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Interactions between copper and cadmium during single and combined exposure in juvenile tilapia *Oreochromis mossambicus*: Influence of feeding condition on whole body metal accumulation and the effect of the metals on tissue water and ion content

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Abstract

Juvenile tilapia (*Oreochromis mossambicus*) were exposed for 96 h to ranges of sublethal concentrations of Cu or Cd, under both fed and non-fed conditions. Exposure to one metal (Cu or Cd) not only resulted in an increased whole body content of the metal exposed to, but also influenced the concentration of the other metal present in the fish. Furthermore, the total amount of Cu and Cd accumulated during exposure to heavy metals was influenced by the nutritional state of the fish. Besides exposure to either Cu or Cd, fish were also exposed to mixtures of Cu and Cd. Results indicated that accumulation during Cu/Cd co-exposure cannot be predicted by simple addition of the effects of single metal exposures. Obviously, complex interaction mechanisms are involved, as was concluded e.g. from the significantly decreased whole body Cd-content of Cu/Cd-co-exposed fish compared to the Cd-content of Cd-exposed fish. This phenomenon was observed in both fed and non-fed fish. Because ionic homeostasis is known to be affected by heavy metals, in this study also whole body water, calcium and sodium content in Cu and/or Cd-exposed fish were determined. The results indicate that also with respect to these parameters the two metals interact. The effects on water and ion appear to be dissociated. The data reveal previously unrecognized effects of interaction of the metals on whole body metal content, water and ion regulation.

Key words: Interaction; Accumulation; Sublethal effects; Copper; Cadmium; Tilapia; *Oreochromis mossambicus*

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1. Introduction

As a result of increased industrialization, contamination of natural freshwaters by heavy metals, such as copper (Cu) and cadmium (Cd), has become a global problem. In the case of essential trace elements, e.g. copper, zinc and nickel, the optimal concentration range for growth and reproduction are narrow, and both excess and deficiency are harmful to fish. Some non-essential trace metals such as mercury, lead and cadmium are toxic at concentrations observed in natural waters (Leland and Kuwabara, 1985; McKim, 1985). To assess the toxic impact of ambient levels of Cu or Cd on fish, the effects on several parameters have been studied. For example, Cu affects the sodium balance (Reid and McDonald, 1988), growth (Lett et al., 1976), and swimming performance in rainbow trout (Waiwood and Beamish, 1978), and reproductive success in bluntnose minnow (Horning and Nieheisel, 1979), whereas Cd affects calcium balance in rainbow trout (Verbost et al., 1987) and induces damage in gill structure of zebrafish, rainbow trout and tilapia (Karlsson-Norrgren et al., 1985; Pratap and Wendelaar Bonga, 1993). It is evident from these studies that increased tissue concentrations of heavy metals induce significant stress in the animals. The key to predict important toxic effects will be the understanding of the relation between accumulation of metals and their physiological or biochemical actions (Sprague, 1971; Grahl et al., 1985).

In most natural waters heavy metals are present as mixtures. The effects of mixtures of metals on aquatic organisms are complex (Hamilton et al., 1987). As a reflection of this complexity, conflicting results have been published on the interaction between Cu and Cd concerning their accumulation and, consequently, their toxicity. Westernhagen et al. (1979) reported an additive effect of Cu and Cd on embryonic survival and hatching success in seawater-herring, whereas Eisler and Gardner (1979) observed synergistic actions of Cu/Cd/Zn mixtures in the killifish, *Fundulus heteroclitus*.

Since there is no clear insight in the interaction between Cu and Cd that determine the accumulation of the metals during combined exposure, in the present study we investigated the influence of Cu-exposure on whole body Cd-content and vice versa in the tilapia *Oreochromis mossambicus*, after single and combined exposure to sublethal concentrations of these metals in the water. Since it has been demonstrated that the feeding condition of the fish affects the adaptation to heavy metals (Collvin, 1985; Segner, 1987; Handy and Eddy, 1990), this study was performed with both fed and non-fed fish. Finally, in order to investigate effects of altered whole body metal content on the ion balance of the fish, we related whole body Cu and Cd concentrations to body water, calcium and sodium content.

2. Materials and methods

2.1. Fish and control water conditions

One-month old tilapia (*Oreochromis mossambicus*) were obtained from own labo-

ratory stock. From 9 days after hatching the fish were kept in 'artificial freshwater' to ensure well-defined control water conditions, with undetectable Cu and Cd levels (detection levels below 0.10 and 0.01 $\mu g\ l^{-1}$, respectively). The artificial freshwater consisted of demineralized water supplemented with 1.3 mM $NaHCO_3$, 0.5 mM $CaCl_2$, 0.06 mM KCl and 0.2 mM $MgCl_2$; pH 7.8. The composition and preparation of the water was based on the EEC instructions for artificial water for use in toxicity studies in fish (EEC Directive 84/449 /EEC Annex 5 method c1: Acute toxicity for fish). The ion concentrations were based on those in Nijmegen tapwater. The water was continuously aerated and filtered. The light/dark regime was 12/12 hours, and the water temperature 26°C. Fish were fed at about 2% (DW/WW) of their body weight per day with commercial tropical fishfood (Micromin™). The Cu and Cd contents of the food were: Cu 5.90 ± 0.21 $\mu g\ g^{-1}$ dry food, and Cd 0.130 ± 0.005 $\mu g\ g^{-1}$ dry food (means ± SE; $n = 6$).

2.2. Experimental design

Three days before the start of the experiment, 15 groups of 10 or 15 fish (average weight 1 g) were placed randomly in polyethylene 4.5-l aquaria filled with artificial freshwater. Depending on the experiment, fish were fed daily (2% DW/WW; all food was eaten within ½ min) or not fed. During the acclimation period, the water in the aquaria was continuously refreshed by means of a flow-through-system (flow rate 0.24 l h^{-1}). The experiments with fed fish were performed prior to those with non-fed fish. The experiments started with the connection, by means of a 16 channel peristaltic pump (Watson Marlow), of each aquarium to its own water-supply (stock-solution of metal) with a well-defined concentration of Cu and/or Cd (added as nitrate; Spectrosol, BDH, England) in artificial freshwater. During the first 6 h of exposure, the flow rate was 0.66 l h^{-1}, followed by 90 h of exposure at a flow rate of 0.24 l h^{-1}. Cu and Cd concentrations in the stock-solutions were monitored daily. During the first 6 hours of exposure, Cu- and Cd-concentrations in the aquaria were monitored every hour, and at least once a day during the rest of the exposure period. After 96 h of exposure, the fish were rinsed in artificial freshwater and immediately killed in icecold CO_2/aceton.

2.3. Determination of water- and tissue-concentrations of Cu and Cd, and of tissue water, sodium and calcium content

Water samples were acidified with nitric acid in a final concentration of 0.1% (v/v). Cu and Cd concentrations in the water samples were determined with a flameless atomic absorption spectrometer (AAS, Philips PU 9200), connected with an electrothermal atomiser (Philips PU 9390X).

Fish were put in vials, weighed and lyophilized. After determination of the dry weight, the tissues were completely destroyed by the following procedure: 1 h at 40°C after addition of 150 μl 65% HNO_3; 1 h at 75°C after addition of 200 μl 65% HNO_3 and subsequently overnight at 110°C. After complete destruction, the samples were dissolved in 4 ml 0.1% HNO_3 (final concentration) and stored at 4°C until metal- and

ion-analysis. Whole body sodium and calcium contents in non-fed fish were determined by means of an inductive coupled plasma (ICP) atomic emission spectrometer (Plasma IL 200, Thermo Electron USA).

Several control procedures were performed: No Cu or Cd could be detected in blank destruction samples, indicating that the vials did not release any of these metals. The contribution to the total body metal content of metal adsorption to the body surface of the fish was negligible: rinsing of metal-exposed fish in a 2 mM EDTA-solution did not change the metal content of fish in comparison with fish rinsed in artificial freshwater (data not shown). No interference between Cu and Cd during the measurements could be detected in water and tissue samples, as determined by adding known amounts of Cu or Cd solutions to the samples. Recovery of the spikes was nearly 100%, whereas no changes in the concentrations of Cu or Cd originally in the samples could be observed. Cu and Cd determinations were performed under standard matrix conditions. The accumulation factor (A.F.) was calculated according to (Holwerda, 1991):

$$\frac{[\mathrm{Me}]_{\mathrm{fw,\,exp}} - [\mathrm{Me}]_{\mathrm{fw,\,control}}}{[\mathrm{Me}]_{\mathrm{water}}}$$

where $[\mathrm{Me}]_{\mathrm{fw,exp}}$ is metal concentration in the experimental group in μg g^{-1} fresh weight, $[\mathrm{Me}]_{\mathrm{fw,control}}$ metal concentration in the control group in μg g^{-1} fresh weight, and $[\mathrm{Me}]_{\mathrm{water}}$ metal concentration in the water in μg l^{-1}.

2.4. Statistical analysis

Data are presented as means ± SE. Differences between groups were tested for significance by the Student's *t*-test for unpaired observations. Each metal concentration was tested in at least two, generally three, separate experiments. Before combining the results of the separate experiments, each group of fish was tested for significance against the corresponding control group within the experiment. Controls did not differ significantly between similar experiments and were therefore pooled.

The metal accumulation in feeding and non-feeding fish were fitted by applying regression analysis. Statistical significance is indicated as follows: $*P \leq 0.05$, $**P \leq 0.02$, $***P \leq 0.01$, and $****P \leq 0.001$.

3. Results

Metal-concentrations in the aquaria gradually increased during the first 18 h of exposure, whereafter the metal-concentrations remained constant during the exposure period (Fig. 1). No difference between the water metal concentrations before and after filtration over a 0.45 μm millipore filter in aquaria with fed and non-fed fish was observed. During the experiments, no mortality or changes in food intake, temperature and pH occurred. Exposure to Cu and/or Cd did not result in significant differences in total body weights between control and metal-exposed fish (results not shown).

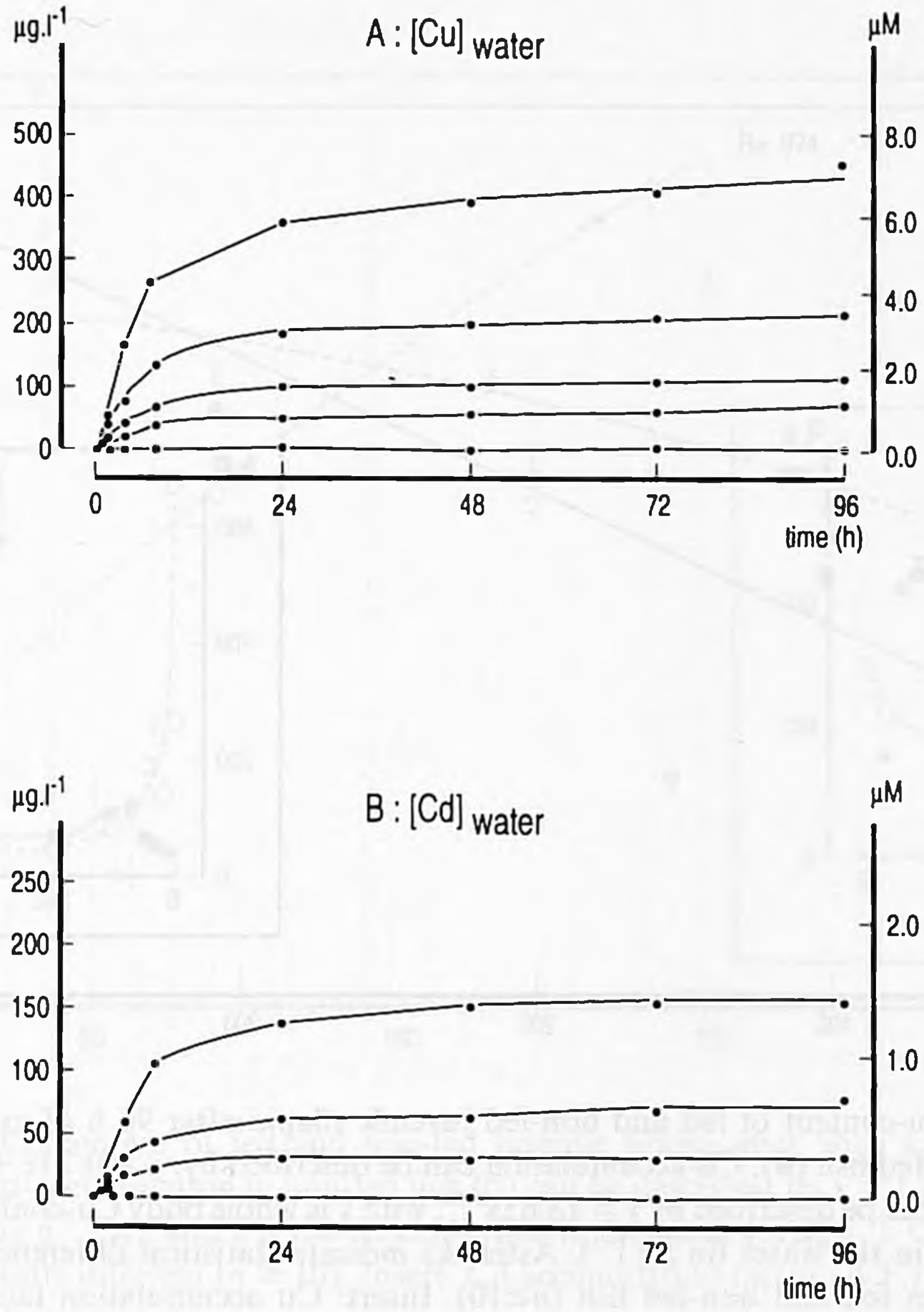


Fig. 1. Cu (A) and Cd (B) concentrations in the water (in μg l^{-1} and μM) during an experiment with fed fish.

3.1. Cu and Cd accumulation

Metal accumulation in fed fish after 96 h exposure to a Cu range of 0–400 μg l^{-1} (Fig. 2) could be described by the linear function $y = 0.23x + 9.44$ ($R = 0.963$), where x is Cu concentration in the water (plateau) in μg l^{-1} and y whole body Cu-content at $t = 96$ h in μg g^{-1} dry weight. Cu accumulation in non-fed fish followed a different pattern. In particular at low Cu concentrations, non-fed fish accumulated significantly more Cu than the fed fish. In non-fed fish Cu-accumulation was fitted best by the non-linear function $y = 13.65x^{0.31}$ ($R = 0.915$). The difference in Cu accumulation between feeding and non-feeding fish was also noticeable in the accumulation factor (A.F.): in fed fish, the A.F. was more or less constant whereas in non-fed fish the A.F. was very high at low Cu-concentrations and decreased with increasing Cu concentrations in the water. The A.F. in 100, 200 and 400 μg Cu l^{-1} exposed fish was similar in fed and non-fed fish (insert Fig. 2).

Cd-accumulation in non-fed fish was best described by the non-linear function

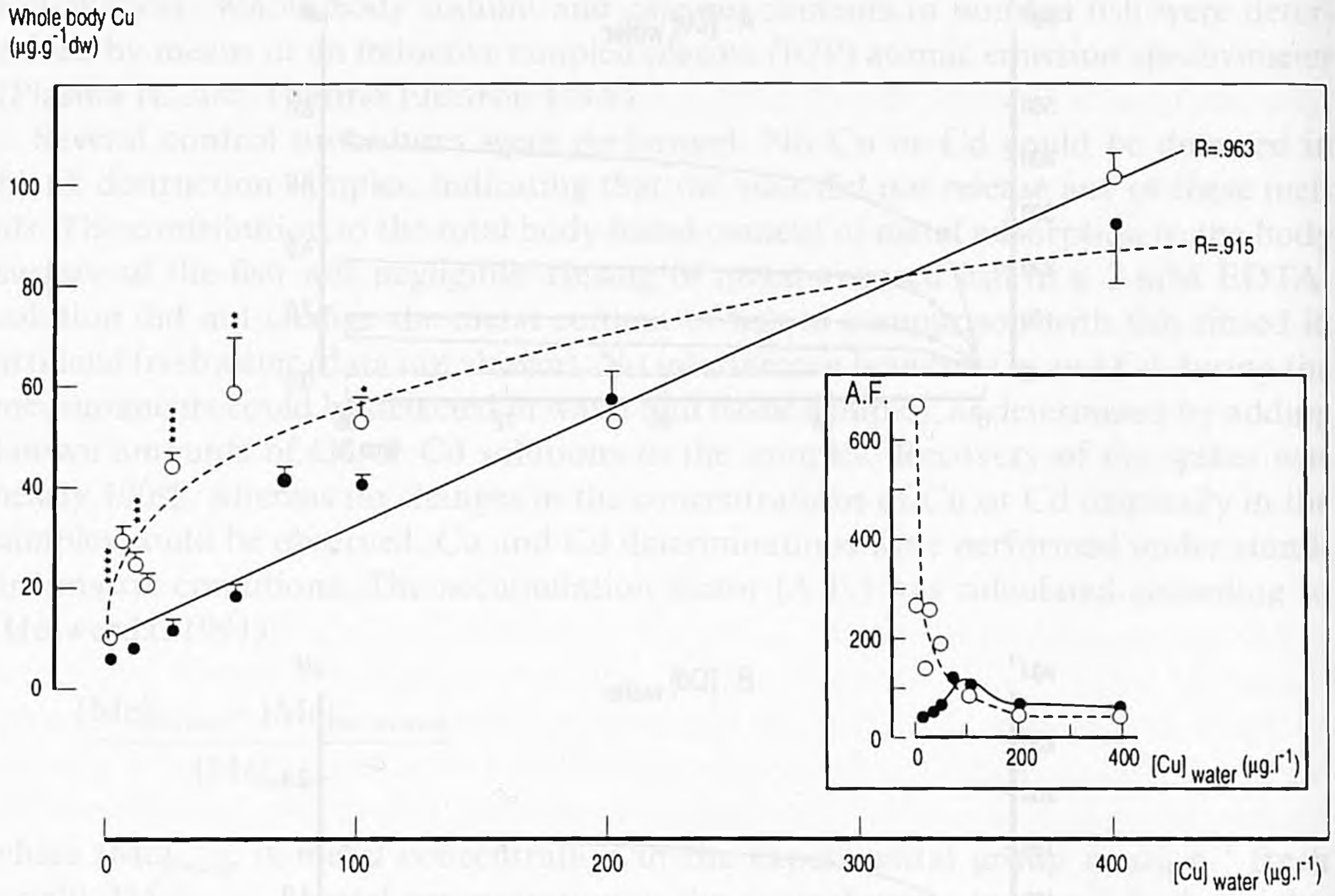


Fig. 2. Whole body Cu-content of fed and non-fed juvenile tilapia after 96 h of exposure to a range of Cu-concentrations. In fed fish (●), Cu-accumulation can be described by: $y = 0.23x + 9.44$; in non-fed fish (○), Cu-accumulation can be described by $y = 13.65x^{0.31}$, with y is whole body Cu-content (in μg g^{-1} dw) and x is Cu-concentration in the water (in μg l^{-1}). Asterisks indicate statistical differences in whole body Cu concentrations between fed and non-fed fish ($n \geq 10$). Insert: Cu accumulation factor (A.F.) after 96 h exposure to a range of Cu-concentrations.

$y = 1.84x^{0.70}$ ($R = 0.974$). Cd-accumulation in fed fish did not differ from accumulation in non-fed fish at the concentrations tested (Fig. 3). The A.F. decreased slightly in the non-fed fish with increasing Cd-concentrations in the water (insert Fig. 3).

3.2. Interactions between Cu and Cd

Fed fish. Two types of interactions between Cu and Cd were studied. Firstly, the effect of Cu-exposure on the Cd-content of fish and of Cd-exposure on the Cu-content of fish (type I). Secondly, the effects of combined exposure on whole body Cu and Cd-content (type II). Both types of interactions were studied in fed and food restricted fish.

Type I interactions in fed fish are shown in Fig. 4. Exposure during 96 h to 20 or 35 μg Cd l^{-1}, resulted in a significant decrease of the Cu-content (Fig. 4A). Fig. 4B shows that exposure to Cu resulted in a significant increase in the whole body Cd-content at all concentrations used.

Type II interactions in fed fish are illustrated by Figs. 5(A) and 5(B). Cu/Cd co-exposure had no consistent effect on whole body Cu-content (Fig. 5A). Compared to

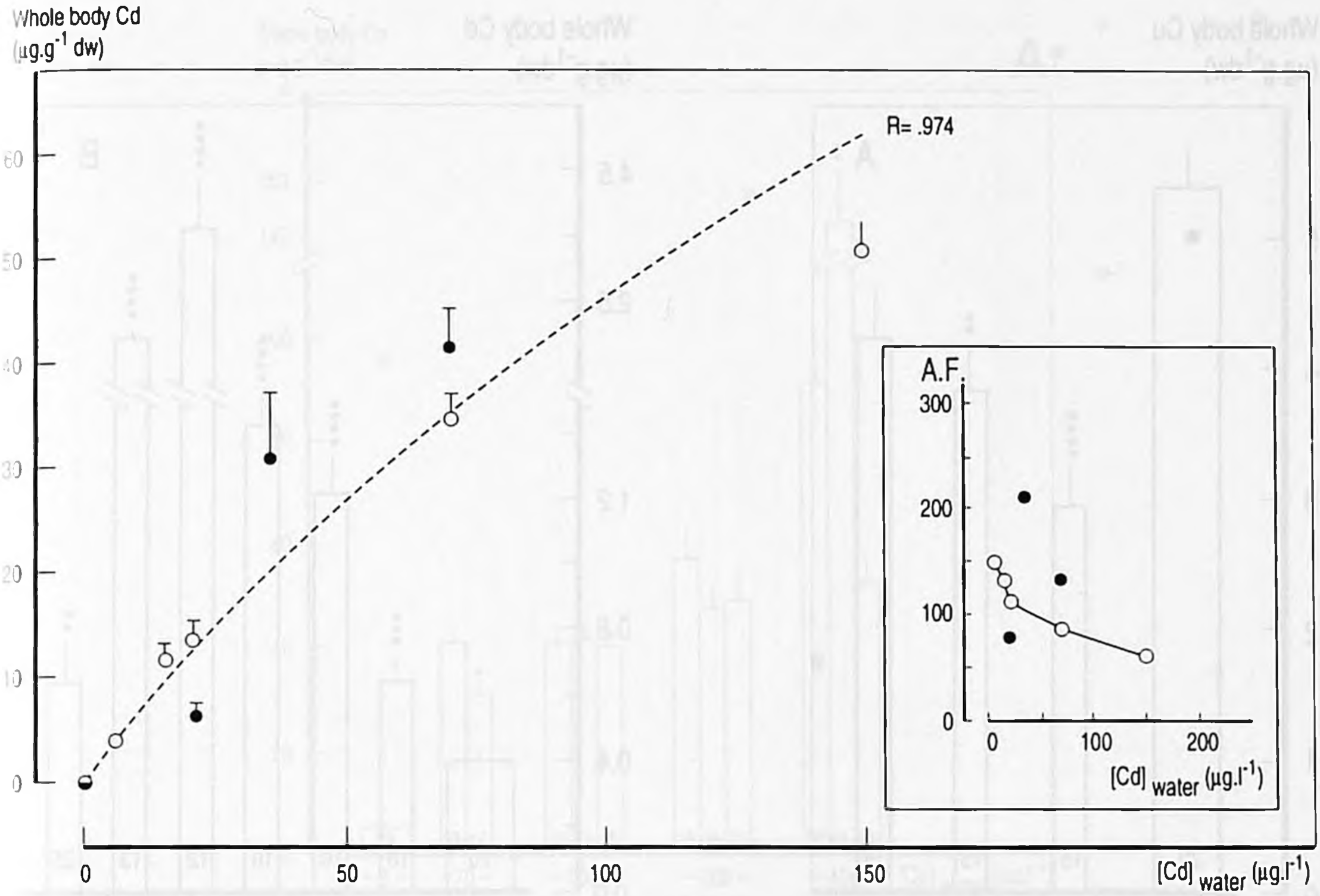


Fig. 3. Whole body Cd-content of fed and non-fed juvenile tilapia after 96 h exposure to a range of Cd-concentrations. Cd-accumulation in non-fed fish (○) can be described by $y = 1.84x^{0.70}$, with y is whole body Cd-content (in μg g^{-1} dw), and x is Cd-concentration in the water (in μg l^{-1}). Cd-accumulation in fed fish (●) is not significantly different ($n \geq 10$). Insert: Cd accumulation factor (A.F.) after 96 h exposure to a range of Cd-concentrations.

the fish exposed to Cu alone, two Cu/Cd combinations (70 μg Cu l^{-1} + 20 μg Cd l^{-1} and 400 μg Cu l^{-1} + 70 μg Cd l^{-1}) decreased the Cu-content in fish while the combination 400 μg Cu g^{-1} + 35 μg Cd g^{-1} increased the Cu-content. Exposure to Cu/Cd combinations resulted in a significant decreased whole body Cd-content when compared to single Cd exposure, with the exception of exposure to the lowest Cd concentration tested (Fig. 5B).

Non-fed fish. Type I interactions in non-fed fish are shown in Fig. 6. Except for exposure to 70 μg Cd l^{-1} (Fig. 6A), Cd-exposure had no effect on the Cu-content. Fig. 6B shows that, in contrast to the results obtained with fed fish, exposure to Cu concentrations above 100 μg l^{-1} resulted in a significant decrease of the Cd-content, whereas lower concentrations (with exception of 5 μg Cu l^{-1}) had no effect.

When compared to single Cu exposure, co-exposure to Cu and Cd had no consistent effect on whole body Cu in non-fed fish (Fig. 7A). In contrast, Fig. 7(B) shows that Cd-exposure in the presence of Cu had a very pronounced effect on the whole body Cd-content. Exposure to nearly all combinations of Cu and Cd used resulted in a significant decrease of the Cd-content in fish when compared to fish exposed to Cd alone.

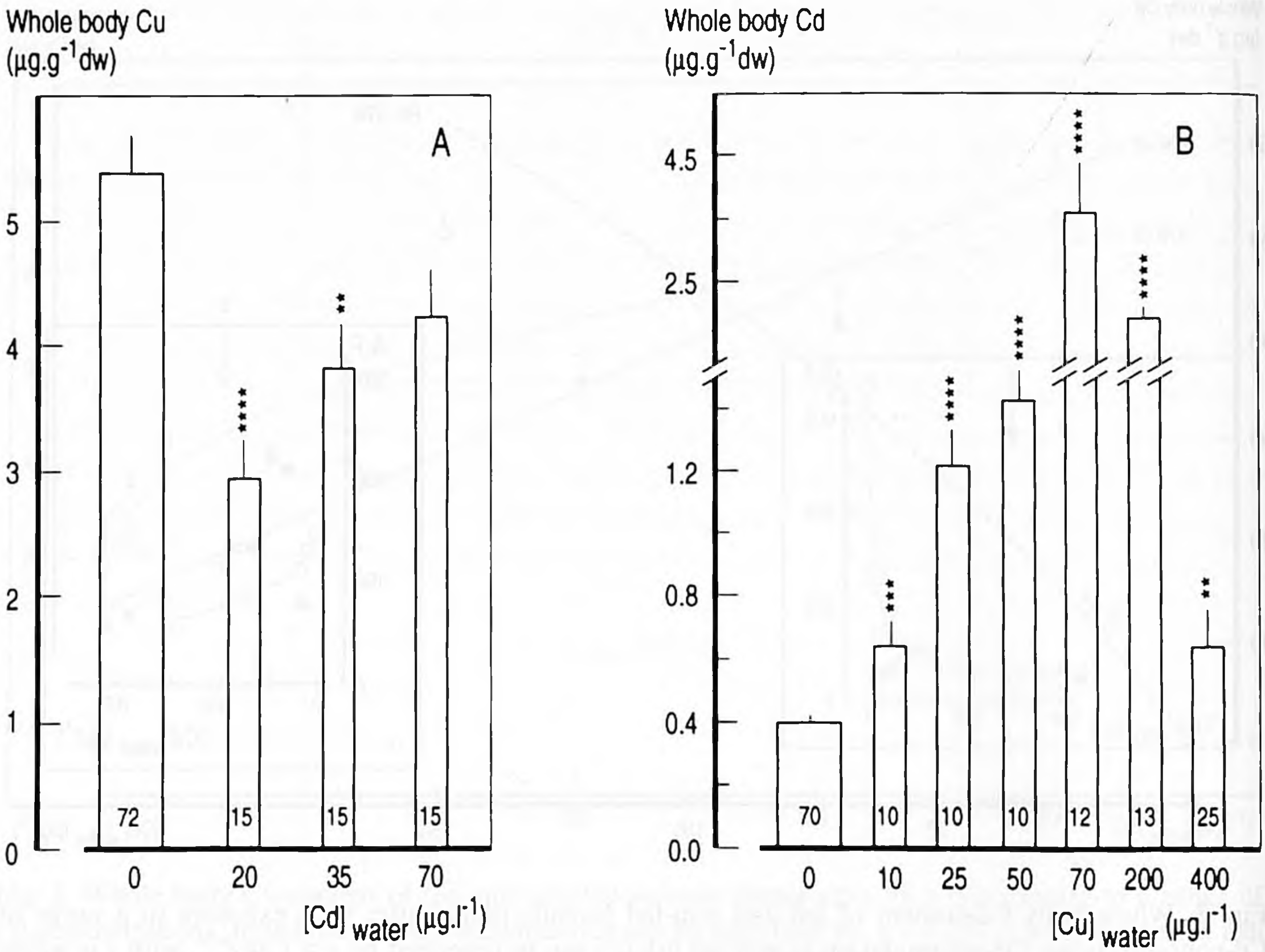


Fig. 4. Whole body Cu-content of Cd-exposed fed fish (A) and whole body Cd-content in Cu-exposed fed fish (B) after 96 h of exposure. Asterisks indicate significant differences between control and metal-exposed groups. The number of fish per group is indicated in the bars.

3.3. Effects of Cu and Cd on water- and ion content

In fed fish, exposure to Cu concentrations higher than 70 μg l^{-1} resulted in a significant, dose-dependent, decrease of their water content (Fig. 8A). Exposure to Cd and combinations of Cu and Cd had a similar effect (Fig. 8B and C). In food-restricted fish, exposure to low Cu concentrations resulted in an increased water content whereas the opposite was found after exposure to higher Cu-concentrations (Fig. 9). In contrast to the decreased water content observed in fed fish after Cd-exposure, in non-fed fish exposure to Cd concentrations between 10 and 70 μg l^{-1} resulted in an increase of the water content of the fish. Fig. 9 illustrates the effect of interaction of the metals on whole body water content. For example, exposure to 100 μg Cu l^{-1} + 70 μg Cd l^{-1} resulted in significantly decreased water content. However, exposure to 100 μg Cu l^{-1} had no effect on water content of fish while exposure to 70 μg Cd l^{-1} resulted in a significant increased water content.

Exposure to Cu and Cd, alone or in combination, did not result in consistent effects on thesodium and calcium content of non-fed fish (data not shown). In the groups with altered average body sodium or calcium content (either expressed as dry or wet

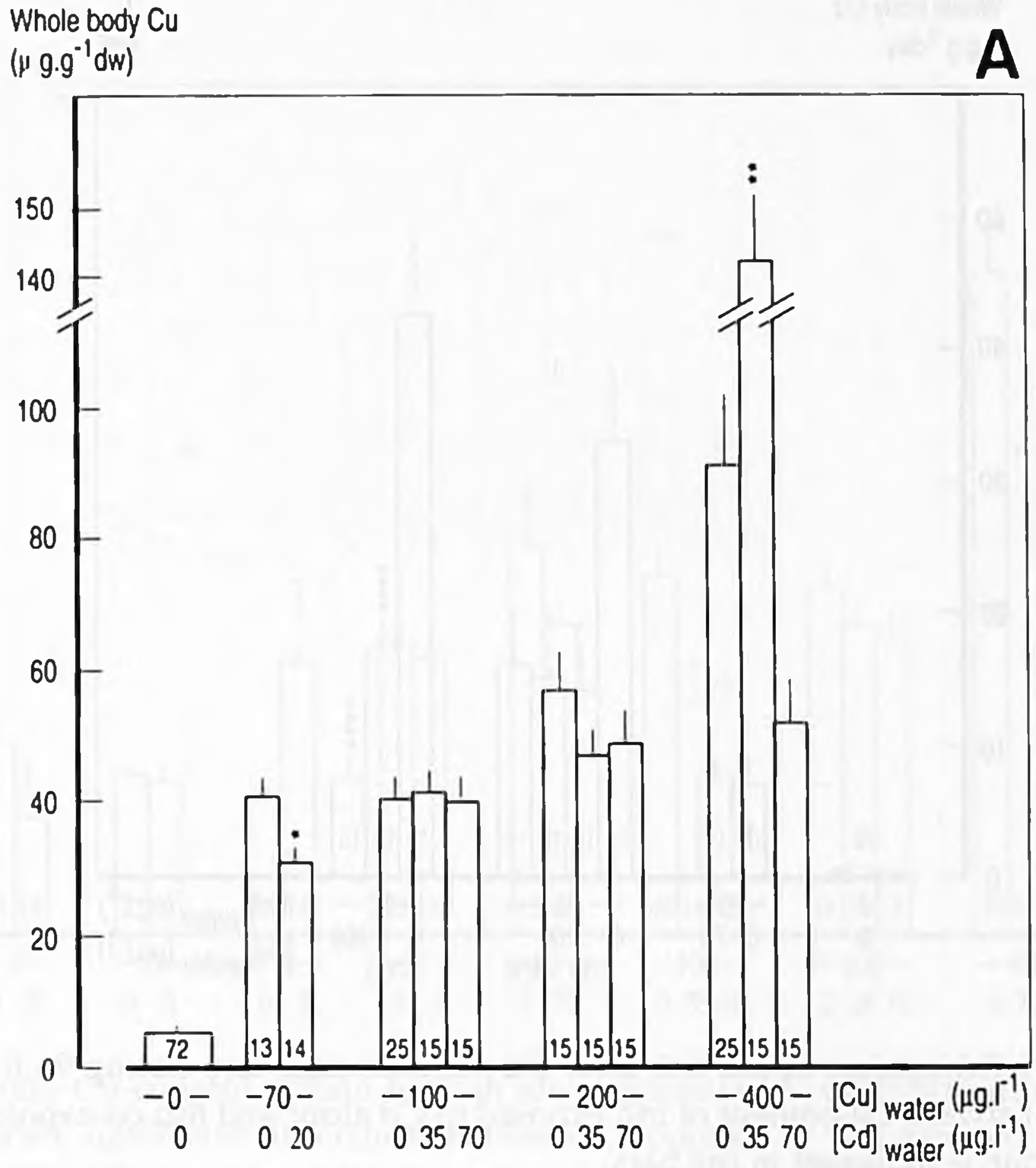


Fig. 5 (A) Whole body Cu-content of fed fish after exposure to combinations of Cu and Cd during 96 h. Asterisks indicate significant differences between Cu-contents of fish exposed to Cu alone and fish co-exposed to Cu + Cd. The number of fish per group is indicated in the bars.

weight), these parameters were not necessarily correlated with whole body water content. To illustrate this, in the fish exposed to 400 μg Cu l^{-1} + 70 μg Cd l^{-1} (Fig. 10C), water content and whole body calcium were significantly decreased. In other groups of fish with a changed water content, no significant changes in whole body sodium or calcium were observed (Fig. 10A and B).

4. Discussion

Two major conclusions can be drawn from the present study. Firstly, interactions between Cu and Cd present in the fish are noticeable both during single and combined exposure to the metals. The interactions result in changed metal and water content of the fish. Secondly, metal-induced effects are also determined by the feeding condition of the fish.

4.1. Cu accumulation in Cu-exposed fish

In accordance with our results with fed fish, Buckley et al. (1984) found a positive

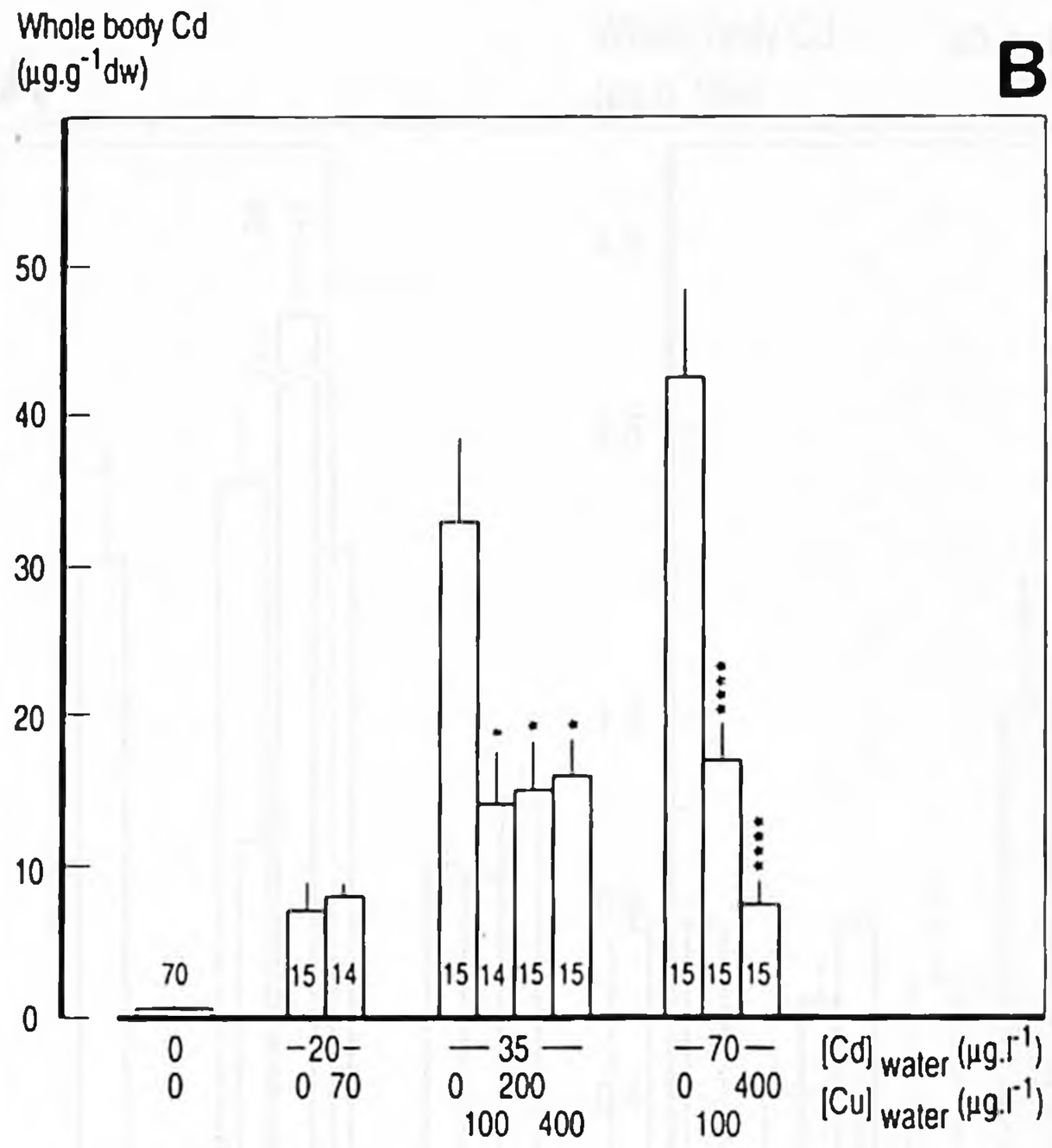
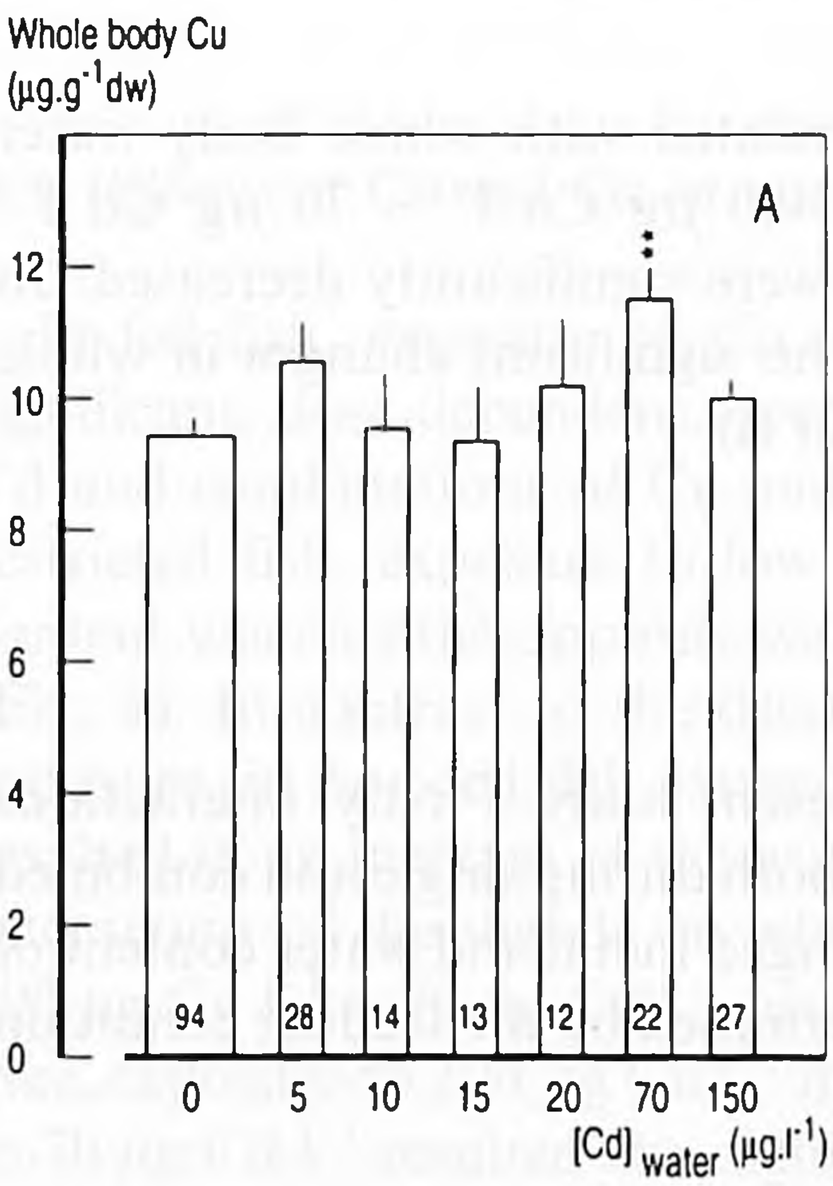


Fig. 5 (B) Whole body Cd-content of fed fish after Cu + Cd co-exposure during 96 h. Asterisks indicate significant differences between Cd-content of fish exposed to Cd alone and fish co-exposed to Cu + Cd. The number of fish per group is indicated in the bars.



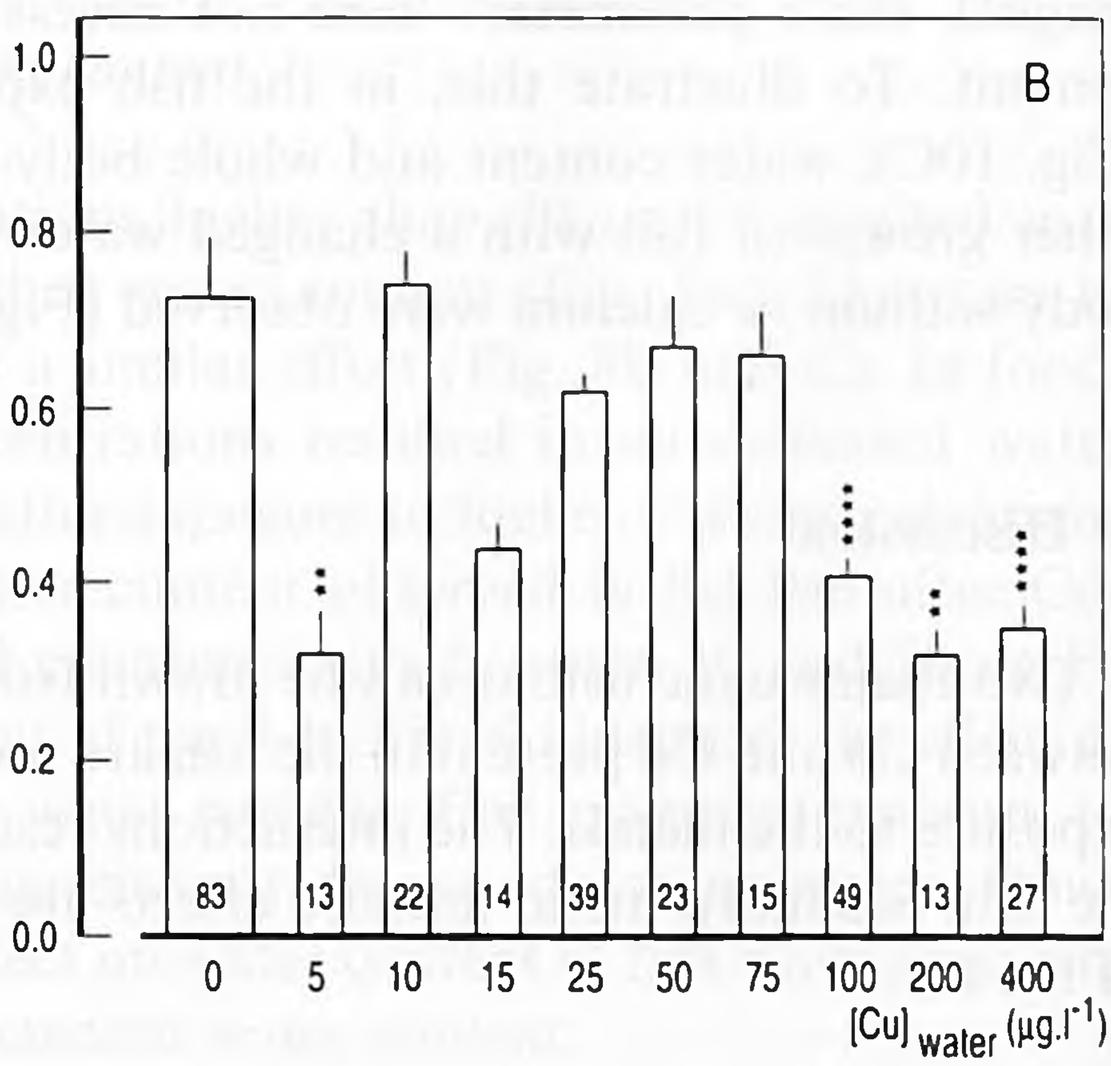


Fig. 6. Whole body Cu-content of Cd-exposed non-fed fish (A) and whole body Cd-content in Cu-exposed non-fed fish (B) after 96 h of exposure. Asterisks indicate significant differences between control and metal-exposed groups. The number of fish per group is indicated in the bars.

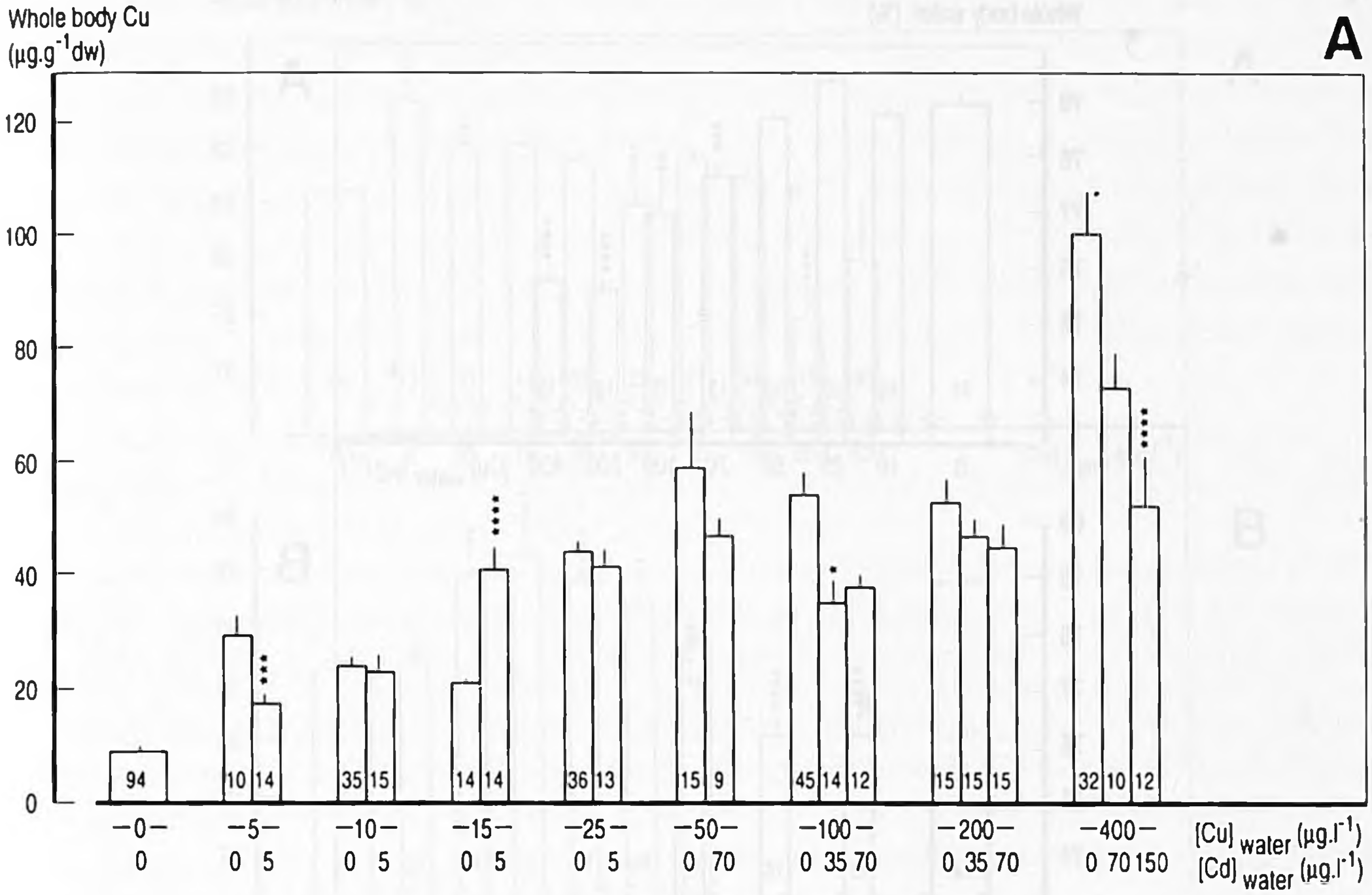


Fig. 7 (A) Whole body Cu-content of non-fed fish after exposure to combinations of Cu and Cd during 96 h. Asterisks indicate significant differences between Cu-content of fish exposed to Cu alone and fish co-exposed to Cu + Cd. The number of fish per group is indicated in the bars.

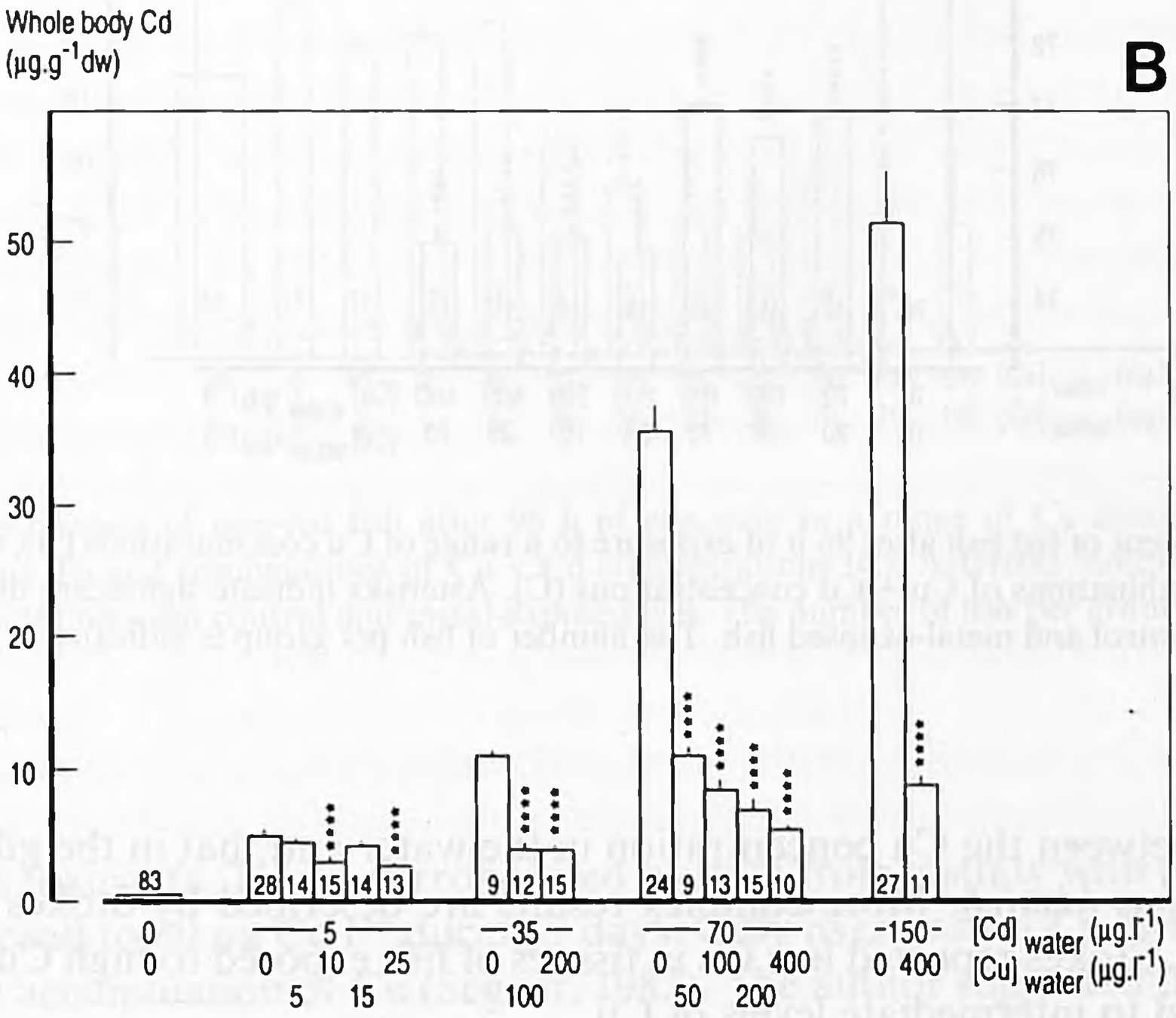


Fig. 7 (B) Whole body Cd-content of non-fed fish after exposure to combinations of Cu and Cd during 96 h. Asterisks indicate significant differences between Cd-content of fish exposed to Cd alone and fish co-exposed to Cu + Cd. The number of fish per group is indicated in the bars.

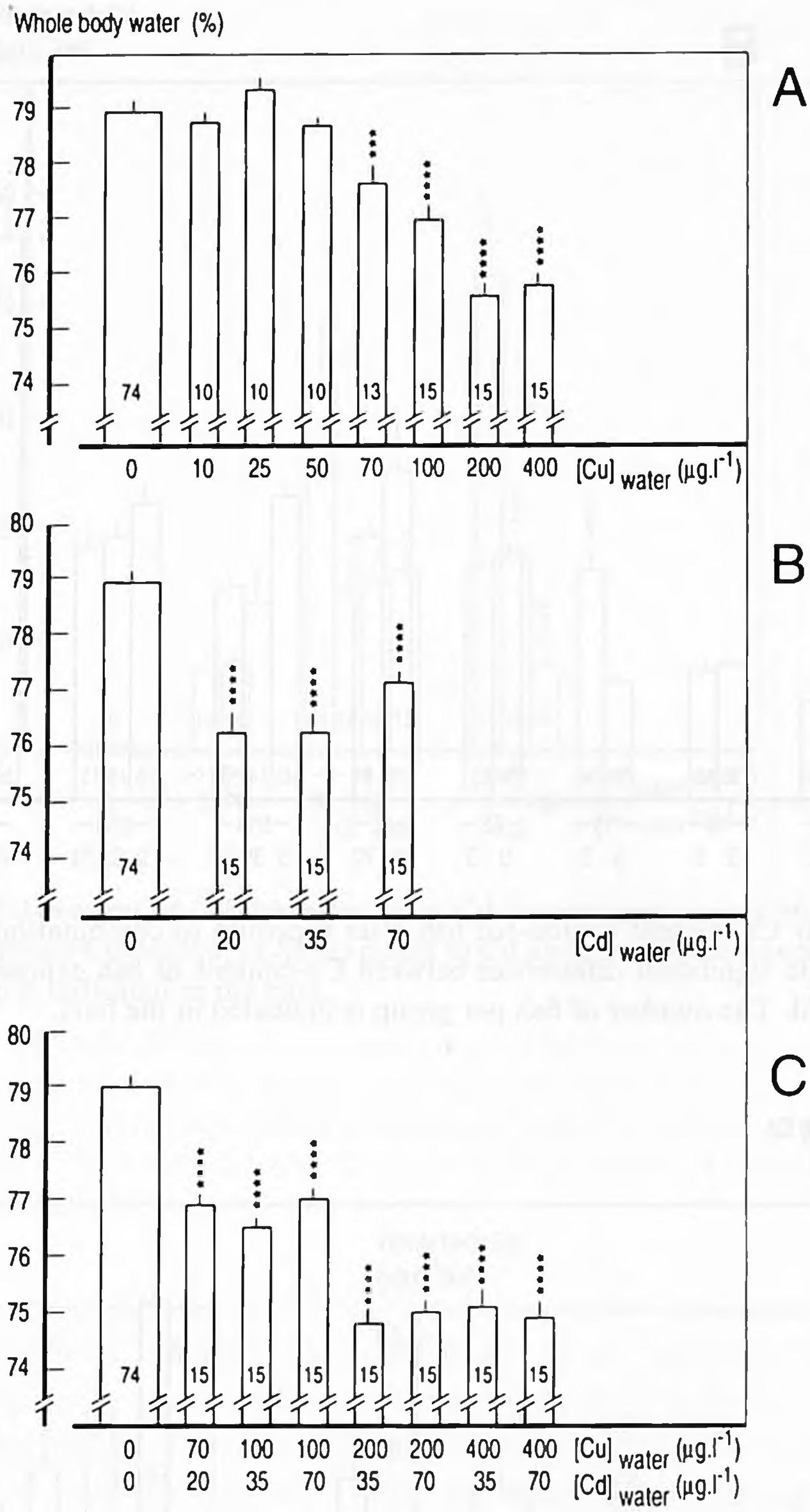


Fig. 8. Water content of fed fish after 96 h of exposure to a range of Cu concentrations (A), Cd concentrations (B) and combinations of Cu + Cd concentrations (C). Asterisks indicate significant differences in % water between control and metal-exposed fish. The number of fish per group is indicated in the bars.

relationship between the Cu concentration in the water and that in the gills and liver of *Oncorhynchus kisutch*. More complex results are described by Stokes (1979) and Segner (1987). Stokes reported less Cu in tissues of fish exposed to high Cu levels than in fish exposed to intermediate levels of Cu.

The accumulation rate, at least in the lower concentration range, was higher in non-fed fish. Two explanations for this phenomenon may be suggested. Firstly, the physiological consequences of food restriction may determine the ability to handle

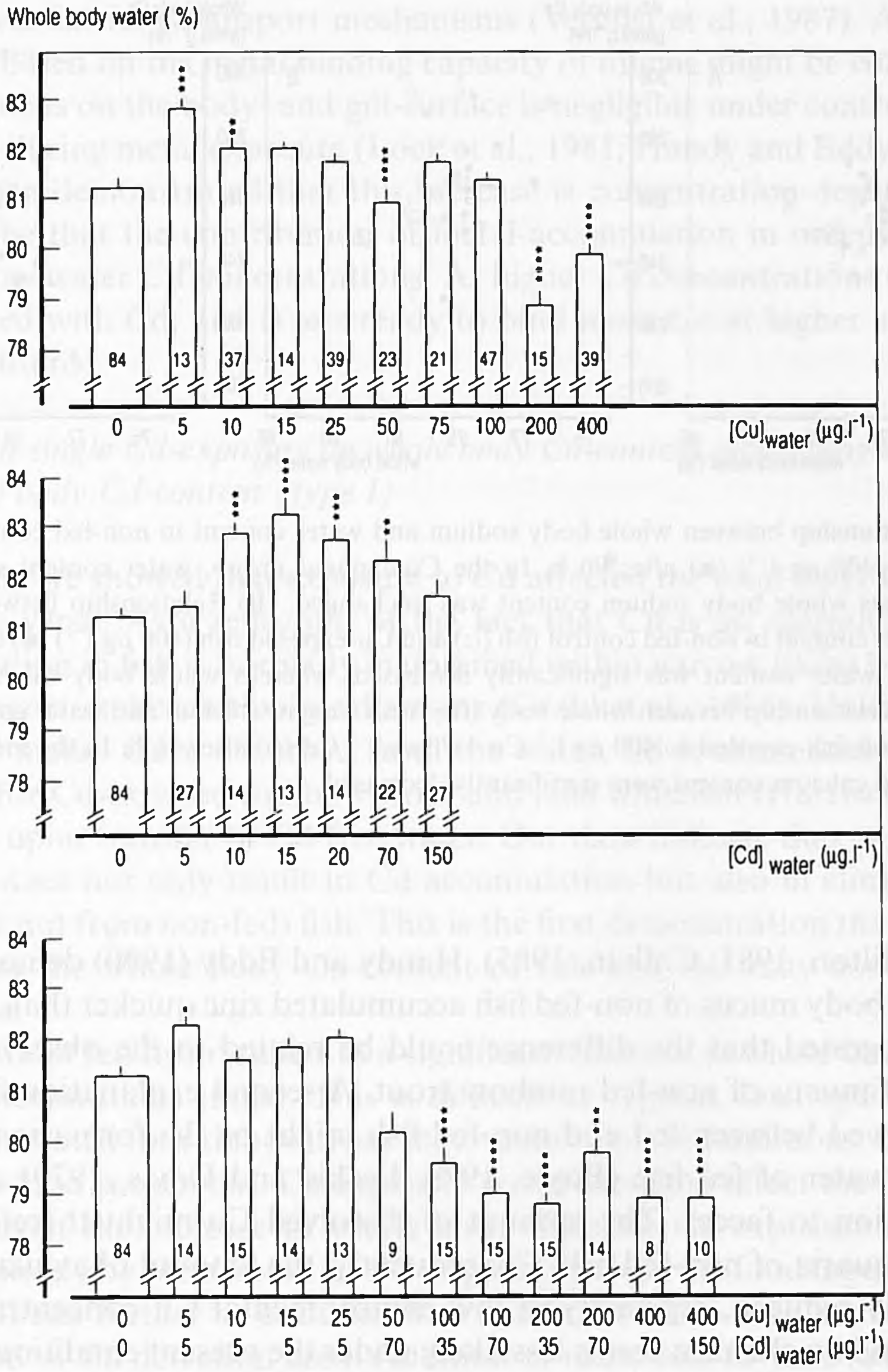


Fig. 9. Water content of non-fed fish after 96 h of exposure to a range of Cu concentrations (A), Cd concentrations (B) and combinations of Cu + Cd concentrations (C). Asterisks indicate significant differences in% water between control and metal-exposed fish. The number of fish per group is indicated in the bars.

exogenous toxicants. This is corroborated by data from a study with fed and non-fed roach exposed to 80 μg Cu l^{-1} during 7 days. Only liver tissue of non-fed fish showed significant accumulation of Cu (Segner, 1987). The author suggested that food-deprived fish lack the ability to regulate transfer of Cu within the body. Diet-related differences in Cu-tolerance were also reported for rainbow trout and perch, resulting in an inreased tolerance to waterborne Cu due to available dietary carbohydrate

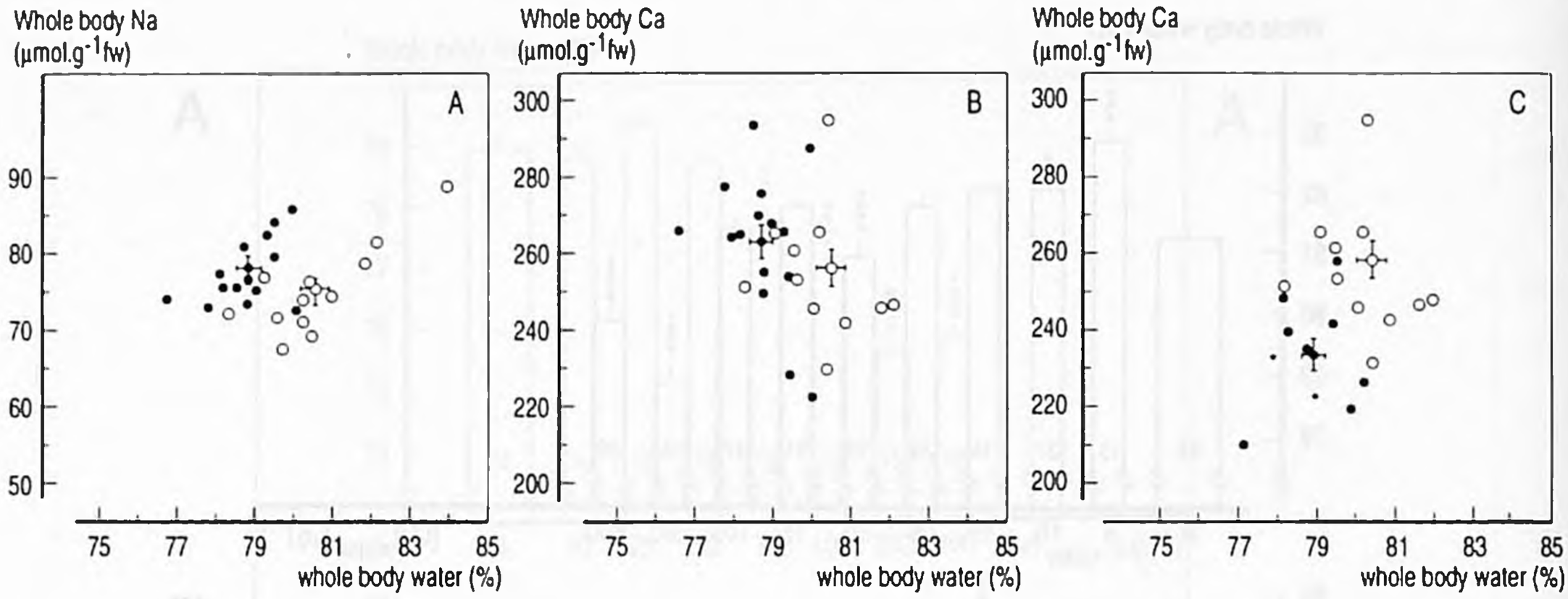


Fig. 10 (A) Relationship between whole body sodium and water content in non-fed control fish (○) and Cu-exposed fish (400 μg l^{-1}) (●) after 96 h. In the Cu-exposed group, water content was significantly decreased, whereas whole body sodium content was unchanged. (B) Relationship between whole body calcium and water content in non-fed control fish (○) and Cu-exposed fish (400 μg l^{-1}) (●) after 96 h. In the Cu-exposed fish, water content was significantly decreased, whereas whole body calcium content was unchanged. (C) Relationship between whole body (fw, fresh weight) calcium and water content in non-fed control fish (○) and fish exposed to 400 μg l^{-1} Cu + 70 μg l^{-1} Cd (●) after 96 h. In the metal-exposed fish water content and calcium content were significantly decreased.

(Dixon and Hilton, 1981; Collvin, 1985). Handy and Eddy (1990) demonstrated that gill tissue and body mucus of non-fed fish accumulated zinc quicker than tissues of fed fish. They suggested that the difference could be related to the observed decreased ion-content of mucus of non-fed rainbow trout. A second explanation for the difference we observed between fed and non-fed fish might be the formation of Cu-complexes in the water of fed fish (Boyle, 1979; Leckie and Davis, 1979) as a result of Cu-complexation to faeces. The amount of dissolved Cu might therefore be lower than that in aquaria of non-fed fish. Consequently, the amount of available Cu could be significantly reduced, especially at low environmental Cu concentrations. However, this second mechanism seems less likely under the present conditions because we could measure no differences in metal concentration after filtration over a 0.45 μm filter.

4.2. Cadmium accumulation in Cd-exposed fish

Our results show that Cd uptake was non-linear, and the accumulation factor decreased with increasing water Cd concentrations. Similar results were reported after exposing rainbow trout alevins (Beattie and Pascoe, 1978) and eggs of Atlantic salmon (Rombough and Garside, 1982) to Cd. A mechanism explaining non-linear Cd accumulation in largemouth bass and bluegill was hypothesized by Cearly and Coleman (1974). They suggested modulation of active transport mechanisms for Cd, which was dependent on the ambient Cd concentration. However, we are unaware of active Cd transport mechanisms. It subsequently has been demonstrated that Cd is

transported via calcium transport mechanisms (Verbost et al., 1987). An alternative explanation, based on the metal binding capacity of mucus might be considered. The amount of mucus on the body- and gill-surface is negligible under control conditions, but increases during metal exposure (Lock et al., 1981; Handy and Eddy, 1990, 1991). It has not been demonstrated that this increase is concentration-dependent. Therefore, it may be that the contribution of metal-accumulation in mucus is most pronounced at low water Cd-concentrations. At higher Cd concentrations mucus will be more saturated with Cd, and is less ready to bind more Cd at higher environmental Cd concentrations.

4.3. Effects of single Cd-exposure on whole body Cu-content and of single Cu-exposure on whole body Cd-content (type I)

In this study we showed that exposure to Cd affected the total body Cu concentration and vice versa. As a reflection of the fact that Cu is an essential element, the Cu-concentration in fish is normally maintained within narrow limits by coordinated uptake, transport and excretion mechanisms (Grahl et al., 1985). Unlike Cu, Cd is a non-essential metal. Once absorbed from the water, Cd is eliminated slowly, as has been shown for Cd-exposed rainbow trout and lake whitefish (Harrison and Klaverkamp, 1989) upon transfer to Cd-free water. Our data indicate that exposure to low levels of Cd does not only result in Cd accumulation but also in elimination of Cu from fed (but not from non-fed) fish. This is the first demonstration that Cd exposure has effects on the whole body Cu-content of fish and this may contribute to the toxicity of Cd.

Cu-exposure of fed fish resulted in a significant increase in whole body Cd content at all Cu concentrations tested. This is difficult to explain. Dallinger and Kautzky (1985) demonstrated for rainbow trout that, under control conditions, most of the Cd derived from food is excreted. Cu exposure could therefore affect the fecal excretion of Cd. However, the increase in whole body Cd after Cu exposure cannot be explained completely by the amount of Cd originating from the food ingested during the experiment. It can further be excluded that this Cd-increase is an artifact introduced by the method of Cd detection used. Addition of nitric acid to vials (control destruction, see Materials and methods section) did not result in any detectable Cu and Cd. Interaction of Cu and Cd during measurement of Cd is unlikely, because absorption spectra of the metals do not overlap. Besides, Cd-increase after Cu exposure is only found in fed fish. Thus, the effect is related to the feeding conditions of the fish during the experiment. This phenomenon needs further study.

In contrast to the results obtained with fed fish, Cu-exposure of non-fed fish resulted in a significant decrease of the whole body Cd content, especially at higher concentrations (>75 μg Cu l^{-1}). This decrease is most likely the result of clearance of previously accumulated Cd and not of the removal of absorbed Cd from the skin- and gill-surface because EDTA-rinsed fish contained as much Cu and Cd as water-rinsed fish (not shown). This observation has not been reported earlier for fish, but is in line with findings of Holwerda (1991) on clams, who reported an accelerated elimination of previously accumulated Cd during Cu-exposure. Release of Cd as a result of Cu

exposure in vitro was also reported for a human carcinoma cell line by Meshitsuka et al. (1983). A possible explanation may lie in the involvement of metallothioneins (MT's). Increased levels of MT's are associated with increased tolerance to toxic metals (Klaverkamp et al., 1984). The amount of MT's also increases during sublethal exposure and MT's are important for the recovery of physiological homeostasis (Giles, 1984). Both Cu and Cd are bound to MT's (Day et al., 1984; Cousins, 1985). However, Cu can displace Cd from MT's (Scheuhammer and Cherian, 1986). Therefore, during Cu exposure, the Cd-binding capacity of MT's decreases (Laurén and McDonald, 1987) and this could explain the observed Cd elimination. The physiological effect of the Cd-release by Cu is not clear. It is possible that this forms an additional burden during exposure to Cu, because the released Cd will be more harmful to the organism than MT-bound Cd.

4.4. Interactions between Cu and Cd during combined exposure

Copper accumulation. The effects observed on Cu-content after Cu/Cd co-exposure are not simply comparable to results obtained after single Cd exposure. This suggests two types of interaction (I and II). Firstly, interaction between Cu already present in the body and the Cd taken up as a result of Cd exposure. Secondly, Cu/Cd interaction during the uptake of Cu as a result of Cu/Cd co-exposure. In addition, our results can also be taken as evidence for the fact that the kind of interaction may, at least in part, depend on the ratio of the metals. This is also demonstrated by the results of Finlayson and Verrue (1982) and Gill et al. (1992). Exposure of the American eel (*Anguilla rostrata*) to 75 $\mu g\ l^{-1}$ Cd resulted in an increased Cu concentration in the kidney, while exposure to 150 $\mu g\ l^{-1}$ Cd decreased the Cu-content of the kidney (Gill et al., 1992).

Cadmium accumulation. In both fed and non-fed tilapia, Cu/Cd co-exposure resulted in a significant reduction of the whole body Cd-content compared to the Cd content of fish exposed to Cd alone. For non-fed fish, this is in line with our results obtained with exposure to Cu alone. For the fed fish, this seems to contradict the increased whole body Cd-content obtained after single Cu exposure. However, this increase was limited when compared to the amounts of whole body Cd after Cu/Cd co-exposure. Our data corroborate the results of Elliot et al. (1986), who reported reduced Cd-accumulation in clams in the presence of 10 or 20 $\mu g\ l^{-1}$ Cu, when Cd was present at 20, but not at 10 $\mu g\ l^{-1}$. Our results also show no effect of Cu co-exposure at the lowest Cd concentration used. The notable decrease in whole body Cd during Cu/Cd co-exposure compared to accumulation during Cd exposure alone, will likely be a combination of increased elimination and decreased uptake of Cd. It is difficult to imagine that the reduced Cd-content of fish exposed to Cu + Cd is the result of elimination alone, because in that case the amount of Cd excreted would be inconceivably high.

4.5. Water and ion content

Both single and combined exposure to Cu and Cd had an effect on the whole body

water content, demonstrating osmoregulation as one of the targets of the metals. In fed fish, these conditions reduced the water content of the fish. However, the effects in non-fed tilapia on water content seem to be complex. For example, exposure to 100 μg Cu l^{-1} had no effect while 70 μg Cd l^{-1} resulted in increased water content. Moreover, exposure to a combination of both metals at these concentrations resulted in a significant decrease of the water content. This apparent complexicity can also be found in the litterature, although information on effects on water content after e.g. Cd exposure is scarce. Increase (in goldfish, McCarthy and Houston, 1976), no effect (in embryos of *Salmo salar*) and inhibition of net water uptake (alevins of *Salmo salar*, Rombough and Garside, 1984) have been reported.

Overall, our results indicate that changes in water content, observed after metal exposure, are not directly related to metal-induced changes in whole body sodium- and/or calcium-concentration. On the basis of comparable results, observed in *Salmo salar*, Rombough and Garside (1984) also concluded that there was no involvement of ion-regulatory processes in the observed changes in whole body water content. In tilapia co-exposed to 400 μg Cu l^{-1} and 70 μg Cd l^{-1}, decreased whole body water content was accompanied by a decreased whole body calcium content compared to controls. These results could imply a relation between ionic- and osmoregulation during Cu/Cd co-exposure, as was suggested by McCarthy and Houston (1976) after Cd exposure of goldfish. However, in other groups of fish, metal exposure only resulted in changes in water content, whereas whole body sodium and/or calcium were not affected or vice versa.

In vitro Cu/Cd co-exposure of cell cultures resulted in an increased toxicity of these metals, as concluded from the protein content of trout hepatocytes (Denizeau and Marion, 1990) and KB cells (Meshitsuka et al., 1983, 1987). Meshitsuka and coworkers suggested that the toxicity of Cd did not depend solely on the amount of Cd absorbed by the cells but also on cofactors such as Cu. An explanation for increased toxicity due to exposure to metal mixtures is given by Irons and Smith (1976), who suggested MT-aggregation as a result of combined exposure, which prevented Cd-sequestration.

From this study can be concluded that the nutritional state of fish influences the amount of metal accumulated during exposure to heavy metals. Furthermore, accumulation and toxicity during Cu/Cd co-exposure cannot be predicted by simple addition of the effects of single metal exposure. Obviously, the complex interaction mechanisms underlying the observed effects, concern previously unrecognized toxic effects of the metals. Also at the cellular level, Meshitsuka and coworkers (1983, 1987) suggested that the toxicity of Cd did not depend solely on the amount of Cd absorbed by the cells but also on cofactors such as Cu.

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