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The Investigation of Enzymatic Activity in the Soils under the Impact of Metallurgical Industrial Activity in Lori Marz, Armenia

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Abstract**—**Beta-glucosidase, chitinase, leucine-aminopeptidase, acid phosphomonoesterase and acetate-esterase enzyme activities in the soils under the impact of metallurgical industrial activity in Lori marz (district) were investigated. The results of the study showed that the activities of the investigated enzymes in the soils decreased with increasing distance from the Shamlugh copper mine, the Chochkan tailings storage facility and the ore transportation road. Statistical analysis revealed that the activities of the enzymes were positively correlated (significant) to each other according to the observation sites which indicated that enzyme activities were affected by the same anthropogenic factor. The investigations showed that the soils were polluted with heavy metals (Cu, Pb, As, Co, Ni, Zn) due to copper mining activity in this territory. The results of Pearson correlation analysis revealed a significant negative correlation between heavy metal pollution degree (Nemerow integrated pollution index) and soil enzyme activity. All of this indicated that copper mining activity in this territory causing the heavy metal pollution of the soils resulted in the inhabitation of the activities of the enzymes which are considered as biological catalysts to decompose organic materials and facilitate the cycling of nutrients.

Keywords**—**Armenia, metallurgical industrial activity, heavy metal pollutionl, soil enzyme activity.

I. Introduction

SOIL quality and its degradation depend on a large number of physical, chemical, biological, microbiological and biochemical properties, the last two are the most sensitive since they respond rapidly to changes. Soil enzymatic activity plays a key role in soil nutrient cycling, its activity is essential in both the mineralization and transformation of organic matters and plant nutrients in soil ecosystem. The activities of enzymes in soil are very sensitive to both natural and anthropogenic disturbances and show a quick response to the induced changes [1].

In soil microbial communities, maintaining critical functions may ultimately be more important than maintaining taxonomic diversity. One essential microbial function in soils is the processing and recovery of key nutrients from detrital inputs and accumulated soil organic matter. This often requires the activity of extracellular enzymes to process complex organic compounds into assimilable subunits (sugars, amino acids, NH_4^+, PO_4^{-3}) [2].

Heavy metals are dangerous group of soil pollutants which are produced from natural processes and anthropogenic sources such as industrial, agricultural, military activities, etc. Changes in enzyme activities resulting from pollution with heavy metals have been well documented by many researchers [3]-[6].

Soils around Shamlugh copper mine in Lori marz, Armenia, due to metallurgical industrial activity, are endangered by heavy metal pollution which may affect soil enzyme activity negatively causing a disturbance in soil nutrient cycling.

The aim of the present study was to investigate enzyme activities in the soils around the copper mining area near Shamlugh town and to assess heavy metal pollution impact on soil enzyme activity.

II. Materials and Methods

The soils around the Shamlugh copper mine and the Chochkan tailings storage facility in Lori marz, Armenia were investigated (Fig. 1).

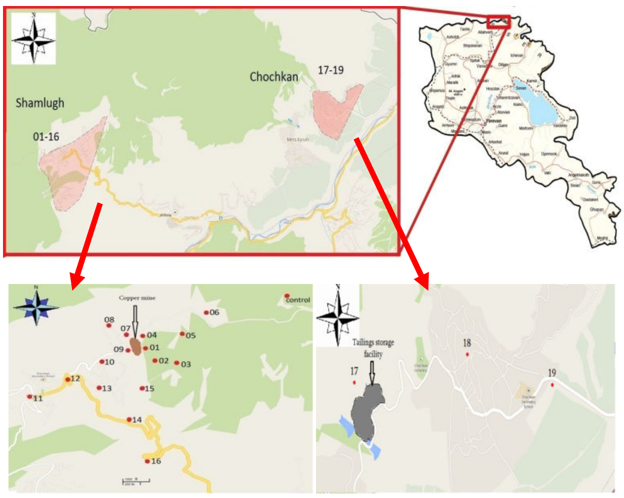

Fig. 1 The locations of investigated territories and sampling sites in Armenia

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Soil sampling, observations were done in September, 2014. Soil samples were obtained from a depth of 0-20 cm. The coordinates of sampling sites were registered by a GPS.

The 76 soil samples were taken from 19 observation sites around the Shamlugh copper mine and the Chochkan tailings storage facility, and 4 soil samples were taken from a control site which was 4 km away from the copper mining area (Fig. 1). The 4 soil samples taken from each site were mixed into each other prior to treatment and analysis.

Enzyme activities in the soils were determined by the standard methods. Beta-glucosidase, leucine-aminopeptidase and acid phosphomonoestearse activities were assayed as described in [7], chitinase activity was determined according to the procedure described in [8], and acetate-esterase activity was measured by a method described in [9].

Fluorescence was measured with “Hitachi F-7000” fluorescence spectrophotometer equipped with a microplate reader. The results of enzyme activities were expressed as nmol 4-MUF g^{-1} soil dry weight h^{-1}.

Heavy metal concentrations in the soils were measured by the standard methods. After the homogenization and removal of unwanted content (stones, plant material, etc.), the soil samples were oven dried at 70°C for 48 h, grounded into powder, sieved with 1 mm mesh and stored in an air tight container for the analysis of heavy metals. The soil samples were digested according to a procedure described in [10].

The concentrations of heavy metals (Cu, Pb, As, Co, Ni, Zn) in the soils were determined by “PG990” atomic absorption spectrophotometer (PG Instruments LTD, UK).

Pollution index of individual heavy metals was calculated by [11], [12]:

$$PI = C_i/S_i \quad (1),$$

where C_i is the determined concentration of each metal in soil, S_i is the background (control) concentration of each metal in soil.

The PI of each metal is classified into five pollution categories which are presented in Table I.

TABLE I
POLLUTION CATEGORIES ACCORDING TO THE PI VALUES

Value	Pollution categories	References
PI≤1	non-pollution	[13]
1<PI <2	low level of pollution	[12]
2≤PI<3	moderate level of pollution	[11]
3≤PI<5	strong level of pollution	[11]
PI≥5	very strong level of pollution	[11]

Nemerow integrated pollution index (NIPI) was calculated as [11], [14]:

$$NIPI = \sqrt{\frac{PI_{avg}^2 + PI_{max}^2}{2}} \quad (2)$$

where PI_{avg} is the average value of the single pollution indices of all heavy metals, PI_{max} is the maximum value of the single pollution indices of all heavy metals.

The average value of the single pollution indices was calculated by [14]:

$$PI_{avg} = \frac{1}{m}\sum_{i=1}^{n} PI_i \quad (3)$$

where PI_i is the single pollution index value of heavy metal i, m is the number of heavy metal species.

The classification of pollution categories according to the NIPI values is presented in Table II.

TABLE II
POLLUTION CATEGORIES ACCORDING TO THE NIPI VALUES

Value	Pollution categories	References
NIPI≤0.7	non-pollution	[11]
0.7<NIPI ≤1	warning line of pollution	[11]
1<NIPI≤2	low level of pollution	[11]
2<NIPI≤3	moderate level of pollution	[11]
NIPI>3	high level of pollution	[11]

Correlation analyses were performed by the Pearson correlation method using Microsoft Excel 2007 software.

III. RESULTS AND DISCUSSION

The results of the study of beta-glucosidase (beta_G), chitinase (chit), leucine-aminopeptidase (leu), acid phosphomonoesterase (acP) and acetate-esterase (ester) enzyme activities in the soils around the copper mining area near Shamlugh town are presented in Fig. 2. Figure shows that beta-glucosidase, chitinase, leucine-aminopeptidase, acid phosphomonoesterase and acetate-esterase activities in the investigated soils were mostly lower than those in the control site. The highest soil enzyme activity was registered in the №6 observation site farthest from the mining area by the direction of the №4-6 sampling sites. The lowest soil enzyme activity was observed in the №16 observation site which was very close to the ore transportation road. Soil enzyme activity rose with increasing distance from the Shamlugh copper mine, the Chochkan tailings storage facility and the ore transportation road (Fig. 2).

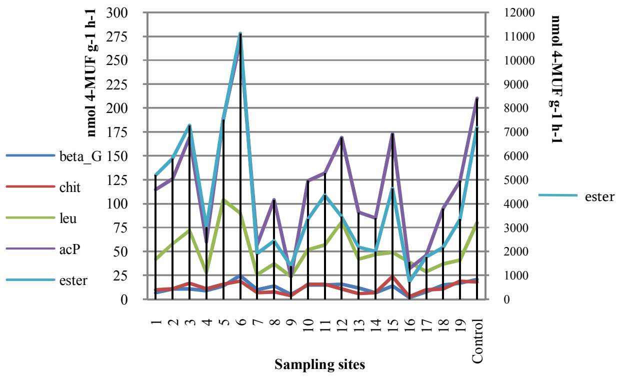


Fig. 2 Beta-glucosidase (beta_G), chitinase (chit), leucine-aminopeptidase (leu), acid phosphomonoestearse (acP) and acetate-esterase (ester) enzyme activities in the soils around the Shamlugh copper mine and the Chochkan tailings storage facility

According to the observation sites, a significant positive correlation between the activities of investigated enzymes in the soils was observed which indicated that the variation of beta-glucosidase (beta_G), chitinase (chit), leucine-aminopeptidase (leu), acid phosphomonoesterase (acP) and acetate-esterase (ester) enzyme activities in different observation sites was mainly conditioned by the same anthropogenic factor (Table III). In this territory, the main anthropogenic factor was metallurgical industrial activity which was the potential source of the heavy metal pollution of the soils.

TABLE III
THE RESULTS OF THE PEARSON CORRELATION ANALYSIS BETWEEN THE ACTIVITIES OF THE INVESTIGATED ENZYMES

	beta_G	chit	leu	acP	ester
beta_G	1				
chit	0.696**	1			
leu	0.676*	0.614*	1		
acP	0.816**	0.766**	0.878**	1	
ester	0.630*	0.658*	0.798**	0.894**	1

*Correlation is significant at the 0.01 level.
**Correlation is significant at the 0.001 level.

The degree of the individual and integrated heavy metal pollution of the soils around the mining area near Shamlugh town was assessed by the pollution index (PI) and the Nemerow integrated pollution index (NIPI) (Table IV).

Investigations showed that the soils were polluted with heavy metals (Cu, Pb, As, Co, Ni, Zn). The soils were highly polluted particularly with copper because of copper mining activity in this territory. High heavy metal pollution degrees were registered in the № 7, 9, 10, 13, 14 and 16 observation sites which were very close to the copper mine and the ore transportation road (Fig. 1, Table IV). The PI and NIPI values in the investigated soils decreased with increasing distance from the copper mine, the tailings storage facility and the ore transportation road (Figs. 1, 3 and 4).

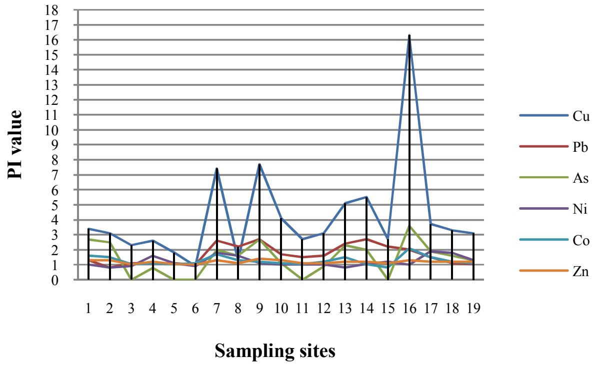


Fig. 3 The PI values in the soils around the Shamlugh copper mine and the Chochkan tailings storage facility

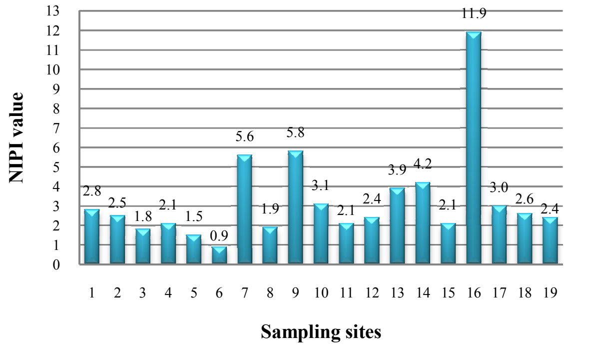
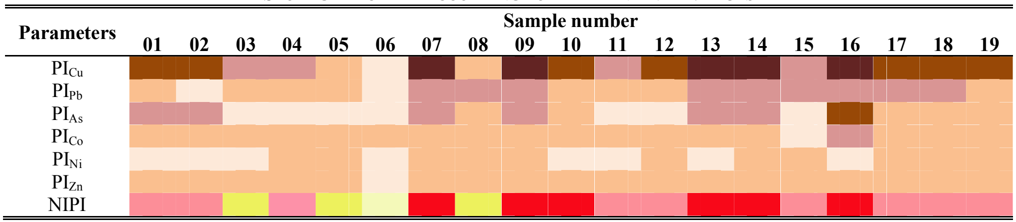


Fig. 4 The NIPI values in the soils around the Shamlugh copper mine and the Chochkan tailings storage facility

TABLE IV
INDIVIDUAL AND INTEGRATED HEAVY METAL POLLUTION DEGREE IN THE SOILS AROUND THE SHAMLUGH COPPER MINE AND THE CHOCHKAN TAILINGS STORAGE FACILITY ACCORDING TO THE PI AND NIPI VALUES



PI - = no-pollution; = low level of pollution; = moderate level of pollution; = strong level of pollution = very strong level of pollution

NIPI - = warning line of pollution; = low level of pollution; = moderate level of pollution; = high level of pollution

Taking into consideration the abovementioned discussion, it's possible to conclude that soil enzyme activity in the investigated territory was affected by heavy metal pollution. To verify this fact, correlation analysis was implemented, the results of which are presented in Table V. Table shows a significant negative correlation observed between heavy metal pollution degree and the activities of the investgated enzymes (Table V). All of this indicated that a decrease in beta-glucosidase, chitinase, leucine-aminopeptidase, acid phosphomonoestearse and acetate-esterase enzyme activities was conditioned by the heavy metal pollution of the soils.

TABLE V
THE RESULTS OF THE PEARSON CORRELATION ANALYSIS BETWEEN NIPI VALUES AND THE ACTIVITIES OF THE INVESTIGATED ENZYMES

	beta_G	chit	leu	acP	ester
NIPI	-0.694***	-0.661**	-0.536*	-0.648**	-0.592**

*Correlation is significant at the 0.05 level.
**Correlation is significant at the 0.01 level.
***Correlation is significant at the 0.001 level.

IV. Conclusions

The results of the study revealed that the activities of beta-glucosidase, chitinase, leucine-aminopeptidase, acid phosphomonoesterase and acetate-esterase enzymes in the soils around the Shamlugh copper mine and the Chochkan tailings storage facility in Lori marz, Armenia were affected by mining activity. Copper mining activity in this territory resulted in the heavy metal pollution of the soils which caused a decrease in the activities of the investigated enzymes that are considered as biological catalysts to decompose organic materials and facilitate the cycling of nutrients.

Thus, the study has confirmed the fact that the heavy metal pollution of soils affects soil quality and fertility by the inhabitation of soil enzyme activity.

Acknowledgment

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