

Germination, root and shoot length as indicators of water quality

**Sonja Gvozdenc, Dušanka Indić, Slavica Vuković, Mila Grahovac,
Milena Vrhovac, Žarka Bošković, Nina Marinković**

*Faculty of Agriculture, Trg Dositeja Obradovića 8,
Novi Sad, Serbia - sonjag@polj.uns.ac.rs*

Abstract: Seed germination, root and shoot length of mustard (*Sinapis alba* L.), cucumber (*Cucumis sativus* L.), barley (*Hordeum vulgare* L.) and maize (*Zea mays* L.) were measured to determine the influence of water quality on plant growth. Parameters were assessed after seven (corn, barley and mustard) and eight days (cucumber). The results indicate that water quality has no effect on germination, since there were no significant differences in germination percentage between treatments of all four plant species. The test plants, however, responded to changes through variability of root length (cucumber and barley) and shoot length (mustard, cucumber and maize). Given the above, germination was the least sensitive to changes in water quality. The results of this study showed that, as regards the assessment of water quality using seed germination as the parameter, deionized water was the most appropriate for mustard, distilled water for cucumber, and distilled deionized water for barley and maize. Regarding root length, deionized water was the most appropriate for mustard, tap water for cucumber, distilled water for barley and deionized water for maize. As regards shoot length, deionized water was most suitable for mustard growth, alkaline water for cucumber, and tap water for barley and maize. Apart from this, one should also consider the presence/absence of significant differences between these treatments, and instructions given by standard protocols.

Key words: plant indicators, seed germination, root length, shoot length, water quality

Introduction

Monitoring the quality of water used in agriculture is becoming increasingly important, particularly given the ecotoxicological point of view, because polluted water, through crop irrigation reaches food chains and indirectly affects human and animal health. Besides the abovementioned, it is necessary to consider the possibility of waste water use in crop irrigation, because it helps reduce the pressure on natural resources of fresh water, which is extremely important in terms of reproducibility of these sources (Kanae 2009). Developed countries intensively promote and improve waste water treatment systems, due to its potential use in agriculture, which, however requires a detailed evaluation of water quality effect on agricultural plants (Tal 2006). To prevent the negative impact of contaminated water on crops, it is important to conduct continuous evaluation of water quality. It is a well-known fact that some plants are very sensitive to high content of pesticides, heavy metals and organic substances in soil and water. They react showing different morphological and physiological changes, thus being successfully used as bioindicators of water contamination, test plants in bioassays for contamination detection and assessment of ecotoxicological risk, and are a crucial part of regulatory programs (Wong and Bradshaw 1982; Ankley *et al.* 1993; Gong *et al.* 2001, Angelopoulos *et al.* 2009, Kungolos *et al.* 2011). A number of standardized phytotoxicity tests refer to germination, root elongation and shoot length as parameters that reliably indicate changes in water quality (OECD 1984, US EPA 1996, AFNOR 1982, 1993). According to Wang, (2001) germination and root elongation, being fast indicative phytotoxicity parameters, have several advantages, including high sensitivity, ease of implementation and assessment, low cost and a convenience for a wide range of substances and large number of samples. The aim of this study was to evaluate suitability of certain physiological and morphological parameters of several cultivated plants (mustard, cucumber, barley and maize), as indicators of water quality, but at the same time to define the optimal water (alkaline, acid, tap, deionized and distilled) for mentioned plant species to serve as control variant in biotests.

Material and methods

In laboratory conditions, assessment was made of the effect of four waters of different quality (pH 11.4 alkaline, acidic pH 4.6, tap, deionized and distilled) on physiological and morphological parameters of mustard (*Sinapis alba* L.) cultivar Torpedo, cucumber (*Cucumis sativus* L.) cultivar Typhoon, barley (*Hordeum vulgare* L.) hybrid Novosadski 525 and maize seed (*Zea mays* L.) hybrid NS 6030. These species are recommended by numerous protocols (OECD 1984, US EPA 1996, AFNOR 1982, 1993) for testing chemicals

phytotoxicity, due to high sensitivity. Twenty-five seeds per replication were placed in plastic boxes (21x15x5cm) on pleated filter paper previously moistened with 25 ml of tested water. The boxes are designed to fit one into another, while the upper one is closed with a plastic lid to prevent evaporation. Seeds are incubated in dark, at a temperature of 20-25 °C for seven days for maize, barley and mustard and eight for cucumber, after which germination (%), root length (cm) and shoot length (cm) measured. These conditions are prescribed by the Regulation on the Quality of Seed of Agricultural Plants (Official Gazette, 2002). The whole experiment was carried out in four replications. The data were processed by statistical software STATISTICA 10, using Duncan's multiple comparison test and non-parametric Kruskal-Wallis test, when distribution of variables deviated from *normal (Gaussian) distribution*. The confidence interval was 95%.

Results and discussion

An important step in assessing water quality is certainly an adequate choice of test organisms and selection of parameters that reliably indicate changes in medium (surface and pore water or sediment). According to several authors (Mahmood *et al.* 2005, Ling *et al.* 2010, Wang *et al.* 2001) germination, root elongation and shoot length are the most authoritative parameters that indicate changes in environmental quality. The results of these tests indicate that the sensitivity of the test plants in tested samples manifested as different intensity of changes in observed parameters. It is also supported by the statement of Liu *et al.* (2005), indicating that the sensitivity of plants to certain contaminants (e.g. heavy metals) in water depends not only on the concentration and type of pollutants, but also on the development stages of plants (germination, emergence, vegetative growth).

Germination

Mustard seed germination (Tab. 1.) was highest in alkaline water (80%), followed by tap, denionized and acidic water (79%), and lowest in distilled water (71%). The results of one-way ANOVA indicate that differences in mustard germination depending on the water quality were not significant ($F=0.623NS$, $p>0.05$). However, one should bear in mind that the minimal germination for mustard seed, defined by the Regulation cited above, is 75%, and was not achieved in distilled water. These results suggest that distilled water is not an appropriate medium for the cultivation of mustard, variety Torpedo. Further research with other cultivars is required in order to exclude possible varietal sensitivity. Cucumber seeds germinated best in acidic water (97%), then in distilled and deionized (95%), tap (91%), and the lowest percentage was

registered in alkaline water (90%), but the variances of these groups were at the same level of significance ($F=1.833NS$, $p>0.05$). The highest percentage of barley seed germination was recorded in distilled water (99%), followed by tap, deionized and acidic water (97%), and the lowest in alkaline water (92%), with no significant difference observed between treatments ($F=0.638NS$, $p>0.05$). Germination of maize seeds was 100% in distilled water, followed by deionized (99%), acidic (97%) and tap (96%) water, and was lowest in alkaline water (92%), but all were at the same level of significance ($H=7.569NS$, $p>0.05$). In the case of maize germination, we used non-parametric Kruskal-Wallis test, due to the deviation of data from normal (Gaussian) distribution.

The results hereby presented indicate that water quality had no effect on germination, since there were no significant differences in values of this parameter between water treatments (alkaline, acidic, tap, deionized and distilled water) of all four plant species. Also, germination of all species, except in the case of mustard in distilled water, was within the norms stipulated by Regulation on the Quality of Agricultural Plants (Mustard-75%, cucumber-80%, barley-88% and maize-90%).

Tab 1. Seed germination (%) differences in water of different quality

Parameters	Water	Plant species			
		mustard	cucumber	barley	maize
Germination (%)	alkaline	80±0.07 a	90±0.03 a	95±0.03 a	92±0.03 a
	acidic	79±0.74 a	97±0.02 a	97±0.01 a	97±0.02 a
	tap	79±0.01 a	91±0.01 a	97±0.02 a	96±0.01 a
	deionized	79±0.04 a	95±0.02 a	97±0.01 a	99±0.01 a
	distilled	71±0.50 a	95±0.02 a	99±0.01 a	100±0.00a
	F value	0.626 NS	1.791 NS	0.660 NS	
	H value				7.569 NS

Average values±SD. Each value with the same letter is at the same level of significance for 95% interval (Duncan's multiple comparison test F values, and Kruskal-Wallis test-H values).

The results achieved in this assay may help in choosing water for the control variant in tests that involve these plants as indicators of water quality. Numerous protocols suggest the use of distilled and deionized water as control variants. The results of this study showed that, for water quality assessment using seed germination as the parameter, the most appropriate water was deionized water for mustard, distilled water for cucumber, and distilled deionized water for barley and maize. Some authors, however, present different results. Bojović et al. (2009) and Srivastava and Singh (2009) reported that distilled water is the optimal variant for control experiments with mustard. Wang et al. (2001) point

out that cucumber germination in deionized water was within acceptable norms, and Mahmood et al. (2005) reported that deionized water was successfully used as a control option in the experiment with maize (% germination 99.5%). Both statements are in agreement with our results.

Root length

Mustard root length was the longest in deionized water (4.32cm), followed by distilled, alkaline, acidic, tap and alkaline waters (2.87, 2.71, 2.36, 2.03cm, respectively) ($H=7.243$ NS, $p>0.05$), but all were at the same level of significance (Tab. 2). In this case we used non-parametric Kruskal-Wallis test, due to data deviation from normal (Gaussian) distribution.

Root length of cucumber plants ranged from 11.46cm in tap water, 9.09cm in alkaline, 8.89cm in distilled, 8.48cm in deionized and 7.38cm in acidic water. However, highly significantly longer roots were registered only in tap water as compared to others ($F=6.858^{**}$, $p<0.001$) which were at the same level of significance. The results of Wong et al. (1982) state the opposite showing that cucumber root growth was reduced in tap water, unless calcium nitrate was added. The longest root of barley was measured in distilled water (14.48 cm), then in deionized (4.37cm), acidic (13.88cm), tap (13.34cm), and lowest in alkaline water (13.05cm). The significance of differences between root length was observed only in distilled and deionized water compared to other variants, which were at the same level of significance ($F=3.054^{**}$, $p<0.001$).

Tab. 2. Root length differences in water of different quality

Parameters	Water	Plant species			
		mustard	cucumber	barley	maize
Root length (cm)	alkaline	2.03±0.16 a	9.09±0.10 a	13.05±0.02 a	10.91±0.06 a
	acidic	2.71±0.23 a	7.38±0.05 a	13.88±0.02ab	13.09±0.05 a
	tap	2.36±0.17 a	11.46±0.03b	13.34±0.03ab	13.67±0.03 a
	deionized	4.32±0.09 a	8.49±0.03 a	14.37±0.02 b	13.72±0.01 a
	distilled	2.87±0.15 a	8.89±0.02 a	14.48±0.02 b	13.41±0.13 a
	F value		6.712 **	3.109 *	1.411 NS
	H value	7.243 NS			

Average values±SD. Each value with the same letter is at the same level of significance for 95% interval (Duncan's multiple comparison test-F values, and Kruskal-Wallis test-H values).

The root of maize was the longest in distilled water, followed by tap, deionized and acidic water, and the shortest in alkaline water (13.72, 13.67, 13.41, 13.09, 10.91cm, respectively). However, all values are at the same level of significance ($F=1.567$ NS, $p>0.05$). According to Mahmood *et al.* (2005),

deionized water was successfully used as a control variant in an experiment with maize varieties, which is in accordance with our results.

The results of this assay indicate that root length when using cucumber and barley as test plants, has good potential in indicating changes in water quality, given that the roots of these species responded to changes in length variability.

Shoot length

Shoot length of mustard as presented in Tab. 3. was significantly higher in deionized (3.37cm) and tap water (3.17cm) as compared to distilled water (2.31cm) ($F = 2.462^*$, $p < 0.05$), while being at the same level of significance in alkaline (2.56cm) and acidic water (2.45cm).

Tab. 3. Shoot length differences in water of different quality

Parameters	Water	Plant species			
		mustard	cucumber	barley	maize
Shoot length (cm)	alkaline	2.56±0.07 ab	10.72±0.05b	12.03±0.02a	3.47±0.07 a
	acidic	2.45±0.13 ab	10.09±0.04b	12.33±0.02a	5.76±0.07bc
	tap	3.17±0.09 ab	8.45±0.03 a	12.59±0.10a	6.43±0.1 c
	deionized	3.37±0.09 b	9.71±0.02 ab	11.17±0.03a	4.65±0.05 b
	distilled	2.31±0.08 a	9.41±0.03a	11.45±0.02a	4.96±0.05bc
	F value	2.426 *	4.410*	0.669 NS	7.505 **

Average values±SD. Each value with the same letter is at the same level of significance for 95% interval (Duncan's multiple comparison test- F values, and Kruskal-Wallis test-H values).

Cucumber plants also expressed sensitivity to changes in water quality, showing variability of shoot length. Given this, the highest growth was recorded in alkaline (10.72cm) and acidic water (10.09cm) which are at the same level of significance, but was significantly longer than shoot length in distilled (9.41cm) and tap water (8.45cm)($F=4.410^*$, $p < 0.01$). The values of this parameter in deionized water (9.71cm) were not significantly different from other variants.

Barley did not express differences in shoot length between treatments, although the longest was recorded in tap water (12.59cm), and lowest in deionized (11.17cm) ($F=0.669NS$, $p > 0.05$).

High variability of shoot length was noted in maize sprouts. The longest was recorded in tap (6.43cm) and acidic water (5.76cm), which were at the same level of significance, but significantly higher than shoots in distilled water (4.96cm), and highly significantly longer than those in deionized (4.65cm) and alkaline water (3.47cm).

In this assay shoot length proved to be a very sensitive parameter that detects changes in water quality, given the fact that mustard, cucumber and maize sprouts showed high variability in length in different waters.

Conclusion

The results obtained in this assay suggest that germination was not affected by water quality, as mustard, cucumber, barley and maize seeds did not express variability values of this parameter. Root length of cucumber and barley, on the contrary, responded to water quality changes through length variability, suggesting good potential in indicating changes in media when these two species are used as test plants. Sensitivity of mustard, cucumber and maize to water quality manifested through variations in shoot length that can be considered a reliable parameter for detection of changes in water quality. The results of this study showed that, for the assessment of water quality, regarding seed germination the most appropriate water was deionized water for mustard, distilled water for cucumber, distilled deionized water for barley and maize. Regarding root length, the most appropriate water for the bioassay was deionized water for mustard, tap water for cucumber, distilled water for barley and deionized water for maize. As regards shoot length, deionized water was the most appropriate water for mustard, alkaline water for cucumber and tap water for barley and maize. Apart from these results, one should also consider the presence/absence of significant differences between these treatments, and instructions given by standard protocols.

References

- AFNOR, X31-201. (1982). Essai d'inhibition de la germination de semences par une substance.
- AFNOR X31-203/ISO 11269-1. (1993) Determination des effets des polluants sur la flore du sol: Méthode de mesurage de l'inhibition de la croissance des racines.
- Angelopoulos, K., Paraskeva, C.A., Emmanouil, C. (2009): Phytotoxicity of olive mill wastewater fractions and their potential use as a selective herbicide, paper work, www.srcosmos.gr/srcosmos/showpub.aspx
- Ankley, G.T., Benoit, D.A., Hoke, R.A., Leonard, E.N., West, C.W. (1993): Development and evaluation of test methods for benthic invertebrates and sediments: Effects of flow rate and feeding on water quality and exposure conditions, Archives of Environmental Contamination and Toxicology, **25**, 12-19
- Ecological Effects Test Guidelines OPPTS 850.4000 Background-Nontarget Plant Testing
- Bojović, B., Đelić, G., Topuzović, M., Stanković, M. (2010): Effects of NaCl on seed germination in some species from families Brassicaceae and Solanaceae. *Kragujevac j. sci.*, **32**, 83-87

- Gong, P., Wilke, B.M., Strozzi, E., Fleischmann, S. (2001): Evaluation and refinement of a continuous seed germination and early seedling growth test for the use in the ecotoxicological assessment of soils, *Chemosphere*, **44**, 491-500
- Kanae, S. (2009): Global warming and the water crisis. *J Health Sci*, **55**, 860–864
- Kungolos, A., Emmanouil, C., Bakopoulou, A., Petala, M. (2011): Bioassays as a means of ecotoxicological evaluation of wastewater quality. *Environ Sci Pollut Res*, **74**, 188-194
- Ling, T., Fangke, Y., Jun, R. (2010): Effect of mercury to seed germination, coleoptile growth and root elongation of four vegetables. *Research journal of phytochemistry*, **4**, 225-233
- Liu, W. H., Zhao, J. Z., Ouyang, Z. Y., Soderlund, L., Liu, G. H. (2005). Impacts of sewage irrigation on heavy metals distribution and contamination. *Environment International*, **31**, 805–812
- Mahmood, S., Hussain, A., Saeed, Z., Athar, M. (2005): Germination of seedling growth of maize (*Zea mays* L.) under varying levels of copper and zinc. *Int.J.Environ.Sci.Tech.*, **2**, No 3, 269-274
- OECD Guideline for Testing of Chemicals 208. (1984). Terrestrial Plants, Growth Test Regulation on the quality of agricultural plants (Official gazette of SFRJ 47/87, 60/87, 55/88, 81/89, "Sl. list SRJ", no. 16/92, 8/93, 21/93, 30/94, 43/96, 10/98, 15/2001 and 58/2002
- Srivastava, A.K., Singh, A.K. (2009): Effects of insecticide profenophos on germination, early growth, meiotic behavior and chlorophyll mutation on barley (*Hordeum vulgare* L.). *Acta Physiol Plant*, **31**, 537-544
- Tal, A. (2006): Seeking sustainability: Israel's evolving water management strategy. *Science*, **313**, 1081–1084
- US EPA, OPPTS Ecological Effect Guideline, 850 Series. (1996).
- 850.4000: Background - Non-target Plant Testing.
 - 850.4025: Target Area Phytotoxicity
 - 850.4100: Terrestrial Plant Toxicity, Tier I (Seedling Emergence)
 - 850.4200: Seed Germination/Root Elongation Toxicity Test
 - 850.4225: Seedling Emergence, Tier II
 - 850.4230: Early Seedling Growth Toxicity Test
- Wang, X., Sun, C., Gao, Shixiang, Wang, L., Shokui, H. (2001): Validation of germination rate and root elongation as indicator to assess phytotoxicity with *Cucumis sativus*. *Chemosphere*, **44**, 1711-1721
- Wong, M.H., Bradshaw, A.D. (1982): A comparison of the toxicity of heavy metals, using root elongation of rye grass, *Lolium perenne*. *New Phytol.*, **91**, 255-261

KLIJAVOST I DUŽINA KORENA I IZDANKA KAO POKAZATELJI KVALITETA VODE

- originalni naučni rad -

**Sonja Gvozdenc, Dušanka IndiĆ, Slavica Vuković, Mila Grahovac,
Milena Vrhovac, Źarka Bošković, Nina Marinković**
*Poljoprivredni fakultet,, Trg Dositeja Obradovića 8,
Novi Sad, Srbija - sonjag@polj.uns.ac.rs*

Rezime

Ispitivana je klijavost semena, dužina korena i dužina izdanka slačice (*Sinapis alba* L.), krastavca (*Cucumis sativus* L.), ječma (*Hordeum vulgare* L.) i kukuruza (*Zea mays* L.) da bi se utvrdio uticaj kvaliteta vode na rast biljaka. Analiza parametara vršena je nakon sedam (kukuruz, ječam i slačica) i osam dana (krastavac). Rezultati su pokazali da kvalitet vode nije uticao na klijavost, jer nisu utvrđene značajne razlike u stepenu klijavosti među varijantama kod sve četiri ispitivane biljne vrste. Međutim, ispitivane biljke ispoljile su reakciju na promenu kvaliteta vode u pogledu varijabilnosti dužine korena (krastavac i ječam) i dužine izdanka (slačica, krastavac i kukuruz). S obzirom na navedeno, utvrđeno je da je klijavost najmanje osetljiva na promene kvaliteta vode. Rezultati istraživanja pokazali su da je, za ocenu kvaliteta vode na osnovu klijavosti semena kao parametra, dejonizovana voda imala najpovoljniji uticaj na slačicu, destilovana voda na krastavac, a destilovana i dejonizovana voda na ječam i kukuruz. U pogledu dužine korena, slačici je najviše odgovarala dejonizovana voda, krastavcu vodovodska voda, ječmu destilovana voda, a kukuruzu dejonizovana voda, a kad je reč o dužini izdanka, na rast slačice najviše je uticala dejonizovana voda, na krastavac alkalna voda, a na ječam i kukuruz vodovodska voda. Osim toga, u obzir bi trebalo uzeti i prisustvo/odsustvo značajnih razlika između tretmana i uputstva data u standardnim protokolima.

Ključne reči: biljni pokazatelji, klijavost semena, dužina korena, dužina izdanka, kvalitet vode