

## SOIL POLLUTION WITH Cu, Zn AND Cd BY NON-FERROUS METAL MINING AFFECTS SOIL-MICROBIAL ACTIVITY OF KASTANOZEMS IN THE MASHAVERA VALLEY

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The topsoils of the Mashavera valley in SE Georgia are heavily contaminated by Cu, Zn, and Cd due to irrigation with river-water loaded with suspended fines containing sulfidic trace metals (Cu, Zn, and Cd). To investigate the ecological impact of this contamination, 128 topsoils from house-gardens and arable land (irrigated either with clean or contaminated water or without irrigation) were sampled. Total content and mobile fractions of Cu, Zn, and Cd concentrations were measured. Soil-microbial parameters (enzyme and respiration activity) were measured in 37 selected samples. In soil irrigated with contaminated water, total contents of Cu, Zn, and Cd were elevated by factors of 2.1, 1.3 and 3.3 as compared to the control. Mobile fractions were elevated by factors of 18.5 for Zn and 16.4 for Cd. Phosphatase and dehydrogenase activity were significantly lower in topsoils irrigated with contaminated water (52% and 30%, respectively, as compared to control), while respiration activity was not affected. Soil fertility and food quality are strongly endangered in the Mashavera valley.

### INTRODUCTION

Trace metal contamination of soils in the vicinity of metal mines and industrial sites is very common in industrial countries. The study area is situated in the region of Bolnisi, about 80 km south of Tbilisi in SE Georgia at the edge of the Lower Caucasus (Fig. 1). It is characterized by a semi-arid climate and very fertile, neutral to weakly

alkaline Chernozems, Kastanozems and Phaeozems [1,2]. The intensive agricultural land use is limited by low annual precipitation (504 mm) and hot, arid summer months. Accordingly, vegetable gardens, vineyards and orchards as well as arable land are irrigated intensively with water supplied by a canal system fed by the Mashavera River [2].

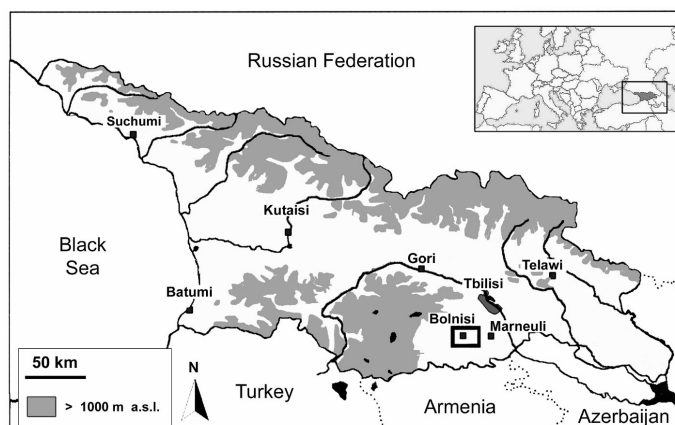


Fig. 1. Location of the study area in the Mashavera valley in SE Georgia [2]

The relatively high Cu, Zn, and Cd contamination of the topsoils due to non-ferrous metal mining in the mountainous area of the middle reaches of the Mashavera valley is already known. The soils show a high sorption capacity for trace metals due to neutral soil pH, high content of

clay and organic carbon ( $C_{org}$ ) resulting in a high effective cation exchange capacity ( $CEC_{eff}$ ) [2]. Therefore a high total content of metals does not necessarily mean a strong negative influence on soil quality. The aims of this study were to investigate the impact on crop production by

measuring the mobile and potential plant available fractions of Cu, Zn, and Cd as well as to test the impact on the habitat function of the soils. Microorganisms are far more sensitive to trace metal stress than soil animals or plants [3]. Negative impacts of metal contaminations on soil-microbial parameters are already well-known (for an extensive review see *Giller et al.* [3] and *Giller et al.* [4] and thus can be useful for assessing the ecological impact of Cu, Zn, and Cd contamination in the Mashavera valley. According to *Filip* [5], soil quality has an impact on productivity and food safety, human and animal health and environmental quality and must be assessed by specific indicators. He recommends, among others, soil respiration and dehydrogenase activity as sensitive parameters to assess soil quality. Due to the fact that only a biologically active soil warrants sufficient food production, *Ellis et al.* [6] states that measurements of microbial activity are more appropriate indices for "soil health" than analyses of the genetic diversity of the microorganisms. *Mathe-Gaspar et al.* [7] measured a decrease of plant and microbial biomass and mycorrhizal infections in soils contaminated with As, Cd, Cu, Pb, Zn and Hg by a former mine in Gyöngyöszori, Hungary.

#### OBJECTIVES AND METHODS

In the mountainous area of the middle reaches of the Mashavera valley, metal sulphide ores in hydrothermally altered volcanic bedrocks of the Madneuli barite-polymetallic deposit have been mined since 1974 [8]. Due to slag heap erosion and waste water from a flotation plant in the Kazreti village, the river is loaded with suspended fines containing sulfidic metals. *Felix-Henningsen et al.* [2] revealed that after decades of irrigation with this water the topsoils of the Mashavera valley became highly contaminated with Cu, Zn, and Cd to such an extent that permitted thresholds for soils used for food production of Germany and other European countries are greatly exceeded. The contamination is caused by the suspended particles of the river water used for irrigation. Strong correlations between irrigation frequency and trace metal contamination were found in the topsoils of the region. Migration of metals into deeper soil layers was found to be negligible. Due to the wavy micro-relief higher areas are flooded to a smaller degree than lower ones, creating tessellated patterns of metal distribution, which leads to a high spatial variability of contamination within an area of arable land. A high proportion of the metals belong to the supply fraction (unspecifically and specifically adsorbed metals). Due to the narrow correlation of this fraction with the mobile and potentially plant available fraction, a high long-term risk potential within the food chain exists. A high risk of transfer of Cu, Zn, and Cd into the food chain can be assumed and was confirmed by a field experiment using *S.oleracea* [9].

The sampled areas were situated in (house gardens) or in the vicinity (market gardening and crop land) of the town of Bolnisi and 15 villages in the Bolnisi district. Soil types according to *WRB* [10] were Hortic Anthrosols (former Kastanozems transformed by human activities; house gardens and most market gardening), Kastanozems (most arable land) and Kolluvisols (some arable land and some market gardening).

Irrigation frequency controls the degree of trace metal contamination and depends on water-management practices of the land owners. Typical frequencies are 2-3 times irrigation per growing season for cereals, whereas vegetables are irrigated up to 2-3 times per week in the growing session. In practice irrigation frequency varies greatly and is not consistent even in the same field. In recent years, considerable arable land was not irrigated due to the ongoing degeneration of the channel distribution system following the dissolution of the Soviet Union. In addition, soil cultivation was not equal in all fields. While most soils were cultivated only to a depth of 20 cm, some Hortic Anthrosols were cultivated up to a depth of 35 cm. Most irrigated soils of the study area are supplied with Mashavera water (labelled as CONT). Nevertheless, some soils are supplied by water from smaller and non-contaminated streamlets, well water or are not irrigated and can thus be used as a control (labelled as: CLEAN). According to *Melikadze et al.* [11], the Poladauri streamlet is loaded with Cu, Zn, and Cd and thus soils irrigated with this water would show enhanced metal contents. This was confirmed by our measurements and these samples also included in CONT.

Sampling was conducted in two field campaigns (October 2009 and March 2010). To investigate Cu, Zn, and Cd in the rhizosphere, composite samples (based on five subsamples) were taken in 128 topsoils (0-20 cm) using an aluminium auger to avoid contamination. Sampling and storing until preparation followed the guidelines of *BBodSchV* [12].

Soil samples for chemical and physical analyses were dried at 40°C, sieved for 2 mm, partially finely ground and stored at room temperature until analysis. The soil samples for soil-microbial measurements were also sieved for 2 mm and stored field-fresh at 4°C until analyses. Soil pH was measured in suspension of soil and 0.01 M CaCl<sub>2</sub> with a ratio of 1:2.5 [13]. Contents of carbonates were determined by the gas-volumetric method using a calcimeter [14]. Total amount of carbon (C<sub>t</sub>) was determined by a C-N-S element analyzer (Elementar). Inorganic C was calculated from the carbonate content by using the factor 0.1199, while the amounts of C<sub>org</sub> result from the difference between C<sub>t</sub> and inorganic carbon. Particle size distribution was determined by a combined sieving (sand and coarse silt fractions) and pipette method (medium silt and clay fractions) after decomposition of carbonates (HCl) and organic matter (H<sub>2</sub>O<sub>2</sub>) and dispersion in Na-Pyrophosphate [15]. The (pseudo)-total content of Cu, Zn, and Cd was extracted with aqua regia (3 parts 32% HCl and 1 part 65% HNO<sub>3</sub>) from finely ground samples [16]. As the total content is not sufficient to determine ecotoxicologically relevant trace metals, the mobile and exchangeable fraction (potentially plant available and easily leachable) was also extracted with 1 M NH<sub>4</sub>NO<sub>3</sub> [17] according to the *BBodSchV* [12]. For quality assurance a certified reference material ("soil 1", round-robin test 2006) supplied by the "Centre for Agricultural Technology Augustenberg" (Karlsruhe) was used for measurement of metals in aqua regia extract and Cu and Zn in NH<sub>4</sub>NO<sub>3</sub> extract. For measurement of Cd in NH<sub>4</sub>NO<sub>3</sub> extract, an internal reference material was used. All extracts were stored in polyethylene bottles until analysis.

Copper, Zn, and Cd in aqua regia,  $\text{NH}_4\text{NO}_3$  and  $\text{HNO}_3$  extracts were measured with an inductively coupled plasma optical emission spectrometer (ICP-OES, Varian 720ES). All analytical determinations were replicated twice.

German thresholds for soils according to *BBodSchV* [12] were used in this study to evaluate the measurements due to the lack of appropriate standards in Georgia. The *BBodSchV* [12] has different types of thresholds. Precaution values depend on soil texture (highest for clayey soil) and aqua regia-extractable metal content. If they are not exceeded, no negative impact can be assumed. On the other hand, trigger and action values are aimed at the mobile fractions. If they are exceeded further steps are required (more detailed investigations, change of land use or remediation).

Alkaline phosphomonoesterase activity (APA) was determined according to *Schinner et al.* [18] because alkaline phosphatase is only produced by microorganisms and soil faunae but not by plants. Five gm of field-fresh soil were incubated for 3 h at  $37^\circ\text{C}$  with 0.1 M phenylphosphate-dinatrium (three replicates and one blank value). The cleaved phenol was measured photometrically at 614 nm using a spectral photometer (T80 UV/VIS Spectrometer, PG Instruments Ltd.).

Dehydrogenase activity (DHA) was measured according to *DIN ISO 23753* [19]. Therefore 5 gm of field-fresh soil were incubated for 16 h at  $25^\circ\text{C}$  with 1 % w/v 2,3,5-tripheyltetrazolium chloride solution (three replicates and one blank value). The released 1,3,5-triphenylformazan

was measured photometrically at 485 nm using a spectral photometer (T80 UV/VIS Spectrometer, PG Instruments Ltd.).

Basal respiration (BR) and substrate induced respiration (SR) were determined according to *DIN ISO 16072* [20] and *DIN ISO 17155* [21], respectively, by measuring  $\text{CO}_2$  flux using an infrared gas analyzer (LI-8100 automated soil  $\text{CO}_2$  flux system, LI-COR Inc.). Ten gm (three replicates per sample) were pre-incubated for 24 h at  $27^\circ\text{C}$  and 60% of the maximum water-holding capacity,  $\text{qCO}_2$  ( $\mu\text{g CO}_2 \text{ g}^{-1} \text{ DM h}^{-1}$ ) was measured (for a time period of 100 sec) (BR). Subsequently, 0.1 g glucose was added and gently mixed. The soil was incubated again for 3 h and  $\text{qCO}_2$  was measured again (SR).

For statistical analyses SPSS 18.0 for Windows was used. Data are normal distributed according to the Kolmogorov-Smirnov test. A two-sided Student's t-test was used to analyze differences between CONT and CLEAN. Pearson correlation coefficients were used to test the impact of the different metals on soil-microbial activity.

## RESULTS AND ANALYSIS

All topsoils were characterized by a neutral to weakly alkaline pH (due to the  $\text{CaCO}_3$  content), and high contents of  $\text{C}_{\text{org}}$  and clay. Soil pH and  $\text{C}_{\text{org}}$  were significantly lower ( $p \leq 0.05$ ) in CONT. Nevertheless differences, especially for pH and  $\text{C}_{\text{org}}$ , were low. Furthermore, soil texture differed ( $p \leq 0.05$ ) between the treatments but can be considered to be uninfluenced by irrigation (Table 1).

**Table 1.** Characterization of the topsoils (0-20 cm) in the Mashavera valley (arithmetic mean and standard deviation)

Type of irrigation	n	pH ( $\text{CaCl}_2$ )	$\text{C}_{\text{org}}$ %	$\text{CaCO}_3$ %	Sand %	Silt %	Clay %
Clean	27	7.50 $\pm 0.14$	2.89 $\pm 0.86$	6.66 $\pm 5.14$	22.18 $\pm 14.42$	36.06 $\pm 5.16$	41.75 $\pm 13.72$
Contaminated	101	7.33** $\pm 0.34$	2.07** $\pm 1.20$	3.35 $\pm 4.52$	14.70* $\pm 9.08$	36.58 $\pm 4.35$	48.72* $\pm 9.07$

\* significant at  $p \leq 0.05$ , \*\* significant at  $p \leq 0.001$

Total contents of Cu, Zn, and Cd are significantly elevated by factors of 2.1, 1.3, and 3.3, respectively, in CONT as compared to CLEAN. Mobile fractions are also elevated significantly by factors of 18.5 for Zn and 16.4

for Cd but not for Cu (Table 2). Both variants show a high range of metal content and also CLEAN partly exceeds precaution values according to *BBodSchV* [12].

**Table 2.** Total (aqua regia-extractable;  $\text{Cu}_{\text{AR}}$ ,  $\text{Zn}_{\text{AR}}$ ,  $\text{Cd}_{\text{AR}}$ ) and mobile (1 M  $\text{NH}_4\text{NO}_3$ -extractable;  $\text{Cu}_{\text{AN}}$ ,  $\text{Zn}_{\text{AN}}$ ,  $\text{Cd}_{\text{AN}}$ ) metal content of topsoils (0-20 cm) in the Mashavera valley (arithmetic mean and standard deviation)

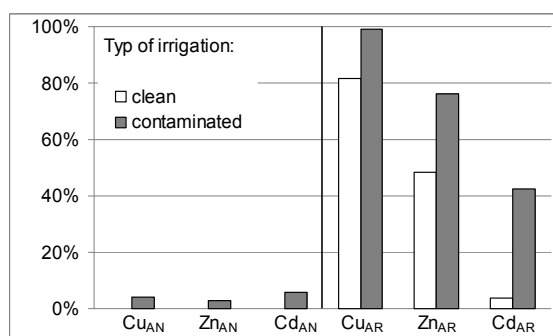
Type of Irrigation	n	$\text{Cu}_{\text{AR}}$ ( $\text{mg kg}^{-1}$ )	$\text{Zn}_{\text{AR}}$ ( $\text{mg kg}^{-1}$ )	$\text{Cd}_{\text{AR}}$ ( $\text{mg kg}^{-1}$ )	$\text{Cu}_{\text{AN}}$ ( $\text{mg kg}^{-1}$ )	$\text{Zn}_{\text{AN}}$ ( $\text{mg kg}^{-1}$ )	$\text{Cd}_{\text{AN}}$ ( $\mu\text{g kg}^{-1}$ )
Clean	27	147.82 $\pm 109.77$	260.19 $\pm 153.88$	0.52 $\pm 0.46$	0.23 $\pm 0.18$	0.02 $\pm 0.04$	0.79 $\pm 0.51$
Contaminated	101	314.99* $\pm 318.32$	343.68* $\pm 243.48$	1.73** $\pm 1.59$	0.36 $\pm 0.35$	0.37* $\pm 0.91$	12.98* $\pm 25.49$
Threshold due to <i>BBodSchV</i> [12]		60 <sup>a</sup>	200 <sup>a</sup>	1.5 <sup>a</sup>	1 <sup>b</sup>	2 <sup>b</sup>	40/100 <sup>c</sup>

\* significant at  $p \leq 0.05$ , \*\* significant at  $p \leq 0.001$

<sup>a</sup>precaution value, <sup>b</sup>trigger value, <sup>c</sup>action value (Cd-accumulating vegetables and bread wheat/ normal crops)

Nevertheless, action and trigger values for arable soils due to *BBodSchV* [12] are only exceeded in CONT (even only in a few cases) and precaution values (for clayey

soils) are exceeded to a higher degree in CONT than in CLEAN, especially in the case of Cd.



**Fig. 2.** Topsoils in the Mashavera valley ( $n = 128$ ) exceeding German thresholds according to BBodSchV [12]

### 3.2 Soil microbial activity

In topsoils chosen for microbial investigations metal content, soil pH and Corg did not differ between WITHOUT and CLEAN, while CONT showed significantly higher

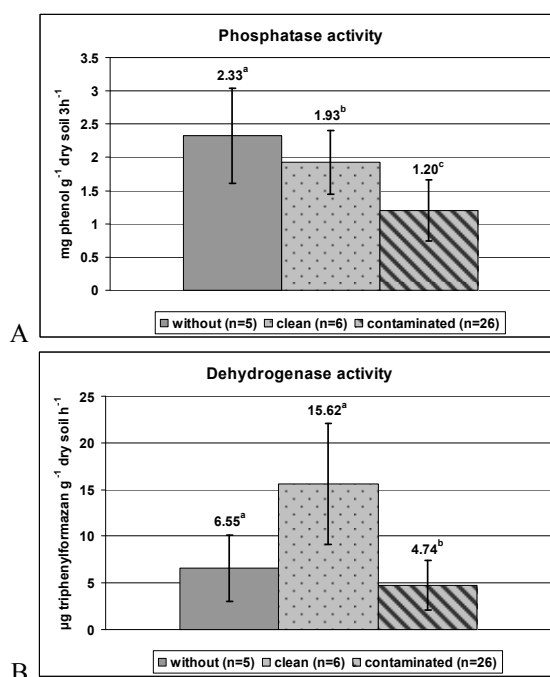
metal and lower CaCO<sub>3</sub> contents as well as lower soil pH and Corg. Soil texture differed heterogeneous between all three treatments (Table 4).

**Table 3.** Total content (aqua regia-extractable; Cu<sub>AR</sub>, Zn<sub>AR</sub>, Cd<sub>AR</sub>) and properties of topsoils (0-20 cm) sampled for soil microbial measurements (arithmetic mean and standard deviation); different letters (a,b,c,d) denote significant differences ( $^{\alpha} p \leq 0.05$ ,  $^{xx} p \leq 0.01$ )

Type of Irrigation	n	Cu <sub>AR</sub> mg kg <sup>-1</sup>	Zn <sub>AR</sub> mg kg <sup>-1</sup>	Cd <sub>AR</sub> mg kg <sup>-1</sup>	pH (CaCl <sub>2</sub> )	C <sub>org</sub> %	CaCO <sub>3</sub> %	Sand %	Silt %	Clay %
Without	5	113.96±1 15.97 <sup>a,d</sup>	196.41± 152.44 <sup>a</sup>	0.45± 0.10 <sup>a</sup>	7.63± 0.06 <sup>a</sup>	3.08± 0.48 <sup>a</sup>	1.04± 0.56 <sup>a</sup>	13.89± 7.90 <sup>a</sup>	35.14± 4.33 <sup>a,d</sup>	50.97± 6.24 <sup>a</sup>
Clean	6	82.24± 32.82 <sup>a,b</sup>	205.96± 102.25 <sup>a</sup>	0.56± 0.28 <sup>a</sup>	7.48± 0.23 <sup>a,bb</sup>	3.20± 0.82 <sup>a</sup>	1.15± 0.66 <sup>a</sup>	30.76± 9.95 <sup>b</sup>	34.76± 2.83 <sup>a</sup>	34.48± 12.17 <sup>b</sup>
Contaminated	26	248.09±1 78.06 <sup>d,c</sup>	321.18± 141.22 <sup>a</sup>	1.37± 0.98 <sup>bb,c</sup>	7.36± 0.35 <sup>bb</sup>	2.00± 0.54 <sup>bb</sup>	0.23± 0.33 <sup>bb</sup>	16.09± 4.54 <sup>a,cc</sup>	39.89± 3.19 <sup>d,bb</sup>	44.02± 5.57 <sup>a,b</sup>

Measurements of enzyme activity showed significant differences in type of irrigation. APA (Fig. 3A) differed for all three types; highest rates for CLEAN and lowest for CONT (52% of CLEAN). Dehydrogenase activity (Fig. 3B) was equal for CLEAN and WITHOUT and significantly lower for CONT (30% of CLEAN).

Cu (aqua regia-extractable) had the highest significant impact ( $* p \leq 0.05$ ,  $** p \leq 0.01$ ) on enzyme activity (= -0.76\*\* for APA and -0.58\* for DHA), followed by Cd (= -0.69\*\* and -0.50\*, respectively), while Zn correlated significantly with APA (= -0.54\*\*) only (Fig. 3).



**Fig. 3.** Enzyme activity in topsoils (0-20 cm) of the Mashavera valley (arithmetic mean and standard deviation) (A) alkaline phosphatase activity (B) dehydrogenase activity; different letters (a,b,c) denote significant differences ( $p \leq 0.05$ )

Respiration activity did not differ significantly for the different types of irrigation, neither for BR nor for SR.

However, SIR correlated ( $= -0.35^*$ ) with total Cd content. (Table 5).

**Table 4.** Respiration activity in topsoils (0-20 cm) of the Mashavera valley (arithmetic mean and standard deviation)

Type of Irrigation	n	Basal respiration ( $\mu\text{g g}^{-1} \text{h}^{-1}$ )	Substrate induced respiration ( $\mu\text{g g}^{-1} \text{h}^{-1}$ )
Without	5	57.82 $\pm$ 27.81	231.08 $\pm$ 96.68
Clean	6	82.31 $\pm$ 69.44	192.32 $\pm$ 69.77
Contaminated	26	62.70 $\pm$ 39.83	202.23 $\pm$ 137.86

It was the aim of the study to investigate the mobile and potential plant available fractions of Cu, Zn, and Cd to evaluate the impact on crop production. Additionally the impact on the habitat function of the soil was measured. Hence soil-microbial parameters were chosen as parameters.

The results of this study conform to *Felix-Henningsen* et al. [2] concerning the elevated trace metal contents in the topsoil with irrigation using Mashavera river water. Aqua regia-extractable Cu, Zn, and Cd were significantly elevated and in most cases exceeded the German precaution values. The high spatial variability of CONT is caused by the tessellated distribution of metal "hot spots" due to the micro-relief, as mentioned earlier.

The fact that thresholds are also partly exceeded in CLEAN can be explained as follows. At first the geogenic background concentrations are close to the thresholds. According to *BBodSchV* [12], elevated trace metal contents on the basis of geogenic background are unproblematic if metals are not expected to be released. An input of Cu, Zn, and Cd can also be caused by deposition of metal containing dust from the slag heaps of the mine. Residents reported a dusty cover on trees and crops in the summer months resulting from dust blown out of the slag heaps. Another source could be the use of ash from domestic fuel as fertilizers, a typical practice in house gardens of the Mashavera valley. While sampling, we noted considerable ash-residues between the crops. Ash from domestic fuel can contain elevated trace metal concentrations if metal-containing products are burned. Hence, this source of contaminations must be considered, too.

The mobile metal fraction extracted by unbuffered salt solutions such as 1 M  $\text{NH}_4\text{NO}_3$  includes mainly metals non-specifically electrostatically bound to negatively-charged colloid surfaces [22]. Accordingly, potentially plant available and soluble HM were determined, which are very important for ecological assessments.

Mobile Cu did not differ significantly between CONT and CLEAN due to the fact that Cu shows a high affinity to organic matter, especially at soil pH  $>6$  [23], and the relatively high Corg content of the topsoils. Due to this, Cu is strongly adsorbed in both variants and its mobile fraction is assumed to be relatively low. Furthermore, dilute salt extracts usually provide good estimates of Cd and Zn concentrations in soil solution, while Cu may differ by orders of magnitude. This is attributed to Cu, in contrast to Zn and Cd, as it appears mainly as complexes with dissolved organic matter (DOM) in soil solution. DOM concentrations are often lower in salt extracts due to dilution effects [24].

It should be pointed out that the relative increase of the mobile fractions of Zn and Cd is at a much higher level (ten times!) than the increase of total content. Thus if only the slightly increased total content is considered, the actual hazard is underestimated in the Mashavera valley.

Nevertheless, despite this clear increase in mobile fractions of Zn and Cd in CONT, only in a few topsoils are trigger and action values exceeded. This can be explained by the high adsorption of metals due to the nearly-neutral soil pH and the high content of Corg and clay resulting in a high CECeff. Hence, in the majority of cases, sorption capacity of the topsoils is still sufficient to avoid high Cu, Zn, and Cd concentrations in soil solution, although 6% of CONT exceeds the action value for Cd. These topsoils should not be used anymore for crop production. Nevertheless, even if  $\text{NH}_4\text{NO}_3$ -extractable metals might pass the thresholds, it cannot be excluded that certain crops can take up critical amounts of metals. Due to this crop production is endangered in the Mashavera valley. Leafy vegetables, a major crop in the study area, show a disproportionately high uptake of trace metals [25]. Hence Cu, Zn, and Cd concentrations of food crops grown in the study area must be investigated in future.

Apart from the intake of trace metals via the food chain, residents of the study area uptake Cu, Zn, and Cd directly from inhalation of dust or hand-mouth contact. Almost half of indoor dust is considered to be derived from contaminated outdoor soil and consumption of home-grown vegetables [26]. According to *BBodSchV* [12], a direct uptake trigger value of 2 mg Cd  $\text{kg}^{-1}$  soil exists for areas used for food cropping and residential zones of children (assumed daily uptake of 0.5-1 gm soil by ingestion). This threshold is exceeded in 33% of CONT but not in CLEAN.

The lower soil pH in CONT could be induced by the acidic irrigation water with pH values sometimes  $<4$  [2] and corresponds well with the lower  $\text{CaCO}_3$  content of CONT. Nevertheless, differences are small and the actual effect on metal mobility should not be overestimated. But it points to another negative impact of irrigation with Mashavera and Poladauri water. An enhanced acidification of the topsoils will result in a reduced sorption capacity in the longterm. This effect is enhanced by the lower Corg content of CONT. Lower Corg contents in topsoils burdened with trace metals seem to contradict the well-known fact that metal stress leads to accumulation of organic matter due to reduced decomposition. This typical effect for forest soils, however, has rarely been observed in agricultural soils [3]. An explanation could be a reduced biomass (due to Cu and Zn concentrations toxic for plant growth) and thus lower input of organic matter in these soils, as noted by *Mathe-Gaspar* et al. [7].

Trace metal toxicity of plants can be predicted from bulk soil properties but for microbes their micro-location relative to hot-spots of C and metals could be an important aspect of their exposure [4].

Soil protection is partly based on soil-microbial parameters, e.g. in Flanders [27] or in England and Wales [28]. The precaution values for Cu, Zn, and Cd of *BBodSchV* [12] are intended to protect soil organisms [29].

In the present study, the response of a microbial community adapted to elevated metal concentrations as a chronic toxicity was measured. This can lead to results totally different from laboratory experiments testing acute toxicity by adding metal salts [3]. A relevant control soil to represent accurately polluted soil in its pristine state is rarely available in field studies. A control soil differing in chemical and physical soil properties can hardly be used to establish the cause of any differences in microbial properties [3]. However, in this study WITHOUT and CLEAN mostly fulfilled the needed pristine requirement. Metal ions may inhibit enzyme reactions by complexing the substrate, by combining with the enzymes active sites or by reacting with the enzyme-substrate complex [30]. Alkaline phosphatase is an extracellular enzyme and is mainly produced to mineralize organically bound P [18] and plays an essential role in the cycling and availability of soil P. Hence it is a good parameter for evaluating soil fertility. In contrast, dehydrogenase is an intracellular enzyme and is involved in metabolism as an oxidoreductase. Its activity reflects the total oxidative activity of soil-microorganism. Accordingly, we investigated the activities of an extra- as well as of an intracellular enzyme. APA and DHA are often used as indicators for trace metal contaminations and especially DHA has been found to be a good indicator. For example *Welp* [31] measured the effective dose, 50%, of different metals on DHA. The effect increased in the order Zn (115 mg kg<sup>-1</sup>), Cd (90 mg kg<sup>-1</sup>) and Cu (35 mg kg<sup>-1</sup>). In this study both enzyme activities were affected by the increased metal contents of topsoils. Also *Stöven* and *Schnug* [32] measured significantly negative correlations between DHA and Cu, Zn, and Cd on arable land contaminated with metals by sewage sludge application. In this study Cu and Cd had a similar negative impact on enzyme activity. *Kizilkaya* [33] measured significantly negative correlation between DHA and total Cu and Cd (= -0.44 and -0.41) and *Wang et al.* [34] measured significant negative correlations between APA and total Cu and Zn (= -0 and -0.84) in topsoil near a copper smelter in Zhejiang province, China. We conclude that elevated Cu, Zn, and Cd contents in topsoils due to mining activity reduce enzyme activity in the topsoils of the Mashavera valley. The results of the respiration measurements mainly do not support the data of enzyme activity measurements. Neither BR nor SR differ significantly between the different types of irrigation. Whereas BR seems to be a sensitive parameter in forest soils, results for agricultural soils are conflicting, maybe because BR depends on the nature of the available substrate. In addition, a high microbial respiration rate is a surprisingly common characteristic of long-term trace metal contaminated soils, maybe due to greater maintenance energy requirements [3]. Furthermore, it must be considered that the high clay and Corg content of the soils in the study area might reduce the metal stress of microorganisms due the fact that effects are normally greater in soils with a light-texture than in clayey soils with a high content of organic matter [3]. However, at least a small negative effect of Cd on SIR was measured.

#### CONCLUSION

The actual ecological impact of the Cu, Zn, and Cd contamination of the topsoils in the Mashavera valley is alarming. Elevated metal concentrations of topsoils point to a high risk of metal transfer into the food chain. Hence crop production and public health are endangered. Soil

microbial enzyme activity is significantly lower in topsoils irrigated with Mashavera and Poladauri water. This points to a disturbed habitat function of the soils. Due to this, the nutrient-cycle might be disturbed and result in reduced yields. However, respiration activity is not affected by elevated metal contents.

Water quality of the Mashavera river and the Poladauri streamlet must be improved and monitored to avoid further contamination and acidification of topsoils. To evaluate the transfer of Cu, Zn, and Cd into the food chain food crops must be investigated. An assessment of the Cd content absorbed by the residents is necessary to determine the actual impact of the mining activities on their health. Furthermore, restrictions of land use or remediation of heavily contaminated topsoils must be considered.

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## ЗАГРЯЗНЕНИЕ ПОЧВЫ Cu, Zn и Cd ГОРНОДОБЫВАЮЩЕЙ ПРОМЫШЛЕННОСТИ И ПОЧВЕННО-МИКРОБНАЯ ДЕЯТЕЛЬНОСТЬ КАШТАНОЗЕМОВ В ДОЛИНЕ МАШАВЕРА

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Верхние слои почвы долины р. Машавера в юго-восточной Грузии сильно загрязнены Cu, Zn и Cd от отходов карьеров горнодобывающей промышленности. Для исследования экологического воздействия этого загрязнения было отобрано 128 образцов верхних слоев почвы из приусадебных садов и пахотных земель (орошаемых как чистой, так и загрязненной водой или без орошения). Были исследованы концентрации валовых и мобильных фракций Cu, Zn и Cd. Почвенно-микробные параметры были измерены в 37 отобранных образцах. В почве, орошаемой загрязненной водой, валовое содержание Cu, Zn и Cd было оценено факторами 2.1, 1,3 и 3.3 по сравнению с контролем. Мобильные фракции были оценены факторами 18.5 для Zn, 16.4 для Cd. Фосфатная и дегидрогеназная активность были значительно ниже в верхних слоях почв, орошаемых загрязненной водой (52% и 30%, соответственно, по сравнению с контролем), в то время как деятельность дыхания не была затронуто. Плодородие почв и качество сельхозпродукции подвергаются сильной опасности в долине р. Машавера.