

**LIVER AND KIDNEY STRUCTURAL AND BIOCHEMICAL  
CHANGES INDUCED IN GOLDFISH (*Carassius auratus gibelio*)  
DURING MANGANESE ACUTE INTOXICATION**

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**ABSTRACT.** Manganese, an essential element in humans and animals, is known to have both protective and toxic effect. Prolonged Mn(II) exposure induces the formation of reactive oxygen species leading to impairment of antioxidant system. The aim of the present study was to evaluate the fish adaptative response to experimental manganese pollution by histological and biochemical methods, with particular attention to oxidative metabolism.

The enzymatic variations and morphological damages simultaneously appeared after acute manganese exposure.

The liver and kidney nuclei were the main pollutant target. The structural changes consisting in karyomegaly, anisokary, pycnosis prove that the metal ion has a deep effect on the nuclear genetic material.

Specially adapted enzymes to prevent the formation of oxyradicals (catalase, glutathione peroxidase, glutathione reductase and glutathione-S-transferase) changed their profiles after acute manganese exposure.

The two different organs have different patterns in antioxidant enzymes activities.

Both liver and kidney were deeply damaged after manganese intoxication, but liver seemed to be more resistant to the pollutant and more efficient in organism detoxification. The changes we observed, could be considered as early indicators of stress status of the animals determined by changed environmental conditions.

**KEY WORDS.** manganese, pycnosis, oxidative stress, catalase, glutathione peroxidase, glutathione reductase, glutathione-S-transferase

## INTRODUCTION

The health of aquatic organisms may be linked to oxidative stress (Di Giulio et al., 1989). The processes by which environmental contaminants may enhance oxidative stress in aquatic organisms are of particular concern. As a consequence of normal oxygen utilization by aerobes, various reactive oxygen species are produced ( $1O_2$ ,  $O_2^-$ ,  $\cdot OH$ ,  $RO\cdot$ ,  $ROO\cdot$ ) (Lackner, 1998). These oxygen species are capable of reacting with critical cellular macromolecules, leading to enzyme inactivation, lipid peroxidation, DNA damage and ultimately cell death. Specially adapted enzymes such superoxide dismutases (SOD; EC 1.15.1.1), catalase (EC 1.11.1.6) and glutathione peroxidases (EC 1.11.1.9) prevent the formation of oxyradicals.

Manganese is an essential element in humans and animals, functioning both as an enzyme cofactor and as a constituent of metalloenzymes. It has been implicated both in the carbohydrate, lipid and sterol metabolism and oxidative phosphorylation.

It accumulates in mitochondria, a major source of superoxide, which can oxidize  $Mn^{2+}$  to the powerful oxidizing agent  $Mn^{3+}$ . It has been suggested that the oxidation of important cell components by  $Mn^{3+}$  is a cause of the toxic effects of manganese (Halliwell and Gutteridge, 1990). The tissues rich in mitochondria and endoplasmic reticulum as the skeleton, liver, skeletal muscle, connective tissue and intestine concentrate manganese.

Depending on the concentration,  $MnCl_2$  is known to have both protective and toxic effect.  $Mn(II)$  is reported to be a scavenger of superoxide radical (Archibald-Fridovich, 1982). It reduces hydroxyl radical to yield  $Mn(OH)^{2+}$  (Chang-Kosman, 1989) and activates a variety of enzymes (West et al., 1974). On the other hand, prolonged  $Mn(II)$  exposure induces the formation of reactive oxygen species leading to impairment of antioxidant system. (Desole et al., 1995)

In our experiments, the liver and kidney were chosen due to their important role in the metabolism of the xenobiotics.

The aim of the present study was to evaluate the fish adaptative response to experimental manganese pollution by histological and biochemical methods, paying particular attention to oxidative metabolism.

## MATERIAL AND METHODS

Freshwater goldfish *Carassius auratus gibelio* of 13.5-16.5cm and 20.0-30.0g in weight, were obtained from The Nucet Fishery Research Station and housed in a 60 l glass aquarium at 25°C. Prior to exposure, the fish were held for 15 days for acclimatization and evaluation of overall fish health under laboratory conditions. For the intoxication experiments, the unfed fish lots were exposed to a final concentration of 0.5mg  $Mn^{2+}/l$ . After one, two, three and respectively seven days the liver and kidney were removed and prepared for histological and biochemical analysis.

### *Histological method*

Small tissue fragments obtained from two years old *Carassius auratus gibelio* were fixed in Bouin three hours long. The tissue samples were embedded in paraffin

and cut in 8µm thick slices which were Hemalaun Meyer-Eosine stained and examined by Olympus light microscope.

### ***Enzymatic assay***

The frozen tissues were homogenized in ten volumes of 0.1M Tris-EDTA buffer, pH=7.4 and centrifuged at 8000xg for 30min at 0-4°C. Aliquots of the supernatant were utilized for enzymatic assays.

Catalase (CAT) activity was assayed by the method of Aebi (1974). Change in absorbance was recorded at 240nm. Catalase activity was calculated in terms of k/min/mg protein, where it is the first order rate constant.

Glutathione reductase (GR) activity was recorded by the Goldberg and Spooner method (1983). The enzyme activity was calculated as µmol NADPH oxidized/min/mg protein.

Glutathione S-transferase (GST) activity was determined by the method of Habig et al. (1974). The change in absorbance was recorded at 340nm and the enzyme activity calculated as nmol CDNB conjugate formed/min/mg protein using a molar extinction coefficient of  $9.6 \times 10^3 \text{ M}^{-1} \text{ cm}^{-1}$ .

Glutathione peroxidase (GPx) activity was assayed according to the method described by Beutler (1984). Oxidation of NADPH was recorded spectrophotometrically at 340nm at 25°C. The enzyme activity was calculated in nmoles NADPH oxidized/min/mg of protein, using a molar extinction coefficient of  $6.22 \times 10^3 \text{ M}^{-1} \text{ cm}^{-1}$ .

The protein estimation was done through the method of Lowry et al. (1951) using Folin reagent and bovine serum albumin (BSA) as standard.

The Student test analysis was used to determine the significance of the differences between the treated fish group and the control one.

## **RESULTS**

### ***Histomorphological tests***

Acute manganese exposure induced significant structural changes in *Carassius auratus gibelio* tissues. Hepatic tissue was affected after 2 days of exposure: some hepatocytes were vacuolized and frequently karyomegaly or less nucleolus nuclei with different affinity for the dyes were observed (Fig.1). Other hepatocytes presented karyorhexic or pycnotic nuclei. The sinusoids containing erythrocytes were dilatated. It seems that manganese pollution induced frequently an eccentric location of the nuclei, hepatocyte vacuolisation likely accumulating glycogen or lipids. Macrophages accumulations frequently appeared (Fig.2).

Serious damages in the renal tissue after only one day of acute exposure on manganese polluted water were noticed. Many necrotic urinary tubules having lesions of cubic or columnar epithelial layer, nuclei modifications (binucleated epithelial cells, pycnotic nuclei, nuclei malformations, anizokaric nuclei, denudated nuclei) can be seen (Fig.3).

### ***Enzymatic assay***

The specific activity of catalase in the kidney significantly decreased after one day of acute manganese exposure but increased after 2 days, attaining a maximum at 3 days intoxication (Fig.4). In the liver this enzyme specific activity increased in the first 2 days and decreased after the 3rd day and then maintained at a high level till 7 days of exposure (Fig.5).

The kidney glutathione reductase specific activity constantly decreased during the first 3 days. In the 7th day of exposure enzymatic activity attained the control level (Fig.6). In the liver, the glutathione reductase activity strongly increased after one day, then it slowly decreased but its level was higher compared to the control (Fig.7).

We noticed a significant decrease in the kidney glutathione peroxidase specific activity after one day which is maintained after 2, 3 and respectively 7 days of exposure (Fig.8). Important fluctuations in the liver glutathione peroxidase profile were noticed. Its activity strongly increased after one day, then, after 2 days it recovered its activity level and attained a higher level compared to the control slowly decreasing in the next hours until the 7th day (Fig.9).

In the kidney, glutathione-S-transferase activity significantly increased in the first day, decreased after 2 and respectively 3 days and was the highest after seven days (Fig.10). The liver enzymatic activity significantly increased after the first day, then slowly decreased until the seventh day maintaining higher than the control (Fig.11).

## **DISCUSSION**

Acute exposure to 0.5 mg/l Mn(II) for one, 2, 3 and 7 days generates a pollutant-induced adaptative response in fish liver and kidney.

The enzymatic and morphological changes simultaneously appeared. The fish liver involved both in metabolic and detoxification processes, is known to be damaged by chemical water pollution and particularly by heavy metals. Aberrant nuclei have been reported in the hepatocytes by other scientists too. In striped bass and in salmon this condition coincided with megalocytic hepatopathy and it was linked to toxic environmental factors (Kent 1988, Groff, 1992).

Once it penetrate the cell, manganese crosses the mitochondrial inner membrane using the  $H^+/Mn^{2+}$  symport, alters the mitochondrial functions and affects the electron transport in the inner mitochondrial membrane. As a consequence, the level of  $H_2O_2$  increases and the synthesis and/or release of liver catalase is stimulated.

In the kidney the significant inhibition of the specific activity of catalase after a day of  $Mn^{2+}$  acute intoxication is possible to appear because, in the glomerular filtrate the xenobiotic is concentrated and can reduce  $Fe^{3+}$  of the hemic group of catalase to  $Fe^{2+}$ . The increased activities after 2, 3 and 7 days of intoxication could appear because of an increase of the catalase synthesis. The inhibition of catalase activity was also observed following  $MnCl_2$  exposure of rat feochromocytoma cells

for one and 3 days. (Seth et al., 2002). The free radical-mediated toxicity of Mn (II) at cellular level involved down-regulation of antioxidants.

In the liver and kidney, the decrease of glutathione peroxidase activity is probably due to the high concentration of hydrogen peroxide, which is inhibitory for glutathione peroxidase and is correlated with the increase of catalase activity .

Glutathione peroxidase is the key enzyme of the antioxidant system under normal and oxidative stress conditions. It is important to notice that among the studied tissues in various species of fish, the liver showed the highest activities of catalase and glutathione peroxidase (Radi et al., 1985; Otto-Moon, 1996; Perez-Campo et al., 1993). The cause of such differences could be due to a high rate of free radical generation in the liver than in other tissues. Catalase required for the detoxification of  $H_2O_2$  is likely inhibited by  $MnCl_2$  instead peroxidase is activated by the same metal. Similar results were obtained by Dimitrova et al.(1994) in *Cyprinus carpio* liver, who noticed that after the combined treatment with zinc and lead for one and 10 days, catalase activity had increased. It seems that both hepatic enzymes operate in a cooperative manner, so while one is inhibited, the other one is activated in order to eliminate  $H_2O_2$ . The idea that the antioxidant enzymes act cooperatively and even in synergy had been suggested earlier by Michiels et al., (1994). Other studies showed that exposure to increased  $CuSO_4$  concentration caused a decrease in catalase activity of liver, but, on the other hand, it stimulated glutathione peroxidase activity in the same tissue of carp (Radi-Matkovich, 1988).

Generally, the oxidative stress causes an elevation in GPx activity. This could reflect an adaptation to oxidative conditions to which the fish had been exposed. Glutathione peroxidase activity progressively increased following manganese exposure, possibly due to the increased production and enzyme-inducing effect of  $H_2O_2$  derived from  $O_2^-$ . Low activity of glutathione peroxidase in the kidney of intoxicated fish, as observed in the present study, demonstrates inefficiency of this organ in neutralizing the impact of peroxides and thus resulting in increased lipid peroxidation.

In the liver, Mn(II) acute intoxication generates a significant increase of the specific glutathione reductase activity after one day of exposure, which can be correlated to GSH necessity, the most abundant cellular thiol, involved in metabolic and transport processes and in the protection of cells against the toxic effects of reactive oxygen species and heavy metals (Meister-Anderson, 1983).

In the kidney the significant decrease of glutathione reductase activity could be due to the oxidation of Mn(II) by  $O_2^-$ , which generates Mn(III) or manganese oxygen complexes which are more oxidants for NADPH than superoxide anion. As a consequence, NADPH sources for this enzyme are diminished. After 7 days, the effect of the toxicant is possibly lowered and the level of NADPH is increased.

Glutathione-S-transferase (GST) is a group of multifunctional enzymes involved in biotransformation and detoxification of xenobiotics. Highly reactive electrophilic components can be removed before they covalently bind to tissue nucleophilic compounds which would lead to toxic effects. The variation profile of glutathione S transferase in liver and kidney is the same as for glutathione reductase,

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suggesting that the content of GSH is essential for the enzymatic activity. GST activity increased consistently in liver because it is involved both in xenobiotic and xenobiotic metabolites detoxification and excretion.

Our results obtained during manganese experimental pollution demonstrated that manganese induced in hepatocytes a hypoxic condition demonstrated by increased LDH activity (not shown data).

It seems that different organs have different patterns in antioxidant enzymes. Antioxidant pattern was different in liver and kidney showing that liver was more resistant to oxidative damage as compared to kidney. The changes we observed, could be considered as early indicators of stress status of the animals determined by the environmental conditions which were changed.

### CONCLUSIONS

The enzymatic variations and morphological damages simultaneously appeared after acute manganese exposure.

The liver and kidney nuclei were the main pollutant target. The structural changes consisting in karyomegaly, anisokary, pycnosis prove that the metal ion has a deep effect regarding the nuclear genetic material.

Specially adapted enzymes for the prevention of the formation of reactive oxygen species changed their profiles after acute manganese exposure.

Both liver and kidney were deeply damaged after manganese intoxication, but the liver seemed to be more resistant to the pollutant and more efficient in organism detoxification.

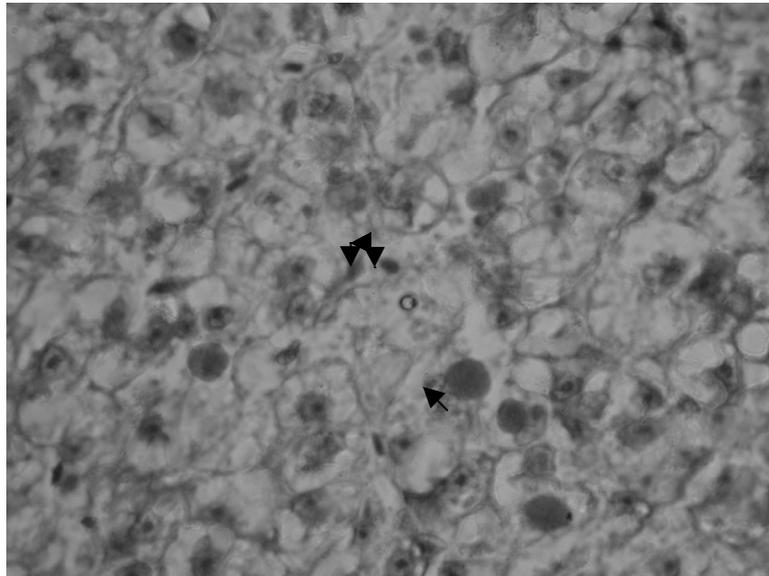
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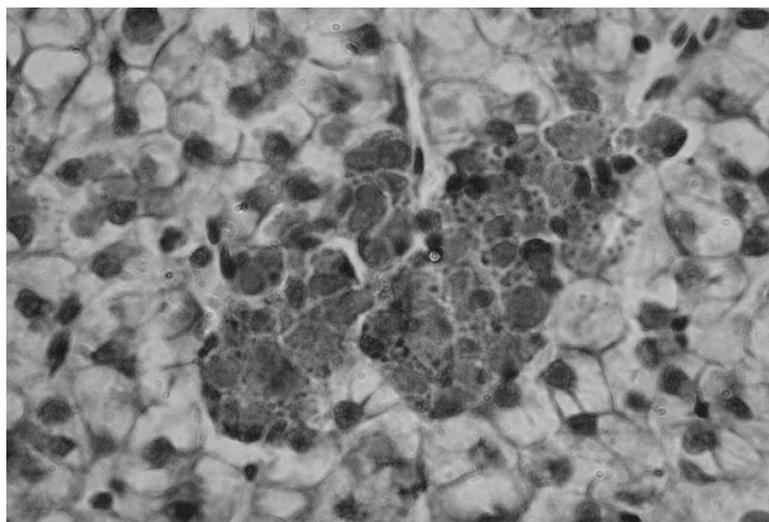
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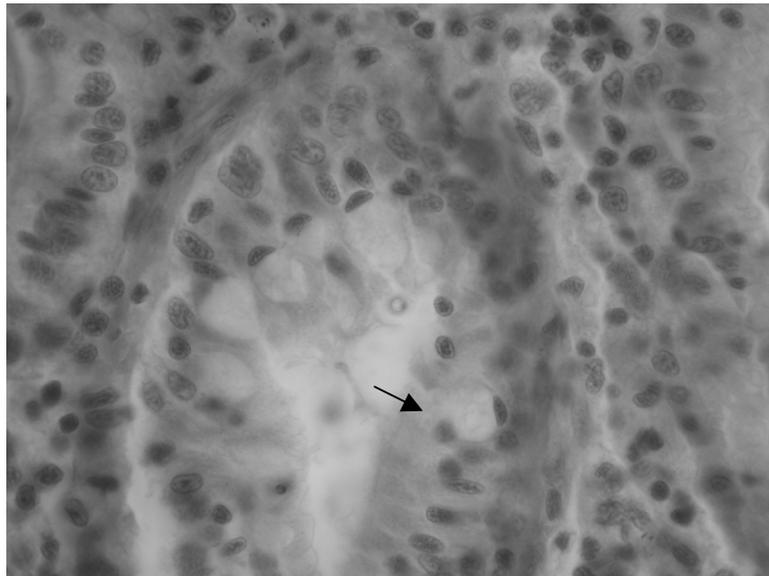
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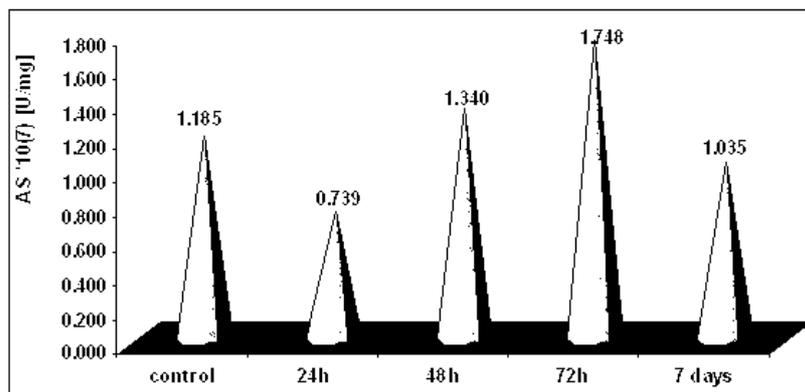
**Fig. 1** *Carassius auratus gibelio* liver after 2 days of manganese acute exposure (H-E).  
Hepatocytes with karyomegaly and picnotic nuclei.



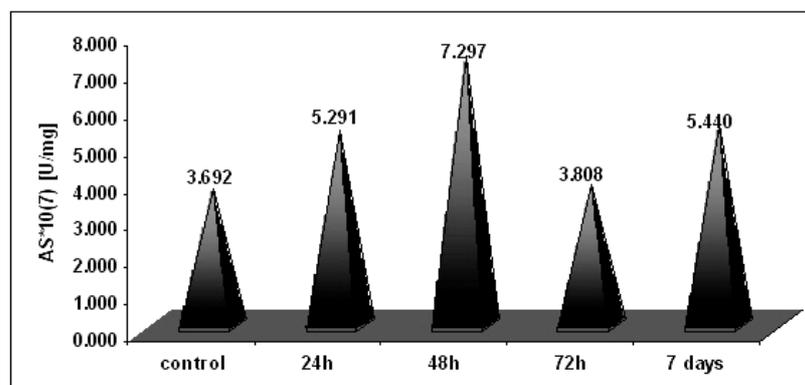
**Fig. 2** *Carassius auratus gibelio* liver after 2 days of manganese acute exposure (H-E).  
Macrophages accumulations



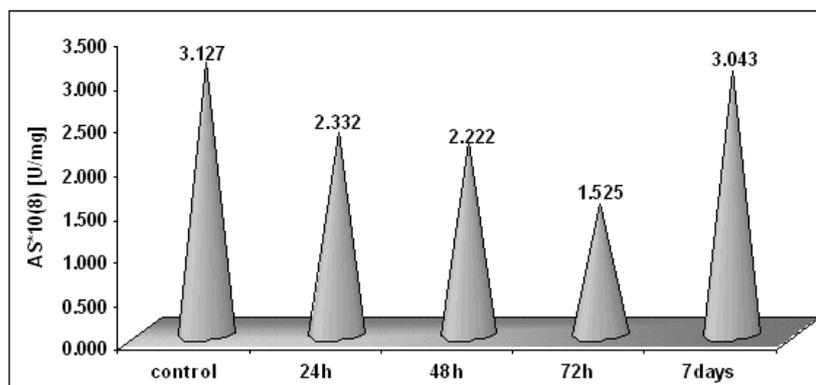
**Fig. 3** *Carassius auratus gibelio* kidney after one day of acute manganese exposure (H-E). Necrotic urinary tubules, with vacuolization (arrow) of cubic or columnar epithelial layer and nuclei changes



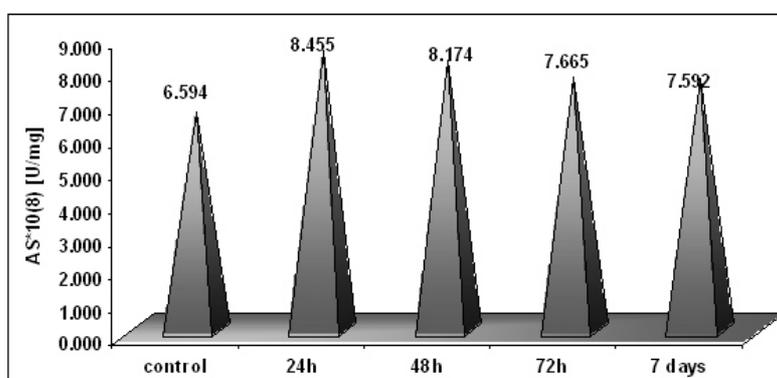
**Fig. 4** The catalase specific activity in kidney of *Carassius auratus gibelio* exposed to 0.5mg  $Mn^{2+}/l$  for 1, 2, 3 and 7 days and control fish.



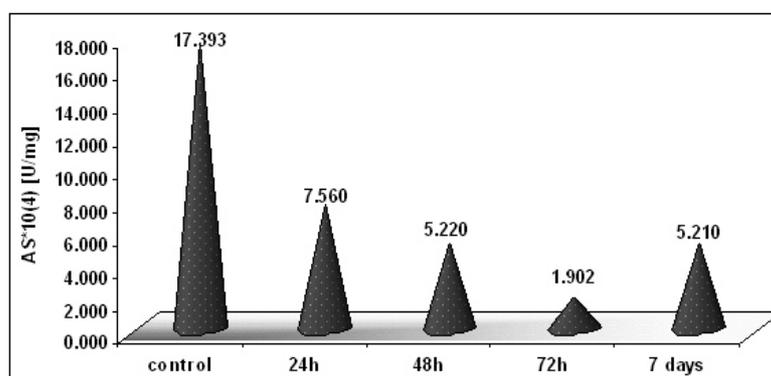
**Fig. 5** Catalase specific activity in liver of *Carassius auratus gibelio* exposed to 0.5mg  $Mn^{2+}/l$  for 1, 2, 3 and 7 days and control fish.



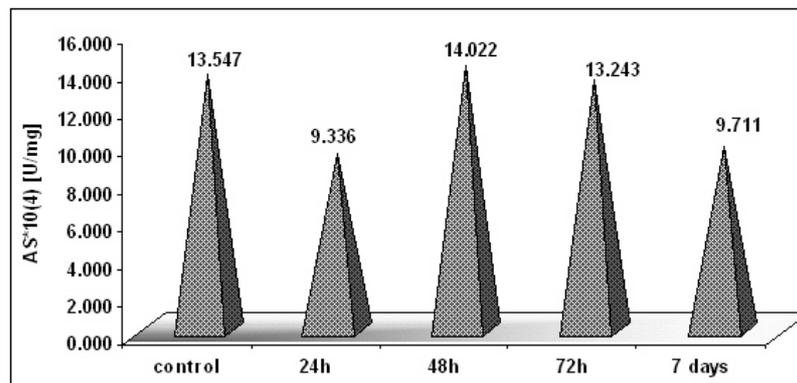
**Fig. 6** Glutathione reductase specific activity in kidney of *Carassius auratus gibelio* exposed to 0.5mg Mn<sup>2+</sup>/l for 1, 2, 3 and 7 days and control fish.



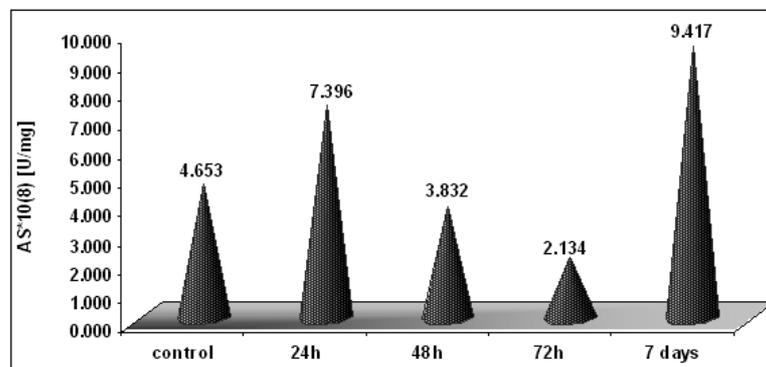
**Fig. 7** Glutathione reductase specific activity in liver of *Carassius auratus gibelio* exposed to 0.5mg Mn<sup>2+</sup>/l for 1, 2, 3 and 7 days and control fish.



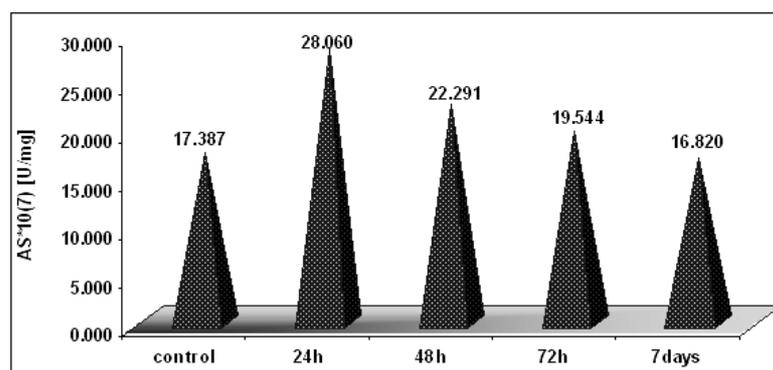
**Fig. 8** Glutathione peroxidase specific activity in kidney of *Carassius auratus gibelio* exposed to 0.5mg Mn<sup>2+</sup>/l for 1, 2, 3 and 7 days and control fish.



**Fig. 9** Glutathione peroxidase specific activity in liver of *Carassius auratus gibelio* exposed to 0.5mg Mn<sup>2+</sup>/l for 1, 2, 3 and 7 days and control fish.



**Fig. 10** Glutathione S-transferase specific activity in kidney of *Carassius auratus gibelio* exposed to 0.5mg Mn<sup>2+</sup>/l for 1, 2, 3 and 7 days and control fish.



**Fig. 11** Glutathione S-transferase specific activity in liver of *Carassius auratus gibelio* exposed to 0.5mg Mn<sup>2+</sup>/l for 1, 2, 3 and 7 days and control fish.