Bull. Environm. Contam. Toxicol. 27, 332-337 (1981)

Some Effects of Polyphenols on Aquatic Plants: I. Toxicity of Phenols in Aquatic Plants

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Industrial wastewater is often polluted with significant amounts of phenolic compounds. Phenols in high concentrations are injurious to algae and aquatic spermatophytes. Some aquatic plants are able to eliminate phenolic compounds (SEIDEL 1963, 1965; WERNER & PAWLITZ 1978). Effects of phenolic compounds on growth and metabolism of aquatic plants are poorly understood. In the present work we use rather sensitive physiological parameters as cytoplasmic streaming and motility to analyze the effects of chemically rather different phenolic compounds.

MATERIALS AND METHODS

Test organisms were cultivated as published: Cyclotella cryptica (WERNER 1969), Dunaliella salina (MILKO 1962), Chlamydomonas reinhardii strain 137 (WERNER & PAWLITZ 1978), Lemna minor (WERNER 1967) and Euglena gracilis (MARCENKO 1972). Nitella sp. from Lake Baikal was grown as described previously (STOM et al. 1974). Elodea canadensis was collected in the Angara river and kept for adaptation at least one week. Vallisneria spiralis was received from the museum collection of the Faculty of Biology, State University (Irkutsk) and cultivated according to STOM (1977). E. canadensis was cultivated at 16°C and L. minor at 24°C in petri dishes (20 cm diameter), both at 800 lux and a light dark regime of 9:15 h.

In the experiments with Nitella sp., E. canadensis, L. minor and V. spiralis, phenols were dissolved in tap water; with algae the phenols were dissolved in the media used for cultivation. In the experiments with E. canadensis and L. minor the media were exchanged after 24 h.

Effects on cytoplasmic streaming in E. canadensis and V. spiralis were analyzed according to STRUGGER (1949) and STOM & KOZHOVA (1976), in Nitella sp. according to STOM et al. (1974).

Chlorophyll fluorescence was recorded with a microspectrofluorometer, made by M. N. SAKSONOV & G. V. TRIPUSOV in this laboratory. It is constructed on the basis of a "FLUO VAL" fluorescent microscope and a "SPECOL" monochromator (Carl ZEISS, Jena, GDR). The instrument has a photo-electronic multiplier (FEU-79 and a microvoltmeter V-623. Fluorescence of the internodes of Nitella sp. was registered at 685 nm, the maximum of chlorophyll fluorescence. Chlorophyll fluorescence in Cyclotella cryptica was studied in a haemocytometer (Gorjaev chamber) with successive measurements at different

0007-4861/81/0027-0332 $01.20

positions in the algal cell.

Phenols were estimated by gas chromatography and polarography (SUSLOV & STOM 1978). Total amount of quinoid products of phenol oxidation was determined potentiometrically (STOM et al. 1972).

RESULTS AND DISCUSSION

With the concentrations studied (10^{-2} and 10^{-4} moles/L), chlorophyll fluorescence in Cyclotella cryptica decreased within 5 min by addition of p-benzoquinone (Fig. 1 and 2). At the 10^{-2} M concentration, fluorescence was reduced to 6.5% of the control (Fig. 1). Other phenolic compounds have a decreasing effect on chlorophyll fluorescence in the following order: p-benzoquinone, hydroquinone, pyrocatechol, phenol, guaiacol, resorcinol.

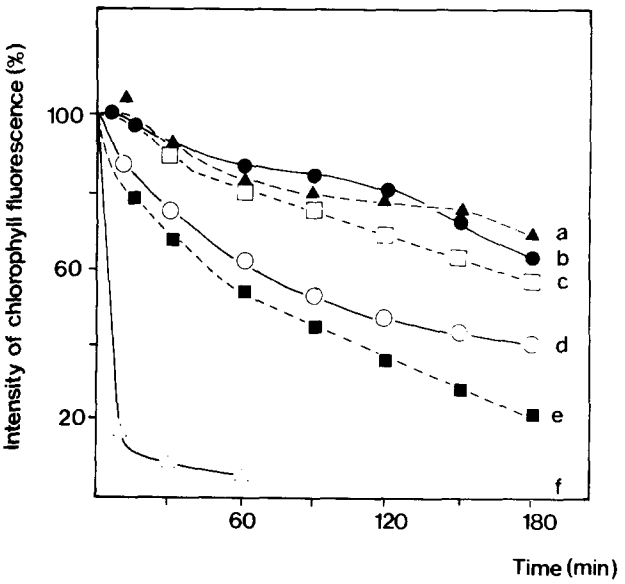

Figure 1. Effect of phenolic compounds at 10^{-2} M concentrations on chlorophyll fluorescence in Cyclotella cryptica after addition to the media of a = resorcinol, b= pyrocatechol, c = phenol, d = guaiacol, e = hydroquinone, f = p-benzoquinone.

A similar order in toxicity was also obtained in experiments on motility of Dunaliella salina (Table 1) and Chlamydomonas reinhardii cells (Table 2) and with the cytoplasmic streaming in Vallisneria spiralis (Table 2). In experiments with Nitella sp. (Table 1) and Euglena gracilis (Table 2) pyrocatechol was more toxic than hydroquinone, otherwise the order remained the same.

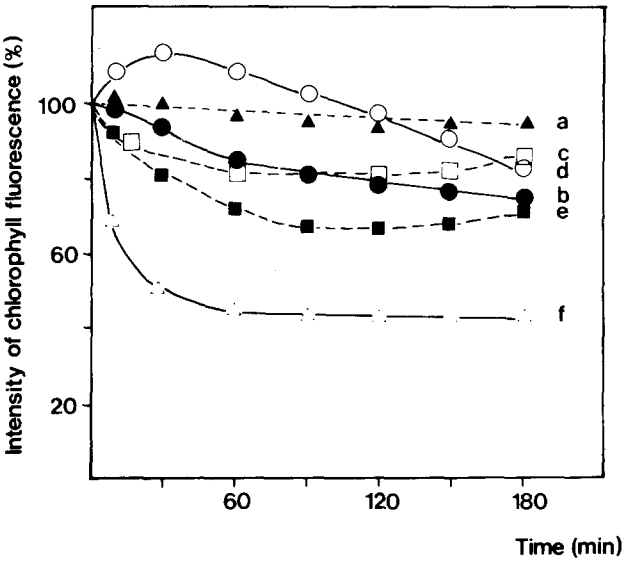

Figure 2. Effect of phenolic compounds at 10^{-4} M concentrations on chlorophyll fluorescence in Cyclotella cryptica after addition to the media. Symbols as in Fig. 1.

Table 1. Concentrations (moles/L) of phenolic compounds with total inhibition (within 15 min) of motility of Dunaliella salina and cytoplasmic streaming in Nitella sp.

	Nitella sp.	D. salina
β-naphthoquinone	$1 \cdot 10^{-4}$	$9 \cdot 10^{-7}$
α-naphthoquinone	$2 \cdot 10^{-4}$	$6 \cdot 10^{-7}$
para-benzoquinone	$2 \cdot 10^{-4}$	$3 \cdot 10^{-5}$
hydroquinone	$2.5 \cdot 10^{-2}$	$3 \cdot 10^{-3}$
pyrocatechol	$2 \cdot 10^{-3}$	$1.2 \cdot 10^{-2}$
resorcinol	$5 \cdot 10^{-2}$	$5 \cdot 10^{-2}$
guaiacol	$3 \cdot 10^{-2}$	$1 \cdot 10^{-2}$
phenol	$3 \cdot 10^{-2}$	$1 \cdot 10^{-2}$
dimethyl hydroquinone	$2 \cdot 10^{-2}$	-
dimethyl pyrocatechol	$2 \cdot 10^{-2}$	$1 \cdot 10^{-2}$
acetic acid	-	$2.5 \cdot 10^{-2}$
malonic acid	$5 \cdot 10^{-2}$	$3 \cdot 10^{-2}$
succinic acid	$2 \cdot 10^{-2}$	-
p-chloromercuribenzoic acid (PCMB)	$3.5 \cdot 10^{-4}$	$3 \cdot 10^{-6}$

Table 2. Concentrations (moles/L) of phenolic compounds with total inhibition within 15 min of motility of Chlamydomonas reinhardii and Euglena gracilis and cytoplasmic streaming in Vallisneria spiralis.

	C. reinhardii	E. gracilis	V. spiralis (leaves)	V. spiralis (roots)
p-benzoquinone	$1 \cdot 10^{-5}$	$2 \cdot 10^{-5}$	-	-
hydroquinone	$5 \cdot 10^{-4}$	$7 \cdot 10^{-2}$	$2.5 \cdot 10^{-2}$	$2.5 \cdot 10^{-3}$
pyrocatechol	$2.5 \cdot 10^{-3}$	$7 \cdot 10^{-3}$	$3 \cdot 10^{-2}$	$8 \cdot 10^{-3}$
resorcinol	$2.5 \cdot 10^{-2}$	$4 \cdot 10^{-2}$	$5 \cdot 10^{-1}$	$5 \cdot 10^{-2}$

Chlorophyll fluorescence, motility of algal flagellates and cytoplasmic streaming of aquatic spermatophytes are physiologically very different processes. Therefore it is somewhat surprising, that toxicity of a large number of phenolic compounds and organic acids on these processes is rather similar (Fig. 3, Table 1).

The mechanism of the inhibition of the phenolic compounds has to be studied in forthcoming experiments. The sensitive reaction of the three physiological parameters studied qualifies them for use of biotests on chemical substances. The lowest effective concentration found (with 100% efficiency) is about $6 \cdot 10^{-7}$ M with α-naphthoquinone (Table 1). By classical chemical methods these concentrations are very difficult to determine

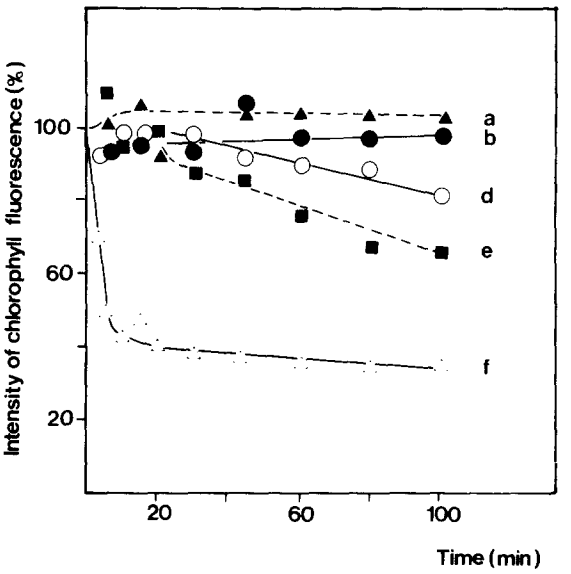

Figure 3. Effect of phenolic compounds at 10^{-3} M concentrations on chlorophyll fluorescence in Nitella sp. after addition to the media. Symbols as in Fig. 1.

Growth of Lemna minor and Elodea canadensis is in general more affected by the phenolic compounds tested than cytoplasmic streaming in Nitella sp. (Table 1 and 3). But again we find a similar order in toxicity of the compounds.

Table 3. Concentrations (moles/L) of phenolic compounds with 50% inhibition of plant multiplication in Lemna minor (within 12 days) and growth of Elodea canadensis (within 9 days).

	L. minor	E. canadensis
p-benzoquinone	$2 \cdot 10^{-4}$	$8.9 \cdot 10^{-5}$
hydroquinone	$7 \cdot 10^{-5}$	$3.9 \cdot 10^{-4}$
pyrocatechol	$1.2 \cdot 10^{-4}$	$2.5 \cdot 10^{-4}$
phenol	$1.8 \cdot 10^{-3}$	$2.5 \cdot 10^{-3}$
resorcinol	$1.5 \cdot 10^{-3}$	$1.3 \cdot 10^{-3}$
dimethyl hydroquinone	$7.5 \cdot 10^{-4}$	$1.4 \cdot 10^{-3}$
dimethyl pyrocatechol	$1 \cdot 10^{-2}$	$1 \cdot 10^{-3}$

Thus, using different algal flagellates and water plants, we can summarize, that phytotoxicity of phenols, meta-isomers and methylated phenols is lower than that of ortho- and para-diphenols. Comparing ortho- and para-isomers of the same substance, the results are not uniform. There are organisms, for which para-isomers are more toxic (Cyclotella cryptica, Dunaliella salina, Chlamydomonas reinhardii, Vallisneria spiralis), and others, for which ortho-isomers are more toxic (Nitella sp., Euglena gracilis, Elodea canadensis).

This suggests that most compounds tested affect unspecifically cell proteins, structural proteins in cell organelles as well as cytoplasmic proteins involved in cell motility and cytoplasmic streaming. However, since we do not understand well the mechanisms of the two processes mentioned, the effects of these unspecific inhibitors are difficult to explain. Cytoplasmic streaming and cell motility are energy consuming processes. Unspecific inhibition of the ATP generating system in the cells therefore certainly will affect these processes (NULTSCH 1974). On the other hand, also an unspecific blocking of SH-groups (e.g., by PCMB) will stop movement of cell organelles such as chloroplasts (SCHÖNBOHM 1972).

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Accepted June 18, 1981