NITROGEN MINERALIZATION

IN MINE WASTE-CONTAMINATED SOILS

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SUMMARY

In this study, the effect of mine wastes on nitrogen mineralization was investigated at two different depths of soil (0-5 cm and 5-15 cm) in waste sites in the surroundings of the Etibank Wolfram Mine Work, Bursa, Turkey. Due to the disorder of the distribution of mining wastes, the pH, $CaCO_3$% and element content (Fe, Mn, Zn, Mg) in the soils around the mine were enriched by mining activity. The investigation was carried out in soils taken from waste-removal pools (WRPs) and from selected sample sites which differed in their distances from the mine works. Waste materials resulted by mining were discharged with water and, they were fall down on two waste removal pools which were constructed as primitive terraces around the mine work. For this reason, elements were most enriched on sandy ground of abandoned pools. Both N-mineralization and nitrification were determined by a standard incubation method under laboratory conditions at 20 °C and 60 % WHC. Mineral nitrogen was analyzed at the initial, the 21st and the 63rd day by micro-distillation method. The net mineral N production was estimated for 21 and 42 days. It was calculated as the potential mineralization rate of organic nitrogen. N-mineralization and net production were the highest at the site furthest from the mine waste site. Significant negative correlations between nitrification and element contents (Fe, Zn, Mn, Mg) of soils were also found. This shows that the mine wastes have a negative effect on the nitrogen mineralization, especially nitrification.

KEYWORDS: contaminated soil, mine wastes, Fe, Zn, Mn, Mg; N-mineralization; nitrification.

INTRODUCTION

Nitrogen mineralization is generally regarded as a key-process in terrestrial ecosystems. Since nitrogen supply often limits plant growth in natural environments, nitrogen availability affects the outcome of species competition and consequently controls the development, persistence and decline of plant communities in many areas [1]. Mineralization in soil and nitrogen uptake by plants are important indicators of the productivity of ecosystems [1-3]. Nitrogen mineralization controlled by the chemical and physical conditions of the soil such as temperature, pH, water holding capacity (WHC %), total N and organic C content, is one of the main ecosystem processes [1, 4].

Persistent contaminants in the environment affect human health and ecosystems. Heavy metal contamination is considered to be one of the main sources of pollution in the environment, since they have a significant effect on its ecological quality [5]. Air, water and soil are polluted by a variety of metals due to anthropogenic activities, which alter the normal biogeochemical cycling [6]. Heavy metals are quantitatively the most important pollutants over the past 300 years [7]. They are known to be toxic to most organisms when present in excessive concentrations, and can disrupt soil microbial processes, sometimes resulting in severe ecosystem disturbance [8].

N transformations have become a major indicator in assessing soil quality and the effect of xenobiotics [8-10]. Heavy metal content of soil is one of the factors effecting the nitrogen mineralization [10-14]. Generally, industrially contaminated soils show a combined toxicity due to the presence of several heavy metals and the effect of single metal contamination is not clear [10]. The effects depend on the respective element, the concentration and the soil conditions [15].

Güleryüz et al. [16] previously determined high heavy metal contents in soils around Etibank Wolfram Mine Work which is located in the Uludağ National Park, Bursa-Turkey. They reported that Wolfram mining activities changed the soil composition locally. Our objective was to examine the influence of mining waste on nitrogen mineralization rates under laboratory conditions (20 °C and 60 % water holding capacity).

MATERIALS AND METHODS

Sampling, experiments, and analyses: Soil samples were collected near the Etibank Wolfram Mine Work located in Mount Uludağ, Bursa, Turkey. Detailed information is given in our previous work [16].

Four sample sites (A, B, C, D) were located north of the mine works. Site A is the nearest (ca. 300 m) and site D is the furthest from the mine work (ca. 2000 m). The sampling was performed in plots of 400 m². Samples were taken from four different places at each sample site; soil samples were taken from two layers (0-5 and 5-15 cm). In addition to these sites, another sample site (WRPs) from waste-removal pool was chosen as a reference to determine whether soil contamination arises from mine work. Soil samples were taken at a depth of 0-10 cm from WRPs. The samples were sifted through with a standard 2-mm stainless steel sieve and then dried in air.

The water holding capacity (WHC %) of the soil samples were analysed by calculating the differences between the fresh and dry weights of materials, which were saturated and then dried at 80 ^{o}C until a constant weight was obtained [17]. Air dried soil samples (100 g) were put into polyethylen bags, moistened with distilled water until the WHC was 60 %. These bags prevent escaping of mineral nitrogen and water (and moisture), but permit the passage of CO_2 and O_2 [18]. The moistened samples were incubated in a biotron apparatus (Heraeus Vötsch, HPS 500) at a temperature of 20 ^{o}C for 9 weeks (63 days).

The mineral nitrogen of the soil was determined by Micro-distillation method [19, 20]. Mineral nitrogen (NH_4^+-N and NO_3^--N) was analyzed at three times during 63 days of incubation: at the beginning of incubation period, at the 21st and at the 63rd day. Incubation period was divided into two main periods: 21-days (between the initial and the 21st day) and 42-days (between the 21st day and the 63rd day). Net mineral nitrogen accumulations were calculated for 21 days (mg N_{min}/100 g dry soil/ 21 days) and for 42 days (mg N_{min}/100 g dry soil/ 42 days). Differences between these values were used to calculate the net miner-

al nitrogen production for each period. Net mineral nitrogen production (mg N_{min}/100 g dry soil/ 21 days and mg N_{min}/100 g dry soil/ 42 days) was then expressed as nitrogen mineralization rates.

Total nitrogen in soil was determined by a Kjeldahl method using salicylic-sulphuric acid and selenium [21]. The $CaCO_3$ (%) and organic carbon (%) soil contents were determined by the Scheibler method and wet incineration method (digestion with concentrated sulphuric acid and titration by $K_2Cr_2O_7$), respectively [21]. The pH of air-dried soil samples was measured with a soil:water ratio of 1:2.5 (in saturated mud) [21]. Ten grams of soil were mixed with 60 ml of 2N HNO_3 for determining the acid-soluble cations (Fe, Mn, Zn, Mg) by flame atomic absorption spectrophotometer [16].

Statistical Analysis: The differences among the sampling sites (A, B, C, D) regarding to the mineral nitrogen values and the net mineralization were tested by analyses of variance. Significant differences between groups were determined using the Tukey HSD (honest significant difference) test. Also, correlation between net mineral nitrogen productivity at the end of 42 days (mg N_{min}/100 g dry soil/42 days) and some soil factors (pH, %C, %N, C/N and element content) was tested. All of the tests were performed at the significance level of α; 0.05, with the Statistica Version 6.0 (Stat Soft Inc. 1984-1995) packet program.

RESULTS

Studied soil characteristics and enriched element contents of sample sites by mining are outlined in Table 1. Soil pH, $CaCO_3$, Fe, Mn, Mg and Zn contents were higher in

TABLE 1 - Soil properties and acid-soluble cautions at two soil layers (0-5 cm and 5-15 cm) at different distances from the Etibank Wolfram Mine Work and waste removal pools (WRP; 0-10 cm).

Soil Depth	Site	Soil characteristics				
		pH	CaCO₃	%C	%N	C/N
0-5 cm (n:4)	A	6.2 ± 0.1	1.35 ± 2.24	0.96 ± 0.44	0.08 ± 0.02	12.1 ± 3.5
	B	6.6 ± 0.2	5.19 ± 0.85	0.93 ± 0.43	0.08 ± 0.03	11.8 ± 2.4
	C	6.3 ± 0.1	2.86 ± 1.73	0.59 ± 0.20	0.08 ± 0.02	7.5 ± 1.1
	D	5.7 ± 0.5	0.09 ± 0.07	2.59 ± 0.79	0.06 ± 0.01	47.2 ± 15.3
5-15 cm (n:4)	A	6.3 ± 0.2	1.64 ± 2.63	1.16 ± 0.76	0.08 ± 0.03	14.1 ± 9.1
	B	6.6 ± 0.1	3.42 ± 2.37	1.26 ± 0.84	0.11 ± 0.07	11.3 ± 1.1
	C	6.4 ± 0.1	3.52 ± 2.29	0.96 ± 0.17	0.14 ± 0.01	7.0 ± 1.1
	D	5.3 ± 0.1	0.10 ± 0.04	2.84 ± 0.38	0.18 ± 0.04	16.2 ± 2.5
0-10 cm (n:3)	WRP	7.9 ± 0.1	6.99 ± 3.03	0.39 ± 0.21	0.06 ± 0.01	7.4 ± 4.8
		Elements (mg/g dry weight)				
		Fe	Mn	Mg	Zn	
0-5 cm (n:4)	A	41.3 ± 31.3	4.1 ± 5.1	33.3 ± 54.6	2.02 ± 3.6	
	B	76.9 ± 2.3	13.0 ± 0.5	197.9 ± 51.4	4.02 ± 0.4	
	C	66.7 ± 13.2	7.5 ± 4.2	189.8 ± 145.9	2.51 ± 1.7	
	D	28.5 ± 9.7	1.7 ± 0.6	1.6 ± 0.3	0.04 ± 0.0	
5-15 cm (n:4)	A	47.4 ± 32.8	4.9 ± 5.2	54.0 ± 58.3	2.26 ± 3.5	
	B	68.6 ± 10.0	11.3 ± 3.2	136.5 ± 65.7	2.67 ± 1.6	
	C	74.1 ± 11.0	6.6 ± 3.3	255.6 ± 161.7	3.67 ± 2.9	
	D	38.2 ± 5.8	1.6 ± 0.7	1.8 ± 0.8	0.02 ± 0.0	
0-10 cm (n:3)	WRP	86.9 ± 2.0	22.4 ± 2.3	408.3 ± 42.0	7.49 ± 0.8	

sites A, B, C and WRP than in site D. But the organic C (%) and C/N ratio were higher in site D. Although the total N content of soils was similar between the sampling sites, the high organic content in soils resulted in the high C/N ratio in site D.

Comparisons of mineral nitrogen (N_{min}) contents at the beginning of the incubation period, after 21 and after 63 days are shown in Figure 1. Initial NO_3^--N values in WRPs were higher than at other sites (1.3 ± 0.1 mg/100 g dry soil). No significant difference among sampling sites (A, B, C, D) was found for values of NO_3^--N at the beginning. However, NH_4^+-N and total N_{min} values at both sample layers were highest in site D and a significant difference for these parameters was found among the sites (Figure 1).

In contrast to NO_3^--N values at the beginning (Figure 1a), a significant difference among sample sites regarding to NH_4^+-N, NO_3^--N and total N_{min} values was found after 21 and 63 days (Figure 1). The concentration of mineral nitrogen at site D was higher than at other sites. It was observed that nitrification was increased, but this tendency was not clear for ammonification in both layers of sample sites. The mineral N value at sites A, B, C close to the mine works was similar to that of the WRPs (Figure 1).

NH_4^+-N accumulation for periods of 21 and 42 days at both soil depths was negative and there was no significant difference among sample sites (Figure 2). However, the difference among sample sites for NO_3^--N accumulation was found to be significant. Net NO_3^--N production was highest at D site. Net NO_3^--N production at other sites was similar to that of the WRPs. Negative NH_4^+-N production affected the total N_{min} production. The difference among sampling sites for total net N_{min} production for 21 days was not significant. However, it was significant for total net N_{min} production for 42 days due to increasing nitrification rates (Figure 2).

TABLE 2 - Simple correlation coefficients between net mineral nitrogen production at 42 days (NH_4^+ and NO_3^-) and soil factors (n=16).

Parameter	r	P^a	$Y = a + bx$
Soil Depth 0-5cm			
NH_4^+ and pH	0.290	0.275402	$Y = -8.330 + 1.165x$
NH_4^+ and %C	0.053	0.846452	$Y = -1.214 + 0.091x$
NH_4^+ and %N	0.196	0.467681	$Y = -2.303 + 16.766x$
NH_4^+ and C/N	-0.019	0.942752	$Y = -1.064 - 0.002x$
NH_4^+ and % $CaCO_3$	0.191	0.478840	$Y = -1.404 + 0.129x$
NH_4^+ and Fe	0.168	0.534061	$Y = -1.662 + 0.106x$
NH_4^+ and Mg	0.090	0.740744	$Y = -1.227 + 0.001x$
NH_4^+ and Mn	0.248	0.353552	$Y = -1.592 + 0.075x$
NH_4^+ and Zn	0.251	0.347533	$Y = -1.469 + 0.173x$
NO_3^- and pH	-0.688	**0.003243**	$Y = 36.332 - 5.174x$
NO_3^- and %C	0.720	**0.001656**	$Y = 1.239 + 2.344x$
NO_3^- and %N	-0.557	**0.024966**	$Y = 10.645 - 89.520x$
NO_3^- and C/N	0.778	**0.000389**	$Y = 1.659 + 0.130x$
NO_3^- and %$CaCO_3$	-0.694	**0.002869**	$Y = 6.300 - 0.882x$
NO_3^- and Fe	-0.664	**0.005019**	$Y = 8.391 - 0.078x$
NO_3^- and Mg	-0.525	**0.036734**	$Y = 5.628 - 0.014x$
NO_3^- and Mn	-0.674	**0.004198**	$Y = 6.723 - 0.384x$
NO_3^- and Zn	-0.718	**0.001748**	$Y = 6.197 - 0.926x$
5-15cm			
NH_4^+ and pH	-0.103	0.705236	$Y = 1.974 - 0.694x$
NH_4^+ and %C	-0.178	0.510672	$Y = -1.283 - 0.650x$
NH_4^+ and %N	-0.329	0.213669	$Y = 0.334 - 20.75x$
NH_4^+ and C/N	0.154	0.569598	$Y = -3.448 + 0.097x$
NH_4^+ and %$CaCO_3$	0.233	0.385076	$Y = -3.018 + 0.340x$
NH_4^+ and Fe	0.084	0.756875	$Y = -3.031 + 0.013x$
NH_4^+ and Mg	0.136	0.615305	$Y = -2.696 + 0.004x$
NH_4^+ and Mn	0.069	0.802189	$Y = -2.620 + 0.052x$
NH_4^+ and Zn	0.189	0.499869	$Y = -2.832 + 0.220x$
NO_3^- and pH	-0.525	**0.036700**	$Y = 27.327 - 3.549x$
NO_3^- and %C	0.677	**0.003955**	$Y = 1.718 + 2.479x$
NO_3^- and %N	0.667	**0.004808**	$Y = 0.231 + 42.042x$
NO_3^- and C/N	0.103	0.703061	$Y = 4.756 + 0.065x$
NO_3^- and $CaCO_3^-$	-0.549	**0.027758**	$Y = 7.265 - 0.799x$
NO_3^- and Fe	-0.291	0.273793	$Y = 8.121 - 0.045x$
NO_3^- and Mg	-0.416	0.109109	$Y = 6.793 - 0.011x$
NO_3^- and Mn	-0.481	0.058997	$Y = 7.910 - 0.367x$
NO_3^- and Zn	-0.571	**0.026351**	$Y = 7.500 - 0.644x$

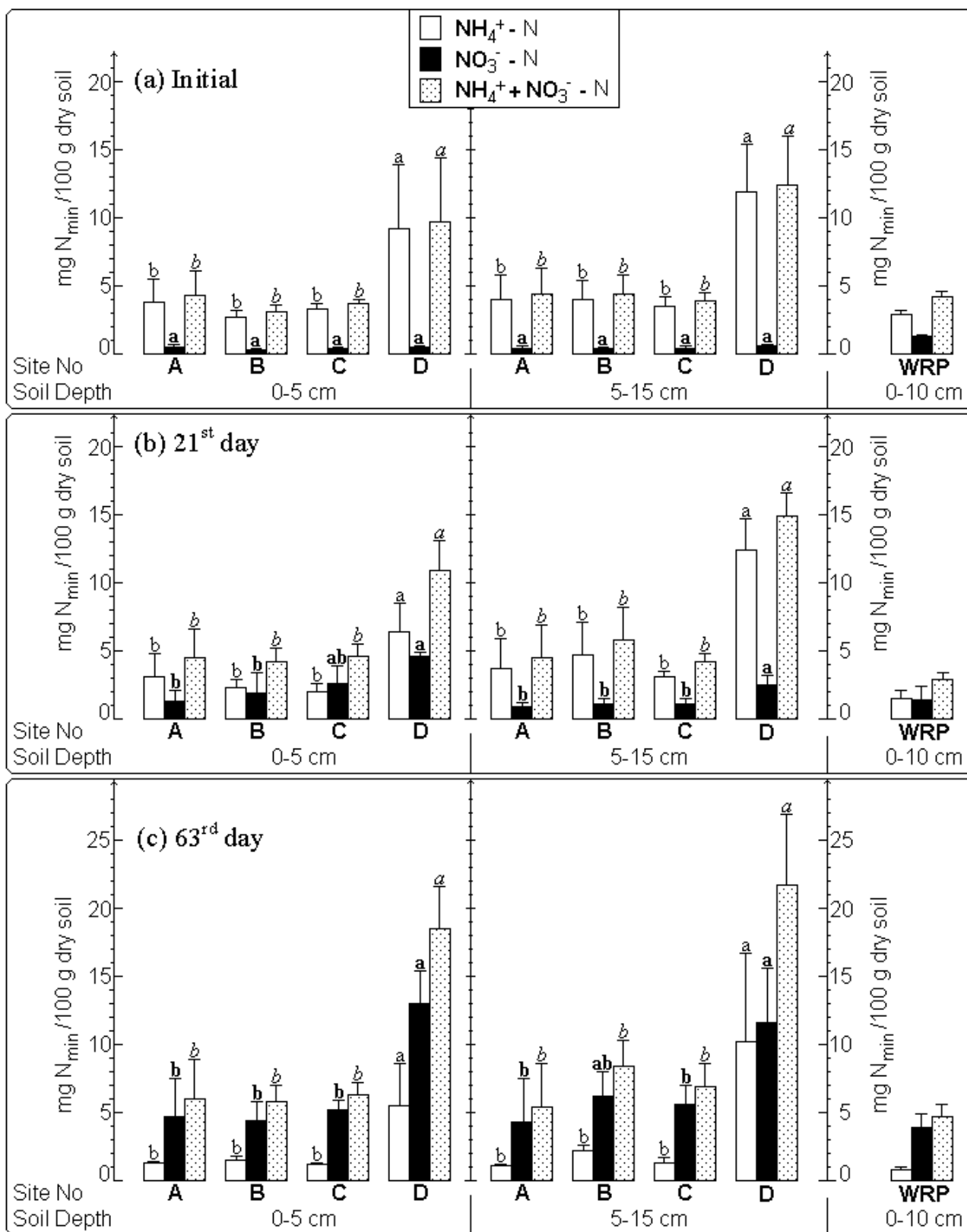[a]Significant *p* values are given in boldface

FIGURE 1 - The comparison of the sample sites (A, B, C, D) and waste removal pools (WRP) regarding to nitrogen mineralization at the initial, after 21 and 63 days of soil incubation. [Difference groups among sample sites (A, B, C, D) are shown by normal letters for ammonium, the boldface letters for nitrate and italic letters for total mineral nitrogen on each bar. Different letters represent the difference groups among the sample sites (*P*<0.05)].
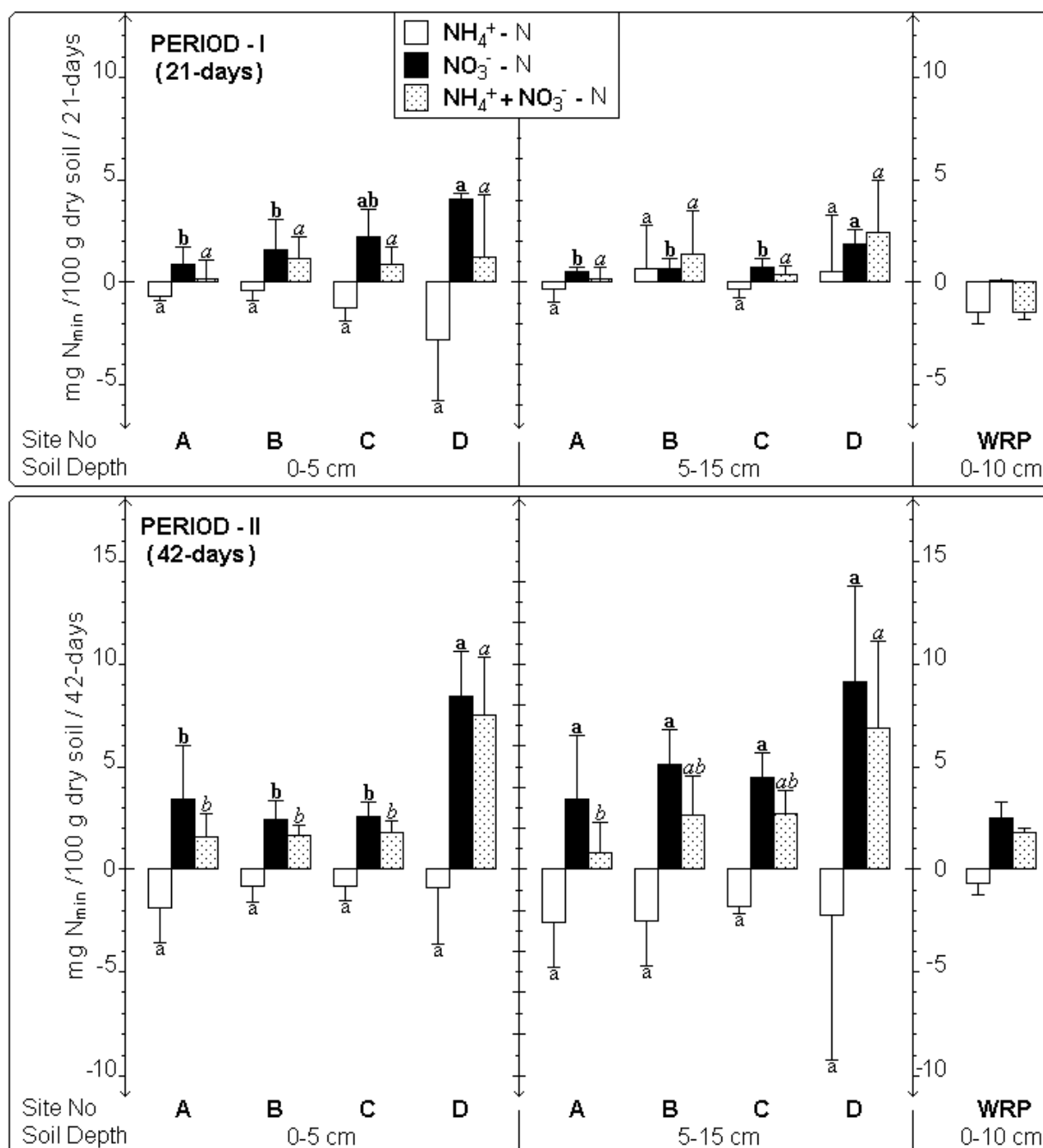
FIGURE 2 - Comparison of the sample sites (A, B, C, D) and waste removal pool (WRP) regarding to the net mineral nitrogen production for an incubation period of 21 days and 42 days (α; 0.05, n=16). [Difference groups among sample sites (A, B, C, D) shown by the normal letters for ammonium, the boldface letters for nitrate and italic letters for total mineral nitrogen on each bar. Different letters represent the difference groups among the sample sites ($P<0.05$)].

The correlations between net N_{min} production after 42 days and some soil factors (pH, C%, N%, C/N, $CaCO_3$ and selected elemental concentrations) were also tested (Table 2). The correlation between NH_4^+-N and the measured soil parameters was not significant in both soil depths. The correlation between NO_3^--N and the soil parameters was different in different soil layers. A significant correlation between NO_3^--N and all soil parameters was found in 0 - 5 cm layer. While the correlation between NO_3^--N and %C and C/N in this layer was positive, it was negative for other parameters (pH, %N, %$CaCO_3$, Fe, Mg, Mn, Zn). In 5-15 cm soil layer, a significant correlation was found for pH, %C, %N, $CaCO_3$ and Zn. This correlation was negative for pH, $CaCO_3$ and Zn, and was positive for %C and %N.

DISCUSSION AND CONCLUSION

Mineral N values (NH_4^+-N and NO_3^--N) and net mineral N production at the end of 21 and 63 days were used to evaluate the results of this study. While NH_4^+-N values decreased, NO_3^- values increased in all sites. We found that NH_4^+-N values highly decreased in the soils of sample sites nearest the mine works (Figure 1.) Decreased NH_4^+-N values can be explained by two ways: Nitrification which converts NH_4^+ to NO_3^- and inhibition of ammonification by heavy metal contamination. However, Komulainen and Mikola [22] reported that concentrations of NH_4^+-N were increased by heavy metals (Cu and Ni). On the other hand, increased NO_3^--N values show that the activities of nitrifying bacteria increased with time. However, interpretation of N-dynamics is complex, and the accumulation of mineral N may reflect either decreased immobilisation or increased mineralization [1, 23]. Nitrogen mineralization is most often referred to as the net sum of two simultaneously occurring, microorganism-mediated processes; the release of N by ammonification and the uptake through N-immobilization. Ammonification releases mineral ammonium (NH_4^+-N) from soil organic matter, while nitrification converts NH_4^+-N to mineral nitrate (NO_3^--N). Immobilization is the processes by which microorganisms take up mineral N from the soil while consuming the carbon (C)-rich organic matter substrate. Microorganisms both consume mineral-N and release it to the soil (i.e., gross immobilization and mineralization, respectively), with mineralized-N either bring re-used by other microorganisms (i.e., net immobilization) or residing in the soil (i.e., net mineralization) if not leached out [1, 24].

NO_3^--N production was used to define the effect of mine work wastes on the nitrogen mineralization rates. Nitrification rate was highest in site farthest from the mine works (site D). Many site conditions such as soil pH, C/N ratio affect the nitrogen mineralization. For instance, there is a general rule that increasing acidity results in the predominance of NH_4^+-N, whereas slight alkalinity and slight acidity (pH 8.0-6.0) leads to formation of NO_3^--N. In addition, one chemical parameter frequently studied in regard to nitrogen mineralization is the C/N ratio of the organic substance. When the element composition was not effected the decomposition processes, the lower C/N ratio, the more nitrogen will be mineralized in the decomposition of a given amount of organic substance [1]. Soil pH values, %N, %C contents and C/N ratios at site D (the farthest from mine work) was different from that of other sites (A, B, C, and WRP) (Table 1). According to Güleryüz et al., [16], the pH variation can be attributed to the content of $CaCO_3$ and it may be due to mine wastes, which indicates that ore was processed under alkaline conditions at the Etibank Mine. Present technology used to obtain low-grade scheelite concentrates under alkaline conditions involves hydrometallurgical processes [25]. While the pH values and C/N ratio in the soils of A, B, C sites and WRP were more likely for mineralization than site D, however net N_{min} production was found to be low at these sites (Figure 2). We therefore conclude that nitrification was inhibited by enriched element contents at these sites. Higher NO_3^--N productivity in both soil layers of site D supported this conclusion.

In addition, the negative correlation between NO_3^--N production at 42 days and some element content suggests the negative effect of the element contents on nitrification. If we assess the correlation between NO_3^--N production and element contents in the 5-15 cm depth of soil, significant correlation can be found only with Zn content. It can be attributed to the different distribution characteristics of these elements in different soil layers. Although it was shown that heavy metals have an adverse effect on the nitrogen mineralization, it can not be generalized to both NH_4^+-N and NO_3^--N production. Our results suggest that NH_4^+-N production is not affected as NO_3^--N by enriched element content. According to some researchers [8, 10, 26] this result indicates that nitrification is more sensitive than ammonification.

N-mineralization is influenced by past land use [27], residue management practices, and environmental factors that alter microbial activity and N use [28, 29]. In addition, Komulainen and Mikola [22] reported that the decrease in soil activity due to heavy metals might be the outcome of the impacts on microbes and/or soil fauna.

This study indicates that changes in soil element composition due to the Wolfram mine wastes soil contamination results in different N-mineralization rates. Many studies support these findings, reporting extreme metal contamination in the vicinity of smelters causes clearly visible effects such as accumulation of deep layers of organic matter on the soil surface through inhibition of the soil microorganisms and soil fauna [30-32].

ACKNOWLEDGEMENTS

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