Biological potential of barley (*Hordeum vulgare*) and radish (*Raphanus sativus*) in water quality monitoring

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Abstract. Quality and impact of water characterized by its high content of certain pollutants used for watering of the tested plants: barley (*Hordeum vulgare*) and radish (*Raphanus sativus*). was tested in laboratory conditions. Water sampling was performed at two locations on the River Douro in Portugal. Physico-chemical analysis of water indicates that. according to Portuguese regulations for water quality, electrical conductivity, ammonia, iron (Fe), magnesium (Mg) and some heavy metals (arsenic (As), selenium (Se)) were in values that exceed the maximum allowable concentration. The pharmaceuticals (paracetamol, naproxen, ibuprofen, hydrochlorothiazide and azithromycin) were registered over the limit of detection in water sample Douro I, contrary to that, in all analyzed water samples pesticide substances were below the detection limit. Increased amounts of As, Se and Mg from the water sample Douro I caused stimulation of fresh & dry weight of radish shoot and its length by 30, 23 and 71% respectively. In contrast, seed germination & germination energy of radish were significantly inhibited by the same water, while in the case of barley there was no significant differences. Douro II water sample significantly stimulated length and fresh weight of roots of barley by 23 and 81%, compared to the control. These effects are attributed to the presence of iron in a greater amount in this water sample. Morphological factors have proved better in relation to physiological factors. An expressed variability of parameters indicates their potential as possible bioindicators.

Keywords: Douro river, pollutants, phyto-indicators, barley, radish

1. Introduction

The steady growth of human population and technological development has resulted in an increase in water demands. Amount of drinking water is decreasing and available quantity is constantly polluted by various factors. Agriculture is one of the major users of the water resources, but also it is one of the main sources of diffuse water pollution. Problems occur due to improper and unprofessional utilization of chemical agents used by farmers. On a large scale production use of chemical resources is necessary but it is crucial to apply them at the right time and in an appropriate concentration. In addition to agriculture the major polluters are: industry, municipal wastewater, waterway traffic, accidents, etc. Water is the most widespread substance on the planet and as such is the foundation of life, but only a small fraction of this amount is available for people needs. In addition to the amount of water that is available it is also important to know its quality. Quality of water is expressed through biological. chemical and physical parameters. To prevent and reduce environmental pollution. it is essential to carry out the continuous monitoring of water quality. Methods that involve the use of cultivated plants as test organisms are extremely important for assessing the contamination of water used in agricultural production, because its results reflect the benefits of the area for cultivation of plants and the use of water for irrigation. The aim of the study was to determine the degree of water pollution through morphological (germination energy, germination) and physiological parameters (length of roots and shoots, fresh/dry weight of roots and shoots) by use of test plants, barley (*H. vulgare*) and radish (*R. sativus*).

2. Methods

In 2014, water sampling was conducted by experts from "Northern Region Water Institute"- IAREN (Instituto da Água da Região do Norte) from Portugal. Water was sampled from two sites along the River Douro in Portugal. Physico-chemical analysis of water was also conducted in Portugal and includes the following parameters: general parameters of water quality. organic compounds. heavy metals. pesticides. pharmaceuticals. For chemical analysis. the following techniques were used: Atomic Adsorption Spectrometry-Flame Technique (EPA Method 7000B). Liquid Chromatography-Tandem Mass Spectrometry (LC-MS-MS). Gas Chromatography Mass Spectrometry (GC-MS) and Inductively Coupled plasma Mass spectrometry (ICP-MS). For the extraction that preceded detection of the presence of pollutants Solid Phase Extraction (SPE) and Accelerated Solvent Extraction (ASE) were used. The maximum allowable quantities (MAC) used in this experiment are stipulated by Portuguese regulations Decreto-Lei nº 103/2010. D.-Lei nº 236/1998 and the EU Directive 2008/105/EC. Evaluation of water quality was performed by use of physiological (germination energy and

germination /%/) and morphological parameters (length of roots and shoots of seedlings /cm/, fresh and dry weight of roots and shoots of seedlings /g/) of the test plants. A filter paper method by ISTA (International rules for seed testing) for 2013 was used. Results for physiological parameters are expressed in percentages. The values of morphological parameters are shown as average values and are processed using the Analysis of Variance (ANOVA) and Duncan's multiple comparison test, in the statistical software R ver. 3.2.2.

3. Results

3.1 Physico-chemical analysis of water

According to the results of physico-chemical analysis. in water sample Douro I electrical conductivity exce-eded maximum allowed values by 7.5x and ammonium by 14x (tab. 1). Electrical conductivity. concentration of salt in the water, is an essential characteristic of water for irrigation and it significantly affects the crops productivity. The primary effect of high salt concentration is reflected in the inability of the plant to uptake water from the soil solution which leads to a physiological drought. The higher the conductivity. the less water is available to plants (Bauder *et al*., 2014). Sample Douro I excess limit values for conductivity, so this water does not meet the required quality for irrigation. Exceeding level of ammonium (1.2x) was also detected in the sample Douro II, however, water from this sample is a less polluted than the Douro I. The results of chemical analysis on the contents of heavy metals and other parameters from the list of priority pollutants. indicates an extremely high amount of selenium (Se), arsenic (As) and magnesium (Mg) in the water from the sample Douro I, in quantities that exceed MAC according to the mentioned regulations. Also high amounts of the iron (Fe) are found in a water sample Douro II (tab. 2).

Most of the heavy metals are extremely toxic because of their solubility in water (Arora et. al., 2008). Today As is no longer in use. but it is necessary to determine it's residual effect. At sufficiently high concentrations. As interferes with critical metabolic processes in plants, which can lead to death (Finnegan and Weihua, 2012). According to the research conducted by Hartikainen *et al*. (2000), high concentrations of selenium in plants also leads to harmful effects. which are reflected in the reduction of biomass and inhibition of seed germination. Excess amounts of Mg^{2+} in the plants might inhibit photosynthesis and plant growth. particularly during dehydration (Shaul, 2002). Toxicity of iron has not been reported under most aerobic plant production systems (Hochmuth, 2011). Increased amounts of As, Se and Mg from the water sample Douro I caused stimulation of fresh & dry weight of radish shoot and its length. so these results are not in accordance with the previously presented claims. But, radish germination energy and germination were notably inhibited by same water, and these results back up mentioned claims. In recent years. the occurrence and fate of pharmaceutically active compounds (PhACs) in the aquatic environment has been recognized as one of the emerging issues in environmental chemistry (Heberer, 2002). The main sources of pollution of surface and ground waters with these compounds are urban and agricultural waste waters or households, hospitals and agricultural lands (Robinson *et al*., 2007). Chemical analysis of pharmaceutical contents. in water sample Douro I, showed that the following pharmaceuticals were registered over the limit of detection: paracetamol, naproxen, ibuprofen, hydrochlorothiazide and azithromycin. The test parameters of sample Douro II were below the limit of detection.

Pesticide substances were also below the limit of detection in all analyzed water samples.

Table 1. Physico-chemical analysis of the general parameters in analyzed water samples

Location	Detected values of general parameters							
	pH	**EC (mS/cm) at 20˚C**	**t°C**	**NO_3^- mgN/l**	**NO_2^- mgN/l**	**NH_3 mgN/l**	**P mgP/l**	**B mgB/l**
Douro I	7.6	**7450**	18.1	4.8	<0.1	**0.7**	0.12	0.5
Douro II	7.6	240	17.5	4.6	<0.1	**0.06**	0.03	<0.1
***MAC**	**5-9**	**≤1000**	**30.0**	**10.0**	**1.0**	**0.05**	**1.0**	**1.0**

Table 2. Content of heavy metals & other elements from the list of priority water pollutants in analyzed samples

Location	Detected values of heavy metals and other elements									
	Cd (µg/l)	**Se (µg/l)**	**As (µg/l)**	**Mo (µg/l)**	**Cr (µg/l)**	**Pb (µg/l)**	**Mn (µg/l)**	**Fe (µg/l)**	**Zn (µg/l)**	**Mg (mg/l)**
Douro I	<0.5	**47**	**15**	<0.01	5	<0.2	11	95	<10.0	**210**
Douro II	<0.5	<2.5	2.6	<0.01	5	<0.2	17	**170**	<10.0	6.2
***MAC**	**5**	**10**	**10**	**50**	**50**	**50**	**100**	**100**	**500**	**50**

**MAC - Maximum allowable concentration*

3.2 Bioassay results - barley and radish

The obtained results (tab. 3-4) indicate differences in tolerance of the test plants according to the parameters which have been detected in the water. Although the differences between control and water samples were significant, values for germination and germination energy were above the prescribed limits for those plant species. Based on this results it can be concluded that physiological parameters (germination energy and germination), in both cases, have not been proven to be good indicators of water quality. while some morphological parameters of barley (length and fresh weight of root) and radish (length and fresh/dry weight of root) that reacted by stimulation may be considered more reliable. Other morphological parameters were not affected by water quality and all values are on the same level of significance. compared to the control. Root length and fresh weight of barley were significantly stimulated by water from Douro II (23 and 81%), compared to the control. Shoot length and its fresh/dry weight of radish were also stimulated by water from sample Douro I (30, 23 and 71% respectively), compared to the control. Differences between the mentioned treatments are statistically significant.

Barley and radish proved that they could be used as good indicators of water quality, which is contaminated with specified pollutants.

Table 3. Water quality influence on physiological parameters

Parameters	Water sample	Barley	Radish
Germination energy (%)	Douro I	95.00 ±1.15 a	94.00 ±1.40 b
	Douro II	94.50 ±1.29 a	99.00 ±0.80 a
	Control	96.00 ±4.32 a	95.75 ±0.96 a
	F value	**0.323 ns**	**26.58 ***
Germination (%)	Douro I	95.75 ±1.70 a	97.75 ±1.70 b
	Douro II	95.25 ±1.70 a	99.00 ±0.80 a
	Control	96.75 ±2.98 a	99.00 ±0.80 a
	F value	**0.475 ns**	**1.47 ***

Table 4. Impact of water quality on morphological parameters

Parameters	Water sample	Barley	%	Radish	%
		Values		Values	
Length of root (cm)	Douro I	8.52 ±1.01 b	88	5.97 ±0.42 a	111
	Douro II	11.9 ±1.66 a	123	5.80 ±0.76 a	107
	Control	9.70 ±1.19 b	100	5.40 ±0.37 a	100
	F value	**6.77 ***		**0.99 ns**	
Fresh weight of root (g)	Douro I	1.03 ±0.11 b	127	0.19 ±0.03 a	119
	Douro II	1.47 ±0.24 a	181	0.22 ±0.04 a	138
	Control	0.81 ±0.20 b	100	0.16 ±0.08 a	100
	F value	**16.48 ***		**1.39 ns**	
Dry weight of root (g)	Douro I	0.11 ±0.004 b	92	0.019 ±0.001 a	61
	Douro II	0.14 ±0.020 a	117	0.016 ±0.002 a	52
	Control	0.12 ±0.010 ab	100	0.031 ±0.032 a	100
	F value	**3.36 ***		**0.84 ns**	
Length of shoot (cm)	Douro I	6.27 ±1.69 b	73	7.02 ±0.31 a	130
	Douro II	10.4 ±3.57 a	122	5.80 ±0.52 b	107
	Control	8.55 ±1.60 ab	100	5.40 ±0.47 b	100
	F value	**2.87 ***		**14.26 ***	
Fresh weight of shoot (g)	Douro I	2.04 ±0.22 b	88	2.13 ±0.15 a	123
	Douro II	2.66 ±0.33 a	115	1.93 ±0.23 ab	112
	Control	2.31 ±0.26 ab	100	1.73 ±0.14 b	100
	F value	**5.21 ***		**5.03 ***	
Dry weight	Douro I	0.20 ±0.02 a	100	0.128 ±0.01 a	171

of shoot (g)	Douro II	0.23 ±0.04 a	115	0.091 ±0.01 ab	121
	Control	0.20 ±0.02 a	100	0.075 ±0.04 b	100
	F value	**1.19 ns**		**3.06 ***	

4. Conclusion

Based on the conducted tests and the results achieved on the influence of water quality (Douro I, Douro II) on the test plants (barley, radish) it can be concluded that:

- In the water sample from the Douro river site I, detected pollutants. in the quantities exceeding MAC according to the Regulations, were: arsenic (As) selenium (Se), magnesium (Mg), ammonia (NH3) and electrical conductivity (EC). The pharmaceuticals (paracetamol. naproxen. ibuprofen. hydro-chlorothiazide and azithromycin) were also detected. Based on the biological test of water quality on phytoindicators. plants reacted in a significant stimulation of the length, fresh/dry weight of shoot (radish), which can be attributed to the presence of selenium and ammonium. In contrast, seed germination & germination energy of radish were significantly inhibited by water sample Douro I, which can be correlated with the increased content of As.

- In a sample of water from the site Douro II in quantities exceeding MAC, iron (Fe) & ammonium were registered and none of the tested pharma-ceuticals. Water significantly stimulated length and fresh root weight of barley. These effects are attributed to the presence of iron in a greater amount in this water sample.

Bioassay test results indicate the different sensitivity of tested plant species and parameters as well as their validity in assessing water contamination. An expressed variability of parameters indicates their potential as possible bioindicators.

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