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Eco-physiological Study on the Influence of Contaminated Waters from the Topolnitza River Catchment Area on Some Crops

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Abstract. The present study is a small part of a program for an investigation of the water conditions in the Topolnitza Dam Lake, Topolnitza River and its catchment area. The sensitivity of seeds and young wheat, sunflower and mustard plants to heavy metal stress was examined at laboratory conditions. Our results showed that seedling growth was more sensitive to heavy metals in comparison to seed germination. The length of shoot and root has been adversely affected due to water contamination when compared to the control. A certain negative effect on the photosynthetic pigments content was registered.

Key words: crops, pollution, heavy metals, Topolnitza River, Bulgaria

Introduction

Mining of minerals and metals is important for the economic or industrial development of society all over the world. In the past century, much of the mining has concentrated on the extraction of such metals as gold, silver, iron, copper, lead, zinc, chromium, mercury, then some others, for example, As, Cd and Se are often enriched in mineral deposits and recovered as by-products. Disposal of mines resulting in waste tailings posed a significant risk to the surrounding environment. Their improper management resulted in the migration of heavy metals to the surrounding environment, contributing to the contamination of soil substrates, destruction of ecological landscape, groundwater pollution and decrease in biological diversity. Metals are currently of much environmental concern due to the fact that they tend to bio-accumulate in food chain and are harmful to humans and animals. The mechanisms of metal toxicity induction are not fully understood for crop plants. Metals may directly or indirectly interfere with the metabolic activities by altering the conformation of proteins for example enzymes, transporters or regulatory proteins by their strong affinities as ligands to sulfhydryl and carboxylic groups (SHARMA & SHARMA, 1996).

The photosynthetic surface area and leaf chlorophyll contents are the major factors determining the total biomass production in plants. Cadmium and lead are reported to have inhibitory effect on photosynthesis, transpiration, carbohydrate metabolism and other metabolic activities (KUPPER *et al.*, 1996; VASSILEV *et al.*, 1998; XU *et al.*, 1998). STOBART *et al.* (1985) have reported that Cd particularly inhibits the chlorophyll biosynthesis and decreases total chlorophyll content and chlorophyll a/b ratio, while Pb toxicity emerges with the disturbance of mitosis, toxicity of nucleoli, inhibition of root elongation due to mitotic effects on the root tip cells of *Brassica juncea*, appearance of chlorosis, inhibition of enzymatic activities and reduction in photosynthesis.

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The Topolnitza River catchment area is rich of mining activities and many metals are persistent in the water basins. These waters are being used by farmers to irrigate crop plants without considering its adverse effects - the metals, presented in them, may accumulate in plants in excessive quantities quite enough to cause the clinical problems in animals and human being consuming these metal rich crops. Hence, monitoring of accumulation and distribution of metals in crop plants cultivated in agricultural land irrigated with water from Topolnitza Dam Lake, Topolnitza River and its catchment area is necessary.

Experiments were planned and conducted to study the influence of contaminated waters from the Topolnitza River catchment area on some crop plants cultivated in laboratory conditions.

Materials and methods

Collection of water samples

Sampling was made in the beginning of October 2011 when is the period of the autumn low flow. Water samples were collected in clean containers (5 l) from selected points of Topolnitza River catchment area, as follows: Site 1 (control) – Micro dam near Borimechkovo village; Site 2 – Topolnitza River, near Lesichevo village; Site 3 – Topolnitza Dam Lake, near the wall; Site 4 – Topolnitza River, near Poibrene village; Site 5 – Topolnitza River, near Petrich village; Site 6 – Topolnitza River, near Chavdar village; Site 7 – Topolnitza River, near the town of Zlatitza; Site 8 – Medetska River, before Topolnitza River (Fig.1).

Water analysis

In situ were measured temperature (BDS 17.1.4.01), pH (ISO 10523:2008) and the conductivity (EN 27888) of the water. Chemical analyses, including Pb (ISO 17294-2), Fe (ISO 6332), Mn (BDS 17.1.4.15), Ni (ISO 17294-2), Cu (ISO 17294-2) and As (ISO 17294-2) content in the collected water samples, were made in the Regional laboratory – Plovdiv.

Bioassays

The experimental bioassays were performed in the laboratory of Ecology, Faculty of Biology, University of Plovdiv. Three crop plants were tested – *Sinapis alba*, *Triticum aestivum* and *Helianthus annuus*. Fifty seeds of each species for each treatment were placed into experimental containers (petri dishes with filter paper for mustard and pots with perlite for wheat and sunflower) and irrigated with 50 ml of the collected water samples. Each treatment was made in triplicate. Irrigation was repeated periodically when needed. The seedlings were harvested after period of one week for *Sinapis alba* and two weeks for *Triticum aestivum* and *Helianthus annuus*.

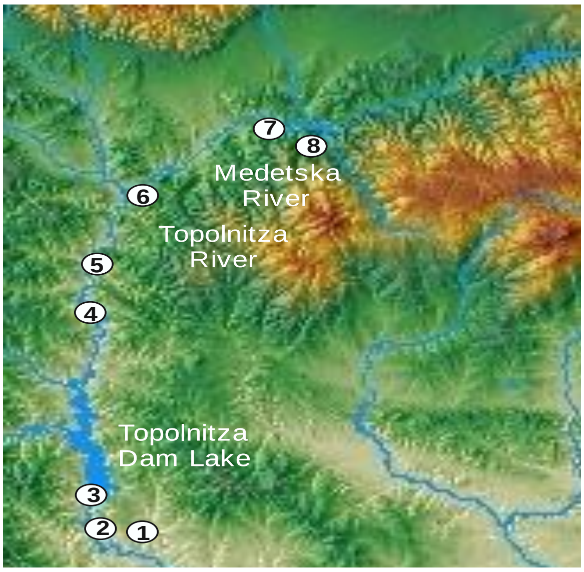

Fig.1. Location of the sampling sites

Estimation of morphometric and physiologic parameters of plants

The following parameters were measured immediately after harvesting: germination of seeds (in %), shoot and root length (in cm), shoot-to-root ratio, chlorophyll content (mg g^{-1} fresh weight) and chlorophyll *a/b* ratio.

Pigment analysis was performed according SCHLYK (1965) after extraction with 90% acetone. Wavelength absorbance was measured at 662 nm for chl *a* and 644 nm for chl *b* in a SPECOL 11 absorption spectrophotometer (Faculty of Biology, University of Plovdiv). Concentrations of chl *a*, chl *b* and chl *a*+*b* were calculated for each sample and expressed on a fresh weight basis (PETROVA, 2011). We reported our results as mean ± SD, where standard deviations were calculated on the base of triplicate analysis of chlorophyll content.

Statistical analysis

To confirm the variability of data obtained and validity of results, all the data were subjected for the statistical analyses using STATISTICA 7.0 package (STATSOFT INC., 2004).

Results and Discussion

Physico-chemical properties of the water

The results from the physic-chemical analysis of the collected water samples showed that the water quality is worst in Site 8 (Medetska River), where the pH was quite acidic (Table 1). The highest conductivity (374.0 µS) correlated with the increased heavy metal content in the same sample.

Table 1. Physico-chemical parameters of water samples

Water sample	Water temperature, °C	pH	EC, µS	Fe, mg l^{-1}	Mn, µg l^{-1}	Pb, µg l^{-1}	Ni, µg l^{-1}	Cu, µg l^{-1}	As, µg l^{-1}
Site 1	19.0	7.5	97.7	-	-	-	-	-	-
Site 2	12.2	6.7	106.3	-	-	-	-	-	-
Site 3	12.6	7.4	111.5	-	-	0.001	0.005	0.005	0.005
Site 4	11.8	6.5	158.2	0.240	0.064	0.006	0.011	0.018	0.090
Site 5	12.2	7.1	137.3	0.540	0.005	-	-	0.005	-
Site 6	12.5	7.3	290.0	0.380	0.171	0.006	0.005	0.048	0.012
Site 7	13.0	7.3	60.5	0.050	0.047	0.025	0.004	0.033	0.008
Site 8	11.1	4.8	374.0	0.131	0.027	0.025	0.040	0.092	0.010

This contamination was significantly lower in the water sample from Site 7, probably due to the dilution effect. There was registered an increment of the pH, conductivity, As and Cu concentrations in Site 6 and Site 5, located hereafter. The results of the As content were highest in water sample from Poibrene Village (Site 4), the conductivity was also quite high. We suppose that in this sampling site some organic pollution has been presented due to an illegal discharging of household waste waters, disposal of livestock manure, etc. The effect of that kind of anthropogenic impact on water quality had been well known in many rivers (VALCHEVA & POPOV, 2007; VALCHEVA & POPOV, 2008). In Site 3 (Topolnitza Dam Lake near the wall) the EC value was lower, that could be explained with some processes like dilution and sedimentation, and close to this was the water sample from the next sampling site - Site 2.

The values of measured characteristics of the water, obtained in this study, were higher in comparison with data from previous research in this region (PETROVA *et al.*, 2011). This could be due both to the different sampling periods, the variances in water debit, and also to the autumn senescence and consequent eutrophication of the rivers.

Germination

Seed germination is a critical phase of the life-cycle in most plant species, particularly in cultivar plants. Stressful conditions exert a strong effect on the dynamics of seed germination and seedlings establishment. There was not observed considerable effect of contaminated waters on the seed germination capacity for wheat and sunflower in comparison with the mustard (Fig. 2). This issue may relate to permeability of seed coat to metal ions. Different degree of permeability led to a different degree of germination inhibition.

No negative effect was observed in the biotest with *Helianthus annuus* seeds. All cases resulted in a stimulation of the germination process, most pronounced in water sample from Site 4 (123%). The lowest values in the biotest with *Triticum aestivum*

(88% in comparison with the control) were found in pots irrigated with water from Site 7, followed by the Site 8 (94%). The mustard seeds showed higher sensitivity and the germination varied from 55% in pots irrigated with water from Site 6, followed by Site 8 (65%), through Site 4 (89%) to Site 5 (91%), Site 7 (93%) and Site 3 (94%).

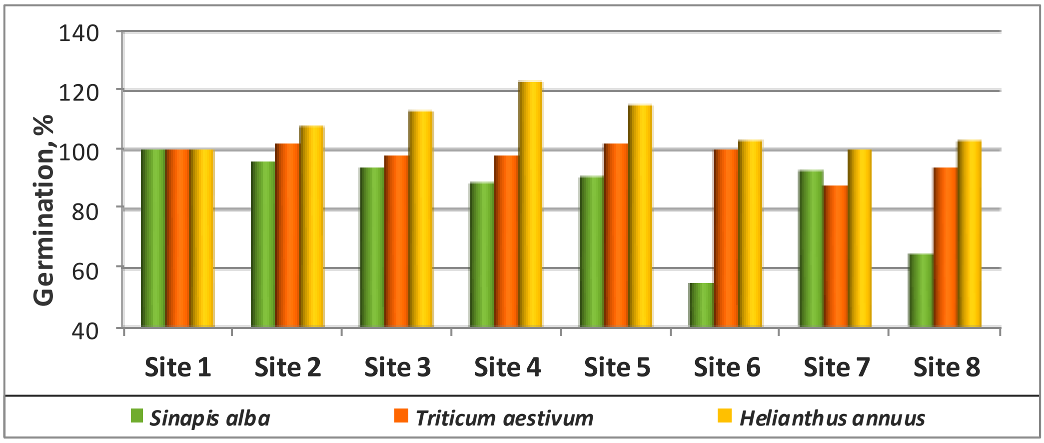

Fig. 2. Germination results for the studied plant species, % in comparison with control sample

Our results can support to the observations described by other authors (FARGASOVA, 1994; WIERZBICKA & OBIDZINSKA, 1998; PANDEY, 2006, 2008; URUÇ & YILMAZ, 2008) of the effect of contaminated with some heavy metals (Ni, Cu, Pb) waters on seed germination of different crop plants.

Growth

The root and shoot length and elongation rate are essential for plants exploring for water and mineral nutrients. Our results showed that seedling growth was more sensitive to heavy metals in comparison to seed germination. The length of shoot and root has been adversely affected due to water contamination when compared to the control (Fig. 3).

As a result from all bioassays conducted, we found the stronger inhibition on the plant growth, both for the aboveground and underground plant parts, in pots irrigated with water from Site 8 (Medetska River). The effect of this water sample was most intense at the root length – we observed a reduction of 95% to wheat seedlings, 93% to mustard seedlings and 87% to sunflower seedlings in comparison with the control plants. Suppression of the shoot growth was also well expressed - 75% (*Sinapis alba*), 70% (*Triticum aestivum*) and 69% (*Helianthus annuus*) shorter seedlings in comparison with the control.

The effect of the tested water samples on sunflower plants growth and development was negative. An exception was the stimulated root elongation in experimental plants irrigated with water from Site 4, which assumed that have been rich in organic matter.

Mustard seedlings had similar shoot length in all cases (except water sample 8). We registered an inhibition of the root growth in pots treated with water from Site 6 (39%) and a positive impact of water samples from Site 2 (169%), Site 4 (132%), Site 5 (129%) and Site 7 (122%) when compared to the control.

We found some stimulation of the root elongation of the wheat seedlings in all bioassays except the Site 8, and an increment of the wheat shoot growth in experimental pots irrigated with water from Site 5 and Site 6. AYDINALP & MARINOVA (2009) studied the effects of heavy metals (Cd, Cr, Cu and Ni) on *Medicago sativa* plants. Their results, similarly to ours, showed that the low concentration of these chemical elements had positive effect on shoot and root growth, but in large quantities they became toxic.

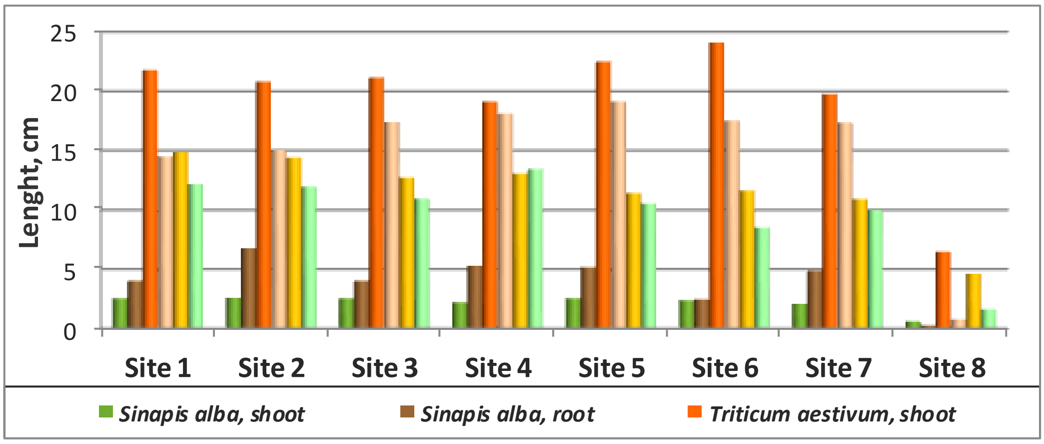

Fig. 3. Shoot and root length of the seedlings (in cm)

The above results were in agreement with the findings of CHEN *et al.* (2003) in soybean, RAI *et al.* (2005) in *Phyllanthus amarus* and XU *et al.* (2008) in garlic. The inhibitory action in root and shoot length might be due to reduction in cell division, toxic effect of heavy metals on photosynthesis, respiration and protein synthesis. These obviously contributed to the retardation of normal growth (KUPPER *et al*., 1996).

HAGEMEYER *et al.* (2002) and MARCHANO *et al.* (2002) also suggested that the morphological and structural effects caused by metal toxicity in plants was due to decrease in root elongation, root tip damage, decrease in root formation, suppression of elongation growth rate of cells, affecting the ultra-cellular structure of meristematic cells and inhibition of the size of plant cells and inter cellular spaces.

Shoot-to-root ratio is a useful parameter for evaluation of the quality of the environment in which plants grow. In our study it was 2.5 (sunflower), 3.5 (mustard) and 5.5 (wheat) times higher in seedlings irrigated with water from Site 8 when compared to the control. These results could be explained with the toxic effect of the water contaminants (mainly heavy metals) and were in agreement with MAMI *et al.* (2011) findings.

The lowest values of this parameter for all three studied plant species we observed under influence of the water samples from Site 4, followed by Site 7 and Site 5 (Fig. 4). An explanation for these results could be found in the mentioned favorable effect of heavy metals in low concentrations on the root extension.

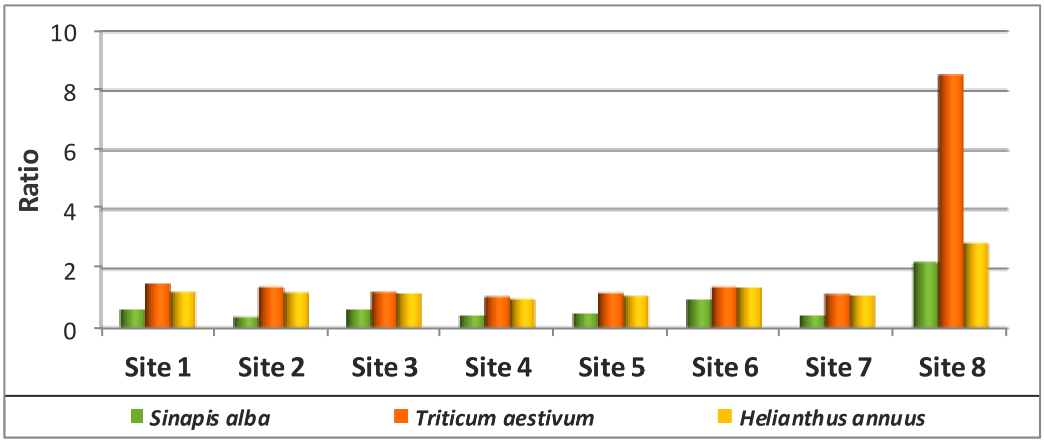

Fig. 4. Shoot-to-root ratio of the seedlings

Pigment content

It is considered that the growth parameters do not always provide enough objective information to phytotoxicity of the environment and therefore in plant bioassays, it is advisable to include functional indicators. Leaf chlorophyll content is an important parameter for testing plant status. For example, it can be used as an index of the photosynthetic potential as well as of the plant productivity (CARTER, 1998). In addition, chlorophyll gives an indirect estimation of the nutrient status (FILELLA *et al.*, 1995) and is closely related to various types of plant stresses (GITELSON & MERZLYAK, 1994).

The pigment analysis indicated that the changes in the levels of photosynthetic pigments in cotyledons varied in wide range (Fig. 5). The strongest inhibitory effect in wheat bioassays was observed at Site 8 - 57% to chlorophyll *a* and 48% to chlorophyll *b* in comparison with the control sample, followed by Site 4 and Site 5. In the rest of water samples an increased concentation was found. Stimulation of the synthesis of chlorophyll in the presence of low doses of zinc and nickel in the middle, accompanied by an increased ratio have been found by other authors (MACIFE & TAYLOR,1992; SARITA & ABHA, 2007) and could explain our results.

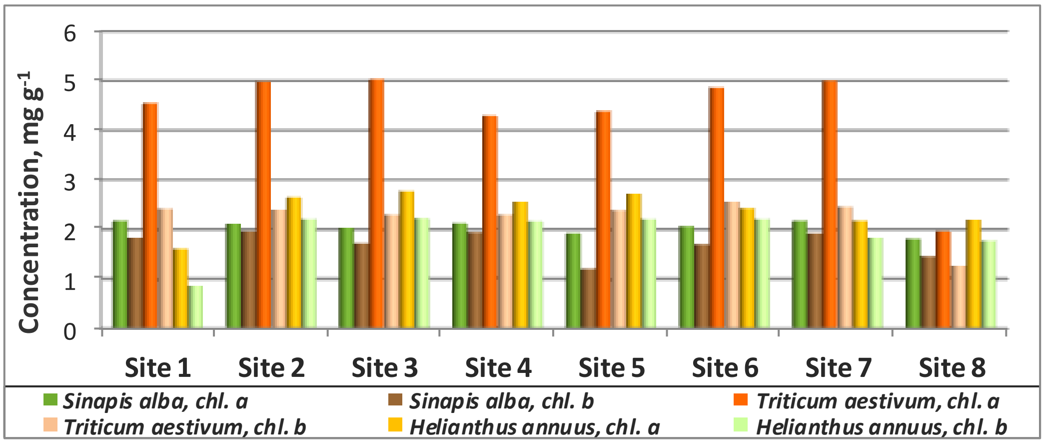

Fig. 5. Chlorophyll content in the leaves of the investigated plants (mg g^{-1} fresh weight)

The bioassay with sunflower plants revealed that the lowest chlorophyll content was determined in the control and in all other cases there was an increment of its concentration – up to 171% to chlorophyll *a* and to 259% to chlorophyll *b* both in water sample from Site 3. Similar to our findings, raise of the level of photosynthetic pigments in sunflower plants were observed by SINGH *et al*. (2004). In the course of their experiment with industrial wastewaters, after the 30th day of exposure the chlorophyll content was higher, however, extend the time the effect became unfavorable and led to a reduction of its amount in comparison with the control.

The mustard seedlings of the water sample 8 were most affected - 17% inhibition of chlorophyll *a* and 21% of chlorophyll *b*, followed by water from Site 5. The change in the amount of both chlorophylls was not clear and there was no statistical reliability.

The change in the chl *a/b* ratio (which had the advantage to be a dimensionless parameter) could be used as more informative indicator in ecological investigations. In all bioassays conducted, the highest ratio values were observed in seedlings with lowest total chlorophyll content – *Sinapis alba* at Site 5, *Triticum aestivum* at Site 3 and *Helianthus annuus* at Site 1 (Fig. 6). The decrease of chlorophyll content may be due to an increase of chlorophyll degradation or to a decrease of chlorophyll synthesis. During the process of chlorophyll degradation, chl *a* is converted in chl *b* (FANG *et al.*, 1998) and this may explain the depression of chlorophyll content together with the increase of the ratio chl *a/b*.

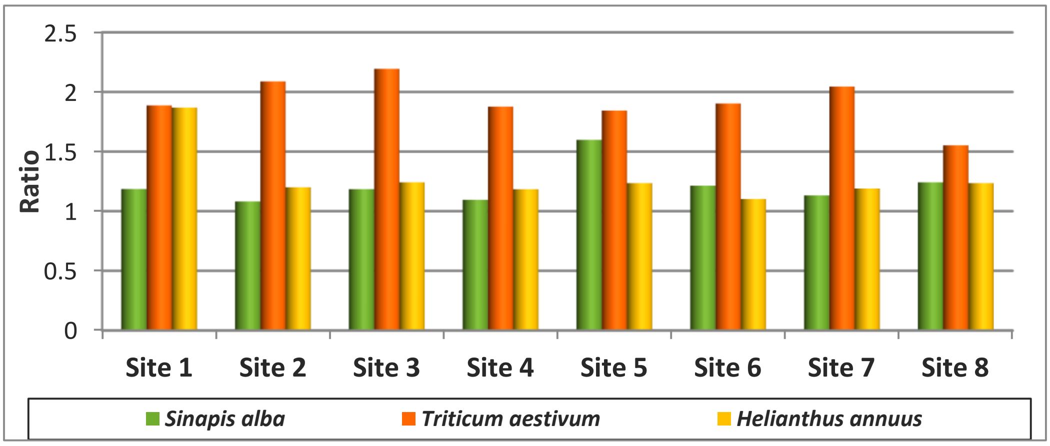

Fig. 6. Ratio chlorophyll *a/b* in the leaves of the investigated plants

Conclusion

The physico-chemical analyses of water samples from Topolnitza River catchment area have revealed that these effluents carry very high toxic metal content and are one of the major sources of soil and water pollution in this area. It indicated that the explored water basins (used for irrigation) and the surrounding agricultural land are not fit for agricultural practices. As a result, it is possible to reduce the biomass production without any visible symptoms of metal toxicity (chlorosis, necrosis, dryness). Further, the study revealed that these crop plants are not fit for consumption by human beings and livestock.

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