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Biological Assessment of the Zapadna Morava River Water used for Integral Food Production

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Abstract: The paper presents the effect of irrigation water of stated quality (based on physico-chemical analysis, phosphatase activity, plankton and ichthyofauna composition) on the number and activity of the microorganisms in alluvium and smonitza under wheat, barley and red clover. The obtained results (depressive impact on the highest number of soil microorganisms and their enzymatic activity) indirectly suggest that the Regulation on watercourse categorization (Official Herald FR Serbia, 5/86) be amended in the part relating to the quality of waters that can be used for plant irrigation.

Key words: food, ichthyofauna, microorganisms, plant, plankton, soil, water quality.

Introduction

Technological revolution increasingly leads to a global biosphere contamination with highly persistent organic and inorganic pollutants, hence to a much higher content of toxic matter than that of MDC.

Toxicants, particularly heavy metals may seriously disrupt the viablility of organisms while binding with sulfide groups of the main enzymes and destroying cell membranes. Any toxic matter in a higher concentration is lethal to microorganisms and higher life forms. The level of toxicity in the compound with heavy metals depends on whether such compound appears in the solution in the form of a free ion, undissociated salt, or may be the constituent of organic or inorganic compounds. Undissociated salts and ions form complex compounds that are usually less toxic than free ions are in equal concentrations. Not only do toxicants enter into the process of matter cycling, but they also remain in this process for quite a long time, displaying a biocidal impact on the activity of soil microorganisms, thereby destroying their microbic system (Duxbury, 1981).

Considering that pollutant's main point of entry into the receiving river and municipal wastewaters is located near urban and rural settlements, unsurprisingly, they pose a serious threat to food 'healthiness' and general development accordingly.

The sources of such an enormous pollution lie in the highly developed countries where the destroying of ozone "screen" started with an accompanying problem of desertification (desert formation), which has spread over some important areas of our country. Therefore, efforts should be made towards: elimination of desertification causes, selection of plants resistant to draught and, building huge irrigation systems. Considering economic and global under-development under present conditions, we shall resort to the third option, although both merits and dismerits exist in terms of the extent, to which polluted watercourses are used for irrigation, and wastewaters for soil fertilization. Some merits refer to soil improvement, enhanced microorganism activity, bettering soil ability to bind with water as well as soil bio-activation. However, dismerits may entail high risks of heavy metals, poisons and pathogenic bacteria encountered in polluted irrigation water, followed by imbalances in the content of nutritional elements (as a rule, the addition of K is considered necessary), some nutritional substances found in undissolved form, ions of heavy metals and sulphur compounds leading to changes in soil microbiocenosis (Jevdokimova, Mozgova, 1976) and others. Polluted irrigation water may adversely affect the evolution of organic matter in the surficial horizons of soil, lowering the contents of humified organic matter and highly polymerized humic acids (Konecka-Betley and Zebrowski, 1978). The process of transforming organic compounds can be sped up using wastewaters, but only during plant growth when the majority of introduced impurities can be microbiologically dissociated.

However, the results on the effects of toxicants from polluted irrigation waters seem contradictory. Future concerns in this regard will contribute to a better understanding of this issue and to a more realistic picture of the relationships within the system neo-chemistry - elements - agriculture (Mandic et al., 1994). This study has acquired in importance for ecological requirements based on environmental protection, all the more because, after entering into the soil, hazardous matter may highly accumulate in some plant parts, continuing to travel to the nutritional chains and, eventually, to human metabolism, thus causing many water-borne diseases, such as methemoglobinemia, cancer, teratogeny and others.

Therefore, our studies of polluted irrigation water used for integral food production had to be approached both directly (chemical properties of water, phosphatase activity, plankton, ichthyofauna) and indirectly (numerousness of diverse microorganism groups and enzymatic activity of alluvium and smonitza under wheat, barley and red clover).

Materials and method

The investigations were conducted at the Microbiological Laboratory of the Faculty of Agronomy in Cacak. The experiment was set up in vegetative containers according to randomized block design with three replications. Soil samples (alluvium, smonitza) were taken in a natural form from the surficial layers of the humus-cumulative horizon. The sowing of wheat, barley and red clover was performed at opimal time, using standard plant densities. Irrigation (experimental series irrigated with polluted, and control ones with pure water), lighting, ventilation and other cultural measures were as usual. Polluted water was taken at the distance 200-300 m from the main collector of industrial and communal wastewaters of Cacak.

Soil samples for microbiological and biochemical analyses were taken aseptically three times during vegetation and immediately tested.

The number of microorganisms was determined on the basis of increased colonies on appropriately chosen nutritional media (ammonifiers on MPA; a total of bacteria on soil agar according to Pochon et al., 1954; azotobacter on Fjodorov's agar; amilolytics on starch and fungi on Capek's agar) and expressed per 1 g of absolutely dry soil. The media were sown, using 0,5 ml dilution 10-6 for ammonifiers and their total number, 0,5 ml dilution 10-5 for amilolytics and fungi, and 0,2 ml dilution 10-2 for azotobacter. The incubation of sown stocks in thermostat at 301oK lasted 7 days for ammonifiers, 5 days for the total of bacteria and fungi, and 2 days for azotobacter and amilolytic microorganisms.

Proteinase activity was determined according to the method of Romeiko (1969) and expressed in gelatinolytic units (10 gelatinolytic units correspond 0,2 ml 4% FeCl. 6H2O) per 1 g of airy dry soil.

Standard methodology was employed to determine main parameters (22) of chemical water quality.

Phosphatase activity of irrigation water was determined, using p-nitrophenylphosphate (Matavulj, 1986), expressed by the index of phosphatase activity (IPA) ...mol p NP/s/dm3, then released from pNPP by the sample enzymatic activity, and calculated, using a molar extinction coefficient (Flint, 1974, cit. by Matavulj, 1986) according to the following formula:

$$\text{IFA } (\mu \text{ mol pNP/s/dm}^3) = \frac{DE \cdot V \cdot C \cdot 100}{v \cdot t \cdot d}, \text{ where,}$$

- DE - the difference in extinction on 420 nm between the trial and control;
- V - the total volume of reaction mixture (cm^3);
- C - molar extinction coefficient;
- v - sample volume (cm^3);
- t - duration of enzymatic activity (sec.);
- d - direction of light through a test tube (cm).

Complying with this procedure, the following formula may be derived:

$$\text{IFA } (\mu \text{ mol pNP/s/dm}^3) = \text{DE} \cdot 33 \cdot 667$$

Qualitative and quantitative composition of plankton communities from irrigation water was evaluated, using standard methodology (Zabelina et al., 1951). So obtained results made it possible to validate saprobity of irrigation water (Pantic-Buck, 1955; Sladecek, 1973).

After fish catch by means of standard hooks and nets, their determination followed (Vukovic and Ivanovic, 1971; Weeler, 1983), with saprobity level established according to Sladacek (1973).

Results and discussion

Chemical analysis of plant irrigation water showed its being overloaded with various pollutants (Tab.1.), primarily those coming from industrial and communal wastewaters. Low concentration of O_2 (1,42%), the values BPK_5 (3,57 mg/l, NO2 (0,775 mg/l), NO_3 (65 mg/l), NH_3 (0,156 mg/l), PO_4 (0,314 mg/l), as well as the use of $KMnO_4$ (340 mg/l) and pH above 7 (7,34) strongly confirm a high intensity of organic matter degradation. Among toxic matters, phenols (0,002 mg/l), mineral oils (0,17 ml/l) detergents (0,09 ml/l), Pb (0,031 mg/l) As(0,008 mg/l) and Ni (0,028 mg/l) were detected.

Tab.1. Physico-chemical properties of water used for plant irrigation

Indices	Calculated value	Indices	Calculated value
pH	7.34	Ca	96.18 mg/l
NH_3	0.156 mg/l	Mg	14.59 mg/l
NO_3	66.00 mg/l	Mn	0.00 mg/l
NO_2	0.775 mg/l	Cu	0.0008 mg/l
$KMnO_4$	340.00mg/l	Zn	0.03 mg/l
O_2	1.42 mg/l	Pb	0.031 mg/l
BPK_5	3.57 mg/l	As	0.008 mg/l
HPK	2.99 mg/l	Ni	0.028 mg/l
Suspended matter	556.00 mg/l	Hg	0.0018 mg/l
Total hardness	18.05 mg/l	Cr^{6+}	0.030 mg/l
Chlorides	1.37 mg/l	Cr (total)	0.042 mg/l
Sulphates	71.70 mg/l	Phenols	0.002 mg/l
Phosphates	0.314 mg/l	Detergents	0.090 mg/l
Fe	0.525 mg/l	Mineral oils	0.170 ml/l

At the level 2,17 μmol pNP/s/dm^3, irrigation water phosphatase activity ranged from II-III class.

The presence of toxic matters is extremely detrimental to irrigation water bio-content (Tab.2.). Phytoplankton community of this water (23 species) consisted of *Chrysopphyta* (14 species), *Chlorophyta* (6 species), *Cyanophyta* (2 species) and *Englenophyta* (1 species), with zooplankton consisting of 2 species - *Rotatoria* (5 species) and *Protozoa* (2 species).

Although beta - mezasaprobic indicators are dominated by the number of species (74,19%), some indications of other saprobic conditions cannot be ignored (Đukic, et al., 1994).

In its composition, the ichthyofauna of the examined waterflow used for irrigation, had 19 fish species from 6 families.Their saprobic value ranged from 1,75 to 2,5. Poorer ecologicity was due to a higher presence of bleak *Alburnus alburnus* (Cakic, 1984; Markovic, Simovic, 1994a) and a somewhat changed population morphology of roach *Rutilus rutilus* inhabiting industrially contaminated waters, which is in agreement with numeorus results (Mironovskij, 1994). Increasing number of some highbred fish species, especially carp *Cyprinus carpio*,pike *Exos lucius*and catfish *Silurus glanis* was obvious (Markovic, Simovic, 1994b).

Tab.2. Composition of the plankton community of the examined part of the Zapadna Morava ecosystem

PHYTOPLANKTON	Saprobic level / the number of species	
Cyanophyta	α / 2	
Chrysophyta	β / 10;	α / 4
Englenophyta	ρ / 1	
Chlorophyta	β / 6	
ZOOPLANKTON		
Protozoa	β / 2	
Rotatoria	β / 4;	α / 1

Except second irrigation date of smonitza under barley, the polluted water used for plant irrigation, significantly reduced the total of microorganisms (Tab.3.) in both types of soils (alluvium, smonitza) under all the three plant cultures (wheat, barley, red clover), which was confirmed by many authors (Malone, Reichle, 1973; Jevdokimova, Mozgova, 1976; Kermen, Pinkiewicz, 1976; Kelling et al., 1976; Ubavic et al., 1993). In contrast, during vegetation, the total number of microorganisms increased, thereby reflecting their ability to transform toxicants into inactive forms and utilize them as energy sources or electron acceptors in the breathing process. In the same period, soil structure was visibly improved, contributing to a better water-air regime balance (Uhrecky et al., 1956) which, in turn, sped up organic matter degradation, thus increasing the total number of microorganisms. Comparing with the two previous dates, lower differences in total microorganism number between the experimental and control variant in both soil types at the end of vegetation, may be explained by partly degraded active toxicants from water (Đukic, 1992). An explanation for the lower absolute values of the total of microorganisms found in smonitza, lies in a better structure, mechanic composition and higher biogenetic potential of alluvium at the expense of a much lower oxido-reduction and thermic potential of the smonitza (Ciric, 1986), which inhibited both, the number and activity of aerobic microorganisms (Misustin, Emcev, 1978).

Polluted irrigation water also led to a decreased ammonifier number in both soil types under wheat and red clover (Tab.3.) except barley, where the number of both microorganisms and ammonifiers depended on the plant culture itself (Misustin, Emcev, 1978). Plant excreta can be highly specific provided some toxic mineral and organic excreta bind in the form of chelates that are less toxic, and in time, entirely harmless.

Tab.3. The effect of polluted irrigation water on the number of individual microorganism groups in the soil during vegetation of wheat, barley and red clover

he stages of sampling		Wheat ∅	Wheat ogled	Barley ∅	Barley ogled	Red clover ∅	Red clover ogled
ALUVIUM							
The total number(10^6) of bacteria	I	238	196	218	200	178	166
	II	468	400	478	396	400	368
	III	766	752	740	738	636	608
Fungi (10^5)	I	14	12	10	6	20	12
	II	14	16	16	30	28	38
	III	46	38	84	74	136	100
Ammonifiers (10^6)	I	54	28	38	40	42	38
	II	242	202	110	194	186	176
	III	452	444	408	430	358	352
Amilolytics (10^5)	I	22	18	32	18	16	12
	II	80	66	84	68	34	25
	III	198	170	160	126	33	52
Azotobacter (10^2)	I	5	2.6	12.6	10	150	50
	II	10	9.4	20	20	750	500
	III	15	15	22.5	27.5	1000	1000
Proteolytic activity	I	11.25	11.20	11.50	10.25	11.50	10.25
	II	12.50	12.50	11.50	12.35	12.65	12.65
	III	20.00	17.00	17.50	17.15	25.00	19.50
SMONITZA							
The total number(10^6) of bacteria	I	144	120	144	120	160	140
	II	502	268	358	384	384	368
	III	696	678	622	596	620	600
Fungi (10^5)	I	14	13	12	13	22	16
	II	24	36	40	40	74	42
	III	72	51	51	74	150	144
Ammonifiers (10^6)	I	56	38	36	44	110	46
	II	188	154	124	166	136	120
	III	440	432	326	300	486	438
Amilolytics (10^5)	I	24	12	20	28	20	15
	II	48	48	60	42	42	39
	III	166	120	98	86	79	74
Azotobacter (10^2)	I	0	0	10	8	50	0
	II	6.8	6.0	5	1.6	500	150
	III	10	15	25	18	500	500
Proteolytic activity	I	7.90	8.00	8.00	8.00	8.50	7.50
	II	11.75	10.95	11.3	11.25	12.2	12.10
	III	15.00	13.25	15.3	15.10	15.9	14.65

Lower effect of irrigation water, established at the beginning of the vegetation, was the consequence of the microbial and microbistatical effect of the toxic matter from water (Doelmann, Haanstra, 1979; Naplekova, 1982; Panikova, Percovskaja, 1982; Shich, Yel, 1985). Within stated dates, microorganisms started degrading toxic matters, which resulted in a higher number of ammonifiers. Similar tendency was confirmed by the results of Munjko (1974). Except smonitza under red clover, the absolute values of ammonifier number proved higher in alluvium, which is accounted for by highly degradable organic toxicants in this soil (Munjko and Mestrovic, 1976), as well as by the compactness of soil aggregates in smonitza, which prevented pollutant diffusion and, for some time, postponed their adverse effects on the ammonifiers.

Due to polluted irrigation water, the decrease in amilolytics in alluvium and smonitza under wheat, barley and red clover, was significant (Tab.3.). During vegetation, they increased in number (Saric, Ristic 1973) except indications of the higher absolute values of their number in the alluvium.

In general, polluted water had a depressive impact on the number of azotobacter cells in both types of soil under all the three crops (Tab.3). The results of sasonal microbiological analysis showed that azotobacter number increased throughout vegetation, except the two first dates when its number highly varied between the experimental and control variant. A considerable decrease in azotobacter number in studied soils can be accounted for by the presence of higher concentrations of heavy metals, detergents, phenols, mineral oils and the other toxicants from polluted water. This finding is supported by many authors (Ismailov, 1983; Guzev et al., 1985; Đukic, 1992; Ubavic et al., 1993). It could be interesting to mention that azotobacter number was equalized in both variants with examined soils at the end of vegetation. This contributes to the fact that, over the time, hazardous matter could have been partly detoxicated. Similarly, Albright and Wilson (1974) pointed out that insofaras microorganisms appear in higher number, they could actively transport heavy metals and chemically modify their lower concentrations as to become non-toxic. The lower number of azotobacter cells in the smonitza was due to its higher content of clay that aggravated its oxido-reduction potential (Tab.3.).

Polluted irrigation water adversely acted on soil fungi only in the smonitza under red clover (Tab.3.). During the vegetation, the number of fungi increased, particularly at the end of vegetation, which is explained by microbiological deactivation of the toxicants from irrigation water, an improved soil structure and a better water-air regime balance (Uhrecky and Zvanovec, 1956). As for fungi, the absolute values of their number proved higher in smonitza under all the three plant cultures.

With minor exceptions, irrigation water caused the fall in proteinase in alluvium and smonitza under wheat, barley and red clover (Tab.3.). The blockages in active enzyme groups caused by toxic matter, may serve as an explanation (Doelman, Haanstra, 1979; Panikova, Percovskaja, 1982). Proteinase was increased during vegetation, most likely as the result of abundant root excreta and rhizospheric microorganisms (Saric, Ristic, 1973; Saric, Đukic, 1985; Đukic 1991a, 1991b). These findings also confirm an experimentally established fact that proteinase was higher in the alluvium.

Conclusion

The results on the effects of polluted irrigation water of stated quality (physico-chemical analysis, phosphatase activity, plankton and ichthyofauna composition) on the microorganism number and activity in alluvium and smonitza under wheat, barley and red clover, suggest the following:

- except ammonifiers and fungi in both soil types under barley, irrigation water displayed a depressive impact on the number of the other groups of microorganisms (total number, amilolytic, azotobacter) as well as on the proteinase in the alluvium and smonitza under all the three crops;

- both, higher number and stronger enzymatic activity (proteinse) during vegetation suggested a much weakened inhibitory effect of irrigation water;

- except fungi, the absolute values of microorganism number and activity of alluvium proved higher than those of the other soils;

- bearing in mind adverse effects of studied water on the soil biological productivity, the Regulation on watercourse classification (Sl.Glasnik of FR Srbia, 5/86) is to be corrected in the part related to the quality of usable plant irrigation waters.

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BIOLOŠKA OCENA KVALITETA VODE REKE ZAPADNA MORAVA ZA INTEGRALNU PROIZVODNJU HRANE

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Agronomski fakultet, Čačak

Rezime

Tehnološka revolucija je uslovila neprekidan rast zagađenosti biosfere različitim tehnogenim materijama organske i neorganske prirode, zbog čega sadržaj toksičnih materija u nekim delovima biogeosfere znatno nadilazi MDK.

Ovim materijama su naročito bogate rečne i kanalizacione vode koje su vezane za veća gradska i seoska naselja, što može biti ograničavajući faktor proizvodnje zdravstveno bezbedne hrane ruralnog i opšteg razvoja.

S toga je u ovom radu praćen uticaj zalivne vode definisanog kvaliteta (na osnovu fizičko hemijske analize, fosfatazne aktivnosti, sastava planktonskih organizama i ihtiopopulacije) na bojnost i aktivnost mikroorganizama u aluvijumu i smonici pod pšenicom, ječmom i crvenom detelinom. Dobijeni rezultati (depresivno dejstvo na najveći broj zemljišnih mikroorganizama i njihovu enzimatsku aktivnost) indirektno ukazuju na potrebu izmene Uredbe o kategorizaciji vodotoka (Sl. glasnik SR Srbije, 5/86), u delu koji se odnosi na kvalitet voda koje se mogu koristiti za zalivanje poljoprivrednih kultura.