Responses of glutathione cycle enzymes and glutathione metabolism to copper stress in *Scenedesmus bijugatus*

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Abstract

Enzymes of glutathione metabolism and GSH content in copper-treated *Scenedesmus bijugatus* cells and the synthesis of metal-binding peptides are reported in this investigation. Progressive depletion of GSH content in the cells was observed with increasing concentrations of copper. There was an increase in the protein thiol content while the non-protein thiol content decreased. There was an initial elevation and later decrease in the hydrogen peroxide level in the cells. Copper stress increased the activities of γ-glutamylcysteine synthetase, GSH S-transferase and GSH-peroxidase and decreased the activity of GSSG-reductase. These results suggest that copper alters the equilibrium between synthesis and utilization of GSH either due to its antioxidant role or by serving as a precursor in the synthesis of phytochelatins. © 2001 Elsevier Science Ireland Ltd. All rights reserved.

Keywords: Copper; Glutathione cycle enzymes; Glutathione; Phytochelatins; *Scenedesmus bijugatus*

1. Introduction

Transition metals like copper have been implicated in oxidative injury involving the initial formation of active oxygen species (AOS) and their subsequent reaction with macromolecules like proteins, lipids, polysaccharides and nucleic acids leading to altered membrane fluidity, loss of enzyme function and genomic damage [1]. AOS cause an imbalance in cellular redox systems in favour of oxidized forms. Of the AOS generated, hydrogen peroxide is the most stable and is a source of the most damaging and reactive hydroxyl radical. H$_2$O$_2$ is scavenged in peroxisomes by catalases and in chloroplasts, the pathway of its detoxification proceeds by the coupling of reduction of H$_2$O$_2$ to the oxidation of GSH via the ascorbate–dehydroascorbate system [2]. The products of oxidative damage initiated by hydroxyl radicals like 4-hydroxyalkenals (membrane lipid peroxides) and base propenals (products of oxidative DNA degradation) are highly cytotoxic. Glutathione S-transferases (GSTs) detoxify such endogenously produced electrophiles by conjugation with glutathione (GSH) as well as by acting as glutathione peroxidases to detoxify toxic base propenals like thymidine hydroperoxide [3].

GSH is associated with stress resistance [4]. It is an important antioxidant in the cellular milieu and is responsible for maintenance of the antioxidative machinery of the cells intact under stress. It is synthesized enzymatically in two ATP-dependent reactions similar in bacteria, fungi, plants and animals [5]: the production of γ-glutamylcysteine, catalyzed by γ-glutamylcysteine synthetase (γ-GCS) and the production of glutathione, catalyzed by glutathione synthetase (GS). It has been proposed that γ-GCS initially reacts with ATP to form a phosphorylated enzyme, which subsequently reacts with L-glutamate to yield a γ-glutamyl-enzyme intermediate. For γ-GCS as well as for GS the presence of Mg$^{2+}$ and K$^+$ is essential to obtain optimal activity.
Glutathione is present in cells in its reduced form (GSH) and most of its proposed functions are related to the thiol group and its use as a reductant. It is oxidized during the antioxidative process to GSSG. A high GSH/GSSG ratio is necessary to achieve optimal protein synthesis in cells. GSSG inhibits protein synthesis by converting an initiation factor into its inactive form. It is re-converted into GSH by glutathione reductase using photosynthetically generated NADH or NADPH [6]. GSH is the main component of the free, low molecular weight thiol pool in cells and is the precursor for enzymatic synthesis of metal-binding peptides with the general structure (γ-glutamylcysteinyl)n glycine [7]. Consumption of GSH for peptide synthesis and in its direct antioxidant role under metal stress leads to activation of its biosynthetic system.

Algae can be used as biosorbents and accumulators of metals. An understanding of the regulatory mechanisms of metal tolerance and the components involved in it, mainly the glutathione cycle, will help in isolation and development of strains for metal removal from aquatic ecosystems. There have been a few reports on glutathione-mediated alleviation of metal stress in higher plants but the number of studies on glutathione synthesis and its role in metal stress in algae is very few and was the reason for undertaking the present study.

2. Materials and methods

2.1. Cell growth and protein extraction

*Scenedesmus bijugatus* was grown under continuous light in Kessler’s medium. Growth was estimated as the increase in Chl a content of cultures over time. Growth curves of copper-treated and control cells were constructed and the percentage decrease in growth rate of treated cells was calculated. Copper content in cells was estimated by atomic absorption spectrophotometry. Mid-log phase cultures were treated with 0, 50, 100 and 200 μM concentrations of CuSO₄ for 3 days. Cells were harvested by centrifugation at 2000 × g for 10 min and were disrupted by grinding in 50 mM Tris–HCl pH 7.4 with the addition of 1 mM PMSF. The extract was centrifuged at 20 000 × g for 30 min and the supernatant was used as the protein source for enzyme assays. Total protein was estimated according to [8].

2.2. γ-Glutamylcysteine synthetase (γ-GCS; EC 6.3.2.2)

The assay was according to [9]. The reaction mixture contained 50 μl each of 0.2 M Na-glutamate, 0.2 M L-aminobutyrate, 40 mM Na₂-EDTA, 0.4% BSA, 100 μl each of 0.2 M MgCl₂, 50 mM Na₂-ATP and 500 μl of 0.2 M Tris–HCl pH 8.2. It was preincubated for 2 min at room temperature and the reaction was started by adding 50 μg of protein. The reaction mixture was incubated at 37°C for 30 min and the reaction was stopped by addition of 100 μl of 50% TCA. The mixture was centrifuged and the supernatant was used for estimation of phosphate content by the phosphomolybdate method. The enzyme activities were represented as μmoles of P₁ liberated/mg protein per h.

2.3. GSSG-reductase (GSSG-R; EC 1.6.4.2)

The assay was carried out according to [10]. The reaction mixture contained 500 μl of 0.2 M NaPO₄ buffer, 100 μl each of 10 mM GSSG, 1 mM NADPH and 180 μl of distilled water. The reaction was started by the addition of 50 μg protein and the NADPH oxidation was recorded as the decrease in absorbance at 340 nm for 10 min. The enzyme activity was represented as μmoles of NADPH oxidized/mg protein per h.

2.4. Glutathione S-transferase (GST; EC 2.5.1.18)

The assay was according to [11]. The reaction mixture contained 500 μl of 0.2 M KPO₄ buffer, 100 μl of 10 mM GSH, 10 μl of 0.1 M 1-chloro-2,4-dinitrobenzene and 390 μl of distilled water. The reaction was started by the addition of 50 μg protein and the increase in absorbance was measured at 340 nm. The enzyme activity was calculated from the absorption coefficient of 9.6 mM⁻¹ cm⁻¹ and represented as mmol/mg protein per h.

2.5. Glutathione peroxidase (GSH-POD; EC 1.11.1.9)

The assay was carried out according to [12]. The reaction mixture contained 100 μl each of 0.5 M KPO₄ buffer, 10 mM Na₂-EDTA, 1.14 M NaCl, 10 mM GSH, 2 mM NADPH, 2.5 mM H₂O₂. The reaction was started by adding 5–6 μl GSSG-re-

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ductase (500 units/2.8 ml), 344 μl of water and 50 μg protein. The disappearance of NADPH was recorded at 340 nm for 10 min. The enzyme activity was represented as μmoles of NADPH oxidized/mg protein per h.

2.6. Measurement of total and non-protein thiol content

Thiol content was measured according to [13]. Cells were homogenized in 0.02 M EDTA in an ice bath.

2.7. Total thiols (TT)

Aliquots of 0.5 ml of the homogenates were mixed with 1.5 ml of 0.2 M Tris buffer pH 8.2 and 0.1 ml of 0.01 M DTNB. The mixture was brought to 10 ml by the addition of 7.9 ml of absolute methanol. Colour was allowed to develop for 15 min. Absorbance of the clear supernatant was read at 412 nm. Total sulfhydryl groups were calculated from the extinction coefficient of 13 100.

2.8. Non-protein thiols (NPT)

Aliquots of 5 ml of the homogenates were mixed with 4 ml distilled water and 1 ml of 50% TCA. The contents were mixed and after 15 min the tubes were centrifuged for 15 min. 2 ml of the supernatant was mixed with 4 ml of 0.4 M Tris buffer pH 8.9, 0.1 ml of DTNB and absorbance was read within 5 min at 412 nm against a reagent blank.

The protein bound thiols were calculated by subtracting the non-protein thiols (NPT) from total thiols (TT).

2.9. Estimation of total, reduced and oxidized glutathione

The estimation of GSH was carried out according to [14]. Cells were homogenized in 0.5 ml of 5% sulphosalicylic acid with liquid nitrogen and centrifuged at 12 000 × g for 10 min. A 300 μl aliquot of the supernatant was removed and neutralized by the addition of 18 μl 7.5 M triethanolamine. A 150 μl sample was used for the determination of total glutathione (GSH + GSSG). Another 150 μl sample was pretreated with 3 μl 2-vinylpyridine for 60 min at 20°C to mask GSH by derivatization and to allow the determination of GSSG alone. Fifty microlitre aliquots of both types of samples were mixed with 700 μl 0.3 mM NADPH, 100 μl 10 mM DTNB and 150 μl of 125 mM NaPO₄–6.3 mM EDTA buffer pH 6.5. A 10 μl aliquot of glutathione reductase (50 U/ml) was added and the change in absorbance at 412 nm was monitored. A standard curve prepared by using GSH and GSSG was used in the calculation of the amounts of total glutathione, reduced GSH (total GSH-oxidized GSSG) and GSSG.

2.10. Measurement of levels of hydrogen peroxide

Levels of hydrogen peroxide were estimated according to [15]. Cells were ground with mortar and pestle at 0°C in the presence of 0.2 N HClO₄ and the slurry was centrifuged at 20 000 × g for 5 min. To remove HClO₄ the supernatant was neutralized to pH 7.5 with 40KOH and the solution was centrifuged for 1 min at 1000 × g. An aliquot of the supernatant was applied to a 1 ml column of AG-1 (ion exchange resin) and the column was washed with distilled water. The eluate was used for the assay of H₂O₂.

The reaction mixture contained 1 ml of eluate, 400 μl of 12.5 mM DMAB in 0.375 M phosphate buffer (pH 6.5), 80 μl of MBTH and 20 μl of peroxidase (0.25 unit) in a total volume of 1.5 ml. The reaction was started by the addition of peroxidase at 25°C and the increase of absorbance at 590 nm was monitored.

2.11. Cu-binding complex in S. bijugatus

The procedure followed was according to [16] with slight modifications. A log phase culture of S. bijugatus was exposed to 200 μM CuSO₄ for 3 days. The cells were harvested by centrifugation at 2000 × g for 10 min. All the following steps were carried out at 4°C with the buffers flushed with gaseous nitrogen just prior to use. The cells were washed thrice and resuspended in buffer A (50 mM potassium phosphate/10 mM 2-mercaptoethanol pH 8.0). The algae were disrupted by sonication for 30 min and the homogenate was centrifuged at 20 000 × g for 30 min. The supernatant was applied to a Whatman DE-52 column (1.5 × 20 cm) equilibrated in buffer A at 90 ml/h. After washing with 2 column volumes of buffer A, the bound material was eluted with 0.5 M NaCl in
buffer A and collected in 4 ml fractions. The eluate was tested for —SH groups with Ellman’s reagent. —SH positive fractions were pooled and brought to 80% saturation with the addition of solid ammonium sulfate. The suspension was centrifuged and dialyzed against buffer A. The dialysate was concentrated by lyophilization and chromatographed on a Sephadex G-50 column (2 × 52 cm) equilibrated in buffer B (10 mM ammonium acetate pH 7.0). Four millilitre fractions were collected and examined for the presence of —SH groups and Cu. Cu content in the fractions was estimated by atomic absorption spectrophotometry. —SH positive and Cu-containing fractions were pooled and lyophilized and were referred to as the Cu-binding material. For comparison of treated and untreated extracts, untreated cells were homogenized and the supernatant lyophilized. The product was redissolved in buffer B for chromatography on Sephadex G-50 as described above.

2.12. Statistical analysis

The results were subjected to statistical analysis by Students’ t-test and the significance levels were determined at both $P < 0.05$ and $P < 0.01$.

3. Results

Growth was found to be adversely affected by copper treatment. There was an extension of lag phase and decrease of 15, 30 and 50% in growth rate, respectively in the presence of 50, 100 and 200 $\mu$M copper (Fig. 1). Copper content in the cells increased with time (Table 1). Total thiol content in 200, 400 $\mu$M and 1 mM copper-treated cells remained almost constant whereas non-protein thiol content decreased (Fig. 2).

GSH content in the cells after 24, 48 and 72 h of copper treatment decreased progressively in all the concentrations tested. The decrease in GSH content was drastic in the first 24 h of copper treatment and gradual after 24 h (Table 2). This may be due to enhanced utilization of GSH and decreased rate of synthesis.

Levels of $\text{H}_2\text{O}_2$ in different stages of treatment were estimated and were found to decrease after an initial period of increase (Table 3). $\text{H}_2\text{O}_2$ level increased in 500 $\mu$M and 1 mM copper-treated cells after 6, 12 and 24 h of treatment and thereafter decreased steadily. $\text{H}_2\text{O}_2$ levels also decreased when treated with exogenous antioxidants like mannitol (MNL), sodium benzoate (SB), butylated hydroxytoluene (BHT) and glutathione (GSH) (Table 3).

The observed decrease in the level of endogenous GSH was taken as a marker of metal-binding peptide synthesis. Copper-binding peptides were isolated from cells treated with 200 $\mu$M CuSO$_4$ for 3 days. Identity was checked with the presence of sulfhydryl groups and copper (Figs. 3 and 4). Bound copper content was estimated by atomic absorption spectrophotometry.

The activity of $\gamma$-GCS, which has an important role in GSH synthesis, showed only a moderate

<table>
<thead>
<tr>
<th>Table 1</th>
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<tbody>
<tr>
<td>Copper content in cells (µg/gm dry wt) after treatment with different concentrations of copper for 0, 24, 48 and 72 h.</td>
</tr>
<tr>
<td>Concentration</td>
</tr>
<tr>
<td>---</td>
</tr>
<tr>
<td>0</td>
</tr>
<tr>
<td>Control</td>
</tr>
<tr>
<td>100 µM</td>
</tr>
<tr>
<td>200 µM</td>
</tr>
</tbody>
</table>
Fig. 2. Total thiol (TT) and non-protein thiol (NPT) content in Cu-treated cells in μmoles/mg chlorophyll. The values are means ± SD of 3 experiments with replicated measurements.

increase of 6 and 18% in 50 and 100 μM treatments and an increase of 51% in 200 μM CuSO₄ treatment (Fig. 5). This may be due to delay in the activation of the biosynthetic part of the GSH cycle, which may occur after endogenous GSH is consumed in the antioxidative process. The presence of 1 mM GSH in the reaction mixture reduced the observed activity of γ-GCS by 50%. This may be due to feedback inhibition of the enzyme by GSH. In homogenates of tobacco cells

Table 2
GSH content in different copper treatments after 0, 24, 48 and 72 h in μmoles/mg chlorophyll. Values are means of three independent experiments with 3 replicates. Values in parentheses represent the S.D.

<table>
<thead>
<tr>
<th>Concentration</th>
<th>Duration (h)</th>
<th>0</th>
<th>24</th>
<th>48</th>
<th>72</th>
</tr>
</thead>
<tbody>
<tr>
<td>Copper treatment</td>
<td>Control</td>
<td>5.78(0.34)</td>
<td>6.23(0.38)</td>
<td>6.59(0.26)</td>
<td>7.04(0.29)*</td>
</tr>
<tr>
<td></td>
<td>50 μM</td>
<td>5.54(0.29)</td>
<td>4.89(0.14)</td>
<td>4.13(0.37)*</td>
<td>3.76(0.24)</td>
</tr>
<tr>
<td></td>
<td>100 μM</td>
<td>5.60(0.26)</td>
<td>4.06(0.09)**</td>
<td>3.63(0.16)**</td>
<td>3.12(0.21)**</td>
</tr>
<tr>
<td></td>
<td>200 μM</td>
<td>5.54(0.1)</td>
<td>3.87(0.13)**</td>
<td>2.37(0.2)**</td>
<td>1.85(0.19)**</td>
</tr>
</tbody>
</table>

* Significant at P<0.05.
** Significant at P<0.01.

synthesis of GSH from constituent aminoacids was inhibited to 50% by GSH concentrations of 30 μM [17].

The activity of GSSG-R showed a significant decrease of 44, 51 and 69%, respectively in cells treated with 50, 100 and 200 μM CuSO₄ (Fig. 5). The rate of decrease in GSSG-R activity was more in 50 μM treatment than in the other treatments. This reflects the decrease in GSH content, which may be due to its oxidation to GSSG by reaction with Cu ions.

The activity of GSH-PX increased significantly by 26, 68 and 123%, respectively in 50, 100 and 200 μM CuSO₄ treatments (Fig. 5). GSH-PX has only recently been reported in plants, especially in algae. Due care was taken not to expose GSH to air and GSSG-reductase was included in the reaction mixture, hence it can be safely assumed that the observed peroxidation of GSH was due to the activity of GSH-PX.

The activity of GST showed an increase of 17, 35 and 78%, respectively in 50, 100 and 200 μM CuSO₄ treatments (Fig. 5). During Cu stress protective mechanisms of the cell are employed, leading to activation of protective enzymes like GST.

4. Discussion

The results obtained in the present study show that the activities of enzymes of GSH metabolism vary considerably in response to copper stress. GSH, being a prominent cellular antioxidant, plays an active role in protecting membranes against free radical damage [4,5]. It is also a precursor of phytochelatins, a class of metal-binding peptides widely reported in plants [7,16]. GSH content decreased to a considerable extent in the presence of copper (Table 2) and this may be one of the mechanisms of toxicity alleviation in Scenedesmus bijugatus. The enzymes of GSH metabolism participate in the H₂O₂-scavenging pathway, thereby maintaining a cellular GSH pool [6].

γ-GCS is the rate-limiting enzyme in GSH synthesis, since the overexpression of γ-GCS rather than glutathione synthetase (GSHS) leads to enhanced GSH level [18]. In Cd-exposed plants γ-GCS mRNA showed a higher increase compared to GSHS showing that γ-GCS was upregulated in response to Cd exposure [19]. In Cd-exposed
Table 3
H₂O₂ levels in cells in different copper treatments after 6, 12, 24, 48 and 72 h in μmoles/mg chlorophyll. Values are means of 5 independent experiments with triplicates.

<table>
<thead>
<tr>
<th>Copper treatment</th>
<th>Duration (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>6</td>
</tr>
<tr>
<td>Control</td>
<td>0.076(0.012)</td>
</tr>
<tr>
<td>200 μM Cu</td>
<td>0.114(0.005)</td>
</tr>
<tr>
<td>300 μM Cu</td>
<td>0.120(0.023)</td>
</tr>
<tr>
<td>1 mM Cu</td>
<td>0.167(0.006)</td>
</tr>
<tr>
<td>500 μM Cu + MNLb</td>
<td>0.068(0.006)</td>
</tr>
<tr>
<td>500 μM Cu + SBb</td>
<td>0.076(0.012)</td>
</tr>
<tr>
<td>500 μM Cu + BHTb</td>
<td>0.080(0.016)</td>
</tr>
<tr>
<td>500 μM Cu + GSHb</td>
<td>0.072(0.013)</td>
</tr>
</tbody>
</table>

* Figures in parentheses denote the S.D.
** Significant at P < 0.01.

Pisum sativum and Zea mays the activities of the enzymes γ-GCS and GSHS increased several fold [20,21]. In tomato cells selected for Cd tolerance γ-GCS activity increased [22]. In Scenedesmus only a moderate increase was observed in the γ-GCS activity in the presence of 50 and 100 μM copper, whereas a significant increase was observed in 200 μM copper treatment (Fig. 5). The moderate increase in activity may be insufficient to replenish the depleted GSH pool which may be due either to phytochelatin synthesis or to direct interaction with Cu ions.

GSSG-R is the key rate-limiting enzyme in the H₂O₂-scavenging pathway in chloroplasts of plants [23]. Although elevated activities of this enzyme have been reported under different kinds of stress [24–26], in Scenedesmus, the activity of the enzyme decreased by 44, 56 and 69% with 50, 100 and 200 μM concentrations of copper, respectively (Fig. 5). The enzyme is sensitive to inhibition by heavy metal ions like Zn²⁺, Cu²⁺ and Fe³⁺ and by compounds that react with –SH groups (like NEM), due to the presence of thiol groups at the active site of the enzyme [27]. Thus, the reduced activity of GSSG-R may be due to inactivation of the enzyme by Cu²⁺ ions which decreases the rate at which GSSG is reduced to GSH. This ultimately leads to a depletion of the cellular GSH pool [25].

Though the activities of γ-GCS were increased several fold, glutathione accumulated only to a limited extent. Enhanced GSH degradation or turnover under stress conditions may be the reason [18]. In this study, a moderate increase in γ-GCS activity coupled to a considerable down-regulation of GSSG-R activity reflects the decrease in GSH content under copper stress. GSSG-R activity may be a limiting factor for the recycling of GSH and thereby operation of the mechanisms.
Fig. 4. Elution profile of fractions from Sephadex G-50 gel filtration column. The peak fractions rich in sulfhydryl groups and Cu after ion exchange chromatography were pooled and the supernatant after 80% ammonium sulfate precipitation was concentrated and loaded onto Sephadex G-50. Four millilitre fractions were collected and sulfhydryl- and Cu-containing fractions were pooled and lyophilized.

of detoxification. GSH, on reaction with oxy radicals generated in the presence of copper, is oxidized to GSSG and a decrease in re-reduction of GSSG contributes to the decrease in cellular level of GSH.

H$_2$O$_2$ is scavenged by GSH-PX in the cytoplasmic compartment [28]. It is a part of the arsenal of protective enzymes present in plants when oxidative stress leads to lipid peroxidation. The Se-dependent and Se-independent forms scavenge inorganic and organic peroxides respectively. It may also function to remove lipid hydroperoxides from membranes as in Euglena [29]. A Se-dependent form was reported in Chlamydomonas reinhardtii [30]. In Scenedesmus, the peroxidation of GSH showed significant concentration-dependent increase (21, 41 and 56%) in the presence of 50, 100 and 200 µM copper (Fig. 5). H$_2$O$_2$ level was found to increase in the initial stages of treatment (Table 3) which may serve as the stimulus for activation of the enzyme. H$_2$O$_2$ levels decreased thereafter showing the elevated activity of the enzyme. Elevated production of harmful peroxides which catalyze peroxidative chain reactions in the presence of copper may be the reason for activation of the enzyme.

GST plays an active role in detoxification. Electrophilic substrates with the help of the enzyme bind to GSH and form GSH-substrate complexes. GST mRNA responds very quickly to oxidative stress [31]. The activity of GST increases in the presence of metals [18]. In Scenedesmus, 15, 26 and 44% increases were observed with treatments of 50, 100 and 200 µM copper (Fig. 5). This may be due to production of endogenous electrophiles like 4-hydroxy alkenals and base propenals, the products of oxidative degradation of lipids and nucleic acids respectively, which act as substrates for the enzyme and their elevated production may be the reason for activation of the enzyme.

The decrease in non-protein thiol content while total thiol content remains almost constant (Fig. 2) shows the increase in protein thiol content and serves as a marker for the synthesis of metal-binding peptides which are reported to have a role in sequestration and metal homeostasis. The peptides, phytochelatins, contain chains of γ-glutamylcysteine units and are rich in sulfhydryl groups. The peptides isolated from S. bijugatus

Fig. 5. Activities of GSH cycle enzymes in control and treated cells 3 days after treatment. The values are means ± SD of three independent experiments with three replicates. The enzyme activities were expressed as: γ-GCS (γ-glutamylcysteine synthetase) in µmoles of P$_i$ released/mg protein per h; GSSG-R (glutathione reductase) and GSH-PX (glutathione peroxidase) in µmoles of NADPH oxidized/mg protein per h; GST (glutathione S-transferase) in mmoles of GSH conjugated/mg protein per h.
were found to contain protein thiols and about 30% of the total copper absorbed by the cells was bound to them. Acidification of the Cu-binding material with HCl was able to free the metal ions from the peptides whereas neutralization led to reconstitution of the Cu-peptide complex. The presence of these peptides in control (untreated) cells was undetectable and their content increased progressively in cells treated with increasing concentrations of Cu. Thus, this mechanism may be the major pathway for detoxification of metal ions in Scenedesmus bijugatus, quite apart from the antioxidative pathway activated to counter the effects of stress.

Thus, the increase in the activities of enzymes of GSH metabolism and the activities of antioxidant enzymes [32] suggests that GSH-ascorbate cycle has a pivotal role in copper detoxification, apart from the role of phytochelatins in the chelation of metals. The inherent or induced tolerance of an organism to a metal determines its ability to take up metal ions and sequester them. So the manipulation of activities of antioxidative enzymes increases tolerance, thereby potentially increasing the uptake capacity of an organism. This approach can be explored as a strategy in metal removal from polluted aquatic ecosystems.

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