems.

KEY WORDS: *mean germination time, germination rate, peak value.*

INTRODUCTION

Global heavy metal contamination of agricultural soil has been identified as one of the main factors reducing crop production (Xin et al., 2022). The history and economy of Western Romania have both been significantly influenced by mining and mineral processing. Romania has been involved in the mining of polymetallic ores and the metal extraction process for more than a century, and before 1990, the extraction of copper ore was among the most significant industrial sectors in Europe (Boros et al., 2021; Stevanović et al., 2021). Copper's mining provides a long list of useful by-products (*i.e.*, cadmium, arsenic, nickel, zinc, and lead) with a variety of uses in farming, building, electrical equipment, and metallurgy. The conditions at the mining waste in Moldova Noua, Romania, show that environmental degradation persisted even after mining activities had ceased. The lives of 15,000 people living near by a mining operation are affected by pollution. The tailing pond is a source of pollution, especially under windy conditions, impacting both the tourist city of Veliko Gradiste in Serbia and Moldova-Noua in Romania, as well as adjacent regions (Isvoran et al., 2021).

There are roughly 84,000 ha of agricultural land in Caras-Severin County, Romania. A total of 2,500 ha in the Moldova Noua area are contaminated with heavy metals and other pollutants due to mining operations (Boros et al., 2021; Ostafe et al., 2021). About 50% of the land is used for cereal farming, 24% for maize farming, 18% for oily crop farming, 9% for potatoes, and 2% for vegetable farming.

It has been reported that heavy metal contamination (*i.e*., Cu, Pb, Zn, and Cd) is the most aggressive type of chemical pollution, affecting nearly 0.9 million ha of soil worldwide (Boros et al., 2021). Out of these, copper (Cu) is one of the main pollutant because of the use of Cu-containing herbicides, insecticides, and fungicides as well as discharges from industrial effluents, sewage sludge, and mining in ecosystems. Above 20–30 mg kg^{-1} levels of Cu, plants become poisoned. According to research on the plant's maize (*Z. mays* L.), lettuce (*Lactuca sativa*), wheat (*Triticum aestivum* L.) and tomato (*Solanum lycopersicum* L.), too much copper in the soil slows plant root growth and interferes with metabolic processes (Xin et al., 2022).

In terms of harvested area, maize (*Zea mays* L.) is the most significant crop in Romania. Between 2 and 3.2 million ha of maize were harvested annually in the past ten years, with an average yield of 1.6 to 4.5 tons of grain per ha (Ion et al., 2013). Thus, the aim of this study is to analyse the condition of Moldova Noua's agricultural fields through various ecotoxicological tests, such as germination of maize.

MATERIAL AND METHODS

Study area and samples collected. The abandoned copper mine in Moldova Noua, Caras-Severin County, Romania, is the focus of the current investigation. Moldova Noua is a town in south-west region of Romania. It is adjacent to the Danube River, which is border between Romania and Serbia. The Mediterranean climate makes Moldova Noua one of Romania's warmest regions. The area is also affected by the powerful Cosava wind, which blows from the N-E and S-W. The mining operations have ceased in 2008, with the remaining tailings pond slowly drying up over time. This abandoned copper mine jeopardises the surrounding environment due to heavy metal pollutants transferred by wind as well as contaminated precipitation seeping through the groundwater. Moldova Noua is currently an agricultural area, providing food for the local population by growing maize, wheat, and vegetables (Boros et al., 2021; Pana et al., 2018; Yáñez-Espinosa et al., 2020).

The sample collection procedure was carried out according to the standard procedures. Samples were collected from surface waters (from the Bosneag, Radimna and Nera rivers), from groundwaters (public wells in the villages near by the former mining area - Coronini, Moldova Veche, Macesti and Divici), sediments from the above-mentioned rivers and from the soils in the vicinity of the copper mine settling pond (FIG. 1). In total, 17 samples were collected in spring 2021.

TABLE 1. Sample ID and location name for all samples

Sample ID	Location name
SW1	Bosneag River from Moldova Noua
SW2	Bosneag River upstream of Moldova Noua
SW3	Radimna River from Radimna
SW4	Radimna River upstream of Radimna
SW5	Nera River from Socol
SW6	Nera River upstream of Socol
GW1	Public wells from Coronini
GW2	Public wells from Moldova Veche
GW3	Public wells from Macesti
GW4	Public wells from Divici
SD1	Sediments from Bosneag River
SD2	Sediments from Radimna River
SD3	Sediments from Nera River
S1	Soil near Bosneag tailings pond
S2	Soil at 200 m from Bosneag tailings pond N-W direction
S3	Soil at 400 m from Bosneag tailings pond N-W direction
S4	Soil at 600 m from Bosneag tailings pond N-W direction

The maize seeds (*Zea mays* L.) were purchased from an agricultural market within the Banat area. *Zea mays* L. seeds ($5 \times 57 = 285$), carefully selected, were immersed in 2% NaOCl for 10 minutes, to obtain a sterile surface. These were then rinsed intensively with distilled water so that no trace of the sterilizing agent remains.

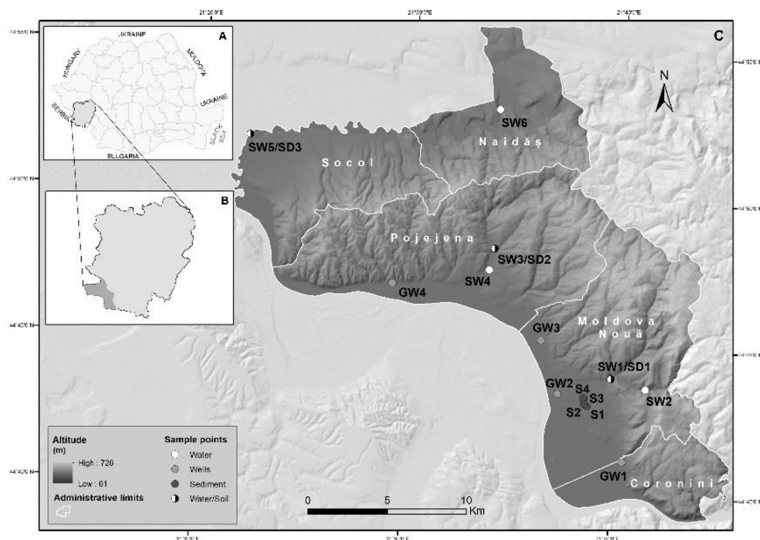

FIG. 1. Distribution of sampling points near the former mine in Moldova Noua

The pH, dissolved oxygen (DO), conductivity (C) and turbidity (T) of all samples have been measured *in situ* using a field multiparameter (Multi 340i, WTW) and a portable turbidimeter (2100Qis, Hach Lange). The heavy metal ion concentration was determined by laboratory analysis using an ICP-MS (Agilent 7700 Series, Agilent Technologies) and an ICP-OES (Spectro Arcos, Spectro, Ametek).

Seed germination. Before germinating the seeds, a sterile filter paper was placed in sterile Petri dishes with a diameter of 90 mm. The experiment started by adding 4 mL of test solution to each filter paper (surface water samples - SW1, SW2, SW3, SW4, SW5, SW6, groundwater samples - GW1, GW2, GW3, GW4, sediment sample SD1, SD2, SD3, soil samples - S1, S2, S3, S4, positive control (C+) - ammonium molybdate tetrahydrate, negative control (C-) - distilled water). Subsequently, 5 seeds were placed in each Petri dish at 10 mm from each other. The experiment was carried out in triplicate, in a day-night cycle over 5 days and at room temperature.

Germination parameters. The behaviour of the seeds was recorded for 5 days, and it was called a germinated seed when an approximately 2 mm radicle emerged from the seed. The following parameters were calculated to assess the seed germination mode: mean germination time (MGT),(Dezfuli et al., 2008; Kulkarni et al., 2007) germination percentage (GP) (Singh et al., 2020), germination rate (GR) (Xin et al., 2022) and germination value (GV) (Ramana et al., 2002) according to the following equations:

$$MGT = \sum(n * d)/N \quad (1)$$

Where:
n = number of seeds germinated on each day
d = number of days from the beginning of the test
N = total number of seeds germinated at the end of the experiment

$$GP = \frac{Number\ of\ seeds\ germinated}{Total\ number\ of\ seeds} * 100 \quad (2)$$

$$GR\ (\%/day) = \frac{GP1}{1} + \frac{GP2}{2} + \cdots \frac{GPx}{x} \quad (3)$$

Where:
GP1 = GP * 100 at the first day of germination, GP2 = GP*100 at the second day of germination.

$$MDG = \frac{Number\ of\ seeds\ germinated}{Total\ number\ of\ seeds} \quad (4)$$

$$PV\ (Tudor\ et\ al.,\ 2017) = \frac{germination\ percent\ cumulate\ on\ each\ day}{Total\ number\ of\ days\ of\ experiment} \quad (5)$$

$$GV = MDG * PV \quad (6)$$

Data analysis. Following the distribution study, an ANOVA analysis with the Dunn's post hoc test was conducted. The difference was only considered as significant if the p value was less than 0.05 ($p < 0.05$). In this work, the data are not displayed. Using PAST software, a Pearson linear r correlation test was used to examine the correlation of the data that was produced. A difference was deemed significant if the p value was less than 0.05.

RESULTS AND DISCUSSIONS

Water quality can be analysed by determining physico-chemical parameters and by determining the concentration of heavy metals in the water body. The surface waters in the Moldova Noua area are within the normal limits of the Romanian legislation (Order No 161/2006 for the Approval of the Normative on the Classification of Surface Water Quality to Establish the Ecological Status of the Water Bodies, 2006) for the concentration of metals, but show elevated values for some physicochemical parameters, such as dissolved oxygen, and turbidity. The high concentration of dissolved oxygen found in surface waters (Nera River and Bosneag River) may be due to the high degree of vegetation, which has adapted to anthropized conditions. Thus, a high level of oxygen concentration may also be due to an inversely proportional relationship between abundant vegetation and a low population of underwater life (Jabłońska-Czapla et al., 2020). Another reason for the high dissolved oxygen value may also be due to the abundant flow that causes turbulence in the river flow as it encounters the air in the atmosphere (Wetzel, 2001). This also produces turbidity in the water and, at the same time, increases the turbidity parameter (Nera River) above the maximum values allowed by the Romanian legislation (Order No 161/2006 for the Approval of the Normative on the Classification of Surface Water Quality to Establish the Ecological Status of the Water Bodies, 2006).

TABLE 2. Surface water general results of physicochemical parameters and heavy metals concentration. Maximum allowed concentration (MAC) are according to 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.(Order No 161/2006 for the Approval of the Normative on the Classification of Surface Water Quality to Establish the Ecological Status of the Water Bodies, 2006)

Parameters	Mean	Min	Max	MAC	Unit
pH	7.79	7.16	8.2	6.5-8.5	
Conductivity	470.83	282	934.00	2500	µS/cm
Dissolved oxygen	11.09	9.25	12.53	< 9	mg/L
Turbidity	4.06	1.2	8.74	5	FNU
Arsenic (As)	2.31	2.1	3.33	< 100	µg/L
Cadmium (Cd)	1.26	0.94	1.47	< 5	µg/L
Chromium (Cr)	2.87	1.7	8.75	< 250	µg/L
Copper (Cu)	3.30	3.3	3.3	< 100	µg/L
Iron (Fe)	153.73	34.39	411.58	< 2000	µg/L
Manganese (Mn)	22.40	14.4	34.38	< 1000	µg/L
Nickel (Ni)	3.60	3.6	3.6	< 100	µg/L
Lead (Pb)	2.55	2.07	2.84	< 50	µg/L
Zinc (Zn)	12.65	6.2	41.49	< 1000	µg/L

Samples of well water had all parameters within normal limits except dissolved oxygen. From the literature, it is known that one of the reasons why oxygen concentration can be increased is the conductivity of the water (the concentration of salts in the water). Even if the conductivity values are in the optimal range, the values are slightly increased, which also influences the increase in oxygen concentration (Liu et al., 2019). This can lead to the maintenance of life, and this is not recommended in well water because microorganisms or even algae can grow in the drinking water that is consumed by citizens.

Sediment samples from the Bosneag River showed elevated values (above normal limits) for the following metals: arsenic, copper, lead, and zinc. Sediment samples taken from the Radimna and Nera Rivers had values within normal limits for all heavy metals analysed. This may also be due to the greater distance of the rivers from the copper mine site in Moldova Noua.

Soil samples were taken from near the settling pond, and the results showed that all samples had values above the limits allowed by Romanian law. The results showed that copper concentrations decreased with distance from the pond. For the rest of the metals, it cannot be said that a trend of increasing metal concentrations was observed. In all soil samples tested, the only element that was within the allowable limits was chromium. The soil type in the Moldova Noua area is a calcareous skeleton lithosol type, with the parent rock being diorite quartz, rich in Cu and Mo (Mihut et al., 2010). The abundant rains in the western part of the country modify the alkalinity of the waters, favouring the absorption of heavy metals from the soil (Liu et al., 2019).

TABLE 3. Groundwater general results of physicochemical parameters and heavy metals concentration. Maximum allowed concentration (MAC) are according to Law No. 458/2002 regarding the quality of drinking water in Romania republished in 2011.(Law No. 458/2002 Regarding the Quality of Drinking Water in Romania Republished in 2011, 2002)

Parameters	Mean	Min	Max	MAC	Unit
pH	6.99	6.90	7.10	6.5 - 9.5	
Conductivity	1058.75	621.00	1717.00	2500	µS/cm
Dissolved oxygen	11.74	11.03	12.29	5	mg/L
Turbidity	0.81	0.62	0.98	5	FNU
Arsenic (As)	2.68	2.1	4.41	10.00	µg/L
Cadmium (Cd)	1.24	1.09	1.33	5	µg/L
Chromium (Cr)	2.56	1.7	5.16	50	µg/L
Copper (Cu)	3.30	3.3	3.3	100	µg/L
Iron (Fe)	50.33	27.51	88.92	200	µg/L
Manganese (Mn)	4.30	3.98	4.69	50	µg/L
Nickel (Ni)	3.60	3.6	3.6	20	µg/L
Lead (Pb)	2.63	2.24	3.41	10	µg/L
Zinc (Zn)	24.55	6.2	68.67	5000	µg/L

TABLE 4. Sediment general results of heavy metals concentration. Maximum allowed concentration (MAC) are according to 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 (Order No 161/2006 for the Approval of the Normative on the Classification of Surface Water Quality to Establish the Ecological Status of the Water Bodies, 2006)

Parameters	Mean	Min	Max	MAC	Unit
Arsenic (As)	11.86	4.62	25.03	17	mg/Kg
Cadmium (Cd)	1.28	0.71	2.43	3.5	mg/Kg
Chromium (Cr)	18.67	11.09	24.44	90	mg/Kg
Copper (Cu)	158.12	23.76	421.04	200	mg/Kg
Manganese (Mn)	549.54	350.77	650.36	None	mg/Kg
Nickel (Ni)	25.11	18.48	30.4	None	mg/Kg
Lead (Pb)	47.93	13.11	111.75	90	mg/Kg
Zinc (Zn)	279.47	38.81	732.58	300	mg/Kg

TABLE 5. Soil general results of heavy metals concentration. Maximum allowed concentration (MAC) are according to 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(Order No 161/2006 for the Approval of the Normative on the Classification of Surface Water Quality to Establish the Ecological Status of the Water Bodies, 2006)

Parameters	Mean	Min	Max	MAC	Unit
Arsenic (As)	45.91	22.26	63.91	5	mg/Kg
Cadmium (Cd)	3.00	1.1	6.85	1	mg/Kg
Chromium (Cr)	14.65	8.28	26.91	30	mg/Kg
Copper (Cu)	1257.80	271.27	1827.68	20	mg/Kg
Manganese (Mn)	657.67	254.44	1045.61	None	mg/Kg
Nickel (Ni)	28.60	19.69	40.77	20	mg/Kg
Lead (Pb)	50.39	30.01	62.16	20	mg/Kg
Zinc (Zn)	603.48	153.52	1492.28	100	mg/Kg

Germination parameters

The measurement of the rate and timing of germination is the mean time germination (MGT). The length of time from the beginning of imbibition to a particular germination percentage is not given by MGT (Soltani et al., 2015). The mean germination time (MGT) for *Zea mays* L. seeds in contact with surface water, groundwater, sediment, and soil samples had varied values, which do not follow a particular trend (Figure 1). The lowest value was recorded for the sample SW1 (Bosneag River from Moldova Noua), which was 1.20% lower than the negative control. The SW3 (Radimna River from Radimna) sample gave a value of 1.28% higher than the negative control. The MGT (mean germination time) is similar for the well water samples, ranging between 3.1 days for GW1) and 2.9 days for GW2. The highest recorded MGT for sediment and soil samples was 3.5 days, as shown by sample S3. The most significant differences ($p < 0.05$) were recorded for positive control (C+), which was significantly different from the rest of the samples by 50%. Samples SW3, SW5, SW6, GW1, GW4, SD3, S2 and S3 were significantly different from negative control (C-) ($p < 0.05$). The SW1 and SW3 samples differed by 22% from the other samples in a significant way ($p < 0.05$). For samples C-, SW2, SW4, GW2, SD2, and S1, there were no differences that were statistically significant ($p > 0.05$).

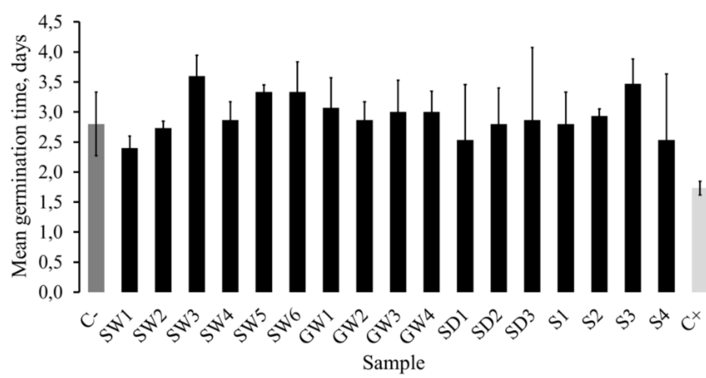

FIG. 2. Mean germination time of *Zea mays L.* seeds in the presence of surface water, groundwater, sediment, and soil samples (mean values n = 3, ± SD). Negative control (C-) is distilled water and positive control (C+) is ammonium molybdate tetrahydrate - $(NH_4)_6Mo_7O_{24}$. The meaning of samples notations on the *ox* axe is given in Table 1.

Literature data showed that the MGT parameters recorded for *Zea mays L.* seeds varied depending on the number of days the experiment was conducted, but there were no major differences for the samples analyzed.(Msuya & Stefano, 2010) The data obtained in this study are in accordance with the literature data: the MGT values for *Zea mays* L. range between 2.03 - 3.53 days;(Dezfuli et al., 2008) higher values (7.52 - 9.01 days)(Muhammad & Majeed, 2014) were also reported. For the determination of MGT in the presence of copper, studies showed lower values with ranges between 1 -2.2

days.(Xin et al., 2022) This demonstrates that our samples are not significantly affected, as none of the metals examined showed increased values, and the groundwater samples even less so. The same cannot be said for the sediment and soil samples, which have lower values than the water samples analysed. This may also be due to the high concentrations of copper.

The germination percentage (GR) for *Zea mays L.* seeds in contact with surface water, groundwater, sediment, and soil samples potentially contaminated from the abandoned copper mining region ranged from 40% to 100% (Figure 2). Germination began in most of the samples on the third day after the experiment was started, except for the C+, which started the germination process on the fourth day. The most significant differences were recorded between the C+ and samples C-, SW3, SW5, SW6, GW1, GW2, GW3, GW4, S1, and S3 ($p < 0.05$), also supported by statistical data determined by Dunn's ANOVA test. Significant differences were also found between sample SW3 and samples SW1, SD1 SD2, S4, and C+, supported by $p< 0.05$. There were no significant differences for samples SW2, SW4, SD3 and S2, having similar values and supported by a p-value > 0.05. There are two special cases for samples SW3 and SW5, which had a 100% germination because these samples had no contact with contaminated seeds during the experiment.

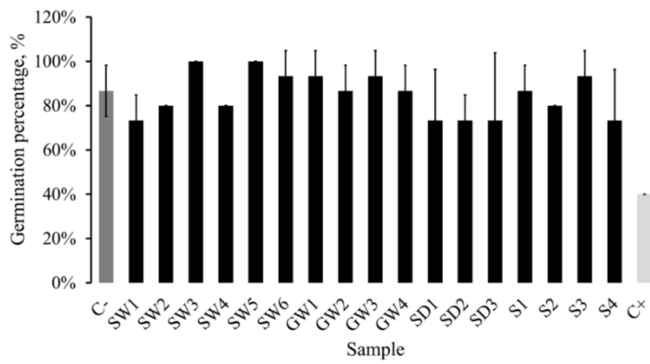

FIG. 3. Germination percentage of *Zea mays* L. seeds in the presence of surface water, groundwater, sediment, and soil samples (mean values n = 3, ± SD). Negative control (C-) is distilled water and positive control (C+) is ammonium molybdate tetrahydrate - $(NH_4)_6Mo_7O_{24}$. The meaning of samples notations on the *ox* axe is given in Table 1.

The literature reports similar results for the germination of *Zea mays* L. seeds, therefore the results reported herein for the germination percentage are in good accord with these findings (Carpýcý et al., 2009). The lowest reported germination percentage is 61% (Dezfuli et al., 2008) and values as high as 94.0 – 99.3% (Msuya & Stefano, 2010). Values exceeding 97% were noted in the presence of Cu^{2+} ions.(Foti et al., 2008; Mahmood et al., 2005) The results obtained in this study showed that metal concentrations in small amounts can accelerate the germination of maize seeds, while large amounts, as in the case of soil samples, can affect germination. This is also supported by data from the literature (Deng et al., 2016).

The term "speed of germination" describes the rate of germination as measured by the total number of seeds that sprout within a given period. Greater and more rapid germination are indicated by high values of these parameters. Only when samples or treatments have similar germinabilities, is it advisable to compare these values (Xin et al., 2022).

The sample SW5 showed the highest germination rate (GR, 31%/day) of maize seeds in contact with potentially polluted water from the Moldova Noua area (surface water sample from the Nera River in Socol) (FIG. 4). Statistical data confirms that sample SW5 gives the most significant differences ($p < 0.05$) from SW1, SW2, SW4, SD1, SD2, SD3, S2, S4 and C+ samples. The lowest germination rate was recorded for sample SD3: 19%/day, which is 48% higher than the positive control. Among the groundwater samples, GW2 recorded the highest germination rate, being significantly different ($p < 0.05$) from SW4, SD1, SD2, SD3, S2, S4 and C+ samples. The samples without significant differences ($p > 0.05$) that show the most similar values are C-, SW1, SW2, SW4, GW4, S2, S4. The sediment samples had the lowest rates, ranging between 19 - 22 %/day.

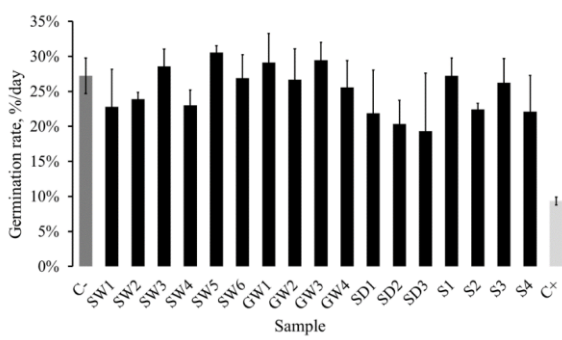

FIG. 4. Germination rate of *Zea mays* L. seeds in the presence of surface water, groundwater, sediment, and soil samples (mean values n = 3, ± SD). Negative control (C-) is distilled water and positive control (C+) is ammonium molybdate tetrahydrate - $(NH_4)_6Mo_7O_{24}$. The meaning of samples notations on the *ox* axe is given in Table 1.

One of the explanations for the variation of germination rate in the water samples is the different content of heavy metals. Certain metals can modify the DNA and other biological organelles, including the plasma membrane, cell wall, and nucleus (Solanki & Dhankhar, 2011). Some reports claim that Zn is a crucial element for all living organisms, including animals, plants, and microbes, whereas Mn plays an important role in secondary metabolism (such as the production of lignin and flavonoids). However, Zn builds up in plant tissues when present in excess, inhibiting growth by oxidizing membranes. It can also reach hazardous levels of 300 $\mu g\ g^{-1}$ in plants. Even that some minerals are necessarily in growth plants process, in our case, some of them are obviously inhibit the germination process. One of this explanation can be the pH of solution when it prepared the metals broth. Or maybe it is not enough the combination between concentration of metals, like in case of sediment samples. The mechanisms inhibiting root growth are not fully understood. The ability of metals to accumulate and interact with specific locations within the cell wall (such as pectins and hemicelluloses), plasma membrane (such as lipids or proteins) as well as the cell nucleus it is most likely responsible for this phytotoxic action (Chamorro et al., 2018).

Tsamo et al. (Tsamo et al., 2022) report that heavy metals and salinity affect the germination of maize seeds. Pb affects seed germination most strongly, followed by Cu and Zn. They also claim the concentration of the heavy metal to be the key factor: at 600 µmol/L germination did not occur, inhibiting most metabolic processes (Tsamo et al., 2022). In our case these concentrations occur only for Cu in soil samples, and these can affect the germination, but at the same time the seed germination needs different ions that help the enzymes implied in germination process (Bityutskii et al., 2002).

The germination value (GV) is the product of the germination percentage (GP) and the peak value of a seed (PV) (Ramana et al., 2002). Thus, if the GP is high and the PV is low, the GV will be low, despite these parameters are strongly dependent on each other (Thomson & El-Kassaby, 1993). For example, the highest GV was recorded for seeds tested in the presence of surface water from the Radimna River – sample SW3 and the Nera River– sample SW5. The high results correspond to those samples that have a 100% GP and the highest PV (*i.e.*, 0.2) recorded in this study. The most significant differences ($p < 0.05$) were recorded for the C+, with 56% of the samples being significantly different from each other. C-, SW3, SW5, SW6, all groundwater samples, and two soil samples: S1 and S3, were all significantly different. Also, samples SW3 and SW5 are significantly different ($p < 0.05$) from samples: SW1, SD1, SD2, S2, and C+. There are also samples that, according to Dunn's ANOVA test, do not show any significant difference ($p > 0.05$) between the values obtained: SW2, SW4, SD3 and S2.

Data analysis

Statistical significance correlation data indicated links between germination parameters and heavy metals. Thus, GP showed statistically correlated data with Cr_{tot} ions (the total chromium ions) and GR with $Cr_{tot.}$, Mn, and Ni ions. Literature data supports that these metals inhibit the germination process by blocking the oxido-reductive system but also by inhibiting the metabolic processes strongly active at the beginning of seed germination (Ma et al., 2021).

For soil and sediment samples, linear regression data show strong links (R^2 is above 0.848) high GP and As^{5+} and Cu^{2+} ions concentration.

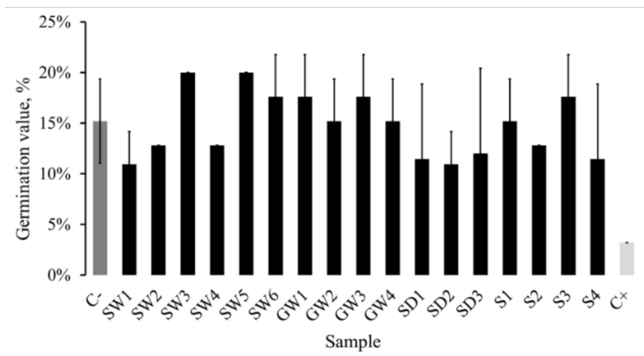

FIG. 5. Germination value of *Zea mays* L. seeds in the presence of surface water, groundwater sediment and soil samples (mean values n = 3, ± SD). Negative control (C-) is distilled water and positive control (C+) is ammonium molybdate tetrahydrate - $(NH_4)_6Mo_7O_{24}$. The meaning of samples notations on the *ox* axe is given in Table 1.

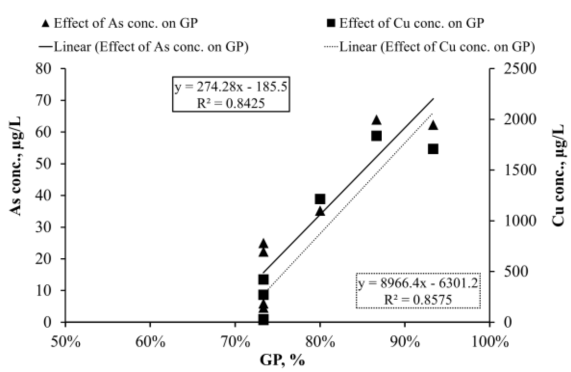

FIG. 6. Linear regression data for soil and sediment samples

TABLE 6. Statistical analysis of correlation of physicochemical parameters and heavy metal concentrations using Pearson Linear r correlation test; probability is shown above the diagonal (statistically significant probability values, $p< 0.05$, are shown with black background for data regarding all samples), statistics of correlation are shown below the diagonal (for the statistically significant probability values, the statistical values of correlation are shown in light grey background if values are positive, and in medium grey background if values are negative).

	MGT[# a]	GP[# b]	GR[# c]	GV[# d]	As	Cd	Cr	Cu	Fe	Mn	Ni	Pb	Zn
MGT					0.938	0.238	0.312	0.730	0.215	0.600	0.362	0.103	0.540
GP	0.864				0.940	0.571		0.853	0.334	0.095	0.079	0.071	0.948
GR	0.631	0.933			0.779	0.955		0.922	0.507			0.104	0.593
GV	0.887	0.991	0.906		0.886	0.534	0.065	0.930	0.295	0.112	0.089	0.066	0.992
As	0.020	-0.020	-0.074	-0.037			0.106		0.483				
Cd	0.302	0.148	0.015	0.162	0.675		0.569		0.585		0.137	0.260	
Cr	-0.261	-0.490	-0.599	-0.457	0.406	0.149		0.126	0.405				0.130
Cu	0.090	0.049	-0.026	0.023	0.982	0.669	0.386		1.000				
Fe	0.430	0.341	0.238	0.368	-0.252	-0.197	0.297	0.000			1.000	0.810	0.438
Mn	-0.137	-0.418	-0.562	-0.399	0.632	0.546	0.791	0.610	0.757				
Ni	-0.236	-0.438	-0.543	-0.425	0.735	0.376	0.892	0.716	8E-13	0.888			
Pb	-0.409	-0.448	-0.408	-0.455	0.599	0.289	0.692	0.511	-0.087	0.720	0.796		
Zn	0.160	-0.017	-0.140	-0.003	0.757	0.951	0.382	0.725	-0.277	0.717	0.590	0.542	

[a] mean germination time, days; [b] germination percent, %; [c] germination rate, %/day; [d] germination value, %;

CONCLUSIONS

This study has looked at series of heavy metal contaminated samples from a former copper mine – region in Romania. The germination parameters were the lowest, and therefore, affected the strongest for the sample from Bosneag River - upstream (SW1) because the Bosneag River is the river that runs along the copper mine. The MGT was averaged for all samples, observing slight variations. The highest value was 3.04 days compared to 2.73 days for the river water and sediment samples, respectively. One of these reasons can be the higher concentration of heavy metals in sediment samples, different the concentration of heavy metals in rivers waters samples. A 90% GP mean was recorded for deep waters, which drops almost 20% (GP mean 73%) for the sediment samples. The sediment samples were also the most affected in terms of germination rate, with an average of 21%/day, one of these reasons to be the higher concentration of metals in sediment by accumulation in time. A similar trend was observed for the germination value, which averaged 11%. For the river and well samples, the results were not significantly different.

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REFERENCES

- Bityutskii, N. P., Magnitskiy, S. v, Korobeynikova, L. P., Lukina, E. I., Soloviova, A. N., Patsevitch, V. G., Lapshina, I. N., & Matveeva, G. v. (2002). DISTRIBUTION OF IRON, MANGANESE, AND ZINC IN MATURE GRAIN AND THEIR MOBILIZATION DURING GERMINATION AND EARLY SEEDLING DEVELOPMENT IN MAIZE. Journal of Plant Nutrition, 25(3), 635–653.
- Boros, B., Roman, D., Ostafe, V., Markovic, R., Gardic, V., Stevanovic, Z., & Isvoran, A. (2021). The effects on human health of non-metallic, semimetals and heavy metals compounds generated by mining activities along the Serbian-Romanian border. Romanian Journal of Ecology & Environmental Chemistry, 3, 61.
- Carpýcý, E. B., Celýk, N., & Bayram, G. (2009). Effects of salt stress on germination of some maize (Zea mays L .) cultivars. AFRICAN JOURNAL OF BIOTECHNOLOGY, 8(19), 4918.
- Chamorro, S., Barata, C., Piña, B., Casado, M., Schwarz, A., Sáez, K., & Vidal, G. (2018). Toxicological Analysis of Acid Mine Drainage by Water Quality and Land Use Bioassays. Mine Water and the Environment, 37(1), 88.
- Deng, B., Yang, K., Zhang, Y., & Li, Z. (2016). Can heavy metal pollution defend seed germination against heat stress? Effect of heavy metals (Cu2+, Cd2+ and Hg2+) on maize seed germination under high temperature. Environmental Pollution, 216, 46–52.
- Dezfuli, P. M., Sharif-zadeh, F., & Janmohammadi, M. (2008). Influence of priming techniques on seed germination behavior of maize inbred lines (zea mays L.). In Journal of agricultural and biological science (Vol. 3, Issue 3, p. 22).
- Foti, R., Abureni, K., Tigere, A., Gotosa, J., & Gere, J. (2008). The efficacy of different seed priming osmotica on the establishment of maize (Zea mays L.) caryopses. J. Arid. Environ., 72(6), 1127.
- Ion, V., Dicu, G., State, D., Fintineru, G., Epure, L., Basa, A., & Toader, M. (2013). Yield Components Of Different Hybrids Of Maize (Zea Mays L.) Cultivated In South Romania Under Drought Conditions. Scientific Papers. Series A. Agronomy, 56, 276.
- Isvoran, A., Roman, D. L., Dascalu, D., Vlad-Oros, B., Ciorsac, A., Pitulice, L., Jonovic, R., Stevanovic, Z., & Ostafe, V. (2021). Human Health Effects of Heavy Metal Pollution in the Cross-Border Area of Romania and Serbia: A Review. Ecological Chemistry and Engineering S, 28(3), 365.
- Jabłońska-Czapla, M., Zerzucha, P., & Grygoyć, K. (2020). Impact of River Water and Bottom Sediment Pollution on Accumulation of Metal(loid)s and Arsenic Species in the Coastal Plants Stuckenia pectinata L., Galium aparine L., and Urtica dioica L.: A Chemometric and Environmental Study. Archives of Environmental Contamination and Toxicology, 79(1), 60–79.
- Kulkarni, M. G., Street, R. A., van Staden, J. (2007). Germination and seedling growth requirements for propagation of Dioscorea dregeana (Kunth) Dur. and Schinz — A tuberous medicinal plant. S. Afr. J. Bot., 73(1), 131.
- Law No. 458/2002 Regarding the Quality of Drinking Water in Romania Republished in 2011, Pub. L. No. No. 552/29.07.2002, Official Journal of Romania (2002). https://legislatie.just.ro/Public/DetaliiDocument/37723
- Liu, J.-J., Diao, Z.-H., Xu, X.-R., & Xie, Q. (2019). Effects of dissolved oxygen, salinity, nitrogen and phosphorus on the release of heavy metals from coastal sediments. Science of The Total Environment, 666, 894–901.
- Ma, X., Li, H., Zhang, J., & Shen, J. (2021). Spatiotemporal Pattern of Acid Phosphatase Activity in Soils Cultivated With Maize Sensing to Phosphorus-Rich Patches. Frontiers in Plant Science, 12, 1.
- Mahmood, S., Hussain, A., Saeed, Z., & Athar, M. (2005). Germination and seedling growth of corn (Zea mays l.) under varying levels of copper and zinc. Int. J Environ. Sci. Te., 2(3), 269. https://doi.org/10.1007/BF03325886
- Mihut, C., Mazare, V., Stroia, M. S., & Dragunescu, A. (2010). PHISICAL AND CHEMICAL PROPERTIES OF SOILS FOUND IN THE VINEYARDS CENTERS TIROL AND MOLDOVA NOUĂ. Research Journal of Agricultural Science, 43(3). file:///C:/Users/pc/Downloads/88.pdf

- Msuya, D., & Stefano, J. (2010). Responses of Maize (Zea mays) Seed Germination Capacity and Vigour to Seed Selection Based on Size of Cob and Selective Threshing. World j. Agric. Sci., 6(6), 683.
- Muhammad, Z., & Majeed, A. (2014). Allelopathic effects of aqueous extracts of sunflower on wheat (Triticum aestivum L.) and maize (Zea mays L.). Pak J Bot, 46(5), 1715. https://www.pakbs.org/pjbot/PDFs/46(5)/22.pdf
- Order No 161/2006 for the Approval of the Normative on the Classification of Surface Water Quality to Establish the Ecological Status of the Water Bodies, Pub. L. No. No. 11/13.06.2006, Official Journal of Romania (2006).
- Ostafe, V., Stepanovic, Z., Sturza, C. P., & Isvoran, A. (2021). A Knowledge Base as a Monitoring System of Environmental State in Mining Operations Areas of the Cross-Border Region of Romania and Serbia. New Front. Chem., 30(1), 1–7.
- Pana, E., Raischi, N., Mitiu, M., Zamfir, Ștefan, Deak, G., & Holban, E. (2018). Analysis of air and soil quality regarding the cross-border impact risk of the mining tailings ponds in Moldova Nouă area. International Journal of Environmental Science and Development, 10, 162.
- Ramana, S., Biswas, A. K., Kundu, S., Saha, J. K., & Yadava, R. B. R. (2002). Effect of distillery effluent on seed germination in some vegetable crops. Bioresource Technology, 82(3), 273.
- Singh, A., Singh, A., Pandey, A., Singh, A., Singh, R., Singh, A., & Yadav, R. (2020). Effect salinity on germination percentage (%) and seed vigour index of rice (Oryza sativa L.). Journal of Pharmacognosy and Phytochemistry, 9, 1130.
- Solanki, R., & Dhankhar, R. (2011). Biochemical changes and adaptive strategies of plants under heavy metal stress. Biologia, 66(2), 195.
- Soltani, E., Ghaderi-Far, F., Baskin, C., & Baskin, J. (2015). Problems with using mean germination time to calculate rate of seed germination. Australian Journal of Botany, 63
- Stevanović, Z., Kovačevi, R., Marković, R., Gardić, V., Vulpe, B. C., Boros, B., & Menghiu, G. (2021). State of the surface waters in cross border region of eastern Serbia and Caras Severin county–Moldova Noua in Romania. Studia UBB Chemia, 66(4), 309
- Thomson, A. J., & El-Kassaby, Y. A. (1993). Interpretation of seed-germination parameters. New Forests, 7(2), 123.
- Tsamo, C., Alanyuy, S., Zama, E., & Tamu, C. (2022). Germination and Survival of Maize and Beans Seeds: Effects of Irrigation with NaCl and Heavy Metals Contaminated Water. Open J. Appl. Sci., 12, 769.
- Tudor, V., Asănică, A., Teodorescu, R. I., Gidea, M., Tănăsescu, C., Tudor, A. D., & Țiu, J. V. (2017). Germination capacity of some Lycium barbarum L. and Lycium chinense Mill. biotypes seeds. Romanian Biotechnological Letters, 22(1), 12191.
- Wetzel, R. (2001). Chapter 9 - Oxygen. In Limnology: Lake and River Ecosystems. In Elsevier/Academic Press (3rd edition, pp. 205–286).
- Xin, X., Zhao, F., Judy, J. D., & He, Z. (2022). Copper stress alleviation in corn (Zea mays L.): Comparative efficiency of carbon nanotubes and carbon nanoparticles. NanoImpact, 25, 100381.
- Yáñez-Espinosa, L., Briones-Gallardo, R., Flores, J., & Álvarez del Castillo, E. (2020). Effect of heavy metals on seed germination and seedling development of Nama aff. stenophylla collected on the slope of a mine tailing dump. International Journal of Phytoremediation, 22(14), 1448.