

Determination of the Effects of Copper (Cu) and Lead (Pb) Heavy Metals on Soil Carbon and Nitrogen Mineralizations

(Penentuan Kesan Logam Berat Tembaga (Cu) dan Plumbum (Pb) pada Tanah Pemineralan Karbon dan Nitrogen)

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ABSTRACT

Heavy metal (HM) pollution has become one of the most important environmental problems of the present day, as a result of the developing industrial activities. Accordingly, it is important to understand microorganism activities in soil ecosystems that have been exposed to HMs for a long time. The aim of this study was to show the potential effects of ores on soil carbon and nitrogen mineralizations which were taken from copper (Cu) and lead (Pb) mines in Balıkesir-Balya and Kastamonu-Küre districts in Turkey. The carbon (C) and nitrogen (N) mineralizations were determined by using the CO₂ respiration method (30 days) and the Parnas Wagner method (42 days) under the controlled laboratory conditions (28 °C, 80% of field capacity), respectively. It was observed that carbon mineralization decreased depending on the dose increase. 250 mg kg⁻¹ treatment with Pb was lower than the control and there was a significant difference between them (P < 0.001). In terms of nitrogen mineralization rate (%), there was no significant difference among all treatments. According to the results, Pb affected microorganisms more negatively; however, the presence of Cu slightly decreased its negative effect. It is possible to conclude that carbon mineralization can be indicator for HM pollution in the soil. However, nitrogen mineralization was not a determining factor at HM pollution in this study.

Keywords: Carbon mineralization; heavy metal; nitrogen mineralization; organic matter; soil pollution

ABSTRAK

Pencemaran logam berat (HM) telah menjadi salah satu masalah persekitaran yang paling penting pada masa kini akibat daripada aktiviti industri yang sedang berkembang. Oleh itu, penting untuk memahami aktiviti mikroorganisma dalam ekosistem tanah yang telah lama terdedah kepada HM. Tujuan kajian ini adalah untuk mendedahkan kemungkinan kesan bijih pada mineral tanah dan mineral nitrogen yang diambil daripada lombong tembaga (Cu) dan timah (Pb) di daerah Balıkesir-Balya dan Kastamonu-Küre di Turki. Pemineralan karbon (C) dan nitrogen (N) ditentukan dengan menggunakan kaedah respirasi CO₂ (30 hari) dan kaedah Parnas Wagner (42 hari) di bawah keadaan makmal terkawal (28 °C, 80% kapasiti medan), masing-masing. Telah diperhatikan bahawa pemineralan karbon menurun bergantung pada peningkatan dos. Rawatan 250 mg kg⁻¹ dengan Pb lebih rendah daripada kawalan dan terdapat perbezaan yang signifikan antara mereka (P < 0.001). Daripada segi kadar pemineralan nitrogen (%), tidak ada perbezaan yang signifikan antara semua rawatan. Menurut hasilnya, Pb mempengaruhi mikroorganisma dengan lebih negatif; namun, kehadiran Cu sedikit mengurangkan kesan negatifnya. Adalah mungkin untuk menyimpulkan bahawa pemineralan karbon dapat menjadi petunjuk untuk pencemaran HM di dalam tanah. Walau bagaimanapun, pemineralan nitrogen bukanlah faktor penentu pencemaran HM dalam kajian ini.

Kata kunci: Bahan organik; logam berat; pemineralan karbon; pemineralan nitrogen; pencemaran tanah

INTRODUCTION

More than 35 metals are exposed in the ecosystem, 23 of them are known as heavy metals (HMs) (Askari et al. 2020; Khalid et al. 2017). The HMs are considered to be one of the most important environmental pollutants all over the world (Ali et al. 2013; Hashem et al. 2017). Copper (Cu), lead (Pb), cadmium (Cd), chromium (Cr), iron (Fe),

cobalt (Co), nickel (Ni), mercury (Hg) and zinc (Zn) are among the frequently encountered and hazardous HMs in various ecosystems including soil (Khalid et al. 2017). The HMs spread to the atmosphere from many different sources such as industry, mining, and fertilizers pesticides. These sources are different processing stages that include natural and anthropogenic factors (Alvarez et

al. 2017). After their release from these sources into the ecosystem, they can interfere with the ecological balance by mixing with dry and wet accumulation into the soil and then underground waters (Fernandes et al. 2007). As parallel with the increasing industrial activities in the modern world, the HMs pollution reached great dimensions over time, and become an enormous global challenge (Hashem et al. 2017).

Numerous studies reported HMs build up in soil and associated risks to soil fertility/quality and biochemical activities such as enzymes and microorganism. Because they might have negative effects on metabolism, morphology and growth rate of various soil microorganisms, the HMs contaminated soils are one of the most important problem in the industrialized world (Yao et al. 2003). The HMs may cause not only a dramatic decrease on activity of the soil microorganisms but also direct toxic effects on their populations (Rajapaksha et al. 2004; Shi & Ma 2017). The presence of HMs in the soil may also have severe effects on biochemical reactions such as carbon mineralization, enzyme activity and nitrification (Dai et al. 2004; Kizildag et al. 2017; Liu et al. 2019; Tang et al. 2020). In addition, soil properties such as clay content, organic matter quality and quantity and pH are also known to be negatively affected by HMs (Fernández et al. 2018; Minnikova et al. 2017; Shahid et al. 2012).

The different mineralogical composition affects the mobility of HMs, hence its bioavailability by soil microorganisms. Investigations on the functioning of soil microorganisms in ecosystems, has an important place in soils exposed to long-term contamination by HMs.

The negative effects of HMs on soil biological and biochemical properties have been reported in many studies (Friedlova 2010; Mikanova 2006; Song et al. 2018; Zhang et al. 2010). These studies indicated that the toxicity of HMs is not always stable but vary depending on their concentrations, form (metal, ion, organic compound), location as well as duration of the action. Among these criteria, the total HMs concentration is considered to be one of the most important factors to evaluate soil pollution (Aka Sagliker & Darici 2004; Askari et al. 2020; Nwuche & Ugoji 2008). The most important indicators of heavy metal pollution in the soil are C and N mineralization that reflects microorganism activities. Knowing of how C and N mineralizations responds to heavy metals are essential to better understand whether soils will react to climate change. Despite their possible high toxicities, the knowledge regarding the effects of HMs on soil C and N mineralization, which is one of the most

important indicators of the soil microorganism activity has been rarely addressed (Dai et al. 2004; Kizildag et al. 2017).

This study was aimed to determine the possible effects of mine ores from Cu and Pb mines on soil carbon and nitrogen mineralizations based on the materials obtained from Balıkesir-Balya and Kastamonu-Küre districts in Turkey.

MATERIALS AND METHODS

The experimental soil samples were taken from the upper 0-20 cm on July 2016 under *Tamarix tetrandra* L. (Tamaricaceae) from Adana, a city located in the East-Mediterranean region of Turkey. The study area is characterized by typical Mediterranean climate where annual precipitation of 663 mm and temperature of 18.7 °C occurred for the last 57 years. The samples were air-dried and sieved using a sieve (2 mm mesh in size), and stored in plastic bags. Powder mine ores from Cu and Pb mines were obtained from Balıkesir-Balya and Kastamonu-Küre districts in Turkey. The size and the morphology of Cu and Pb were observed by Scanning Electron Microscope (SEM) and metal contents by Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES).

The soil texture was determined by the Bouyoucos hydrometer (Bouyoucos 1951), whereas field capacity (%) by a vacuum pump at 1/3 atmospheric pressure (Demiralay 1993) and pH with a WTW Inolab 720 pH-meter in 1:2.5 soil-water suspension (Jackson 1958). The total organic carbon (TOC) and the total nitrogen (TN) contents of the soil (%) were determined by modified Walkley and Black and Kjeldahl methods, respectively (Duchaufour 1970). Some physical and chemical properties of the soil are presented in Table 1. The soil was classified as sandy clay loam (SCL) texture, and pH was found to be slightly alkaline. Cu and Pb ores used in this study are in amorphous-shape and their SEM images are given in Figure 1. Cu and Pb contents of mine ores were found 3.47 ± 0.01 (%) and 6.76 ± 0.02 (%), respectively.

The effects of six treatments that consist single and combined (Cu+Pb) uses of Cu and Pb at their two different concentrations (100 and 250 mg kg⁻¹), on carbon mineralization were investigated in the study. These treatments were carried out by mixing them into soil by 80% of field capacity, and then incubated at 28 °C for 30 days (Schaefer 1967). Three replicates were used for each treatment.

CO₂ derived from microbial activities was absorbed in saturated Ba(OH)₂ solution (40 mL) by using beakers

TABLE 1. Some physical and chemical properties of *Tamarix tetrandra* L. soil (mean \pm SE, n=3)

Characteristics	Mean		SE
Sand	52.04	\pm	0.39
Silt	15.26	\pm	0.64
Clay	32.70	\pm	0.55
Texture type	Sandy Clay Loam (SCL)		
Field capacity (%)	22.20	\pm	0.69
pH	7.62	\pm	0.01
C (%)	1.83	\pm	0.02
N (%)	0.14	\pm	0.02
C/N ratio	13.14	\pm	0.56

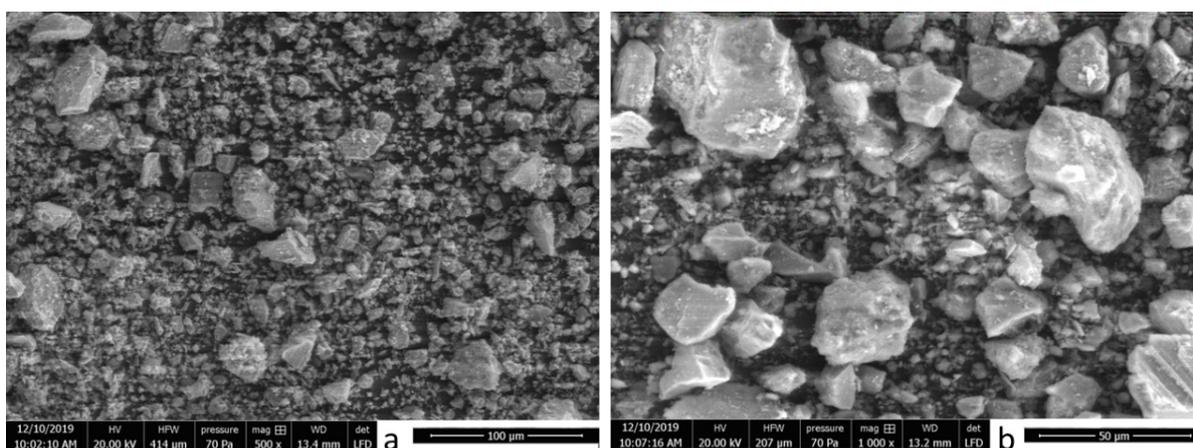


FIGURE 1. The SEM image of (a) Cu and (b) Pb ores used in the experiment

that was placed in the center of the soils in closed incubation vessels. The mixtures were transferred to an incubator set at 28 °C for 30 days. The amount of CO₂ was measured by titration using an oxalic acid at three-day intervals (Benlot 1977). The blank vessels were served as control. The rate (%) of carbon mineralization was calculated by dividing the cumulative amount of C (CO₂) produced in 30 days into total organic carbon.

Whereas, for nitrogen mineralization, the soils with mixed Cu, Pb and Cu+Pb humidified with distilled water to 80% of their field capacity were placed in 750 mL open incubation vessels and transferred to the

incubator set at 28 °C for 42 days. In order to determine weight loss, the vessels were weighed three times a week, and distilled water was added to compensate for water loss. Soil samples were taken from each vessel on 1st and 42nd days following the applications. The samples were used to measure the concentrations of mineral nitrogen (NH₄-N and NO₃-N) by using the Parnas-Wagner method (Gökçeoğlu 1979; Lemée 1967). Thus, each soil sample was mixed with 200 mL 1 N CaCl₂ and shaken for one hour followed by a distillation. The nitrogen mineralization ratio (%) was calculated by dividing the amount of mineral nitrogen (NH₄-N and NO₃-N) on 42nd day of the incubation period by the total organic nitrogen.

A Repeated Measures (General Linear Model) analysis was performed to determine the differences in carbon and nitrogen mineralizations in the soils with different HM treatments (Cu, Pb and Cu+Pb), and their different doses (100 and 250 mg kg⁻¹) (Kleinbaum et al. 1998). Three replicates were used for each combined soil for statistical comparisons. Data were analyzed by a series of analyses of variance. Differences between data were assumed significant at $P < 0.05$. All statistical analyses were carried out using SPSS 21.0.

RESULTS AND DISCUSSION

CO₂ production of Cu at a dose of 100 mg kg⁻¹ was found to be higher compared to 250 mg kg⁻¹, and no significant difference was observed between them (Figure 2). Additionally, 250 mg kg⁻¹ treatment with Pb was significantly lower than the control ($P < 0.001$, Figure 3). Microorganisms reacted more sensitively to 100 and 250 mg kg⁻¹ doses at which both elements combine (Cu+Pb), and they decreased their activities (Figure 4). This decrease was more significant at 250 mg kg⁻¹ ($P < 0.001$).

The highest carbon mineralization rate was observed in the control (1.13%) followed by Cu (1.04%), Pb (0.97%), and Cu+Pb (0.98%) treated soils at 100 mg kg⁻¹ (Figure 5). A significant difference was found between combined (Cu+Pb) treatments at 100 mg kg⁻¹ and 250 mg kg⁻¹ ($P < 0.05$). This result, which was close to control in the soil with 100 mg kg⁻¹ Cu, decreased in the soil with Pb, but it did not change much in the soil with Cu+Pb.

Results regarding nitrogen mineralization showed that the amount of NH₄-N was higher than NO₃-N on the 1st day in the control group. Whereas, an increase in NO₃-N

was observed on the 42nd day. A similar behavior was found in the presence Cu and Pb at 100 mg kg⁻¹ ($P < 0.001$), and their nitrogen mineralization rates were found to be higher compared to the control (Table 2). A significant difference was observed between Cu treatments at 100 mg kg⁻¹ and at 250 mg kg⁻¹ on the 42nd day in terms of NO₃-N contents ($P < 0.001$). While the NH₄-N production increased and NO₃-N decreased in the soil treated with 250 mg kg⁻¹ Cu on 42nd day, an opposite situation was observed in the soil treated with Pb. Whereas the nitrogen mineralization rate was approximately the same as the control in the soil with Cu, the lower rate was observed in soil with Pb.

In the soil with 100 mg kg⁻¹ Cu+Pb, the amount of NH₄-N was high on the 1st and 42nd days, and NO₃-N increased. The nitrogen mineralization rate was found to be slightly lower in this soil compared to the control. In the soil with 250 mg kg⁻¹ Cu+Pb, the amount of NH₄-N was decreased by 50% on 42nd day. However, a slight increase was observed in amount of NO₃-N during the incubation period. These changes occurred at much lower rates at 100 mg kg⁻¹ doses. The lowest nitrogen mineralization rate was determined as 0.51% in soils treated with 250 mg kg⁻¹ Cu+Pb and it was found to be significantly difference from other treatments ($P < 0.05$, Table 2).

According to the results, the cumulative carbon mineralization of the control, Cu, Pb, and Cu+Pb treated soils gradually increased during the incubation period. However, it was observed that microorganism activity decreased depending on the dose increase. Microorganisms were found to be less affected by the presence of Cu compared to Pb and they reacted more sensitively to 100 and 250 mg kg⁻¹ doses at which both elements combine (Cu+Pb). And, the microorganism activities decreased in

TABLE 2. NH₄-N, NO₃-N (mg kg⁻¹, the day of first and 42nd) and N mineralization (%) values of different treatments of Cu, Pb and Cu+Pb (mean ± SE, n=3)

	1. day						42. day						N min rate		
	NH ₄ -N			NO ₃ -N			NH ₄ -N			NO ₃ -N					
	(mg kg ⁻¹)			(mg kg ⁻¹)			(mg kg ⁻¹)			(mg kg ⁻¹)			(%)		
Control	9.13	± 0.19	a	3.40	± 0.21	ab	4.93	± 0.37	A	5.43	± 0.77	ab	0.74	± 0.08	ab
Cu100	9.30	± 0.44	a	3.33	± 0.66	ab	5.37	± 0.67	A	6.47	± 0.66	a	0.85	± 0.09	a
Cu250	6.50	± 0.75	ab	2.47	± 0.29	ab	6.83	± 1.05	A	3.43	± 0.38	bc	0.73	± 0.05	ab
Pb100	7.63	± 0.70	ab	3.77	± 0.29	ab	5.80	± 0.98	A	5.17	± 0.38	ab	0.78	± 0.09	ab
Pb250	5.87	± 0.79	b	4.70	± 0.29	a	3.97	± 0.45	A	4.80	± 0.15	ac	0.63	± 0.02	ab
Cu+Pb 100	7.83	± 0.75	ab	2.67	± 0.84	ab	6.17	± 0.19	A	3.83	± 0.20	bc	0.71	± 0.03	ab
Cu+Pb 250	8.63	± 0.84	ab	2.40	± 0.31	b	4.37	± 0.43	A	2.83	± 0.35	c	0.51	± 0.01	b

Different letters in the same column indicate significant differences ($p < 0.05$)

the combined treatment (Cu+Pb). Furthermore, the interesting thing was the reaction/sensitivity of microorganisms to the presence of every two elements, although they are difficult to dissolve in water. This result clearly indicated that microorganism activities might be adversely affected by mixing mine sites, industrial or domestic wastes in soil.

Pb was more negative effect than Cu on soil microorganisms. However, the presence of Cu slightly decreased the negative effect of Pb. Nwuche and Ugoji (2008) found that carbon mineralization of soils treated with copper (Cu) and zinc (Zn) was higher in comparison with the combination of both elements. Also, they reported that the respiration rate of the soil microbial populations was inhibited by the metals. While the respiration rate was 2.51-2.56 μg of C g^{-1} at the beginning, it was declined to 0.98, 1.08, and 1.61 μg of C g^{-1} in the Cu:Zn, Cu and Zn treated soils by the end of the incubation period, respectively. Walpola and Yoon (2012) showed that cadmium (Cd) decreased carbon mineralization compared to zinc (Zn) in soils treated with Cd and Zn. They reported that it was related to the tolerance and adaptation of the microorganism

community in the soil to the metal. Furthermore, the type of HM and ED_{50} (ecological dosages) were determined as important factors on the soil microorganism activity by Liao et al. (2005). Carbon mineralization is considered as an important and sensitive indicator of HM pollution in soils (Kizildag et al. 2017; Minnich & McBride 1986; Nwuche & Ugoji 2008; Rother et al. 1982).

The nitrogen mineralization decreased due to increased HM concentrations. Similarly, some authors reported that increased HM concentrations inhibited nitrogen mineralization rates (Stuczynski et al. 2003; Vasquez-Murrieta et al. 2006). Cela and Sumner (2002), observed that Pb did not inhibit nitrification. Whereas, a significant decrease was reported in Cu treatment (> 3.8 mg water-extractable Cu kg^{-1} soil). Because, Cu is an element that may enter into the oxidative enzyme compositions, and it changed the result in this way (Festa & Thiele 2011; Kosolapov et al. 2004). According to present results, the nitrogen mineralization in *Tamarix tetrandra* soils was more sensitive to 250 mg kg^{-1} Pb and Cu+Pb combination compared to Cu, as in carbon mineralization.

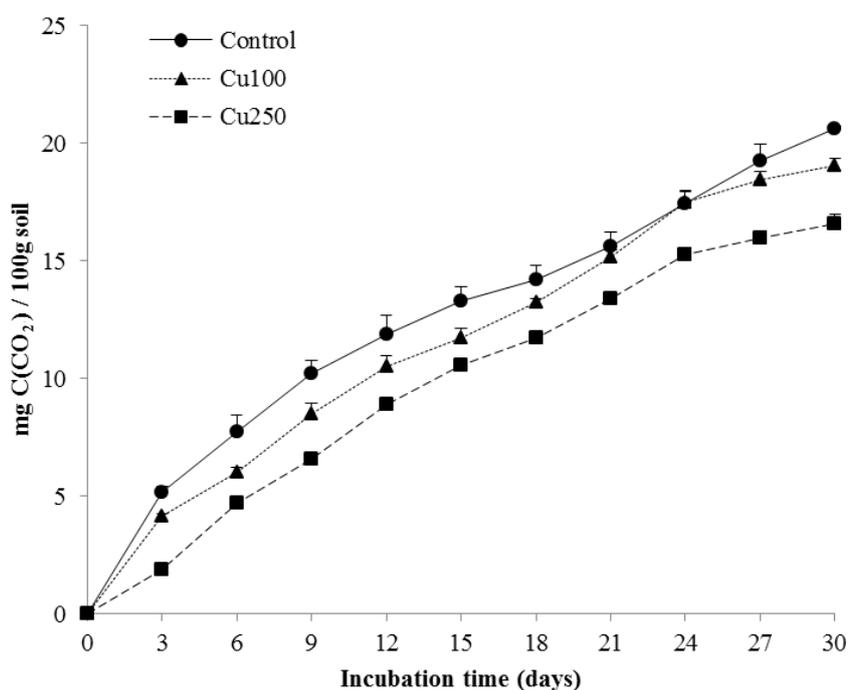


FIGURE 2. Cumulative carbon mineralization in *Tamarix tetrandra* soil mixed with 100 and 250 mg kg^{-1} Cu [mg $\text{C}(\text{CO}_2)/100$ g, (mean \pm SE, $n=3$)]

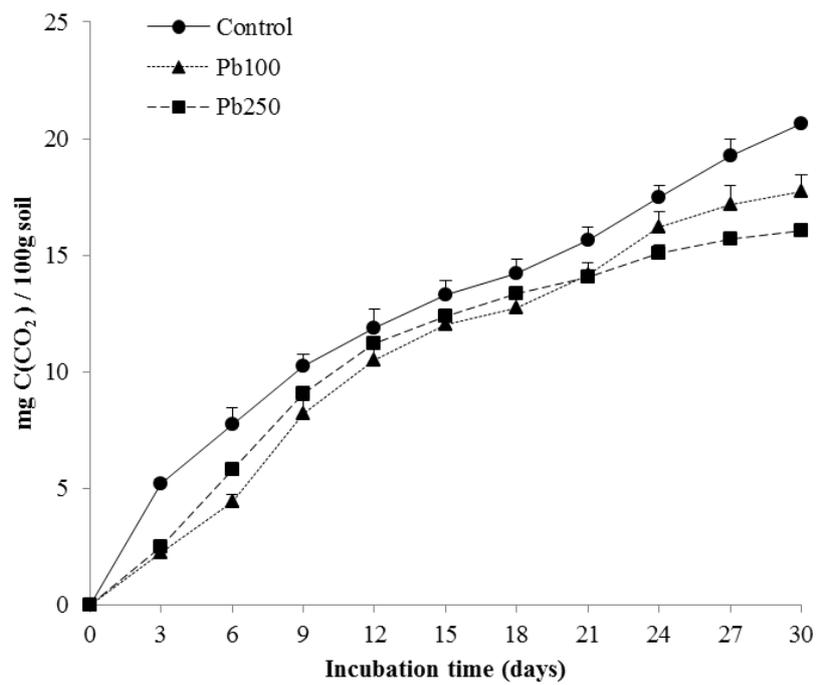


FIGURE 3. Cumulative carbon mineralization in *Tamarix tetrandra* soil mixed with 100 and 250 mg kg⁻¹ Pb [mg C(CO₂)/100 g, (mean ± SE, n=3)]

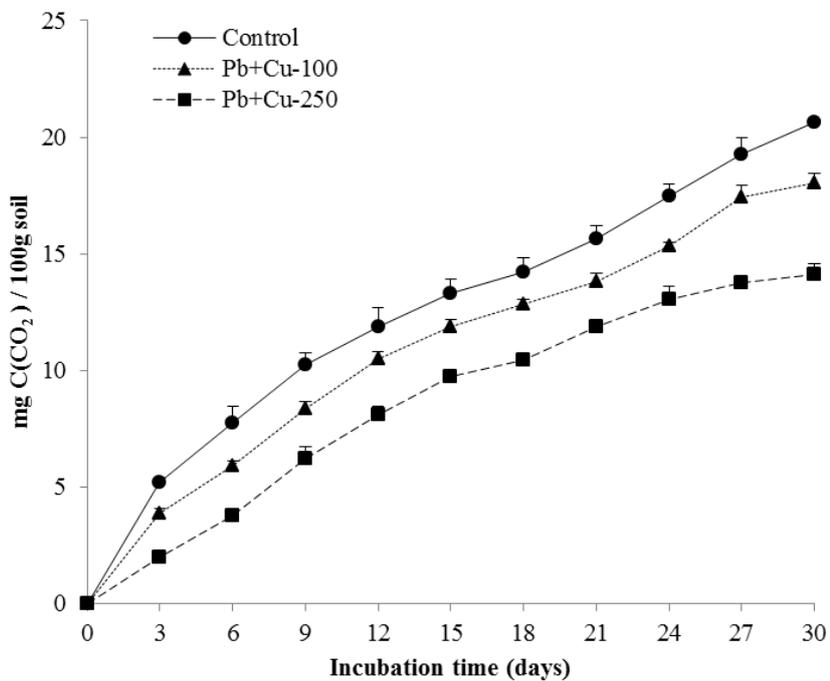


FIGURE 4. Cumulative carbon mineralization in *Tamarix tetrandra* soil mixed with 100 and 250 mg kg⁻¹ Cu+Pb [mg C(CO₂)/100 g, (mean ± SE, n=3)]

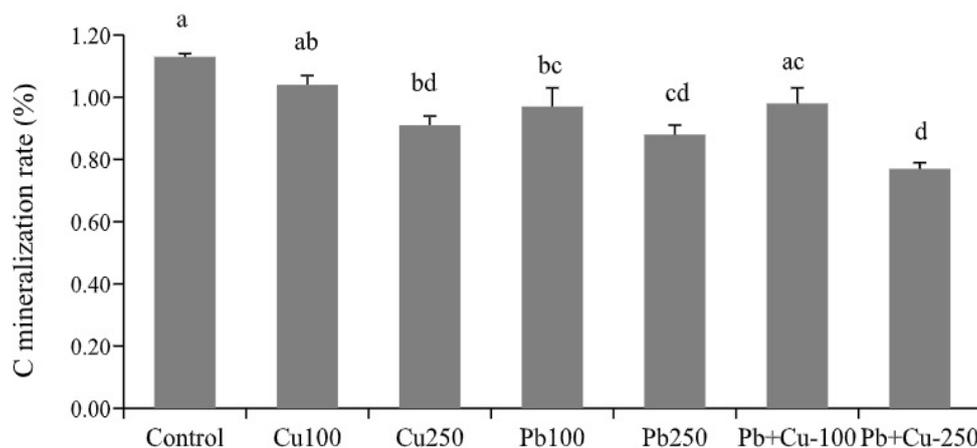


FIGURE 5. Carbon mineralization rate in Cu, Pb and Cu+Pb treatments mixed into *Tamarix tetrandra* soil [mg C(CO₂)/100 g, (mean ± SE, n=3)]

CONCLUSION

According to the current results, Cu and Pb had an adverse balance of the soil. Cumulative carbon mineralization decreased with increased HM concentration. The inhibitions of carbon and nitrogen mineralizations were found to be higher in the soil treated with Pb compared to the soil treated with Cu. Accordingly, it is possible to say that carbon mineralization can be indicator for HM pollution in the soil. However, nitrogen mineralization was not a determining factor at HM pollution in this study. This study can be applied with certain metals, especially in soils that are exposed to or are at risk of being exposed to different metals, and sensitivity levels to different combinations and doses can be determined.

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