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Content of biogenic amines in *Lemna minor* (common duckweed) growing in medium contaminated with tetracycline

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ABSTRACT

Aquatic plants are continuously exposed to a variety of stress factors. No data on the impact of antibiotics on the biogenic amines in duckweed (*Lemna minor*) have been available so far, and such data could be significant, considering the ecological role of this plant in animal food chains. In the tissues of control (non-stressed) nine-day-old duckweed, the following biogenic amines were identified: tyramine, putrescine, cadaverine, spermidine and spermine.

Based on the tetracycline contents and the computed EC values, the predicted toxicity units have been calculated. The obtained results demonstrated phytoxicity caused by tetracycline in relation to duckweed growth rate, yield and the contents of chlorophylls a and b. The carotenoid content was not modified by tetracycline. It was found that tetracycline as a water pollutant was a stress factor triggering an increase in the synthesis of amines. Tetracycline at 19, 39 and 78 μM concentrations increased biogenic amine synthesis by 3.5 times. Although the content of tyramine increased fourteen times with the highest concentration of the drug (and of spermidine – only three-fold) the increase of spermidine was numerically the highest. Among the biogenic amines the most responsive to tetracycline were spermine and tyramine, while the least affected were putrescine and spermidine. Despite putrescine and spermidine being the least sensitive, their sum of contents increased five-fold compared to the control. These studies suggest that tetracycline in water reservoirs is taken up by *L. minor* as the antibiotic clearly modifies the metabolism of this plant and it may likely pose a risk.

1. Introduction

Water pollution is a major global problem which creates a necessity for constant monitoring of water resources on all levels. It was estimated that contaminated water is the main reason of diseases and deaths accounting for 4% of global mortalities and 5.7% of global disease frequency (Kjellstrom, 1986; Murata et al., 2004; Prüss et al., 2002). In India, 80% of the health issues come from water-borne diseases – caused by drinking contaminated water or using it for hygienic practices (Sengupta et al., 2008). Over 80% of underground water in China is polluted according to a government report (Wu et al., 2015). In 2010, the Ministry of Environment Protection's "State of Environment Report" indicated that 40.1% of China's rivers were unfit for human contact (Grade IV-V+) and 57.2% of the monitored groundwater was badly or very badly polluted (State of Environment Report, 2010). Although this problem is particularly severe in developing countries, it affects developed nations too. For example in a 2004 report on the quality of water in the USA 44% of studied river miles, 64% of lake acres and 30% of estuary sq. miles were described as polluted (Fact Sheet, 2004).

Water pollution affects the whole communities of organisms, including plant and animal inhabitants of water biotopes. In almost all cases not just single populations and species are affected but the whole ecosystems. Various products of human activities (like copper, zinc, manganese, boron and phosphorus, manure, fertilizers, pesticides, detergents, as well as industrial, agricultural and municipal wastes, hospital wastewater, chemicals, pharmaceuticals) pollute rivers, lakes, oceans, aquifers and ground waters (Meck et al., 2006; Vitaku et al., 2013; Knauer, 2016; Walukow, 2016; Zhang et al., 2016).

Abbreviations: TC, tetracycline; Ir, percent inhibition of growth rate; Iy, percent reduction yield; EC_x, effect concentration for the inhibition of growth rate at the level of x % (x = 10 25 50 90); FM, fresh mass; DM, dry mass; BAs, biogenic amines; PAs, polyamines; Cad, cadaverine; Put, putrescine; Spd, spermidine; Spm, spermine; Tyr, tyramine.

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High amounts of medicines are measured in communal wastes, surface and ground waters and even in drinking water (Mompelat et al., 2009). Antibacterial chemotherapy has been applied in aquaculture for over 60 years. In aquaculture antibacterial chemicals have been used mainly on fish farms for therapeutic purposes and as prophylactic agents (Shao, 2001; Hernández Serrano, 2005). In Asian countries, where aquaculture is an important industry, from 500 to 600 t of antibiotics are used prophylactically annually (Moriarty, 1999). The residues of pharmaceuticals can in fact have harmful or even toxic effects on aquatic biota (Orias and Perrodin, 2013).

Tetracycline (TC) is one of the most commonly used antibiotics worldwide (Gao et al., 2012) and since it does not undergo complete mineralization in biodegradation and chlorination processes (Sánchez-Polo et al., 2015), its concentration in waters ranges from 0.225 to 2.25 μM or even amounts to 11.25 μM (Campagnolo et al., 2002; Baquero et al., 2008). If found in water, it affects aquatic organisms to which it is not targeted, such as aquatic vascular plants. It has been proven that 2.5 μM of TC inhibits the growth of *L. gibba* by 50% (Brain et al., 2004). *L. minor* exposed to medicines shows both morphological and biochemical disturbances. If exposed to fluoroquinolones, it has a lower content of photosynthetic pigments and accumulates soluble carbohydrates as osmotic protectants. Nevertheless, ciprofloxacin at 1.25 mM is lethal to *L. minor* (Sikorski et al., 2014). Therefore, aquatic pollutants can cause harmful effects on aquatic species i.e., animal and plants.

Biogenic amines (BAs) are divided into aliphatic, aromatic and heterocyclic amines. Aliphatic amines include polyamines (PAs) – cadaverine (Cad), putrescine (Put), spermidine (Spd) and spermine (Spm). Aromatic amines include tyramine (Tyr). PAs are most often found in the eukaryotes, but recent studies indicate that Spm is found only in angiosperms, whereas thermospermine is found throughout the plant kingdom (Naka et al., 2010; Vera-Sirera et al., 2010). Put, a diamine, has been said to be the most important PA since it is a precursor for other PAs (Parimalan et al., 2011).

PA concentration in plant cells is determined not only by the rate of their synthesis but also by their transport, conjugation, degradation and it can play a significant role in the adaptation mechanism of *Potamogeton crispusm*, species of aquatic plant native to Eurasia, under Cd stress (Yang et al., 2010). Such studies most often exploit cultivated plants and only a few publications have focused on the evaluation of BA (biogenic amine) content in aquatic plants, including *L. minor*. To date, it has been shown that BAs accumulate in *L. minor* exposed to Roundup herbicide (Kielak et al., 2011). There are, however, no reports available on the impact of antibiotics on the content of BAs in *L. minor*, a plant which forms no doubt an important link in the animal food chain.

Our previous studies demonstrated that seedlings of land plants (lupin, pea, soybean) take up antibiotics (tetracyclines, fluoroquinolones) from soil (Piotrowicz-Cieślak et al., 2010; Adomas et al., 2013). In duckweed, as a water plant, xenobiotics are absorbed through all surfaces of the frond. The OECD (2006) guideline 221 for the testing of chemicals recommends checking *Lemna* sp. only with the growth inhibition test procedure. Because BAs of plants participate in many important life processes of, as has been demonstrated in recent years, our aim was to find out what, and how many, BAs are present in duckweed. However, the main focus of our studies was to observe the response of duckweed BAs to increasing tetracycline concentrations because there are no such data in the available literature. Our aim was to find out if BAs are a good parameter for the detection of early tetracycline phytotoxicity. In addition, we described the effect of various concentrations of tetracycline on the morphological characteristics recommended by the OECD, as well as on the pigment contents. The aim of this study was to evaluate the effect of increasing TC concentrations on the growth and yield of duckweed biomass and to identify and assess the contents of BAs.

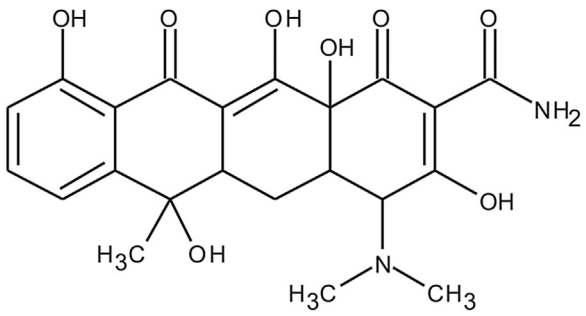

Fig. 1. Chemical structure of tetracycline (TC).

2. Materials & methods

2.1. Plant material

L. minor plants used in the present investigation were taken from the collection at the Department of Plant Physiology, Genetics and Biotechnology, University of Warmia and Mazury in Olsztyn Poland.

2.2. Chemicals

Tetracycline CAS Number 60-54-8, ≥98.0% (NT) was purchased in Sigma Aldrich company (Fig. 1).

2.3. Lemna test

The toxicity of TC (Sigma-Aldrich) to *L. minor* was tested according to the draft OECD 221 (2006) guidelines for testing chemicals. *L. minor* was grown in 10 ml of Steinberg medium in plant growth chamber (ALL–Round–Al 185–4) illuminated with fluorescent lights (140 μmol photon m^{-2} s^{-1} PAR) in a light-to-dark cycle of 16 h/8 h (mean maximum temperature during daytime was 20 °C, and during night time 16 °C) for seven days. The response of *L. minor* to TC concentrations of 0; 0.15; 0.3; 0.6; 1.2; 2.4; 5; 9; 19; 39; 78 μM was determined by percent inhibition of growth rate (Ir), percent reduction in yield (Iy), chlorophyll a and b, carotenoids, BAs content, fresh and dry mass of plant. Ir and Iy were calculated based on the number of fronds and frond area of duckweed according to OECD (2006). FW of new fronds and dry weight of all plants in the sample were determined. Frond area was measured using the computer program Lucia 5.0. Chlorophyll a, b and total carotenoid contents were analyzed spectrophotometrically according to Lichtenthaler and Wellburn (1983).

2.4. Biogenic amine (BA) assay

BAs were extracted from plant material with cold 5% hydrochloric acid (Bouchereau et al., 2000). The extracted plant material was shaken for 1 h and then centrifuged at 16.000*g* for 30 min at temperature of 4 °C. The supernatants were filtered through a 0.22 μm pore nylon membrane syringe filter (Filter-Bio, China) and stored at −20 °C. The filtrate was analyzed by ion-exchange chromatography using amino acid analyzer AAA400 (Ingos, Prague, Czech Rep.). BAs were separated at 76 °C on a 70 × 3.7 mm column filled with Ostion Lg ANB (Ingos, Prague, Czech Rep.) and then eluted from the ion-exchange column with two pH 5.65 sodium citrate buffers with the addition of 1.0 and 2.6 M sodium chloride. The quality and quantity of the BAs were assayed with post-column ninhydrin