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Substrate specificities of cytosolic glutathione-S transferases in five different species of the aquatic macrophyte *Myriophyllum*

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Summary

We report about the general biotransformation activity based on the activities of glutathione S-transferases (GST´s: EC 2.5.1.18) on different model substrates, of five species of the macrophyte genera *Myriophyllum* (*M. hippuroides, M. mattogrossense, M. aquaticum, M. quitense, M. verticillatum*), under controlled conditions of water quality, temperature and photoperiod. The model substrates for the GST´s we employed were 1-chloro-2,4-dinitrobenzene (CDNB), 4-nitrophenyl-2-nitro-4-trifluoromethyl phenyl ether (Fluorodifen, FL), 2,4-dichlor-1-nitrobenzene (DCNB), para-nitrobenzoylchloride (pNBC), 1,2-epoxy-3-(4-nitrophenoxy)propane (EPNP) and ethacrynic acid (EA). All species showed response to all GST conjugating substrates in the cytosol, except *M. hippuroides* and *M. verticillatum* which showed no conjugation reaction with DCNB.

The use of different model substrates give an insight on basic biotransformation capacity of the different macrophyte species. This might indicate which species may have enhanced ability to handle xenobiotics of different molecular structure.

Under maintenance in our standard testing medium and conditions the biotransformation profile of all analyzed species show that highest GST-activities were measured using ethacrynic acid and fluorodifen as conjugating substrates. This suggests that they are well equipped to conjugate xenobiotics of different structures.

Introduction

Glutathione S-transferases (GST´s) are an ancient enzyme superfamily involved in the detoxification metabolism in animals and plants (MARRS, 1996; DIXON et al., 2001). GST´s are mainly detected in the cytosol of cells but also microsomal forms exist (MORGENSTERN and DEPIERRE, 1988; HAYES and PULFORD, 1995). Beside their catalyzing role in the conjugation of a large variety of electrophilic substrates to the co-substrate glutathione (GSH), GST´s exhibit many other functions like peroxidase-, isomerase-activities, inhibition of Jun N-terminal kinase, protection against H_2O_2 induced apoptosis (SHEEHAN et al., 2001). The plant GST enzyme family is divided into five different classes, based on the classification proposed for mammalian GST, e.g. analogous to the alpha, mu, pi, theta, sigma (FROVA, 2003). The plant specific GST´s are phi (φ) and tau (τ), whereas theta (θ) and zeta (ζ) class GST´s are also found in other organisms (DIXON et al., 2002). Recent investigations also found a lambda (λ) class GST in *Arabidopsis thaliana* (DIXON et al., 2002). In plants with incomplete or unavailable genome information the current numbering system is based on the order of discovery of the GST genes for each class in the given plant species (EDWARDS et al., 2000).

In the past years lots of investigations have been carried out regarding GST-based biotransformation, mainly in crop plants (LAMOUREUX, 1989). Only few studies involve plants of the aquatic environment (BYL et al., 1994; ROY et al., 1996; PFLUGMACHER and STEINBERG, 1997; PFLUGMACHER et al., 1999a, b). Aquatic macrophytes are primary producers hence they are important for oxygen production, the nutrient cycle, sediment stabilization in the aquatic ecosystem and furthermore macrophytes provide habitat and shelter for other aquatic organisms (MOHAN and HOSETTI, 1998; ROZAS and ODUM, 1988). The aquatic macrophyte genera *Myriophyllum*, has a cosmopolitan distribution. The most studied *Myriophyllum* species are non-native, invasive plants and are considered as noxious, like *M. aquaticum* and *M. spicatum* (MADSEN et al., 1991; MADSEN, 1994) for example in Lake George (New York) and in the state of Florida. Lots of studies have been carried out in order to remove them from the ecosystems because of their competition with other indigenous plants species resulting in reduction of natural diversity, e.g. because they grow earlier in the season and therefore reduce light penetration necessary for the growth of native species. The native species of this genus are interesting for biomonitoring purposes, because they have two possible uptake routes for pollutants, one by the roots from the bioavailable concentrations in the sediment interstitial water and the other directly from the water column (SCHRENK et al., 1998; TURGUT and FOMIN, 2002). Aquatic plants possess a complex and efficient enzymatic system. Part of this is the biotransformation system of xenobiotics and other toxic compounds which has been divided into three steps: phase I activation, phase II conjugation and phase III compartimentation in plants, which is equivalent to excretion in animals (SANDERMANN, 1994; PFLUGMACHER and STEINBERG, 1997). The broad strategy of plants when confronted with xenobiotics is enabling its insertion into standard metabolic cycles (KVESITADZE, 2001). Lots of investigations have been focused on the application of the measurements of biochemical pathways in plants in order to propose them as biomarkers for different sources of pollution, reviewed by LYTLE and LYTLE (2001). The underlying principle behind the use of biomarkers is that all toxic effects start with a relation between the toxic chemical and some biochemical structure in a living organism (ST-CYR et al., 1997). Biomarkers have been defined as „a biological response to a chemical or chemicals that gives a measure of exposure, and sometimes, also of toxic effect" (PEACKALL and WALKER, 1994; HANDY et al., 2003). When we have in mind that biomarkers are gaining importance within pollution monitoring in aquatic ecosystems and/or as early warning system (LINDSTRÖM-SEPPÄ et al., 2001, FERRAT et al., 2003) and the potential of the macrophyte genus *Myriophyllum* for biomonitoring, we come to the conclusion that more research is needed to understand biotransformation of xenobiotics and oxidative stress metabolism of different species of this aquatic plant.

The aim of this study is to compare the basic biotransformation system profiles and biotransformation substrate specificities from plants under controlled conditions. We assessed the phase II biotransformation activity of the soluble glutathione S-transferases (GST) in five different species from the aquatic macrophyte genera *Myriophyllum*: *M. hippuroides, M. mattogrossense, M. quitense, M. aquaticum* and *M. verticillatum*, in order to give more understanding of species specific biotransformation of GST and its isoforms. Different substrates were tested in order to verify the ability of these plants to enzymatically conjugate the different molecules. Moreover substrate specificity is one criterion for GST classification.

Material and methods

Plant Material and test conditions

Macrophytes from the species *Myriophyllum quitense*, *Myriophyllum aquaticum*, *Myriophyllum hippuroides* and *Myriophyllum mattogrossense* were purchased at Aqua Global (Dr. Jander & Co. OHG, Seefeld, Germany) and *Myriophyllum verticillatum* was collected from Liepnitzsee, Germany. All species were identified according to CASPER and KRAUSCH (1980) and ORCHARD (1981) and verified through ITIS classification standards (http://www.itis.usda.gov) in order to reach current taxonomic status of species. All plants were acclimatized in medium containing de-ionized water, $CaCl_2$ [0.2g/L], $NaHCO_3$ [0.103g/L] and sea-salt [0.1g/L], under same photoperiod and temperature, for two weeks in aquariums. Photoperiod was set at 14:10 hours light: dark cycle of and temperature at 20 ± 1°C. After this period for standard testing conditions, 5g plants of each species were shock frozen in liquid nitrogen, and then stored at –80 °C until enzyme extraction.

Enzyme Preparation (protein extraction)

Enzyme extraction was done according to PFLUGMACHER and STEINBERG (1997) with minor modifications. 5g of each species were ground to fine powder using liquid nitrogen. This tissue-powder was homogenized with sodium-phosphate buffer (0.1M, pH 6.5), containing 20% glycerol, 1.4mM dithioerythritol (DTE) and 1mM ethylenediaminetetraacetic acid (EDTA), in a relation 2:1 v/w. Separation of microsomes was achieved by centrifugation at 100000 x g for 60 min. The proteins of the soluble fraction were concentrated by ammonium precipitation cut between 35% and 80% saturation followed by centrifugation at 30000 x g. The resulting pellet was re-suspended in 1 ml of 20 mM sodium-phosphate buffer (pH 7.0) and desalted by gel filtration on NAP-10 columns (Amersham Pharmacia, Germany), resulting in 1.5 ml protein extracts. Immediately after extraction the protein extracts were shock frozen using liquid nitrogen, and then stored at –80 °C until enzyme activity assays.

Enzyme Activity Assay and Protein Determination

Activities of glutathione-S-transferase (GST: EC 2.5.1.18) were measured testing different substrate specificities in order to identify different taxonomic distribution patterns. GST activities were measured in the soluble (cytosolic) fraction applying described methods using 1-chloro-2,4-dinitrobenzene (CDNB), 4-nitrophenyl-2-nitro-4-trifluoromethyl phenyl ether (Fluorodifen, FL), 2,4-dichlor-1-nitrobenzene (DCNB), para-nitrobenzoylchloride (pNBC), 1,2-epoxy-3-(4-nitrophenoxy)propane (EPNP) and ethacrynic acid [2,3-Dichloro-4-(2'-methylenbutyryl-) phenoxy acetic acid] (EA) as substrates (HABIG et al., 1974; SCHRÖDER et al., 1992; BOOTH et al., 1961; SCHRÖDER et al., 1990; FJELLSTEDT et al., 1973). Total protein content was quantified according to BRADFORD (1976) using bovine serum albumin as standard. The above mentioned substrates were selected in order to reveal the conjugation potential of the GST's from different *Myriophyllum* species, to different groups of toxic compounds (e.g. herbicides, epoxides, alkenals), in order to show which macrophyte species may have enhanced ability to handle with different sources of external and / or internal cell stress.

Results

Glutathione S-transferase on substrate specificity

Highest biotransformation activities of all GST conjugating substrates were obtained by using ethacrynic acid resulting in values reaching up to 543.71 [nKat/mg protein] in *M. aquaticum* followed in decreasing order of activity by *M. mattogrossense* 488.52 [nKat/mg protein], *M. verticillatum* 451.67 [nKat/mg protein], *M. quitense* 377.23 [nKat/mg protein] and *M. hippuroides* with 350.64 [nKat/mg protein] of enzyme activity (Fig. 1). After ethacrynic acid the second highest GST activities were measured using the herbicide fluorodifen as conjugating substrate obtaining maximum values of 102.03 [nKat/mg protein] in *M. mattogrossense* followed in decreasing order by *M. verticillatum* 87.26 [nKat/mg protein], *M. quitense* 71.55 [nKat/mg protein], *M. hippuroides* 48.73 [nKat/mg protein] and *M. aquaticum* with lowest values 8.82 [nKat/mg protein] (Fig. 2). Third in ranking was the substrate 4-Nitrobenzoylchloride (pNBC) obtaining highest values in *M. aquaticum* with highest values of 32.06 [nKat/mg protein] followed in decreasing order by *M. quitense* 14.30 [nKat/mg protein], *M. hippuroides* 10.55 [nKat/mg protein], *M. mattogrossense* 7.92 [nKat/mg protein] and *M. verticillatum* with 2.99 [nKat/mg protein] of activity (Fig. 3). Fourth in ranking was the substrate 1,2-Epoxy-3-(4-nitrophenoxy)propane (EPNP) reaching values of 20.39 [nKat/mg protein] in *M. hippuroides* being followed in decreasing order by *M. aquaticum* 19.72 [nKat/mg protein], *M. verticillatum* 10.86 [nKat/mg protein], *M. mattogrossense* 10.75 [nKat/mg protein] and *M. quitense* with 7.71 [nKat/mg protein] of kinetic activity (Fig. 4). Next in ranking was the most known GST conjugating substrate 1-chloro-2,4-dinitrobenzene (CDNB) for the cytosol resulting highest values in the species *M. aquaticum* with 18.96 [nKat/mg protein] of activity followed by *M. verticillatum* 17.21 [nKat/mg protein], *M. hippuroides* 13.20 [nKat/mg protein], *M. quitense* 13.11 [nKat/mg protein] and *M. mattogrossense* with 11.22 [nKat/mg protein] (Fig. 5). Lowest enzyme activities for GST in the cytosol were measured using 2,4-Dichlor-1-nitrobenzene (DCNB) as conjugating substrate. Highest activities for this substrate were found in *M. aquaticum* with 7.91 [nKat/mg protein] followed by *M. mattogrossense* 3.15 [nKat/mg protein] and *M. quitense* with 1.27 [nKat/mg protein]. No activity could be detected in *M. hippuroides* and *M. verticillatum* (Fig. 6).

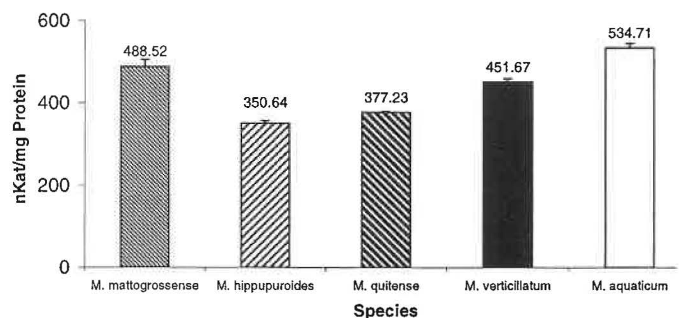

Fig. 1: Specific conjugating activity of cytosolic glutathione S-transferase on ethacrynic acid (EA).

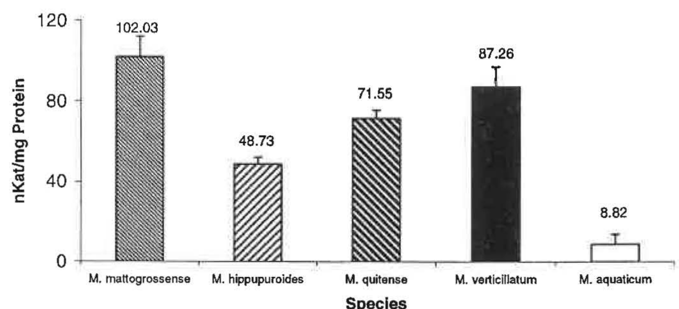

Fig. 2: Specific conjugating activity of cytosolic glutathione S-transferase on 4-nitrophenyl-2-nitro-4-trifluoromethyl phenyl ether (Fluorodifen).

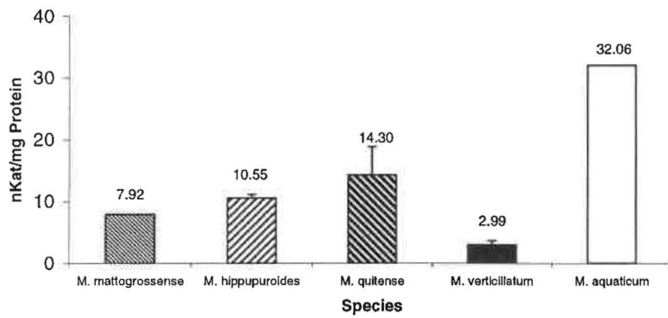

Fig. 3: Specific conjugating activity of cytosolic glutathione S-transferase on para-nitrobenzoylchloride (pNBC).

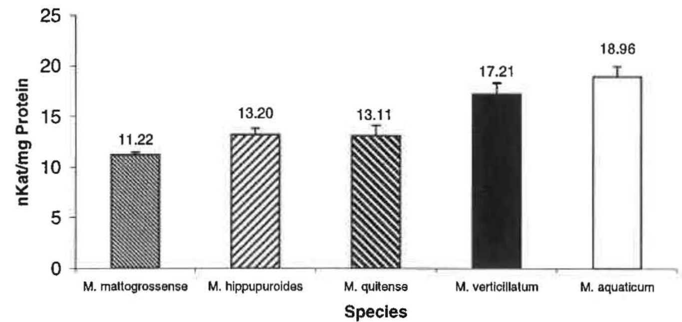

Fig. 5: Specific conjugating activity of cytosolic glutathione S-transferase on 1-chloro-2,4-dinitrobenzene (CDNB).

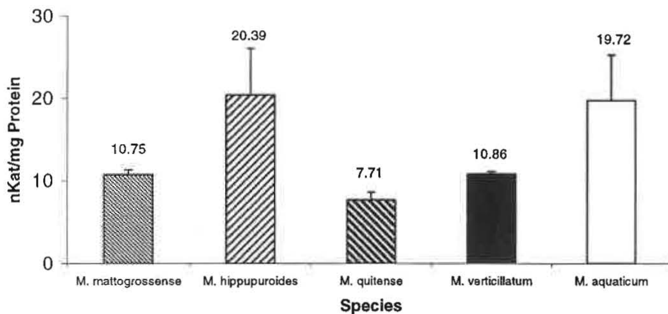

Fig. 4: Specific conjugating activity of cytosolic glutathione S-transferase on 1,2-epoxy-3-(4-nitrophenoxy)propane (EPNP).

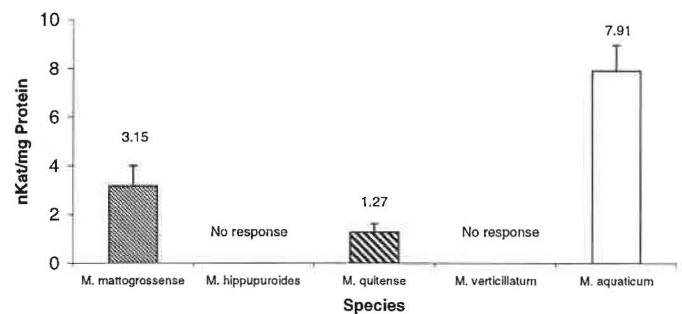

Fig. 6: Specific conjugating activity of cytosolic glutathione S-transferase on 2,4-dichlor-1-nitrobenzene (DCNB).

Discussion

As mentioned above, the soluble glutathione S-transferases in plants are classified into 5 classes (φ, τ, θ, ζ, and λ), where phi and tau are plant specific, the classes theta, zeta and lambda are also found in other organisms (DIXON et al., 2002). Nowadays all classification criteria for GST isoenzymes are based on genome sequence, chromatography, immunological and crystallographic studies (FROVA, 2003; SHEEHAN et al., 2001; DIXON et al., 2002), but one of the most important criteria to proof their functionality still remains in the analysis of their substrate specificity. GEORGE (1994) summarized the catalytic characteristics of rat cytosolic GST, proposing some substrate specific enzyme activities as diagnostic for some isoforms (Tab. 1). For biomonitoring purposes neither the classification nor nomenclature of biotransforming agents (in this case detoxifying enzymes) are as important as the effective capacity of biotransformation of organisms in relation to handle different types of stressing compounds (e.g. specific substrates) in order to reflect for example the plants health status and/or the pollution grade of its surrounding.

Summarizing the results of the different substrate specific biotransformation activities of GST´s, we can observe similarity in the GST profile pattern of the different applied substrates in *M. mattogrossense* and *M. quitense* (Fig. 7). The only difference is that *M. quitense* had a lower activity towards fluorodifen than *M. mattogrossense*, but more activity when pNBC was applied. *M. hippuroides* and *M. verticillatum* show the same profile for ethacrynic acid but vary in the case of EPNP and fluorodifen. That leads us to the conclusion that these four species have a similar potential to handle ethacrynic acid, but they differ in their conjugating capacity for epoxides and herbicides. *M. aquaticum* has highest enzyme activities for the applied substrates (ethacrynic acid, pNBC, CDNB and DCNB) but lowest activity towards the herbicide fluorodifen. This species showed a different activity profile than the other analyzed *Myriophyllum* species.

Comparing our results the specific conjugation of GST for all measured substrates, ethacrynic acid reaches almost 80% of all measured enzyme activities (Fig. 7). Ethacrynic acid (EA) is a phenylacetic acid derivate which contains an electrophilic group similar to α-β-alkenals produced under conditions of oxidative stress in some organisms (GRONWALD and PLAISANCE, 1998). This fact leads us to the assumption that the analyzed *Myriophyllum* GST isoforms might be basically involved in detoxification of oxidative stress generated compounds. Oxidative stress is generated through an increase of reactive oxygen species (ROS) e.g.: $O^{\cdot-}$, $OH^{\cdot}$, OH^{-}, H_2O_2 and organic peroxides in organisms. Several sources of ROS in untreated plants exist, for example: in photosynthesis through electron transport chain; photorespiration through carbon assimilation; respiration in plant mitochondria; but ROS are also produced under adverse conditions such as high light intensities, drought and salt stress, low and high temperature exposure, heavy metals, UV radiation, xenobiotics, mechanical and physical stress and pathogen attack (DAT et al., 2000; BLOKHINA et al., 2003). It is described that the tau (τ) class of the GST family in plants is mainly involved in oxidative stress (FROVA, 2003), but is also involved in herbicide detoxification (DIXON et al., 2003).

The second highest enzyme activities, in all analyzed *Myriophyllum* species except *M. aquaticum*, were detected applying fluorodifen (4-nitrophenyl-2-nitro-4-trifluoromethyl phenyl ether) as substrate. Fluorodifen is a diphenylether herbicide and is mainly detoxified by the τGST class (DIXON et al., 2003). On the other hand phi (φ) class GST´s are characterized by their enhanced detoxification activities for other herbicides like glyphosate, alachlor, atrazine (JAIN and

Compound Name	Molecular Structure	Corresponding Class
CDNB [1-chloro-2,4-dinitrobenzene]	NO_2, Cl, NO_2	chlorinated benzenes (algaecide)
DCNB [1,2-dichloro-4-nitrobenzene]	Cl, NO_2, Cl	chlorinated benzenes
EPNP [1,2-epoxy-3-(4-nitrophenoxy) propane]	O, H_2C— CH— CH_2— O, NO_2	epoxide
pNBC [para-nitro benzoylchloride]	O, C, Cl, NO_2	aromatic ketone
Ethacrynic acid [2,3-Dichloro-4-(2'-methylenbutyryl-) phenoxy acetic acid]	CH_2, H_3C, O, O, COOH, Cl, Cl	phenylacetic acid derivate
Fluorodifen [4-nitrophenyl-2-nitro-4-trifluoromethyl phenyl ether]	NO_2, F_3C, O, NO_2	diphenyl ether (herbicide)

Tab. 1: Glutathione S-transferase substrates used in the laboratory for activity determination and the corresponding molecule class represented by these substrates.

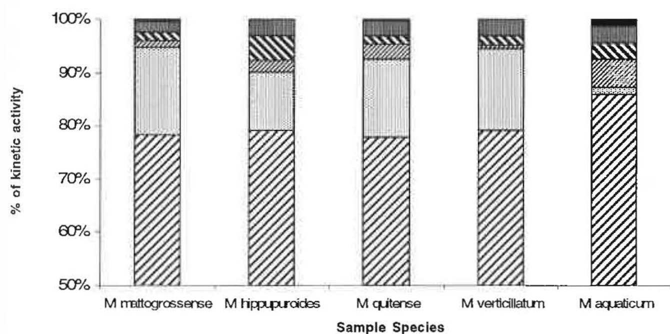

Fig. 7: Biotransformation profile - Percentage of specific GST conjugating substrate activity in different *Myriophyllum* species. [EA = 2,3-Dichloro-4-(2-methylenbutyryl-) phenoxy acetic acid (ethacrynic acid); FL = 4-nitrophenyl-2-nitro-4-trifluoromethyl phenyl ether (Fluorodifen); pNBC = para-nitrobenzoylchloride; EPNP = 1,2-epoxy-3-(4-nitrophenoxy)-propane; CDNB = 1-chloro-2,4-dinitrobenzene; DCNB = 2,4-dichlor-1-nitrobenzene]

BHALLA-SARIN, 2001; MAUCH and DUDLER, 1993; reviewed by FROVA, 2003 and SHEEHAN et al., 2001) and are in general highly active towards chloroacetanilide and thiocarbamate herbicides (DIXON et al., 2003). The activities of φ and τ class GST protect the plants from herbicide damage and can be used as herbicide indicating biomarkers (FUERST et al., 1993; SCALLA and ROULET, 2002; DIXON et al., 2003). Best results could be achieved if the same or similar herbicide, which is causing environmental pollution, is applied as substrate for GST activity determination of the affected organism. The other applied substrates only gather around 10% of the substrate specific enzyme activity, including the universal model substrate CDNB, which is mainly used for the measurement of GST activities in plants as well as in animals. Comparing the results for GST-CDNB specific activities with those obtained by EA and fluorodifen we observe that GST-CDNB activities are one order of magnitude lower than GST-EA activities and 4 times lower than GST-fluorodifen activities. In conclusion we assume that the GST spectrum of the analyzed macrophytes from the genera *Myriophyllum* are mainly equipped to handle oxidative stress through the conjugation of compounds originated via lipid peroxidation and/or the direct conjugation of herbicides. For the measurement of GST activity in macrophytes and higher plants, we suggest to include ethacrynic acid and fluorodifen, beside the traditional model substrate CDNB. The measurement of GST activities applying these substrates in the studied *Myriophyllum* species should provide a good biomonitoring tool, because they give response to different sources of stress. All investigated species might be used for passive biomonitoring purposes, but we recommend the use of endemic plant species for active biomonitoring, due to the invasive character of the non native species of the macrophyte genera *Myriophyllum*.

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References

BLOKHINA, O., VIROLAINEN, E., FAGERSTEDT, K.V., 2003: Antioxidants, oxidative damage and oxygen deprivation stress: a review. Ann. Bot. 91, 179-194.

BOOTH, J., BOYLAND, E., SIMS, P., 1961: An enzyme from rat liver catalyzing conjugations with glutathione. Biochem. J. 79, 516-526.

BRADFORD, M., 1976: A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye-binding. Anal. Biochem. 72, 248-254.

BYL, T.D., SUTTON, H.D., KLAINE, S.J., 1994: Evaluation of peroxidase as a biochemical indicator of toxic-chemical exposure in the aquatic plant *Hydrilla-verticillata*, Royle. Environ. Toxicol. Chem. 13, 509-515.

CASPER, S.J., KRAUSCH, H.D., 1980: Pteridophyta und Anthophyta, 1. u. 2. Teil. In: Ettl, H., Gerloff J., Heynig, H. (eds.), Süßwasserflora von Mitteleuropa, Vol. 23 & 24. Gustav Fischer Verlag, Stuttgart, New York.

DAT, J., VANDENABEELE, S., VRANOVA, E., VAN MONTAGU, M., INZE, D., VAN BREUSEGEM, F., 2000: Dual action of the active oxygen species during plant stress responses. Cell. Mol. Life. Sci. 57, 779-795.

DIXON, D.P., COLE, D.J., EDWARDS, R., 2001: Cloning and characterization of plant theta and zeta class GST´s: Implications for plant GST classification. Chem-Biol. Interact. 133, 33-36.

DIXON, D.P., LAPTHORN, A., EDWARDS, R., 2002: Plant glutathione transferases. Genome Biol. 3, 3004.1-3004.10.

DIXON, D.P., MCEWEN, A.G., LAPTHORN, A.J., EDWARDS, R., 2003: Forced evolution of an herbicide detoxifying glutathione transferase. J. Biol. Chem. 278, 23930-23935.

EDWARDS, R., DIXON, D.P., WALBOT, V., 2000: Plant glutathione S-transferases: enzymes with multiple functions in sickness and in health. Trends Plant Sci. 5, 193-198.

FERRAT, L., PERGENT-MARTINI, C., ROMEO, M., 2003: Assessment of the use of biomarkers in aquatic plants for the evaluation of environmental quality: application to seagrasses. Aquat. Toxicol. 65, 187-204.

FJELLSTEDT, T.A., ALLEN, R.H., DUNCAN, B.K., JACOBY, W.B., 1973: Enzymatic conjugation of epoxides with glutathione. J. Biol. Chem. L248, 3702-3707.

FROVA, C., 2003: The plant glutathione transferase gene family: genomic structure, functions, expressions and evolution. Physiol. Plantarum 119, 469-479.

FUERST, E.P., IRZYK, G.P., MILLER, K.D.: 1993: Partial characterization of glutathione-s-transferase isozymes induced by the herbicide safener benoxacor in maize. Plant Physiol. 102, 795-802.

GEORGE, S.G., 1994: Enzymology and molecular biology of phase II xenobiotic conjugating enzymes in fish. In: Malins, D.C., Ostrander, G.K. (eds.), Aquatic Toxicology: Molecular, biochemical and cellular perspectives, 37-85. Lewis Publishers, Boca Raton, USA.

GRONWALD, J.W., PLAISANCE, K.L., 1998: Isolation and characterization of glutathione S-transferase isozymes from sorghum. Plant Physiol. 117, 877-892.

HABIG, W., PABST, M.J., JAKOBY, W.B., 1974: Glutathione S-transferase: The first step in mercapturic acid formation. J. Biol. Chem. 249, 1730-1739.

HANDY, R.D., GALLOWAY, T.S., DEPLEDGE, M.H., 2003: A proposal for the use of biomarkers for assessment of chronic pollution and in regulatory toxicology. Ecotoxicology 12, 331-343.

HAYES, J.D., PULFORD, D.J., 1995: The glutathione S-Transferase supergene family: Regulation of GST and the contribution of the isoenzymes to cancer chemoprotection and drug resistance. Critical Rev. Biochem. Molecular Biol. 30, 445-600.

JAIN, M., BHALLA-SARIN, N., 2001: Glyphosate-induced increase in glutathione S-transferase activity and glutathione content in groundnut (*Arachis hypogaea* L.). Pestic. Biochem. Phys. 69, 143-152.

KVESITADZE, G., GORDEZIANI, M., KHATISASHVILI, G., SADUNISHVILI, T., RAMSDEN, J.J., 2001: Some aspects of the enzymatic basis of phytoremediation. J. Biol. Phys. Chem. 1, 49-57.

LAMOUREUX, G.L., RUSNESS, D.G., 1989: Propachlor metabolism in soybean plants, excised soybean tissues, and soil. Pestic. Biochem. Phys. 34, 187-204.

LEWIS, M.A., 1995: Use of freshwater plants for phytotoxicity testing: a review. Environ. Poll. 87, 319-336.

LINDSTRÖM-SEPPÄ, P., URBAN, K., HONKALAMPI-HÄMÄLÄINEN, U., ROY, S., 2001: Biochemical responses in aquatic plants as markers of environmental contamination. Atla-Altern. Lab. Anim. 29, 277-282.

LYTLE, J.S., LYTLE, T.F., 2001: Use of plants for toxicity assessment of estuarine ecosystems. Environ. Tox. Chem. 20, 68-83.

MADSEN, J.D., SUTHERLAND, J.W., BLOOMFIELD, J.A., EICHLER, L.W., BOYLEN, C.W., 1991: The decline of native vegetation under dense Eurasian water-milfoil canopies. J. Aquat. Plant. Manage. 29, 94-99.

MADSEN, J.D., 1994: Invasions and declines of submersed macrophytes in Lake George and other Adirondack lakes. Lake Reserv. Manage. 10, 19-23.

MARRS, K.A., 1996: The functions and regulation of glutathione S-transferases in plants. Annu. Rev. Plant. Phys. 47, 127-158.

MAUCH, F., DUDLER, R., 1993: Differential induction of distinct glutathione-s-transferases of wheat by xenobiotics and by pathogen attack. Plant. Physiol. 102, 1193-1201.

MOHAN, B.S., HOSETTI, B.B., 1998: Lead toxicity to *Salvinia natans* grown in macrophyte ponds. J. Ecotoxicol. Environ. Monit. 8, 3-5.

MORGENSTERN, R., DEPIERRE, J.W., 1988: Membrane-bound Glutathione Transferases. In: Sies, H., Ketterer, B. (eds.), Glutathione Conjugation: Its Mechanism and Biological Significance, 157-174, Academic Press, London.

ORCHARD, A.E., 1981: A revision of South American *Myriophyllum* (Haloragaceae), and its repercussions on some Australian and North American species. Brunonia 4, 27-65.

PEAKALL, D.B., WALKER, C.H., 1994: The role of biomarkers in environmental assessment (3), Vertebrates. Ecotoxicology 3, 173-179.

PFLUGMACHER, S., SCHRÖDER, P., 1995: Glutathione S-transferases in trees: Inducibility by various organic xenobiotics. Z. Pflanzenernähr. Bodenk. 158, 71-73.

PFLUGMACHER, S., STEINBERG, C., 1997: Activity of phase I and phase II detoxication enzymes in aquatic macrophytes. J. Appl. Bot. 71, 144-146.

PFLUGMACHER, S., GEISSLER, K., STEINBERG, C., 1999: Activity of phase I and phase II detoxication enzymes in different cormus parts of *Phragmites australis*. Ecotox. Environ. Safe. 42, 62-66.

PFLUGMACHER, S., SPANGENBERG, M., STEINBERG, C.E.W., 1999: Dissolved organic matter (DOM) and effects on the aquatic macrophyte *Ceratophyllum demersum* in relation to photosynthesis, pigment pattern and activity of detoxication enzymes. J. Appl. Bot. 73, 184-190.

ROY, S., SEN, C.K., HANNINEN, O., 1996: Monitoring of polycyclic aromatic hydrocarbons using 'moss bags': Bioaccumulation and responses of antioxidant enzymes in *Fontinalis antipyretica Hedw*. Chemosphere 32, 2305-2315.

ROZAS, L.P., ODUM, W.E., 1988: Occupation of submerged aquatic vegetation by fishes: testing the roles of food and refuge. Oecologia 77, 101-106.

SANDERMANN, H. Jr., 1994: Higher plant metabolism of xenobiotics: The „green liver" concept. Pharmacogenetics 4, 225-241.

SCALLA, R., ROULET, A., 2002: Cloning and characterization of a glutathione S-transferase induced by an herbicide safener in barley (*Hordeum vulgare*). Physiol. Plantarum 116, 336-344.

SCHRENK, C., PFLUGMACHER, S., BRÜGGEMANN, R., SANDERMANN, Jr. H., STEINBERG, C.E.W., KETTRUP, A., 1998: Glutathione S-Transferase

activity in aquatic macrophytes with emphasis on habitat dependence. Ecotoxicol. Environ. Safe. 40, 226-233.

SCHRÖDER, P., LAMOUREUX, G.L., RUSNESS, D.G., RENNENBERG, H., 1990: Glutathione S-transferase activity in spruce needles. Pestic. Biochem. Physiol. 37, 211-218.

SCHRÖDER, P., PFLUGMACHER, S., RENNENBERG, H., 1992: Biomarker für organische Schadstoffe in Fichten (*Picea abies* L.): Dynamik des Entgiftungsenzymes Glutathione S-transferase. J. Appl. Bot. 66, 174-179.

SHEEHAN, D., MEADE, G., FOLEY, V.M., DOWD, C.A., 2001: Structure, function and evolution of glutathione transferases: implications for classification of non-mammalian members of an ancient enzyme superfamily. Biochem. J. 360, 1-16.

ST.CYR, L., CATTANEO, A., CHASSÉ, R., FRAIKIN, Ch.GJ., 1997: Technical evaluation of monitoring methods using macrophytes, phytoplankton and periphyton to assess the impacts of mine effluents on the aquatic environment. K1A 0G1. Canada Centre for Mineral and Energy Technology, 555 Booth Street, Ottawa, Ontario, Canada.

TURGUT, C., FOMIN, A., 2002: The ability of *Myriophyllum aquaticum* (Vell.) verdcourt in the uptake and the translocation of pesticides via roots with a view to using the plants in sediment toxicity testing. J. Appl. Bot. 76, 62-65.

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