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Bioaccumulation and Localization of Exogenous Cadmium in a Teleost by Electron Microscopy (TEM) and its Specific Quantitation by Electron Probe X-ray Microanalysis (EPMA)

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Abstract—A cadmium bioconcentration study was carried out in a fresh water teleost, *Colisa fasciatus*, to study the bioaccumulation kinetics and fate of exogenous cadmium (Cd) in biological tissues. Study shows that on exposure of the fish to a sublethal concentration of cadmium in test water, Cd uptake results in its bioconcentration in gills, liver and muscle tissues. To explore whether the accumulated Cd reaches the membranes or inside the cells, transmission electron microscopy (TEM) of the thin sections of tissues was done after histochemical localization of Cd in cells by modified SST method. TEM studies of sections of gills, liver and muscle tissues showed the deposits of exogenous Cd (visualized as dense clouds) in biological cells. This suggests the presence of free or loosely bound Cd on the membranes and inside the cells, which in the presence of Na_2S is converted into insoluble metal sulfides. Electron probe X-ray microanalysis (EPMA) studies confirmed the presence of Cd on the membrane surface as well as inside the cells of bioindicator organs suggesting involvement of membrane transport of exogenous Cd inside the cells and its deposition as loosely bound insoluble metal complexes.

Introduction

Electron probe X-ray microanalysis (EPMA) or analytical electron microscopy is a relatively new method of analysis at ultra-structural levels. The major contribution of X-ray microanalysis to cell biology is in understanding the mechanisms that control various cellular processes. In EPMA, identification and localization of main elements of the periodic table in the biological specimen is accompanied with specificity, sensitivity and spatial resolution sufficient to permit measurements of element concentration in a single cell as well as in an organelle. X-ray microanalysis of naturally occurring elements in biological specimens could resolve 10^{-18} g in an ultra-thin section under favorable conditions.[1,2] EPMA reveals depositions of heavy metals present on the surface or within the cytoplasm. X-ray disperse analyses have thus been used to determine incipient concentration of toxic metals after exposure of organisms to metal ions.[3] Linton et al.[4] compared laser and ion microprobe detection sensitivity in biological microanalysis and inferred that micro-probe detection is a very competitive technique available for certain toxic heavy metals.

To facilitate detailed information, EPMA has also been coupled with scanning, transmission and scanning transmission electron microscopy. Ion distribution in normal as well as in pathological cell systems of both plant and animal specimens has been studied using the EPMA technique. Certain histochemical methods have been successfully used with transmission electron microscopy (TEM) and EPMA for studies on ion distribution in pathological cells.

Autometallography (AMG) is one of these histochemical processes by which specific metals, metal sulfides and selenides are visualized. It involves a process of catalyzing the reduction of silver ions on their surfaces.[5,6] With high specificity, traces of metals have been demonstrated in tissues and cells that had previously not been suspected of containing metals. AMG has been successfully used to demonstrate:

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(a) exogenous trace metals — gold,[7] silver,[8,6] and cadmium;[9]
(b) endogenous trace metals — zinc;[5]
(c) silver enhanced metal markers of macromolecules and enzymes:

- colloidal gold particles,[10]
- copper sulfide accumulations associated with cuprolinic blue method.[11]

EPMA verifications of electron dense metal accumulations, identified by AMG in the ultra-thin sections, have been shown for cadmium,[9] for gold in lysosomes,[12] and for mercury (by the concomitant presence of mercury and silver.[13]

Present study

Bio-concentration, TEM and EPMA studies were carried out on thin sections of gills, liver and muscle tissues of a fish, *C. fasciatus*. Objective of the study was to:

1. study the bioconcentration of Cd in a bioindicator test fish, *Colisa fasciatus*;
2. histochemically localize Cd in gills, liver and muscle of exposed teleost fish with silver sulfide technique (SST) and TEM;
3. scan, probe and quantify Cd at the membrane surfaces and inside the specific cells of gills, liver and muscle tissues with EPMA, and
4. study the distribution of major monovalent (Na, K) and divalent (Ca, Mg, Zn, Fe, S) cations at specific sites in the normal and Cd exposed tissues.

Experimental Methodology

Bioconcentration studies

The experiment work for bioconcentration studies was carried out in a glass aquarium (50 L). It contained tap water, which was initially charged with sublethal concentrations of Cd(II) ((Cd as $Cd(NO_3)_2$). The experimental fish were acclimatized in tap water (30 days) and exposed to 1.00 mg l^{-1} Cd. A total of 50 specimens were exposed to experimental conditions. Fish and test water (tap water charged with Cd) were drawn (initially 8 h (three times), and then every fifth day) for analysis. Fish tissue (gills, liver and muscles) samples were prepared,[14] and digested with a flux of HNO_3–H_2O_2.[15] Metal concentrations were determined using an inductively coupled plasma–emission spectrometer (ICP–AES) and reported on a dry weight basis. Experimental work was carried out at 24 ± 2 °C. The experiments were conducted in triplicate and the reported values are an average of three values. In the different experiments, blanks (control) were run and the corrections applied wherever necessary.

Transmission electron microscopy (TEM) and EPMA of tissues. The test fish were exposed to sublethal concentrations of cadmium (1.00 mg L^{-1}) for 30 days in glass aquarium under simulated conditions. The fish tissue (gills, liver and muscles) were exorcised and samples were prepared for light and electron microscopic studies.[16]

Localization of Cd

Localization of Cd in fish tissue was attempted in gills, liver and muscles at cellular level by the modified silver sulfide technique.[5] Precipitates of Cd sulfides were viewed on an transmission electron microscope at specific cellular sites of gills, liver and muscle samples at various magnifications.

TEM of tissues

The preparation of the specimen involved the following steps.

Fixation. Fish tissue (gills, liver and muscles) were exorcised and were preserved in formaline. Fish were alive when the tissues were taken out. For TEM studies, tissues were washed with distilled water and were exposed to 0.1% Na_2S in 0.1 M phosphate buffer (pH 7.4) for 10 min. Tissues were fixed later with 1% gluteraldehyde in 0.1 M phosphate buffer (pH 7.4) for 90 min. Tissue samples were rinsed in phosphate buffer (0.1 M, pH 7.4) for 5 min.

Dehydration. Tissues were dehydrated in acetone stepwise in a concentration series given here: with 30% (v/v) acetone — 15 min, 50% (v/v) acetone — 10 min, 70% (v/v) acetone — 10 min, 90% (v/v) acetone — 10 min, 100% (v/v) acetone — 10 min, 100% (v/v) acetone — 15 min. The dehydration was carried out at room temperature in capped plastic vials.

Embedding. Epoxy resin (araldite embedding medium) with the following composition was used as media: component — Araldite Cy_{212} (epoxy monomer) — 29.0 gm, Dodecyl succinic anhydride (hardener) — 24.0 gm, 2,4,6 tridimethylaminophenol (DMP30 (d_4O_{64}) (accelerator) — 0.5 gm. The process was carried out in a fume cupboard. The components were mixed and allowed to stand for 10 min. It facilitated the air bubbles to escape resulting in a homogeneous media. The embedding medium was added to the vials for block preparation. Polymerization was carried out at 65 °C for 48 h. The blocks were removed and allowed to cool at room temperature.

Microtomy and ultra-microtomy. Blocks were trimmed to produce a flat pyramid for microtomy. Thin sections (0.5 μm) were cut for light microscopy and were also fixed on glass slides for EPMA. The sections (70–90 nm) were raised on copper grids for developing and staining for the visualization of Cd by modified SST.

Developing and staining. The sections were developed in complete darkness for 10 min in silver nitrate/hydroquinone medium[5]. 100 mL of medium contained: 60 mL gum arabic (20% in distilled water), 10 mL citrate buffer (pH 3.5), 15 mL hydroquinone (0.85 g in 15 mL), 15 mL silver nitrate (0.11 g in 15 mL). Grids were rinsed in distilled water to free the excess developer and stained

with uranyl acetate for 10 min. The stain was prepared as: uranyl acetate–solid p.a., distt. water — 100 ml. The solution was stored for 24 h, centrifuged and the supernatant was stored at −4°C. The grids were finally washed to free the excess stain by dipping them in water and the excess of water was removed from the surface of the grid.

Viewing. The TEM of the specimen were taken on a Transmission Electron Microscope fitted with a 35-mm camera.

Electron probe microanalysis of ions in fish tissue

For EPMA, the samples of fish tissue (0.5 μm thick) were fixed on glass slides, developed in a Danscher developer and coated with carbon. Cadmium was quantified at specific sites of gills, liver and muscle cells. Results of metal ion concentrations in gills, liver and muscle at specific sites of EPMA analysis are presented in weight percentage. Other specific conditions include: current — 1.00×10^{-10} A; voltage — 10 kV; probe diameter — 0.1 and 5.0 μm. Standards supplied by SPI Supplies Division of Structure Probe Inc., Canada were used for X-ray microanalysis. The metals present in layout of standard mounts were: Cd — 100.00%; S — 13.74%; Ca — 21.73%, Mg — 13.18%, P — 18.50%, Zn — 0.20%; Na — 3.01%; K — 4.18%; Fe — 6.32%.

Instrumentation and reagents

The metal concentrations were determined by an ICP–AES (model Plasmalab 8440 with Labtam 3000 series Computer, Australia). pH measurements were made on a digital pH meter DPH 500 (Global). The Century portable water analyzer measured conductivity. Nitrate and dissolved organic carbon (DOC) in water samples were analyzed on a segmental flow analyzer (model Skalar 20/40).

TEM of fish tissue was carried out on Transmission Electron Microscope, Phillips CM 10 model (magnification — up to×450,000, resolution — 0.3 nm). Blocks of plastic resin embedded specimens were processed using block trimmer Reichert TM-60, and glass knife maker. Ultramicrotome-Reichert OMU was used for processing the tissue sections of 0.5 μm for light microscopy. Ultra-thin sections of 70–90 mm thickness were raised on copper grids with Ultra-microtome-Reichert Jung Ultra-Cut-E.

An electron Probe X-Ray microanalyzer EPMA, (model JXA 8600M, Jeol, Japan) was used for quantitation of metals at selected sites in fish tissue (gills, liver and muscles). Carbon coating of thin sections (0.5 μm) of fish tissue samples for EPMA analysis was done using a Jeol JEE-4 vacuum-evaporator (Jeol Ltd., Japan). The instrument is equipped with plural computers DECLS1-11/73 for coefficient control of its electron optical stage, automatic crystal change X-ray spectrometer and an X-ray measurement system. The application programs available for wave length dispersive spectrometers include quantitative analysis (trace element analysis) with ZAF and Bance and Alee corrections and thus facilitate analysis of elements and the results are normally obtained as wt%.

The chemicals used in the experiments were of AR grade from E. Merck (India). The stock solutions of the metal ions were standardized by the usual complexiometric titrations. For bioconcentration studies in the laboratory, glass aquariums were used as test chambers.

Results and Discussion

Results presented herein on TEM and EPMA carried out on thin sections of gills, liver and muscle tissues of the fish have been discussed in terms of bioconcentration, localization and quantitative analysis of Cd at a specific cellular level and the variations of other metabolic cations.

Bioconcentration of cadmium in *C. fasciatus*

Bioconcentration and organ distribution of Cd in gills, liver and muscle tissues of fish were recorded every 8 h (three times) initially for 24 h and later every fifth day (Table 1). Cadmium appeared in gills and liver in the first observation after 8 h. In the initial 24 h, gills and liver accumulated 8.4 and 6.8 μg g^{-1} Cd, respectively. Muscle tissue accumulated relatively low Cd concentration (2.6 μg g^{-1}). Initial concentration in muscle tissue was recorded after 16 h.

Concentration of Cd in bioindicator tissues increased with time and bioconcentration of Cd up to 39.6, 23.0 and 7.7 μg g^{-1} in gills, liver and muscle tissues was recorded in 25 days. Studies show that bioconcentration and organ distribution of Cd presented herein is a function of time and organ tissue. Bioconcentration increases with time and Cd is accumulated in different concentrations at specific sites (Table 1).

Changes in the physico-chemical characteristics of the test water and concentration of Cd in the exposure medium were regularly recorded during the 25-day observation period. The details are given in Table 2. Cd in the test water decreases which shows its bio-uptake.

Table 1. Bioconcentration of cadmium in fish tissue (unit — μg g^{-1} dry weight)

Time	Bio-concentration in fish tissue		
	Gills[a]	Liver[a]	Muscle[a]
Control	0.0	0.0	0.0
8 h	4.8	1.2	—
16 h	6.2	3.8	0.6
24 h	8.4	6.8	2.6
5th day	15.6	9.6	2.8
10th day	16.8	13.8	3.6
15th day	28.2	16.2	6.8
20th day	N.A.	N.A.	N.A.[b]
25th day	36.9	23.0	7.7

[a]Mean of four samples ($n = 4$).
[b]NA, not analyzed.

Table 2. Variability of physico-chemical parameters and concentration of Cd in test water

Time	Parameters		Initial Cd — 1.0 mg L^{-1})		
	Cadmium (μg L^{-1})	pH	DOC[b](mg i^{-1})	Conductivity (mmhos)	Hardness (mg L^{-1})
0 h	1022	7.3	8.38	0.418	138
24 h	820	7.4	9.24	0.618	144
5 days	698	7.5	12.68	0.742	146
10 days	716	7.5	14.28	0.796	144
15 days	686	7.7	15.38	0.812	150
20 days	N.A.[a]	7.8	14.26	0.802	154
25 days	638	7.9	16.24	0.842	162

[a]N.A., not analyzed.
[b]DOC, dissolved organic carbon.

Localization of Cd in fish tissues

In *C. fasciatus*, gills have five pairs of gill arches with gill filaments on the first four pairs. On the upper and lower surfaces of each filament, rows of closely packed leafy structures are present and are called secondary lamellae. Gills have two distinct regions (i) gill head region, and (ii) gill lamellae region. The head region consists of an outer layer, which possesses epithelial cells. The inner side of the gill head region consists of a number of small cells. Abductor muscles present in this region consist of muscle fibers. Secondary lamellae have epithelial layers, the endothelium of which consists of a series of pillar cells. Smooth muscle cells and mast cells remain present in the primary and secondary gill lamellae.[17]

The transmission electron micrograph of the gill head region and gill lamellae region of the unexposed (Fig. 1) and Cd exposed (Fig. 2) fish shows that the exposed tissue have silver intensified inclusions of Cd at selected sites in mast cells, smooth muscle cells and on the membranes of the epithelial cells which are not seen in unexposed fish. A cellular view of liver and muscle tissues of the unexposed teleost is presented as Figures 3 and 5. In the Cd exposed teleost, Cd was visualized as silver amplified electron dense inclusions inside the cells of liver plate (Fig. 4) and on the membrane surfaces of the muscle cells showing its absorption (Fig. 6). Limitations of these transmission electron micrographs are that they do not exhibit required contrast, probably due to araldite embedding and shrinkage of tissues. This, however, indicates that the exogenous Cd can be visualized as dense clouds on TEM by the modified SST method.

The SST process employs a developer with a low pH, silver nitrate as silver ion donator and a protecting colloid, gum arabic. The developer involves the use of small amounts of hydroquinone as reduction molecules. The SST results in reduced silver ion magnifications at specific sites with gum arabic reducing the autocatalytic activity in the developer itself and the catalytic activity of the zone between the developer and the surface of the section.[6,8] However, SST visualizes only a certain fraction of heavy metals represented by free or loosely bound metal that in the presence of Na_2S is converted into insoluble metal sulfides. The metal that is strongly bound to organic/inorganic ligands may not be sulfidated and remains histochemically invisible.

The visualization of the fraction of Cd in fish tissue presented herein support Danscher and Norgaard[18] in that SST could be used for the demonstration of trace amounts of certain heavy metals at ultra-structural level and that the metal sulfides can be shown by the technique. In their investigation, George et al.,[9] using the precipitation reactions (sulfide for heavy metals), reported precipitation of Cd as a sulfide in bivalve tissues. They also reported that precipitation reactions result in a better localization with a loss of 14% as compared to

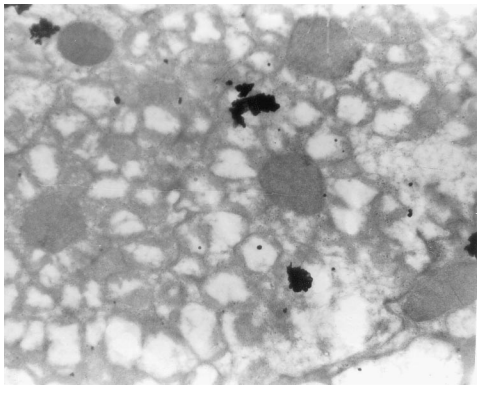

Figure 1. Transmission electron micrograph of gills of control (unexposed to Cd) fish (magnification — 4500×).

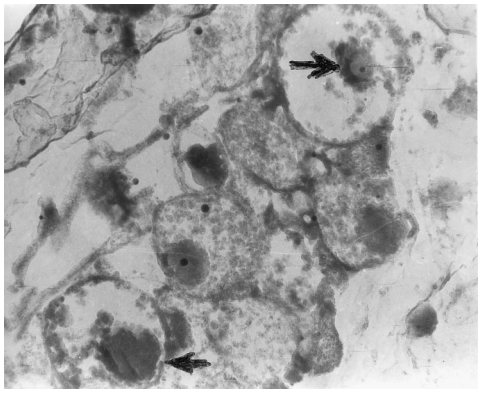

Figure 2. Transmission electron micrograph of gills of fish exposed to Cd — Cd inclusion in gill lamellae cells (→) (magnification — 7000×).

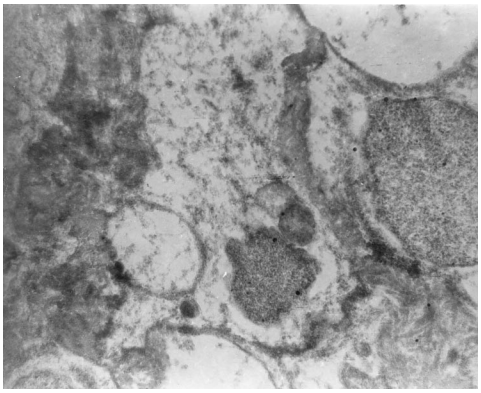

Figure 3. Transmission electron micrograph of liver plate of control fish (unexposed to Cd) (magnification — 9600×).

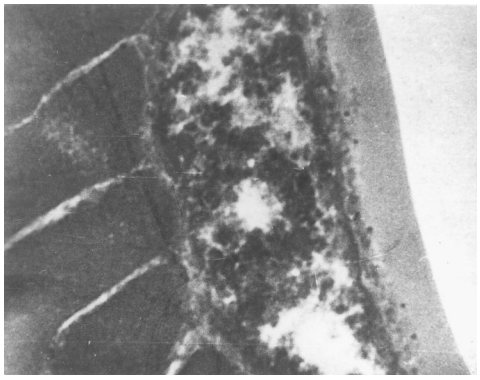

Figure 5. Transmission electron micrograph of skeletal muscle of control fish (unexposed to Cd) (magnification — 7400×).

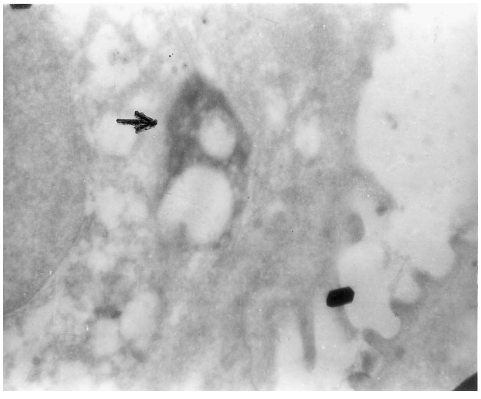

Figure 4. Transmission electron micrograph of liver plate of fish exposed to Cd — showing inclusions (→) (magnification — 9600×).

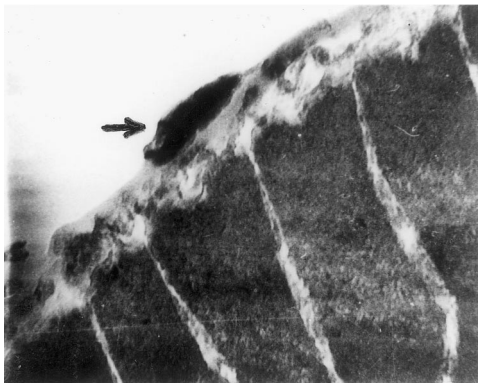

Figure 6. Transmission electron micrograph of skeletal muscle of fish exposed to Cd — showing Cd adsorption on membrane (→) (magnification — 7400×).

28% when the bivalve tissues were processed without precipitant.

Results are also supported by the findings of Seigal[19] that excessive uptake results in bioconcentration from precipitation of insoluble metal complexes through the activities of membrane associated sulfate reductases or through the biosynthesis of oxidizing agents such as H_2O_2. The reduction of sulfate to sulfide and the diffusion of H_2O_2 provide highly reactive means by which metals can be precipitated.[20] In SST, metal sulfides are responsible for the precipitation of metallic silver and result into silver enhanced visualization. Simpson and Dom[21] investigated several heavy metal cations as prospective ionic tracers and reported metal deposits with reproducible results.

Electron probe X-ray microanalysis of fish tissue

Cadmium was scanned and probed at selected sites in gills, liver and muscle tissues of Cd exposed *C. fasciatus* and were compared with that of unexposed tissues. All the samples studied by EPMA were mounted on glass slides, which were coated with carbon film to make it conducive to minimize charging effects. The control (sections of the tissues not exposed to Cd but mounted on glass) does not show the presence of Cd and no Cd was detected in the glass background. The other cations detected and quantified include Na, K, Ca, Mg, S, P, Fe and Zn. The results of quantitative analysis are presented in weight percentage of the probed area.

In the gill head region, three sites were scanned. These include the outer membrane of epithelium, the inner membrane of the epithelial cells and inside the gill head region. In the gill lamellae region, the probe position was on the outer membrane of primary lamellae, base membrane and endothelium of secondary lamellae. The results obtained from the EPMA are presented (Tables 3 and 4). Normal distribution of cations (S, Ca, Mg, Zn, Na, K, P and Fe) is incorporated with their redistribution (concentration variation at the same sites on the exposure of fish to sublethal concentrations of Cd).

Results of the cation distribution (Tables 3 and 4) show that Cd is not present in unexposed fish gills and other cations constitute a wt% of 6.810, 11.286 and 6.695, respectively, at the outer surface and inner surface of epithelial cells and inside the gill head region. In the gill lamellae region, the cations scanned constitute a fraction of 12.844, 10.579 and 8.479 (w% of the area) at the outer membrane of primary lamellae, base membrane and endothelium of secondary lamellae, respectively.

EPMA of the gills exposed to Cd shows exogenous metal at selected sites. The concentrations (average

Table 3. Microanalysis of gill head cells of Cd exposed and control fish[a]

Metals analysed	Distribution of cations in gill head cells					
	Probe position (Probe diameter)					
	Outer membrane 0.1 μm		Inner membrane 0.1 μm		Inside gill head region 5.0 μm	
	Unexposed	Cd exposed	Unexposed	Cd exposed	Unexposed	Cd exposed
Cadmium	—	0.012	—	0.028	—	0.018
Sulphur	0.086	0.320	0.104	0.275	0.216	0.098
Calcium	1.605	3.258	2.622	1.341	1.550	0.941
Magnesium	1.020	2.030	1.706	0.850	1.142	0.479
Zinc	0.028	0.140	0.144	0.210	0.239	0.211
Sodium	3.835	6.369	5.821	2.619	2.998	2.679
Potassium	0.236	0.462	0.326	0.157	0.182	0.083
Phosphorous	—	0.166	0.563	—	0.290	0.106
Iron	—	0.033	—	0.097	0.078	0.068

Table 4. Microanalysis of gill lamellae cells of Cd exposed and control fish[a]

Metals analysed	Distribution of cations in gill lamellae cells					
	Probe position (Probe diameter)					
	Outer membrane 0.1 μm		Inner membrane 0.1 μm		Inside gill lamellae region 5.0 μm	
	Unexposed	Cd exposed	Unexposed	Cd exposed	Unexposed	Cd exposed
Cadmium	—	0.026	—	0.062	—	0.036
Sulphur	0.173	0.113	0.167	0.046	0.245	0.230
Calcium	3.295	2.521	2.344	3.528	2.059	3.643
Magnesium	2.146	1.445	1.308	1.933	1.345	2.239
Zinc	0.132	0.023	—	0.166	—	0.181
Sodium	6.495	3.805	5.052	5.935	4.019	6.729
Potassium	0.436	0.288	0.286	0.428	0.324	0.411
Phosphorous	0.122	0.325	1.358	0.360	0.399	—
Iron	0.045	0.180	0.064	0.038	0.088	0.049

[a]Mean of I, II, III and IV analyses; units — wt% of area probed.

wt%) of Cd in the gill head region of the exposed fish were 0.012, 0.028 and 0.018 at the probed sites. However, the concentrations of Cd in the gill lamellae region were 0.026, 0.062 and 0.036 at the three probed sites, respectively (Tables 3 and 4). In liver, three sites of liver plates were probed. These were outer surface, inner surface and inside the liver plates (including liver cells). The cation distribution of the liver plate is given in Table 5. In the Cd-exposed organisms, the concentrations of Cd were recorded at all the three sites of liver and the concentrations were 0.019, 0.053 and 0.044 (Table 5). Probe position in the skeletal muscle cells was fixed at the outer surface, inner surface and inside the muscle cells. Table 6 includes the results of cations present in the probed area. In the Cd-exposed teleost fish, Cd was found at all the three sites with concentrations of 0.033, 0.049 and 0.039, respectively.

Results presented herein show that the distribution and concentration of cations in the tissues are very site specific. Varying concentrations of Cd are recorded by EPMA at various sites. The in vivo concentration of Cd may be larger than what is detected by EPMA. EPMA, however, confirms compartmentation of exogenous Cd at the cellular level. The findings on the localization, distribution and concentration of membrane bound and other cellular cations of metabolic importance are supported by earlier investigations.[22,23]

Hopkin and Martin[22] reported that the specimens of *Onicus asellus* of Cd, contaminated sites contained about 0.5% Cd (dry weight). They reported the distribution of metals within the hepatopancreas of *Onicus asellus* (crustacean) by AAS, TEM and X-ray. Woodlice from the contaminated sites have also been shown to have Cd deposits on the membrane of the cells. It was found that the membrane of the cells was lined with fine deposits of electron dense material in which Cd was detected by X-ray microanalysis.[22] It was also shown that Cd concentration in the test fraction of *Onicus asellus* was 65 μg g^{-1} dry weight.

Prosi[24] investigated the pathways of metal uptake and their final storage for Cd, Pb and Cu in limnic isopods. He reported identification of Cd and Pb at subcellular level using X-ray microanalysis and histochemical methods. Cd peaks were detected by electron microscopy and microanalysis as electron dense deposits consisting of cadmium–oxine complexes in endothelial cells of cornea of pregnant rats.[25] Further, various issues of common winkles (*Littorina littorea*) experimentally exposed to cadmium chloride were examined using light and electron microscopy and their elemental composition was detected by X-ray microanalysis and histochemistry.[26] They reported traces of Cd in the cytoplasm of excretory cells. X-ray microanalysis revealed that concretions of basophilic cells were the

Table 5. Microanalysis of liver plate cells Cd exposed and control fish[a]

Metals analysed	Distribution of cations in liver plate cells					
	Probe position (Probe diameter)					
	Outer membrane (0.1 μm)		Inner membrane (0.1 μm)		Inside gill lamellae region (5.0 μm)	
	Unexposed	Cd-exposed	Unexposed	Cd-exposed	Unexposed	Cd-exposed
Cadmium	—	0.019	—	0.053	—	0.044
Sulphur	0.271	0.325	0.128	0.075	0.207	0.261
Calcium	2.169	2.756	2.725	2.594	1.828	2.247
Magnesium	2.127	1.300	1.810	1.433	1.623	1.434
Zinc	0.068	0.149	0.070	0.160	0.028	0.094
Sodium	4.309	3.073	5.816	4.373	2.741	4.824
Potassium	0.314	0.256	0.326	0.304	0.221	0.297
Phosphorous	—	0.381	0.215	0.643	0.600	1.140
Iron	0.063	0.053	0.133	0.058	0.089	0.179

[a]Mean of I, II, III and IV analyses; units — wt% of the area probed.

Table 6. Microanalysis of skeletal muscle cells of the Cd-exposed and control fish[a]

Metals analysed	Distribution of cations in skeletal cells					
	Probe position (Probe diameter)					
	Outer membrane (0.1 μm)		Inner membrane (0.1 μm)		Inside gill lamellae region (5.0 μm)	
	Unexposed	Cd-exposed	Unexposed	Cd-exposed	Unexposed	Cd-exposed
Cadmium	—	0.033	—	0.049	—	0.039
Sulphur	0.173	0.113	0.167	0.046	0.245	0.230
Calcium	3.295	2.521	2.344	3.528	2.059	3.643
Magnesium	2.146	1.445	1.308	1.933	1.345	2.239
Zinc	0.132	0.023	—	0.166	—	0.181
Sodium	6.495	3.805	5.052	5.935	4.019	6.729
Potassium	0.436	0.288	0.286	0.428	0.324	0.411
Phosphorous	0.122	0.325	1.358	0.360	0.399	—
Iron	0.045	0.180	0.064	0.038	0.088	0.049

[a]Mean of I, II, III and IV analyses; units — wt% of the area probed.

sites for Cd inclusions. Low amounts of Cd were also found in the granules of epithelial mantle cells rich in sulfur.[26]

Literature is enriched with the reports on the concentration of major mono and divalent cations with their analysis at various membrane surfaces of different model systems.[27] Sauer and Watabe[27] reported Zn accumulations in the lysosome-like structures in the estuarine teleost, *Fundulus heteroclitus* when examined using histochemical (timm sulfide silver technique) and X-ray microanalytical techniques. Element analysis of K, Ca, Fe and Zn were reported in the lysosome-like structures. The concentrations were 14.3, 20.3, 1.0, 1.4 in control and 13.9, 15.4, 0.4 and 10.0 in the fish exposed to Zn. The element analysis for K, Ca, Fe and Zn in the osteoblast cytoplasm of the control and Zn-exposed tissues were 23.8, 17.5, nil (control) and 21.2, 17.0, nil and 0.5 $\mu g\ g^{-1}$ dry wt, respectively. The element analysis in these structures has a relatively high metal content in the surrounding cytoplasm.

Results on metabolic ion distribution as observed in the present study shows that distribution of cations at various sites (Tables 3–6) in the unexposed and exposed cells to sublethal concentrations of Cd is site specific and it would be premature to state that introduction of toxic metal ions (Cd) in fish tissue replaces other cations for its accumulation.

Conclusions

The study presented herein on bioconcentration of cadmium in teleost *Colisa fasciatus* shows that on exposure of the teleost fish to sublethal concentration of Cd in test water, Cd uptake results in its bioconcentration in gills, liver and muscle tissues. Bioconcentration of Cd is a function of time and is site-specific. It was observed that the accumulated Cd precipitates on the membrane and inside the cells as insoluble metal complexes, perhaps through the activities of membrane associated sulfate reductases or through the biosynthesis of oxidizing agents. TEM studies of the sections of gills, liver and muscle tissues confirmed that the deposits of exogenous Cd could be visualized on TEM by the modified SST method as dense clouds. This represents free or loosely bound Cd on the membranes and inside the cells which in the presence of Na_2S is converted into insoluble metal sulfides. Further studies by electron probe X-ray microanalysis of these specific sites confirmed the presence of Cd on the membrane surface as

well as inside the cells of bioindicator organs, suggesting involvement of membrane transport of exogenous Cd inside the cells and its deposition as insoluble metal complexes.

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