

INFLUENCE OF SOME ABIOTIC FACTORS ON THE ACUTE TOXICITY OF CADMIUM TO *CYPRINUS CARPIO*

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ABSTRACT

Hardness of water had significant effect on the acute toxicity of cadmium to common carp, *Cyprinus carpio*. The 96h LC₅₀ and safe application rate increased from 43.17 and 22.77 mg l⁻¹ in soft water (0.9 mM Ca²⁺ l⁻¹) to 310.48 and 177.66 mg l⁻¹, respectively, in very hard water (6.0 mM Ca²⁺ l⁻¹). In medium hard and hard water, 96h LC₅₀ values were 48.39 and 116.45 mg l⁻¹. When sediments were included in the medium hard, hard and very hard water treatments, the 96h LC₅₀ were 111.20, 133.71 and 334.47 mg l⁻¹, respectively. Among these values, the one for medium hard water with sediment treatment was significantly higher than medium hard water treatment; values for the other two treatments were non-significant when compared with respective water treatments. Sediment was able to reduce the acute toxicity of cadmium mainly due to the complexation of cadmium with dissolved organic carbon (DOC). At the lower hardness level, cadmium complexed with DOC and the acute toxicity was reduced significantly. At higher hardness, most of the DOC sites were occupied by calcium and the acute toxicity of cadmium was not significantly reduced in hard water with sediment and very hard water with sediment experiments in comparison to respective water treatments.

Keywords: Cadmium, hardness of water, sediment, *Cyprinus carpio*

INTRODUCTION

Cadmium is a common pollutant distributed in air, water, soil and even in living organisms. It is next only to mercury as the most notorious of heavy metal pollutants. Like mercury (and unlike several other heavy metals), cadmium is not essential for plants or animals (UNEP, 1989). The sources responsible for cadmium contamination are mainly of anthropogenic origin. According to an estimate, approximately 500 t of cadmium enter the

environment annually as a result of natural weathering and about 2000 t are released annually as a result of human activities (Abbasi *et al.*, 1998). Cadmium is mainly used in industries involved in electroplating nickel, cadmium battery manufacture, pigment manufacture, galvanising, plastic manufacture, alloy manufacture and glass manufacture. Effect of cadmium toxicity on aquatic organisms (Sherman *et al.*, 1987; Khangarot and Ray, 1987) and fish (Chambers, 1995; Hollis *et al.*, 2000;

Zohouri *et al.*, 2001; McGeer *et al.*, 2000a, 2000b) is well documented. In fish, it causes hypocalcaemia, malformation of spine, and affects growth and reproduction (WHO, 1992). Majority of the studies related to the establishment of toxicological effect of a chemical to fish did not include the soil part, though it is a must in the natural condition. Different physicochemical parameters of water and soil can affect the toxicity of pesticides and heavy metals in aquatic ecosystems (Datta and Das, 2001, 2002; Datta *et al.*, 2001, 2002a, 2002b, 2002c, 2002d, 2002e, 2003; Datta, 2003). Keeping these things in mind, the present study was undertaken in laboratory condition with two objectives, (a) to study the effect of hardness of water on acute toxicity of cadmium to common carp and (b) to understand the effect of soil sediment on acute toxicity of cadmium to common carp.

MATERIAL AND METHODS

Test Chemical, Test Animal and Test Container

Cadmium nitrate [$\text{Cd}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$] manufactured by Ranbaxy Fine Chemicals Ltd., India, was chosen as the test chemical. Advanced fry of common carp (*Cyprinus*

carpio var. *communis*) (av. total length: 3.5 ± 0.5 cm, av. weight: 0.65 ± 0.25 g, $n = 70$) was chosen for the experiment. Round cylindrical plastic buckets and glass jars of 20 l capacity were used as test container for water and water with sediment experiments, respectively. Simple aquarium aerators with two outlets purchased from the local market were used for aeration.

Test Water

Experiment was conducted at different levels of water hardness. These were prepared by mixing distilled water ($0.10 \text{ mM Ca}^{2+} \text{ l}^{-1}$ of total hardness), drinking water ($0.9 \text{ mM Ca}^{2+} \text{ l}^{-1}$) and tap water ($6.0 \text{ mM Ca}^{2+} \text{ l}^{-1}$) in different proportions. For routine testing of total hardness by standard complexometric titration, Aquamerck Compact Laboratory (E-Merck, Germany) was used. According to the manual provided with this compact laboratory, water can be classified into four different groups on the basis of its total hardness as described in Table 1. So the water used for the experiment were: $0.90 \text{ mM Ca}^{2+} \text{ l}^{-1}$ (soft water), $1.90 \text{ mM Ca}^{2+} \text{ l}^{-1}$ (medium hard water), $2.90 \text{ mM Ca}^{2+} \text{ l}^{-1}$ (hard water) and $6.0 \text{ mM Ca}^{2+} \text{ l}^{-1}$ (very hard water). Salinity was calculated from the equation: salinity ($\%$) = $0.03 + [\text{density of water at } 25^\circ\text{C} \times \text{chlorinity (g l}^{-1})]$.

Table 1: Classification of water on the basis of its total hardness

Categories of water	Hardness		
	$^\circ\text{d}$	$\text{mM Ca}^{2+} \text{ l}^{-1}$	$\text{mg CaCO}_3 \text{ l}^{-1}$
Soft	< 7	< 1.3	< 125
Medium hard	7-14	1.3-2.5	125-250
Hard	14-21	2.5-3.8	250-375
Very hard	> 21	> 3.8	> 375

In this experiment, only hardness of water was manipulated, alkalinity and salinity levels changed automatically. Experiment was not conducted in reconstituted water to simulate the natural field condition where associated chemical factors (salinity, alkalinity, etc.) with the increase the level of hardness are always present.

Sediment and Humus

The sediment was collected from different pollution-free areas of West Bengal and finally, soil from 24 Parganas (North), which contains 24.88% clay, 21.00% silt and 3.01% organic matter was used. For humus, well-decomposed dried powdered farmyard manure was used.

Water Analysis

Standard methods were used for the routine analysis of pH, dissolved oxygen, free CO₂, total alkalinity and salinity (APHA, 1998).

Acclimatisation of Test Animals and Range Finding

Healthy, disease-free common carp was brought to laboratory from the Fish Cultivate Centre, Naihati, and kept in 50 l tap water in polyvinyl chloride (PVC) tubs. For acclimatisation, 250 fry were kept in each tub in 40 l of water with continuous aeration and regular exchange of water at an interval of 24 hours. Fish were fed at 3% of the body weight with formulated fish feed EPIC (West Bengal Dairy and Poultry Development Corporation Limited). The feed was given in three split doses in morning, afternoon and night. The test fish were transferred to 50-l PVC tubs containing 40 l of water of desired hardness for a period of

24 hours with continuous aeration. During this phase, no feed was given to the fish. After this, the fish were transferred to the experimental tubs. Range finding test was conducted with five fish per chamber to find out the concentration at which 10 to 100% mortality was observed. For this, different concentrations were selected with a factor of ten from each other. Two replications were maintained per treatment and the experiment was carried out up to 96 hours. The above experiments were carried out following the standard methods (APHA, 1998).

Definitive Tests

Acute toxicity tests with different level of water hardness: Continuous aeration was given to maintain the aerobic condition and dissolved oxygen level between 7 and 8 mg l⁻¹. Four treatments, *i.e.*, soft water (T1), medium hard water (T2), hard water (T3) and very hard water (T4), were undertaken for common carp and one treatment of very hard water (T5) for catla, *Catla catla*. Six to eight concentrations per treatment, three replications per concentration and 10 l of test water in each container/replicate were taken. Fry were maintained for 96 hours during the test. Range of total cadmium concentrations for different treatments from which test concentrations were chosen after the range finding tests were: for soft water, 30-65 mg l⁻¹; medium hard water, 30-90 mg l⁻¹; hard water, 80-200 mg l⁻¹ and for very hard water, 200-500 mg l⁻¹. Static bioassay test was carried out following the standard methods (APHA, 1998). Parameters such as dissolved oxygen, free CO₂, pH, total alkalinity, total hardness and salinity were measured daily at the same time. Along

with mortality, the behavioural changes of fry were also recorded.

Acute toxicity tests with sediment

Three treatments, *i.e.*, medium hard water (T5), hard water (T6) and very hard water (T7), were taken up for the experiment with sediment. Hardness of water in the sediment treatment was kept the same as that of the corresponding water treatment for comparison of the effect. The same method described above was used with following modifications. To get a realistic level of particulate and dissolved organic carbon in aqueous phase, 100 g of powdered sediment (97 g soil and 3 g humus) were used (Datta *et al.*, 2002d). The surface soil contains 5% organic matter on an average. This value increase up to 30% under poorly drained condition (Brady, 1989). As the soil contained only 3.01% organic matter, 3 g well decomposed, dried, powdered farmyard manure was added to increase the organic matter content above 5%. The powdered sediment was added to 10 l of water and the desired concentration of the toxicant was mixed. The fish were released after 8 hours when the transparency of the water was more than 15 cm. Ranges of total cadmium concentrations in different treatments from which test concentrations were chosen were: for medium hard water, 50-250 mg l⁻¹; hard water: 75-250 mg l⁻¹ and for very hard water, 250-550 mg l⁻¹.

Analysis of Soil

Textural analysis of soil was carried out for different soil samples collected from different places before choosing the final soil sample for experiment. For this, mechanical analysis of soil was carried out

by soil hydrometer. Per cent organic carbon of the soil sample was estimated following Wakley and Black method (Chopra and Kanwar, 1991). Particulate and dissolved organic carbon in the aqueous phase was also estimated by Wakley and Black method with slight modifications (Datta *et al.*, 2002d, 2003). Dissolved organic carbon (DOC), which was estimated to be 7.5 ± 1.5 mg l⁻¹, was analysed after collecting the water sample from the experiments with sediment and filtered to remove the particulate matter. For total organic carbon (TOC) estimation, water sample from the same experiment was used without filtration. The difference between TOC and DOC was particulate organic carbon (POC), which was found to be 22 ± 2.0 mg l⁻¹.

Statistical Analysis

Per cent mortality of fry during different exposure durations were transformed into probit value and log values of different concentrations were computed through probit analysis (Finney, 1971) to obtain the regression equation and LC₅₀ values at 24, 48, 72 and 96 hours. Regression equations, regression coefficients (R²), 96h LC₅₀ values, 95% confidence limits for 96h LC₅₀ values, application factor (AF) and safe application rate (SAR) were calculated in all conditions. AF in different test conditions was found out by multiplying the 96h LC₅₀ value with 0.1 (Sprague, 1971). SAR was calculated from the equation $LC_{50} \times (LC_{10}/LC_{95})$, of Basak and Konar (1997). Analysis of variance (ANOVA) and Student's 't' test were carried out to find the significance between different treatments; 24h, 48h, 72h and 96h LC₅₀ data were taken as four replications in each condition and average of these four replications was taken as

average LC₅₀ value of that particular condition. Conclusion of statistical analysis was drawn after comparing the mean LC₅₀ values in different treatments with the least significant difference by t-test at 5%. For testing the effect of exposure time on the toxicity, LC₅₀ values of all conditions at every 24-h interval were taken as replications. MS Excel 2000 in Windows 98 computer operating system was used for the above analysis.

RESULTS AND DISCUSSION

Different physicochemical parameters during the experiment are presented in Table 2. It was found that salinity level changed from that of fresh water to oligohaline kind of brackish water. Values of total hardness, pH, alkalinity and salinity showed transient increment in different test conditions due to the addition of cadmium.

Behavioural changes were significantly prominent at the higher concentrations of test medium. During the initial stages of experiment, fish exhibited erratic swimming behaviour and dashed to the sides of the containers. As the test period prolonged, activity of fish was reduced greatly. Fish surfaced frequently to gulp air, followed by sideward movements. During the later stages of experiment, fry swarm to the upper layer and remained lethargic, respiring slowly prior to death.

Acute toxicity data of cadmium in different conditions are presented in Table 3. It was observed that the regression coefficients in different conditions are in the range of 0.69 to 0.92, which signify a good correlation between the observed and expected data. Hardness of water had very significant impact on the acute toxicity of cadmium to common carp fry ($P < 0.05$). The 96h LC₅₀ values in soft, medium hard, hard and very hard water were 43.17, 48.39,

Table 2: Physicochemical parameters (mean ± SD, n = 24) of different kinds of water used for the experiment

Parameter	Control				Treatment			
Total hardness								
(mM Ca ²⁺ l ⁻¹)	0.9 ± 0.05	1.9 ± 0.10	2.9 ± 0.10	6.0 ± 0.10	1.0 ± 0.05	2.1 ± 0.10	3.3 ± 0.10	6.7 ± 0.10
Total alkalinity								
(mg CaCO ₃ l ⁻¹)	94 ± 2.0	150 ± 2.0	190 ± 3.0	334 ± 4.0	96 ± 2.0	154 ± 3.0	196 ± 3.0	334 ± 4.0
Salinity (‰)								
	0.084 ± 0.015	0.102 ± 0.018	0.282 ± 0.018	1.022 ± 0.018	0.086 ± 0.017	0.105 ± 0.020	0.286 ± 0.021	1.027 ± 0.023
pH	7.7 ± 0.1	7.7 ± 0.1	7.8 ± 0.1	7.9 ± 0.1	7.8 ± 0.1	7.9 ± 0.1	8.0 ± 0.1	9.1 ± 0.1
O ₂ (mg l ⁻¹)	8.0 ± 0.3	7.8 ± 0.4	7.5 ± 0.3	7.4 ± 0.4	7.8 ± 0.3	7.6 ± 0.3	7.2 ± 0.4	7.2 ± 0.3
CO ₂ (mg l ⁻¹)	5.5 ± 1.0	7.5 ± 1.5	8.0 ± 1.5	10.0 ± 2.0	6.5 ± 1.5	8.5 ± 1.5	9.5 ± 1.5	11.3 ± 2.0

116.45 and 310.48 mg l⁻¹ (Fig. 1). AF values were in the range of 4.31 – 31.04 mg l⁻¹, while SAR values were in the range of 22.77 – 177.66 mg l⁻¹ in soft to very hard water treatments (Table 3). It was found after statistical analysis (Table 4) that water treatment could produce significant impact at higher hardness levels (above medium hard water or in hard and very hard water), while sediment was able to reduce acute toxicity of cadmium to common carp significantly in combination with water of

lower hardness level (below hard water level or from medium hard water). In sediment with medium hard water, sediment with hard water and sediment with very hard water, 96h LC₅₀ values were 111.20, 133.71 and 334.47 mg l⁻¹; AF values were 11.12, 13.37 and 33.44 mg l⁻¹ and SAR values were 45.43, 70.66 and 194.03 mg l⁻¹ (Table 3). Exposure time could not produce any significant impact up to 96 hours on the acute toxicity of cadmium ($P = 0.05$).

Table 3: Acute toxicity of cadmium to common carp in different test conditions

Parameter	Soft water (T1)	Medium hard water (T2)	Hard water (T3)	Very hard water (T4)	Medium hard water + sediment (T5)	Hard water + sediment (T6)	Very hard water + sediment (T7)
regression equation	$y = 12.47x - 15.40$	$y = 9.43x - 10.89$	$y = 12.63x - 21.10$	$y = 14.94x - 32.24$	$y = 7.52x - 10.40$	$y = 10.56x - 17.46$	$y = 12.37x - 26.23$
Regression coefficient (R ²)	0.75	0.69	0.92	0.70	0.80	0.83	0.70
96h LC ₅₀ (mg l ⁻¹)	43.17	48.39	116.45	310.48	111.20	133.71	334.47
95% confidence limits (mg l ⁻¹)	42.74–43.74	47.68–49.16	115.22–117.88	307.92–313.93	109.53–113.81	132.11–135.77	330.81–338.00
AF (mg l ⁻¹)	4.31	4.87	11.64	31.48	11.12	13.37	33.44
SAR (mg l ⁻¹)	22.77	23.69	68.32	177.66	45.43	70.66	194.03

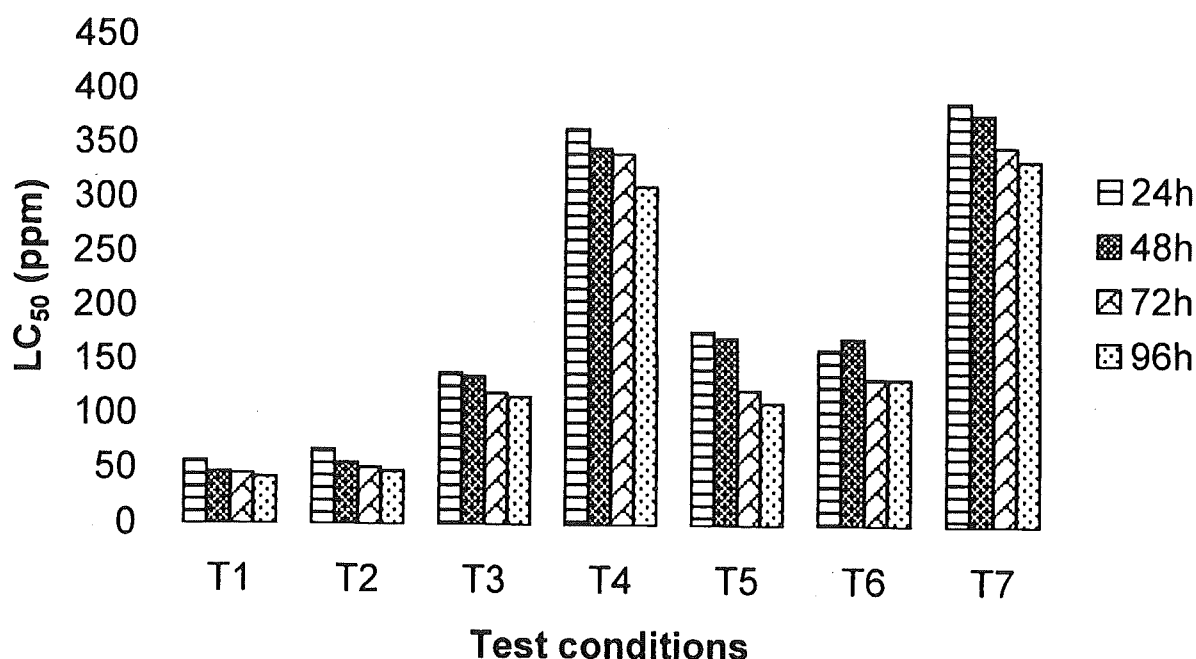


Fig. 1. LC₅₀ values of cadmium to common carp under different test conditions

Table 4: Summary of statistical analyses

Test parameter	Fcal	Fcrit	P-value	Lsdt at 5%	Average LC ₅₀ (mg l ⁻¹)	Test result
Effect of water hardness	407.84	3.49	2.45E-12	20.76	T1: 48.7; T2: 56.0 T3: 127.5; T4: 340.1	T4>T3> T2 = T1
Effect of sediment	131.90	2.77	1.56E-13	32.15	T2: 56.0; T3: 127.5 T4: 340.1; T5: 145.5 T6: 149.8; T7: 361.9	T5 > T2 T6 = T3 T7 = T4
Effect of exposure time	0.16	2.90	0.91	Non- significant	24 h: 232.5; 48 h: 223.7 72 h: 205.1; 96 h: 192.5	No significant effect up to 96 h

It is well known that the toxicity of a chemical compound depends on its concentration and duration of exposure to test species. Further, these effects are influenced more or less by the resistance of the test organisms and the variations of external factors. Therefore, when the test organism and duration of exposure are the same for all the chemicals tested, the effect will vary mainly due to the external factors. Early life stages of fish provide the most conservative estimates of potential toxic effect (Jones, 1964). In the present study, therefore, the fry of *C. carpio* were exposed to different concentrations of the test chemical cadmium.

Effect of hardness of water

It was observed that with the increase in hardness of water (from 0.9 to 6.0 mM Ca²⁺ l⁻¹), toxicity of cadmium to *C. carpio* decreased and the effect was more prominent at higher hardness levels. It is proposed that two kinds of reactions are responsible for cadmium toxicity in water medium (without any sediment) to aquatic

organisms: first, the competition reaction between cadmium and calcium at the gill receptor site and second, the precipitation of cadmium by water hardening cations, e.g., calcium and magnesium. The competing reaction with calcium was more prominent in lowering the uptake of cadmium through chloride cells (Ca²⁺ channels) of the gills up to the medium hard water level. After this as the hardness of water increased, the receptor sites of the gills were almost saturated with calcium and the competition reaction could not produce much increase in effect as observed by Craig *et al.* (1999). They found that cadmium accumulation by larvae of the aquatic insect *Chironomus staegeri* was inhibited by 46 to 88% as the Ca²⁺ concentrations were increased above a control (0.1 mM Ca²⁺) to 1-10 mM Ca²⁺. The inhibition was more pronounced up to 2.5 mM aqueous Ca²⁺ concentration and after that, the inhibition was very slow up to 10 mM Ca²⁺. The simplest explanation for this effect is that the two ions compete for passage through the same site of entry;

ionic radii of Cd^{2+} and Ca^{2+} are quite similar at 0.97 and 0.99 Å respectively (Pauling, 1960). After the medium hard water level, precipitation of cadmium as colloidal CdCO_3 increased as the hardness increased and was mainly responsible for further reducing the toxicity of cadmium. Carroll *et al.* (1979) found that precipitation influenced the LC_{50} values slightly and that not more than 40% of the cadmium present was precipitated. No precipitation was detected at 15 mg l^{-1} , at which 90-100% mortality was observed in waters low in calcium. At the same concentration, little or no mortality was observed in waters high in calcium. Sherman *et al.* (1987) observed that mortality of fathead minnows was low (0-10%) during the 96-h test period in pond water due to higher pH and water hardness, which produced supersaturated conditions resulting in the rapid formation of nontoxic CdCO_3 precipitate and more rapid decrease in Cd^{2+} concentration as compared to bioassay in the laboratory. The other reason for the lower toxicity of cadmium in hard water may be the increase in the number of chloride cells due to the acclimatisation in the same water as observed by Calamari *et al.* (1980). They postulated that the presence of a higher number of chloride cells in fish acclimated to hard water would explain the lower sensitivity to cadmium. A detoxification mechanism based on the increase in the number and activity of chloride cells should, therefore, be independent of the hardness and rather related to the presence of metals. On the contrary, the possibility of action by cadmium could depend upon the role of calcium in regulating gill permeability. The decreased toxicity of cadmium with the

increase in hardness of water was also observed by Chapman and Dunlop (1981), Part *et al.* (1985), Pascoe *et al.* (1986), Nakagawa and Ishio (1989), WHO (1992) and Jackson *et al.* (2000), which supports present observation.

It was observed in the present experiment that the total hardness of water and other related parameters (*e.g.*, total alkalinity, salinity, etc.) changed in the treatment with respect to the control due to the addition of the test chemical cadmium. McCarty *et al.* (1978) also observed several parameters (*e.g.*, pH, total alkalinity, conductivity) exhibit transient and/or sustained variation of a cadmium concentration-dependent toxicity to gold fish, *Carassius auratus*, in soft ($20 \text{ mg CaCO}_3 \text{ l}^{-1}$) and hard water ($140 \text{ mg CaCO}_3 \text{ l}^{-1}$). Cadmium concentrations were reasonably stable in soft water. Water quality was not greatly altered except for the total hardness. During hard water trials, there were transient increases in the amount of particulate cadmium present and sharp decrease in total cadmium levels.

Effect of sediment on the toxicity of cadmium

Sediment was able to reduce the acute toxicity of cadmium. This might be mainly due to the complexation of cadmium with DOC. The source of DOC in this experiment was organic matter already present in soil (3.01%) and the dissolution of externally-added humus (3.0 g), which was estimated to be $7.5 \pm 1.5 \text{ mg l}^{-1}$ equivalent to natural level of DOC, *i.e.*, 8 mg l^{-1} (Richards *et al.*, 1999). Cadmium and calcium compete for DOC-binding sites. At lower hardness levels (lower Ca^{2+} concentrations), cadmium

complexed with DOC and the acute toxicity of cadmium was reduced significantly in medium hard water with sediment in comparison to medium hard water. At higher Ca^{2+} concentration, most of the DOC sites were occupied by calcium and the acute toxicity of cadmium was not significantly reduced in hard water and very hard water with sediment experiments (Table 4) in comparison to respective water treatments. In hard water, the added hardness cations, especially Ca^{2+} , effectively competed with Cd^{2+} for available binding sites in the dissolved organic matter (DOM). Simultaneously, Ca^{2+} ions interfered also with the uptake of Cd^{2+} either competing in transport through cell membranes or by reducing the membrane permeability. An increase in water hardness decreased the measured binding coefficient of cadmium to DOM. Combined effects of DOM and water hardness on toxicity of cadmium were also observed by Penttinen *et al.* (1998) using *Daphnia magna*. Cadmium was significantly less toxic in humic lake water ($\text{DOC } 19.6 \text{ mg l}^{-1}$) than the reference water ($\text{DOC} < 0.2 \text{ mg l}^{-1}$). The reduced toxicity of cadmium in lake water was due to complexation with DOC. The decrease in acute toxicity of cadmium in sediment treatment might also be due to adsorption of Cd^{2+} on the negatively charged soil colloids (clay, humus, and iron and manganese oxyhydroxides) and POC. The addition of humus also acted as a source of POC in the test medium, which was estimated to be $22 \pm 2.0 \text{ mg l}^{-1}$. Under the aerobic condition, clay colloids, FeOOH , MnOOH and POC are the dominant binding phases in sediments (Tessier *et al.*, 1996).

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