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Duckweed as a Test Organism for Eco toxicological Assessment of Wastewater

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Abstract: ***A chemical-specific approach is not sufficient to characterize the wastewater quality. Therefore, it is essential to use biological test systems with living cells or organisms that give a global response to the quantity of micropollutants present in the wastewater sample. Duckweed can be used in eco toxicological assessment of wastewater to monitor heavy metals and other aquatic pollutants, because duckweed may selectively accumulate certain chemicals. The plants possess certain physiological properties like small size, rapid growth (almost double in two days) between pH 5 and 9, and vegetative propagation, which make them an ideal test system. The objective of the present study is to evaluate the sensitivity of duckweed plant towards selected parameters for screening and biomonitoring complex effluent samples. In the various studies the possible phyto- and genotoxic effects of partially treated industrial effluent waters were investigated. Obtained data from various studies demonstrate the relevance of duckweed as sensitive indicators of water quality.***

Keywords: Wastewater, Micro pollutant, Duckweed, Eco toxicological assessment, Genotoxic effects.

1. Introduction

Heavy metal pollution in waters is a very serious environmental problem with potentially harmful consequences for agriculture and human health. The modern agricultural practices and the industrial activities have polluted waters with great amounts of heavy metals and other micro pollutants. Sources of anthropogenic metal contamination include smelting of metalliferous ore, electroplating, gas exhaust, energy and fuel production, the application of fertilizers and municipal sludges to land, and industrial manufacturing (Raskin et al. 1994; Cunningham et al. 1997; Blaylock and Huang, 2000). Heavy metal contamination of the biosphere has increased sharply since 1900 (Nriagu, 1979) and poses major environmental and human health problems worldwide (Ensley, 2000).The presence of enormous number of potentially polluting substances contained in waste waters from municipal and environmental sources, needs a dependable source that can provide the information about water quality. Duckweed (Lemna minor) is used in many studies to monitor various types of pollutants. The plants possesses physiological properties (small size, rapid growth between pH 5 – 9, and vegetative propagation), that make duckweed an ideal test system. Among the developmental parameters, the most commonly assessed in ecotoxicological test systems, are growth parameters (Wang and Freemark 1995). The ideal plants for ecotoxicological studies should possess the ability to tolerate and accumulate high levels of heavy metals in their harvestable parts, while producing high biomass. Many species of macrophytes are used for this purpose. One of the most commonly used aquatic plant is duckweed belonged to the family of Lemnaceae. In particular, Duckweed is a free-floating, aquatic perennial plant that forms a rapidly-expanding mat of foliage (to 1/4" tall) on water surface, able to remove and accumulate large amounts of pollutants principally through the fronds (Zayed et al. 1998). Thus, this study was carried out to evaluate the ability of duckweed plant to be used as a test organism for ecotoxicological studies.

2. Review Work

There are several studies that supported the fact that most Lemna spp. show an exceptional capability and potential for the uptake and accumulation of heavy metals as well as metalloids, surpassing that of algae and other aquatic macrophytes. Many studies support Lemnaceae (duckweed) for its greatest capacity in heavy metal removal as well as organic matter removal which make this plant an ideal plant for ecotoxicological stydies. Sandra Radic et al., 2009 conducted a study on Lemna minor L.In their study, standardized protocol ISO/DIS 20079 (2004) was applied and the duckweed plants are exposed to a toxicant over a period of 7 days, to observe the growth inhibition effect on the plant. Duckweed growth was determined by measuring frond number (FN), fresh weight (FW, biomass) and dry weight (DW), according to the ISO 20079 test protocol. The frond number was scored at the start of the experiments (t0) and 7 days after (t1). All the parameters were studied e.g. the photosynthetic pigment contents, Lipid peroxidation, Heavy metal, Chlorophyll a (chl a), b (chl b) and carotenoid contents and the activity of POD were measured according to the standardized methods. The results obtained suggest that phyto- and genotoxicity tests with L. minor should be used in the biomonitoring of municipal, agricultural and industrial effluents because of their simplicity, sensitivity and cost-effectiveness. According to ecotoxicological study performed by Ivana Soukupova et al. 2010 with duckweed, according to the standard EN ISO 20079, the 7-day toxicity test conducted at 24 ± 2oC in test vessels containing a minimum of 100 ml of test solution and 3-frons plants, which shows the effect of various doses of two defined referent toxicants: potassium chloride (3; 4; 7; 10 and 15 g.l-

1) and 3.5 – dichlorphenol (1.5; 2.1; 3; 4.2 and 5.9 mg.l-1) on Lemna minor. From the resulting values of 168 hours lasting growth inhibition test using water bioindicator Lemna minor (168hEC50) for two referent toxicants they observe a good correlation between conventional (100 ml) test and microbiotest (10 ml). So it clearly shows that microbiological tests for assessing toxic effect of chemicals or other hazardous substances are a suitable alternative to commonly used ecotoxicological biotests. Stefan Gartiser et al., 2010 also supported the fact that the Lemna test would be a suitable method which also detects inhibitors of photosynthesis and is not disturbed by wastewater colouration as in case of algal test due to the complex relationship of colouration and inhibition and the smooth dose–effect relationship or even promotion of algae growth often observed. In the described research, the wastewater samples from paper mills are assessed with regard to persistency (P), presence of potentially bio-accumulative substances (B) and toxicity (T).The growth inhibition was determined by both determining the frond numbers and the frond area after an incubation time of 7 days at defined light conditions (85–135µE m−2s−1 photosynthetically active radiation) at 24±2°C with an imagine analysis system (Scanalyzer, LemnaTec, Germany). Each dilution state was tested in three replicates, the control vessels in six replicates. For the testing of dark-coloured test solutions compared to the algae growth inhibition test, the Lemna test has the advantage of light absorption and thereby resulting growth inhibition is irrelevant. According to test result, duckweed proved to be more sensitive of the two endpoints i.e. frond numbers and frond area. A comparative study of relative sensitivity of three species of Lemna (Lemna gibba, Spirodela polyrhiza and Lemna minor) to compound comprising hexavalent chromium and bivalent zinc as potential toxic metals was done by Vladimír DVOŘÁK et al. 2012. Synthetic Solutions of K2Cr2O7 (200 mg/dm3) and ZnSO4 • 7H2O (100 mg/ dm3) were used as stock standards for the experiment and alternative growth inhibition tests and the standard growth inhibition test with L. minor were performed according to the biotest based on the ISO 20079 protocol. The results showed that the chromium and zinc sensitivity decrease in the order: D. magna > S. polyrhiza > L. gibba > L. minor and D. magna > L. gibba > L. minor > S. polyrhiza, respectively. L. gibba proved the most suitability for assessment of environmental quality due to its highest sensitivity from the tested duckweeds. On the other hand, it proved that the duckweed species may be used for efficient elimination of toxic pollutants and thus may ultimately serve as phytoremediation agents in the natural environment. The Lemna and Spirodela are among the most standardized test organisms in aquatic ecotoxicology (EPA.1996, OECD. 2002). Another study performed by K.P. Sharma et al., 2007 clearly described the fact that advantage of biological test such as on Lemna is even detection of eutrophic potential of wastewaters, as noted at their higher dilutions when toxicity of textile wastewaters (untreated and treated) and their ingredient chemicals was quantified in terms of their chemical characteristics and end point growth responses of duckweed in short-term bioassays. Duckweeds (Blinova, 2000) and submerged macrophytes (Lee et al., 1998; Rai et al., 2003; Kumar and Prasad, 2004) are the test materials for monitoring toxicity of man-made chemicals on primary producers in the aquatic ecosystems, which have an additional advantage of detecting even stimulatory role of pollutants. However, duckweed such as Lemna is the first choice of ecotoxicologists because it is wide spread, fast growing and reproduce faster. It is sensitive to many pollutants, which are assimilated from the growing medium (or aquatic environment) through the underside of the leaf (Greenberg et al., 1992; Becker et al., 2002; Sharma et al., 2006). Another study noted that (Martin Mkandawire et al.,2004), L. gibba can also be used as a preliminary bioindicator for arsenic transfer from substrate to plants and might be used to monitor the transfer of arsenic from lower to higher trophic levels in the affected ecosystem. L. gibba L. can also be used for arsenic phytoremediation of mine tailing waters because of its high accumulation capacity as showed in the study. In this earlier study, Mkandawire and Dudel (2002) showed that this floating macrophyte is capable of accumulating relatively large uranium quantities. Most members of the Lemna genus are used as model plants for phytoremediation, nutrient and metal uptake studies, and bioassays (Ensley et al., 1996).

3. Conclusion & Future Scope

Duckweed is a small free floating macrophyte that can be transported to uncontaminated areas with flowing water. Hence, investigations on the remobilisation and biomineralisation mechanisms of micropollutants in duckweed are required. The results obtained from various studies suggest that phyto- and genotoxicity tests with duckweed should be used in the biomonitoring of municipal, agricultural and industrial effluents because of their simplicity, sensitivity and cost-effectiveness. From the presented study, we conclude that duckweeds are important early warning indicators for the assessment of contaminated ecosystems due to their propensity to accumulate pollutants and biomonitoring potential. More research is needed to address the further development of duckweed technologies.

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