Integrated use of histological and immunohistochemical biomarkers of different fish species for assessing pollution in Crisul Negru river, Romania

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AIM OF RESEARCH

- **distribution of heavy metals contaminants** in the gills, liver and kidney of the 3 fish species

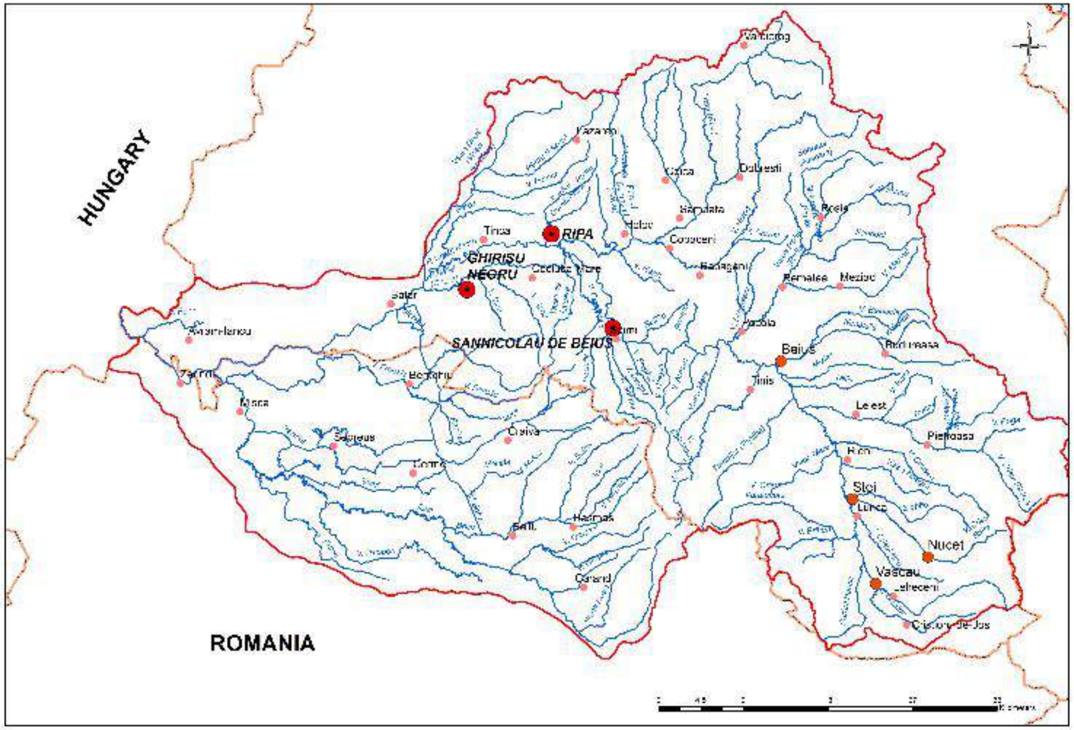
- Correlation between heavy metals bioaccumulation and **structural injuries** induced in the gills, liver and kidney via histopathological and immunohistochemical investigations

- **Structural recovery** of tissular damage by PCNA analysis

- **Ultrastructural analysis of injuries** in gills, kidney and liver by electron microscopy

MATERIAL AND METHODS

- One sampling sites along along Crisul Negru River was investigated (Grisul Negru)
- Sampling took place during one day in June, 2013) and individuals were caught by electro-fishing:
- *Barbus barbus (BB)*
- *Chondrostoma nazus (CN)*
- *Squalius cephalus (SC)*



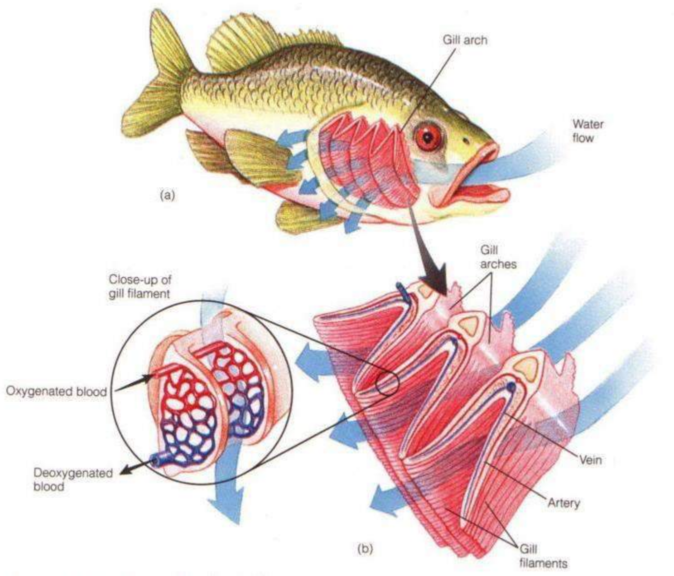
Gills , liver and kidney **histology** : H&E, trichrome stain

IHC and WB: TNFalpha, PCNA

Electron microscopy – liver and kidney *(Chondrostoma nazus)*

Bioaccumulation: Fe and Zn – atomic spectroscopy 4100 MP-AES; Cu, Pb and Cd - 200 series AA 240 ZAA Atomic absorption spectrometer equipped with the GTA 120 graphite tube atomizer (Agilent Technologies)

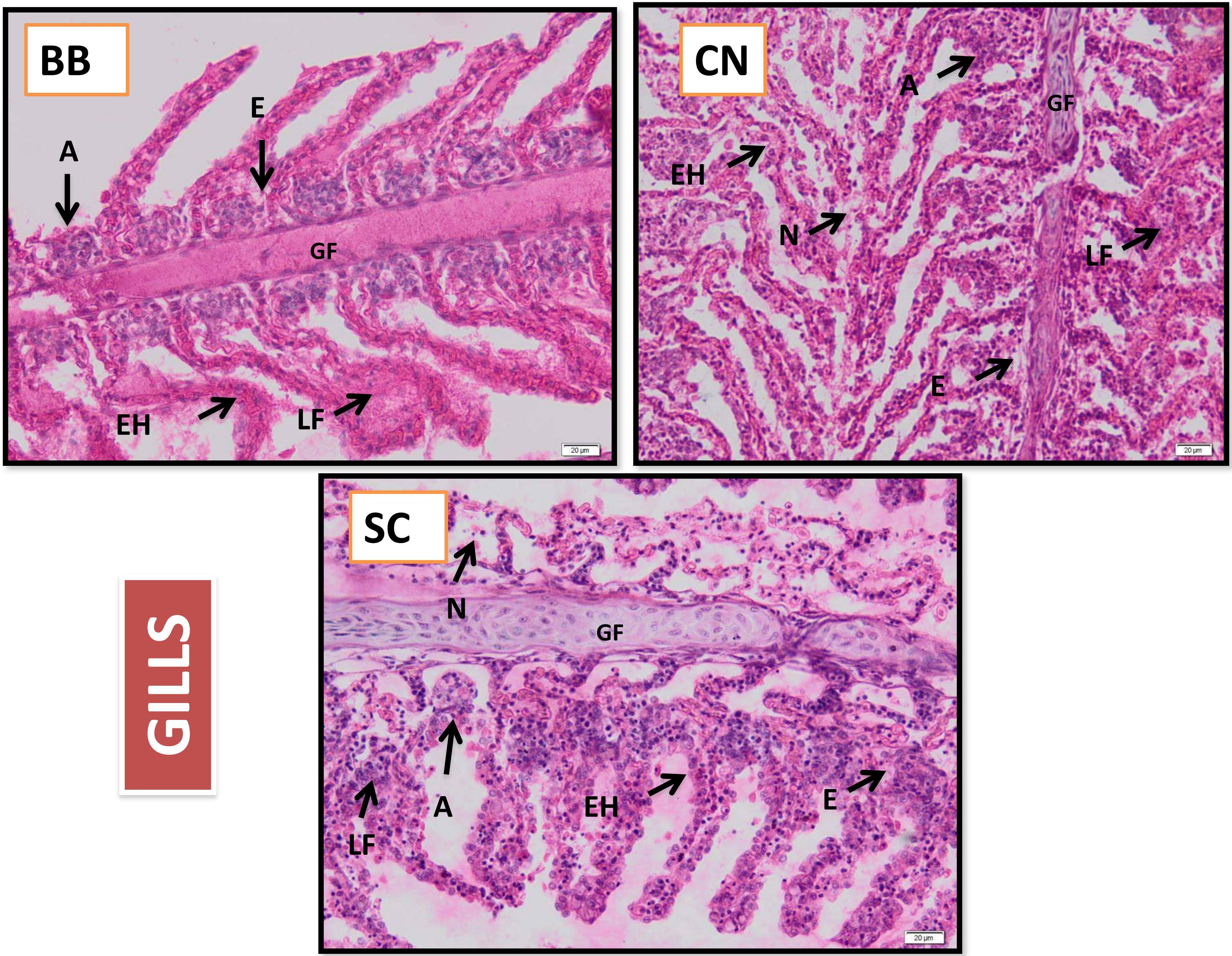
- ✓ The gills are in permanent contact with environmental pollutants and are the major target for metal toxicity in fishes



- ✓ Gills are important organs for the uptake and release of xenobiotics

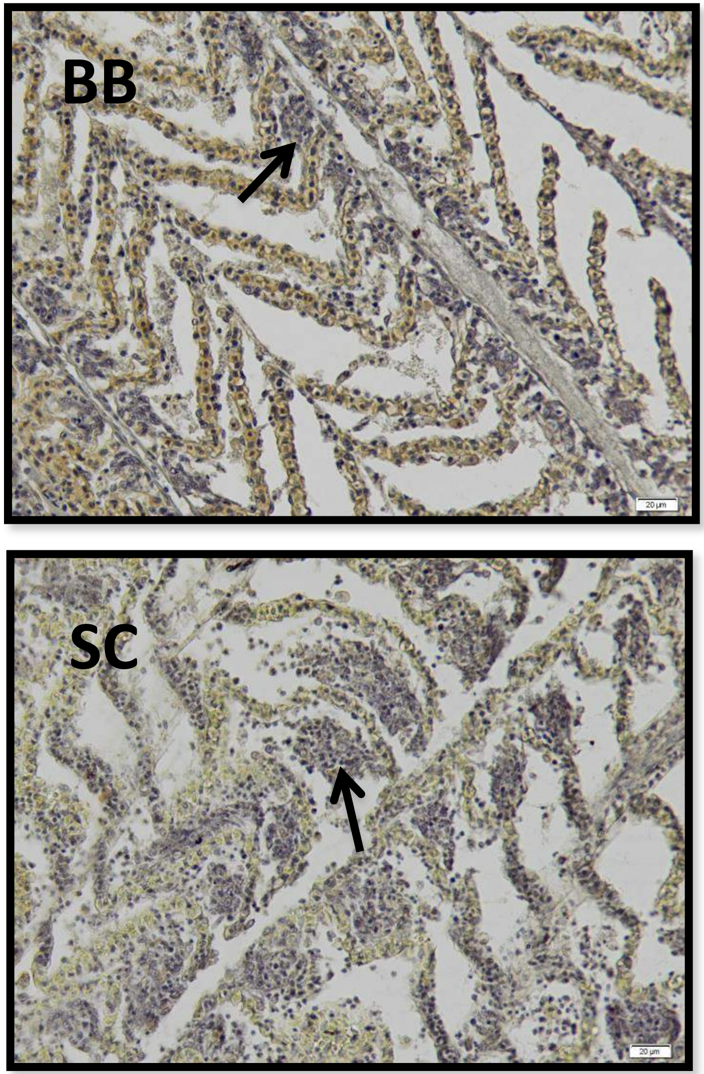
- ✓ Metals exert their toxic effects mainly on the **branchial epithelium** which could affect vital functions due to their active role in respiration, acid-base regulation, osmoregulation and excretion

- ✓ Metals have the ability to induce a redox cycle through the Fenton reaction by catalyzing the degradation of hydrogen peroxide, generating highly reactive hydroxyl radicals that may disrupt the molecular structure of cell membranes

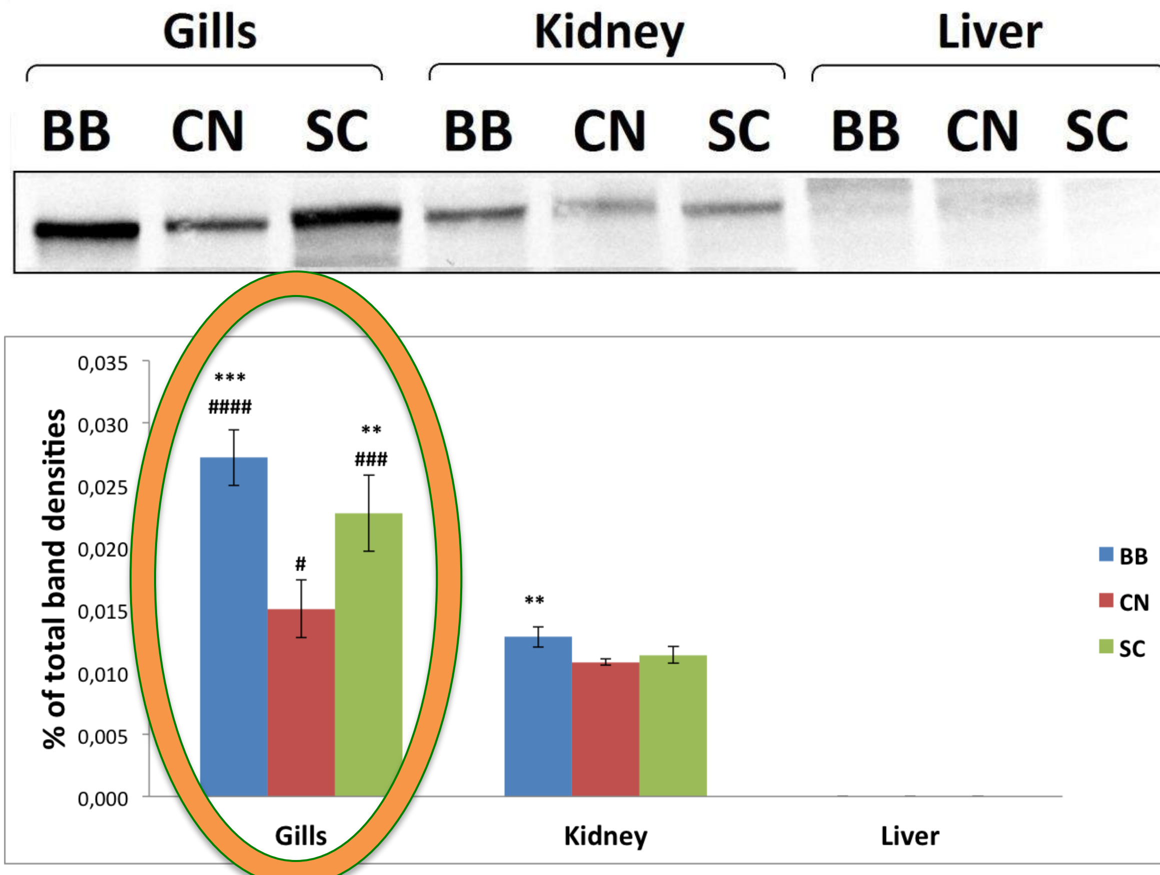


BB
A
E
GF
EH
LF
CN
A
GF
EH
N
LF
E
SC
N
GF
A
EH
E
LF
GILLS

TNF α



BB
CN
SC



Gills
Kidney
Liver
BB CN SC
BB CN SC
BB CN SC
% of total band densities
0,035
0,030
0,025
0,020
0,015
0,010
0,005
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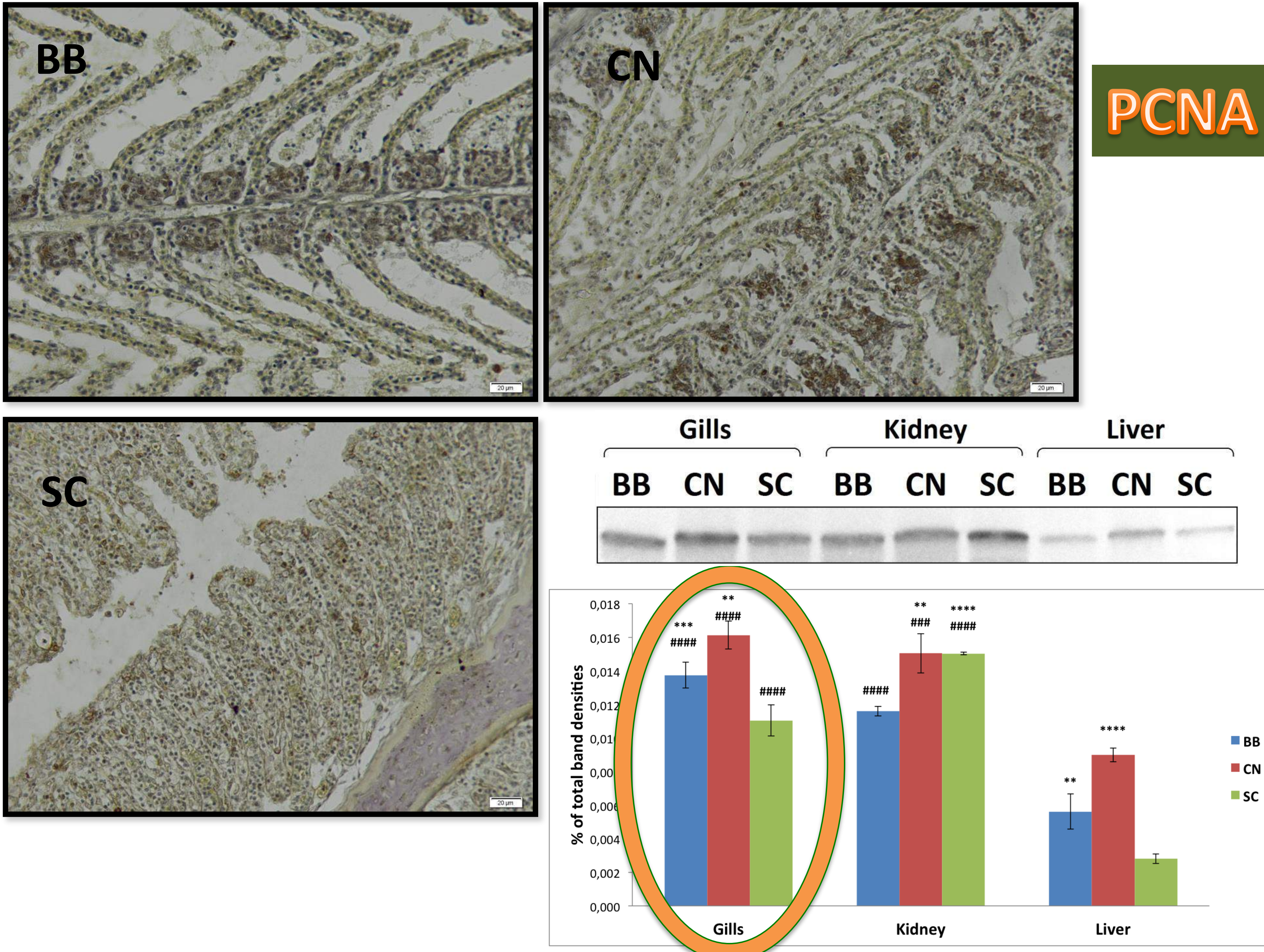
Gills
Kidney
Liver
BB
CN
SC



BB
CN
SC
PCNA
Gills
Kidney
Liver
BB CN SC BB CN SC BB CN SC
% of total band densities
0,018
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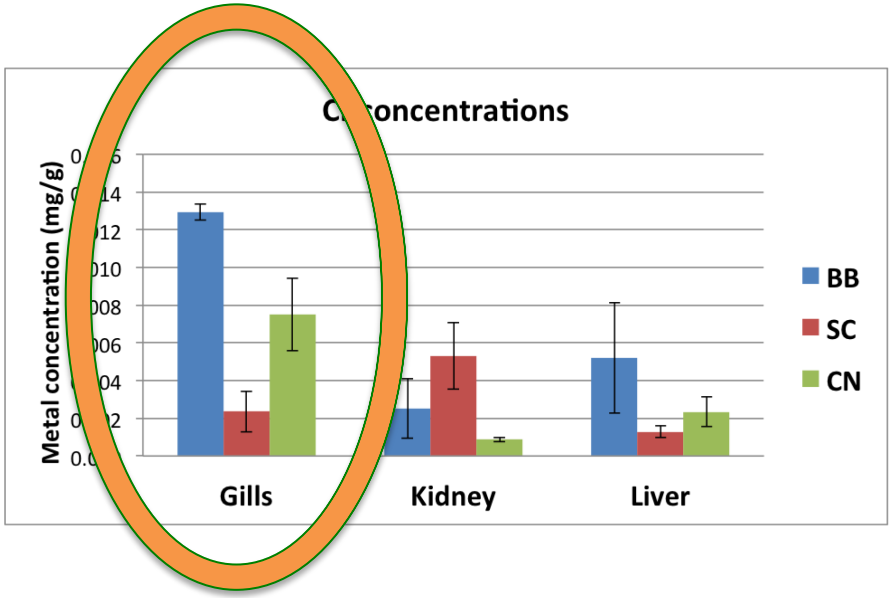
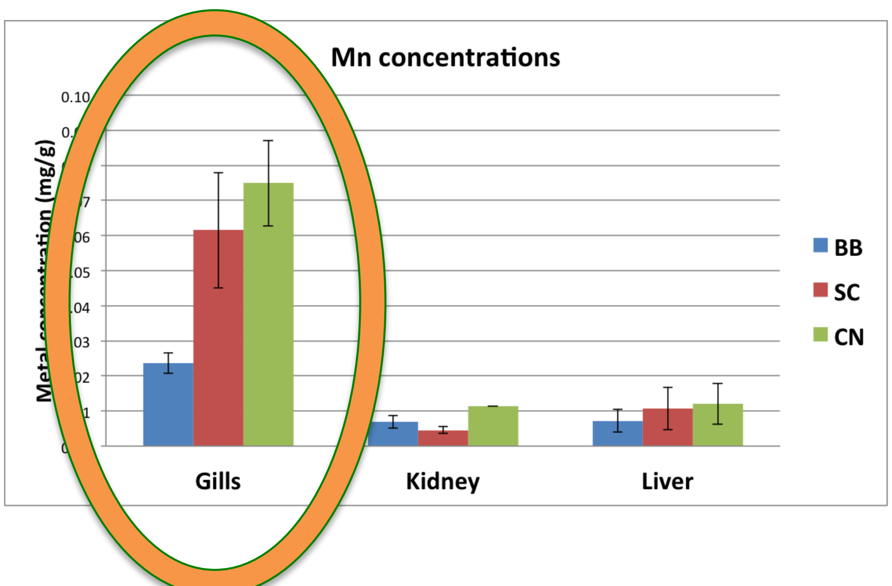
Gills
Kidney
Liver
BB
CN
SC

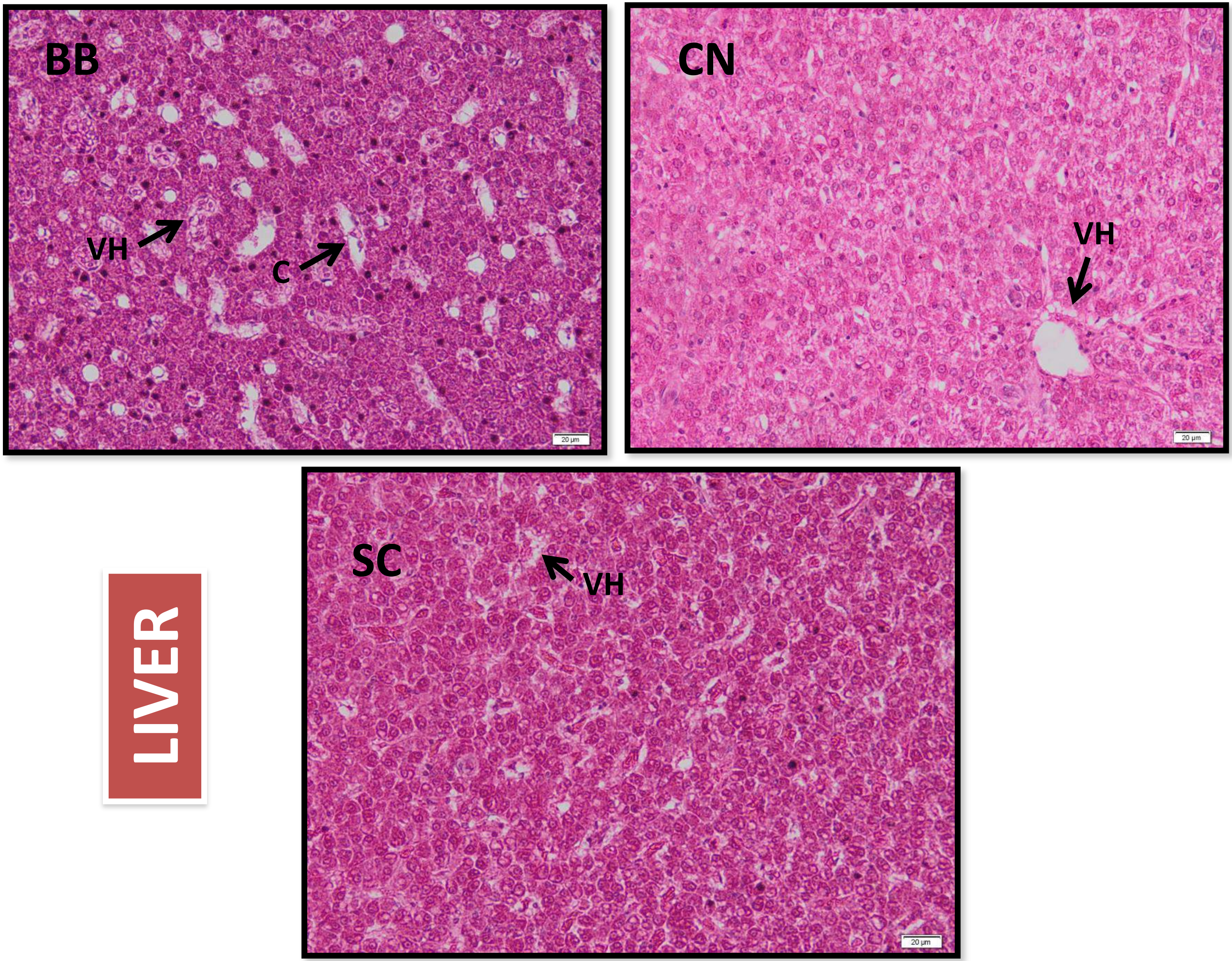
Heavy metal bioaccumulation

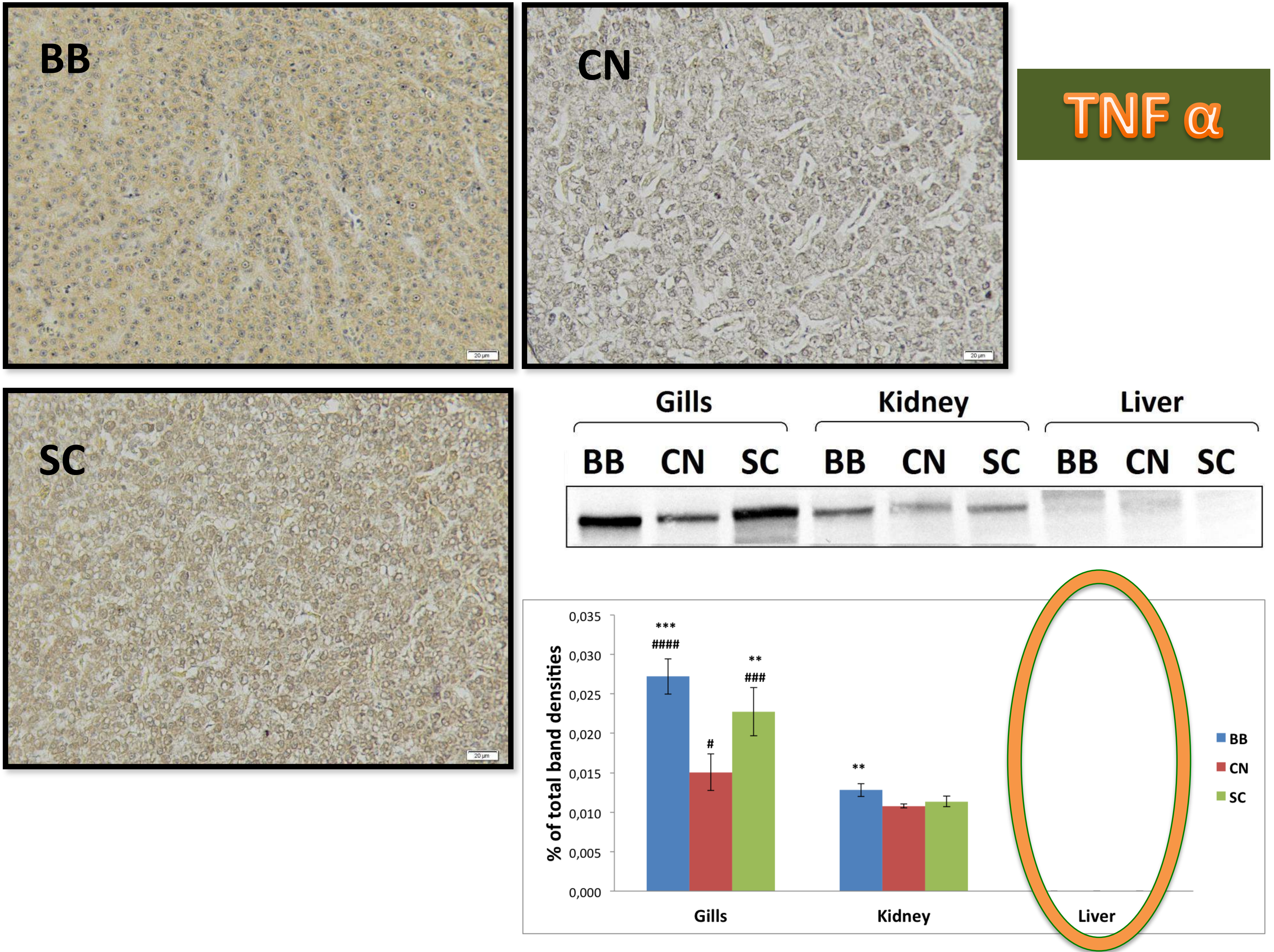


Fish exposure to chromium ions could affect gill morphology (Mishra and Mohanty, 2009) and that might impair respiration and oxygen supplementation to tissues.



Mn is essentially accumulated in organelles like the mitochondria, whereas concentrations in the cytoplasm are relatively low. While such concentrations are maintained by metal transporters in the membrane, its elimination from mitochondria is as low process, which involves active transport (Gavin et al.,1999). Excessive loads of dissolved organic matter lead to greater demand for oxygen, reducing dissolved oxygen levels in water streams (Winemiller etal.,2008).


BB
VH
C
20 µm
CN
VH
20 µm
SC
VH
20 µm
LIVER



TNF α
BB
CN
SC
Gills
Kidney
Liver
BB CN SC BB CN SC BB CN SC
% of total band densities
0,035
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Gills
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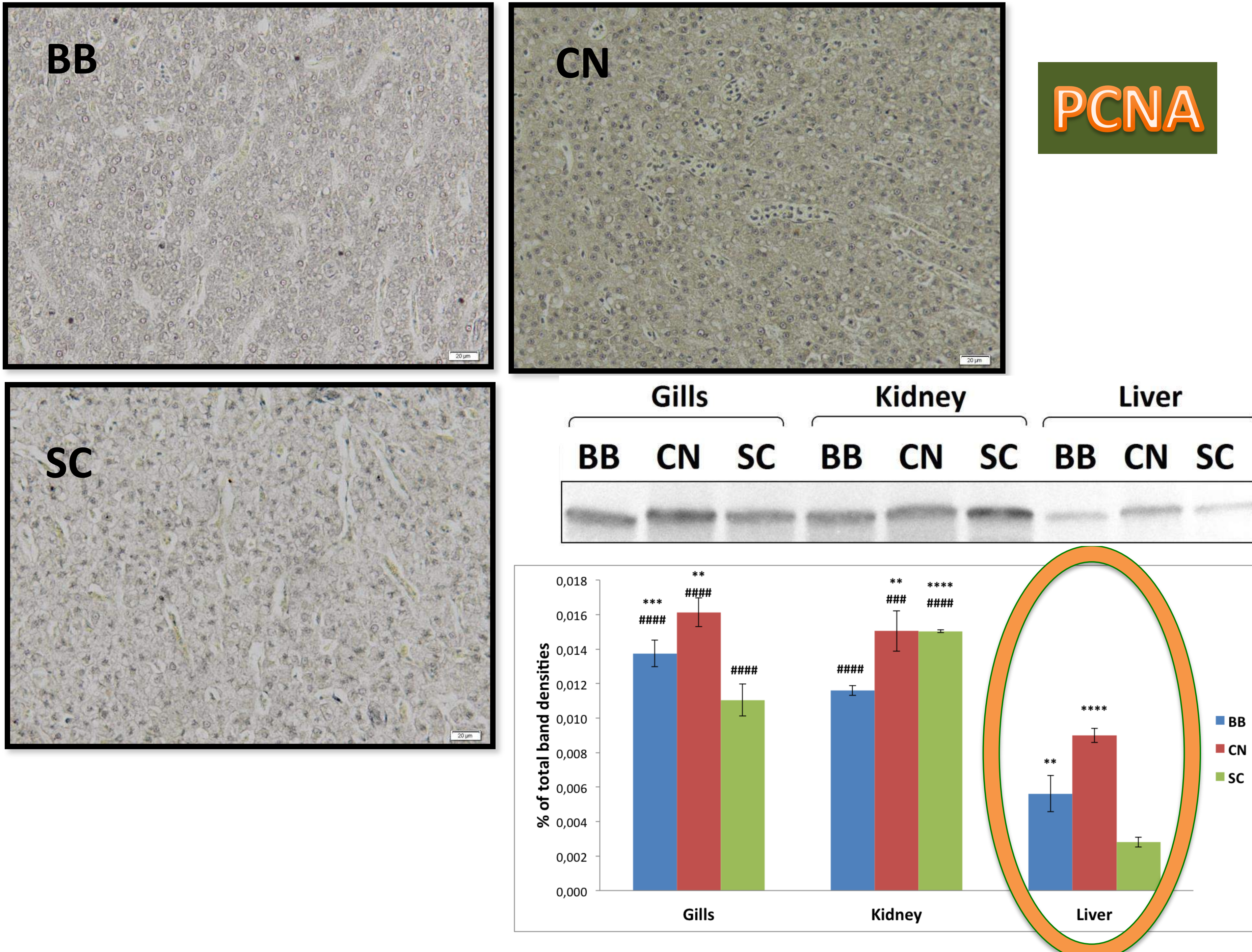
BB
CN
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BB CN SC BB CN SC BB CN SC
% of total band densities
0,018
0,016
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0,012
0,010
0,008
0,006
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Gills
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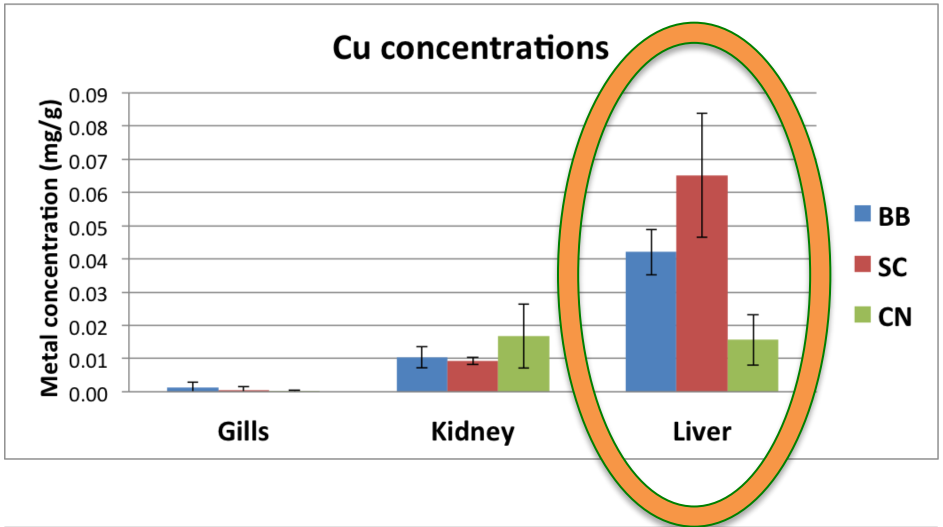
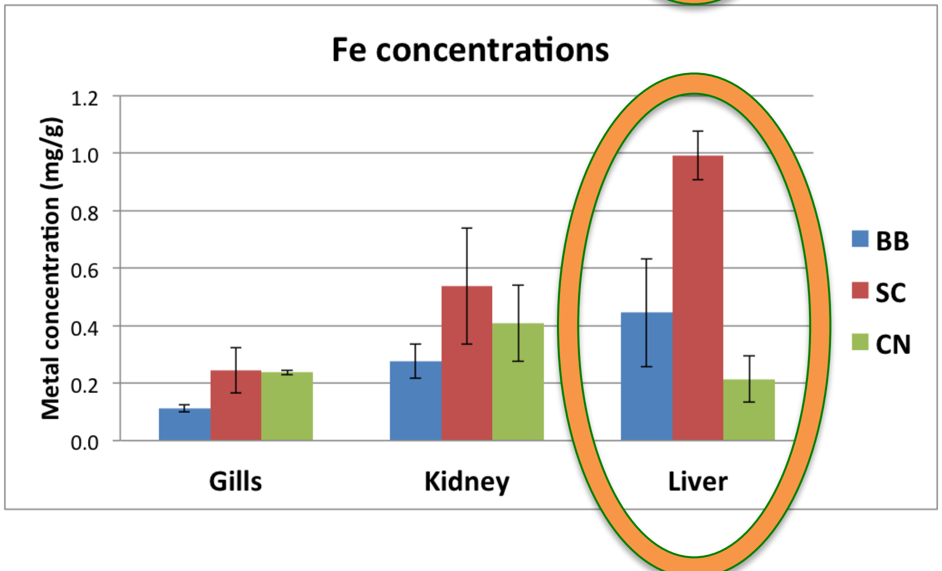
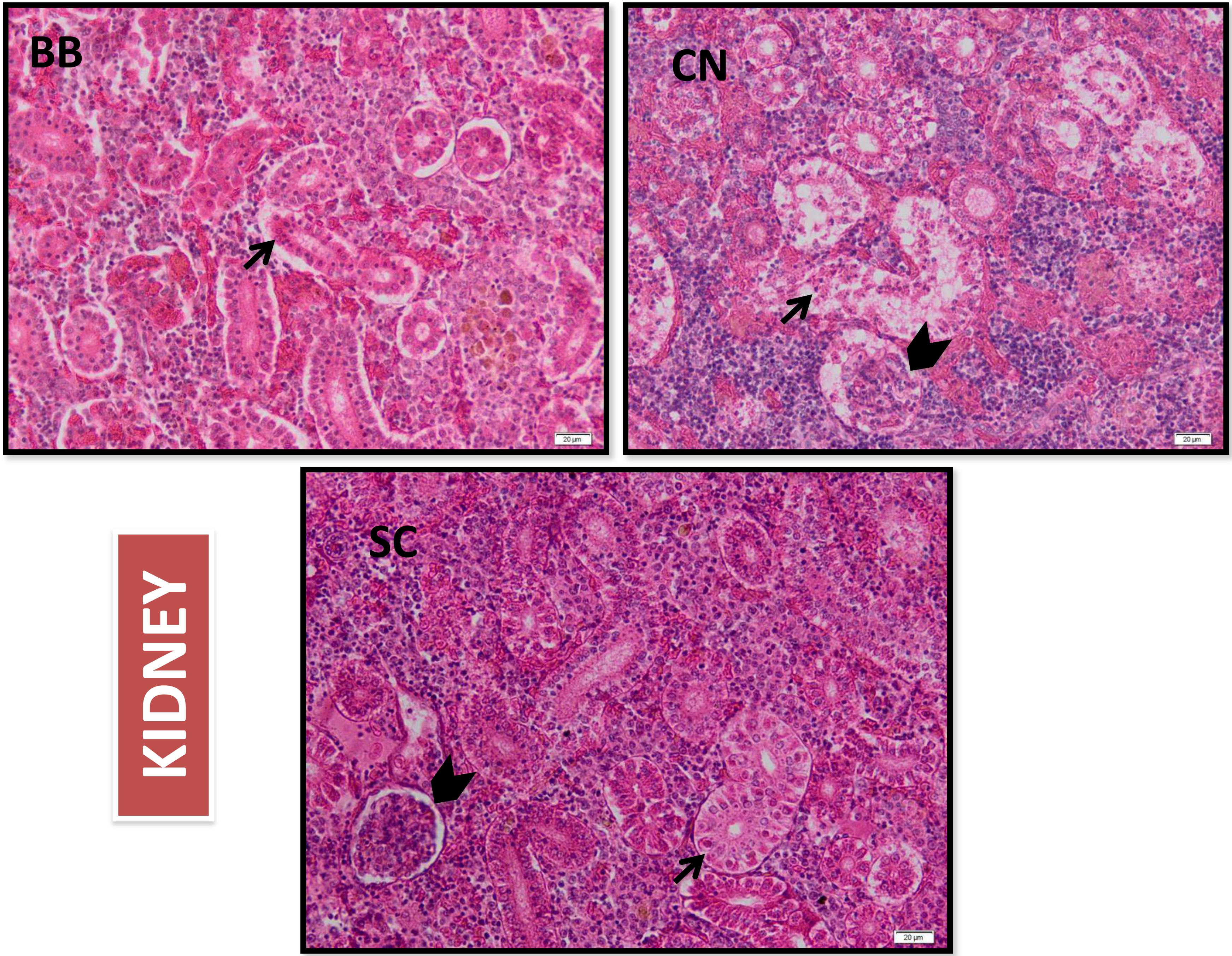
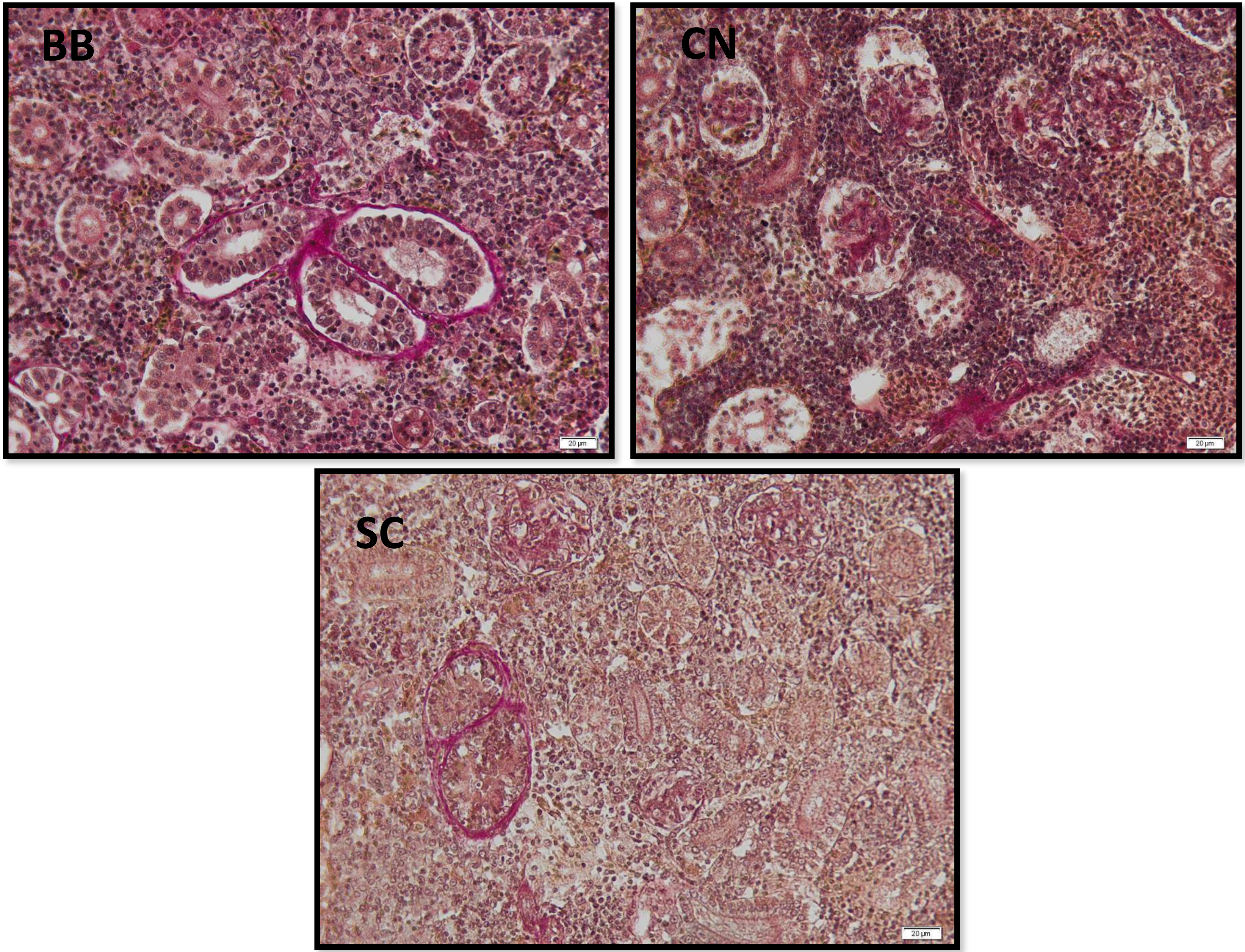
Heavy metal bioaccumulation



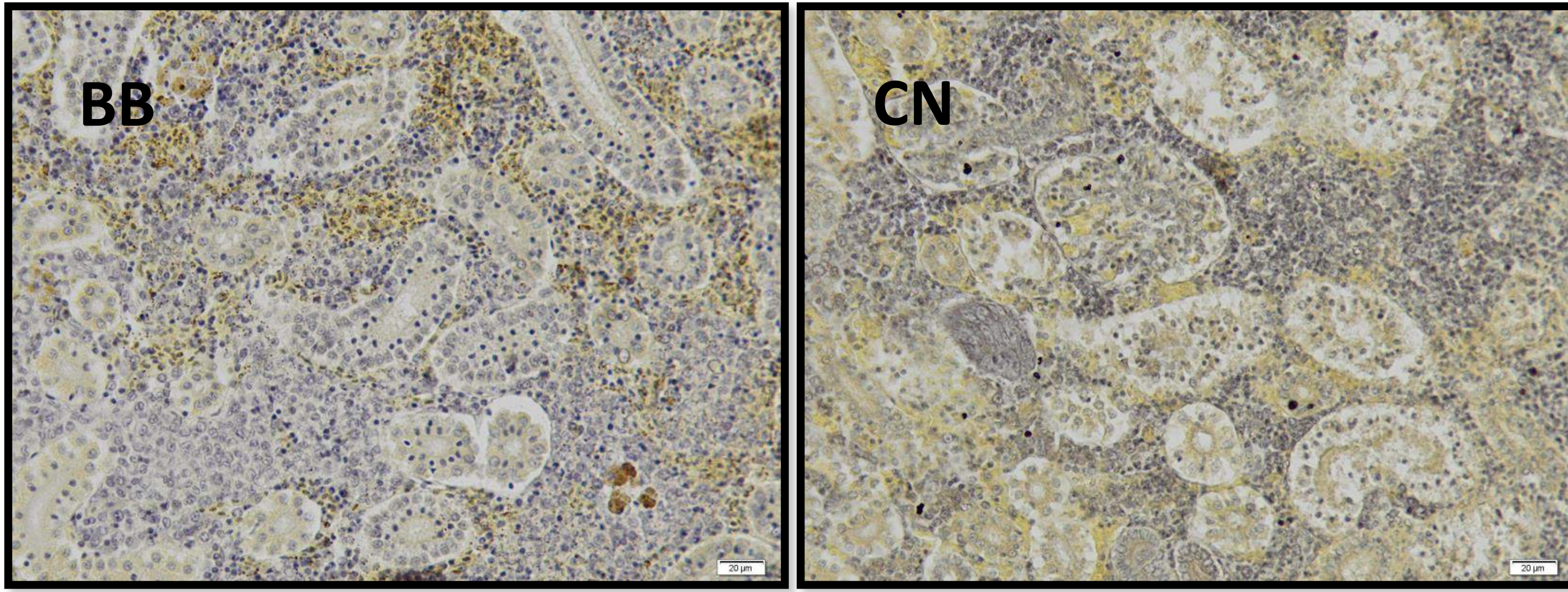


Copper and iron are essentials for fish at low concentrations, but it is toxic at high concentrations (Macomber and Imlay, 2009)

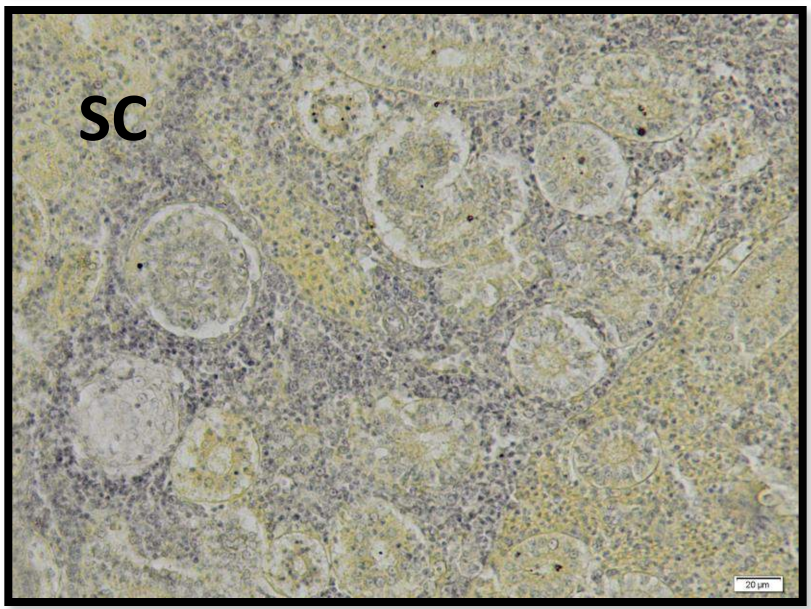

BB
CN
SC
KIDNEY
20 µm
20 µm
20 µm


BB
20 µm
CN
20 µm
SC
20 µm

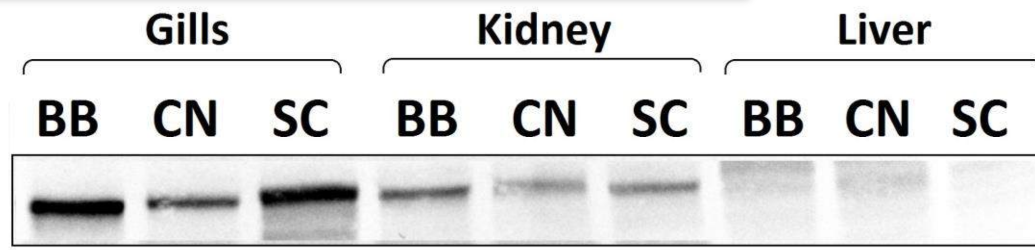
TNF α



BB
CN
20 µm



SC
20 µm



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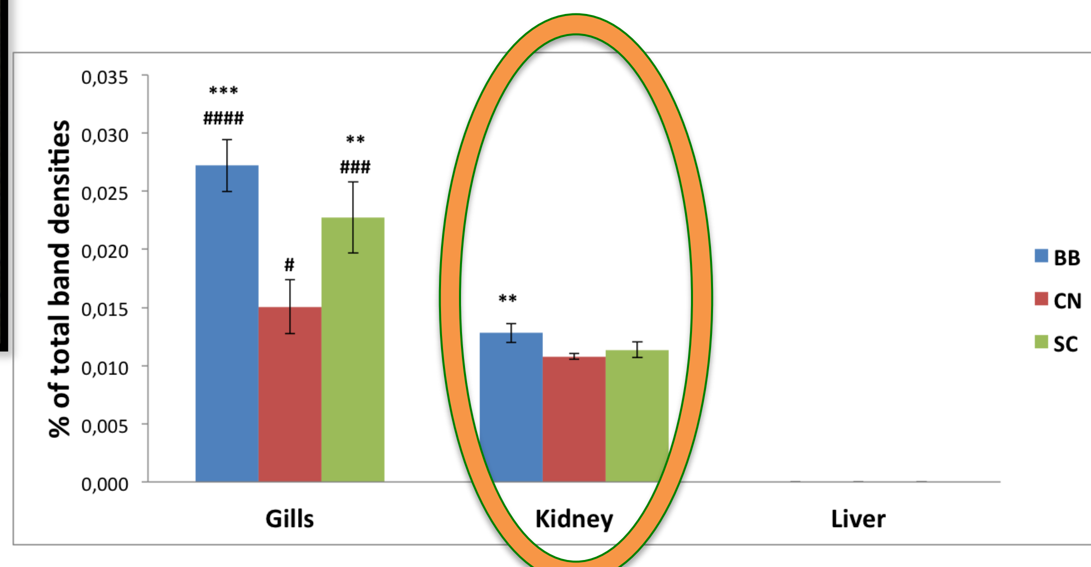
CN
SC
BB
CN
SC



% of total band densities
0,035
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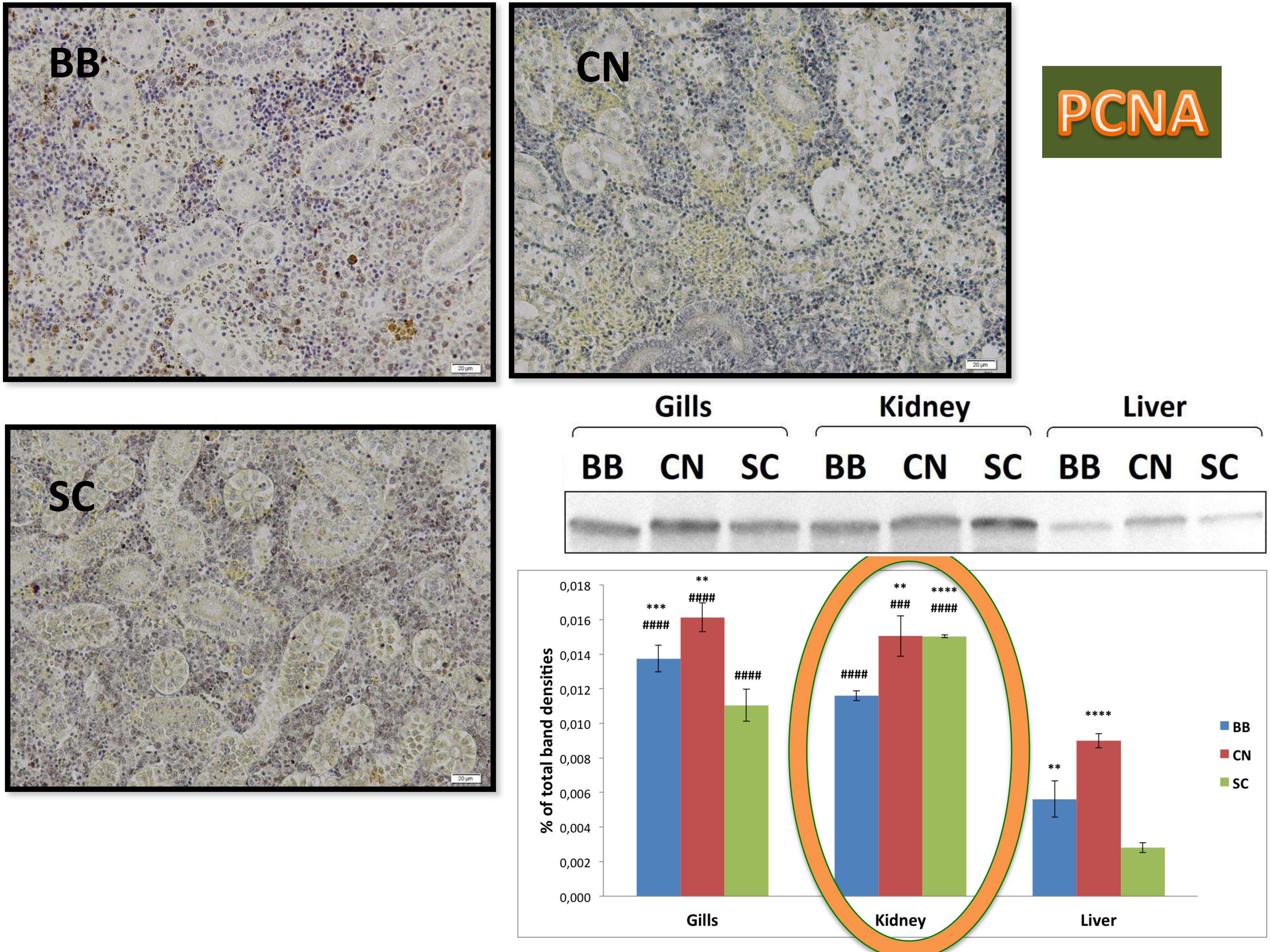
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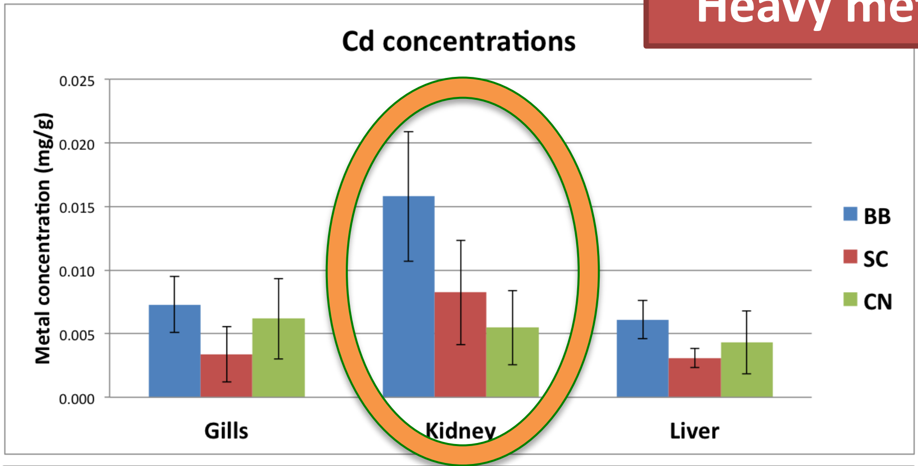
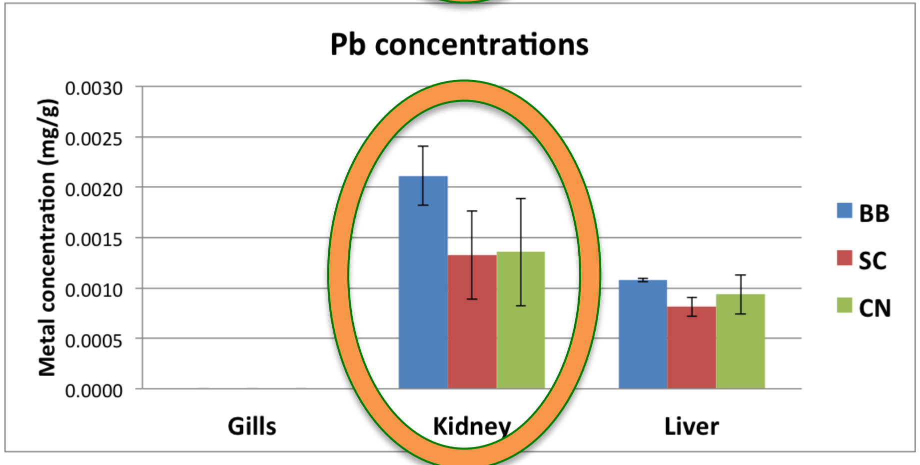
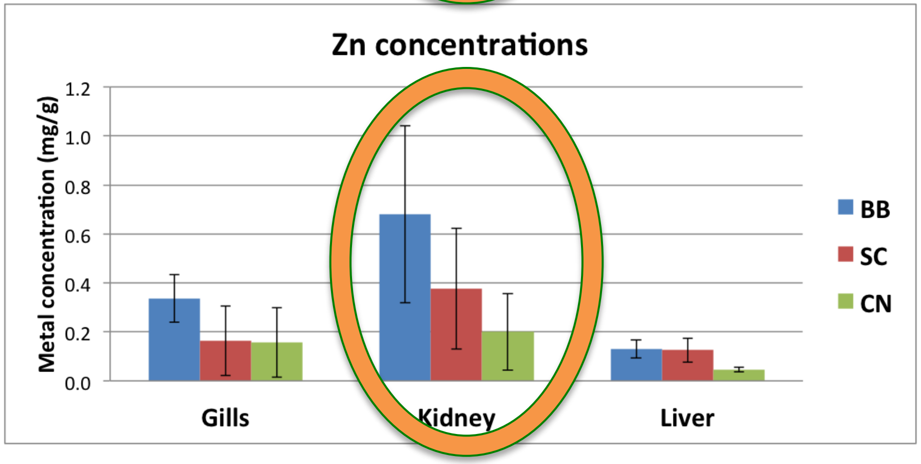
Heavy metal bioaccumulation







Cadmium is first transported to the liver through the blood, where it is bound to metallothioneins (MTs) to form complexes that are transported to the kidneys

These are excreted by the glomeruli and reabsorbed by the tubules, resulting in the release of Cd into epithelial cells (Nordberg, 2009).

Metal accumulates in kidneys, damaging filtering mechanisms and affecting structure depending on the exposure time and dose (Costa et al., 2013).

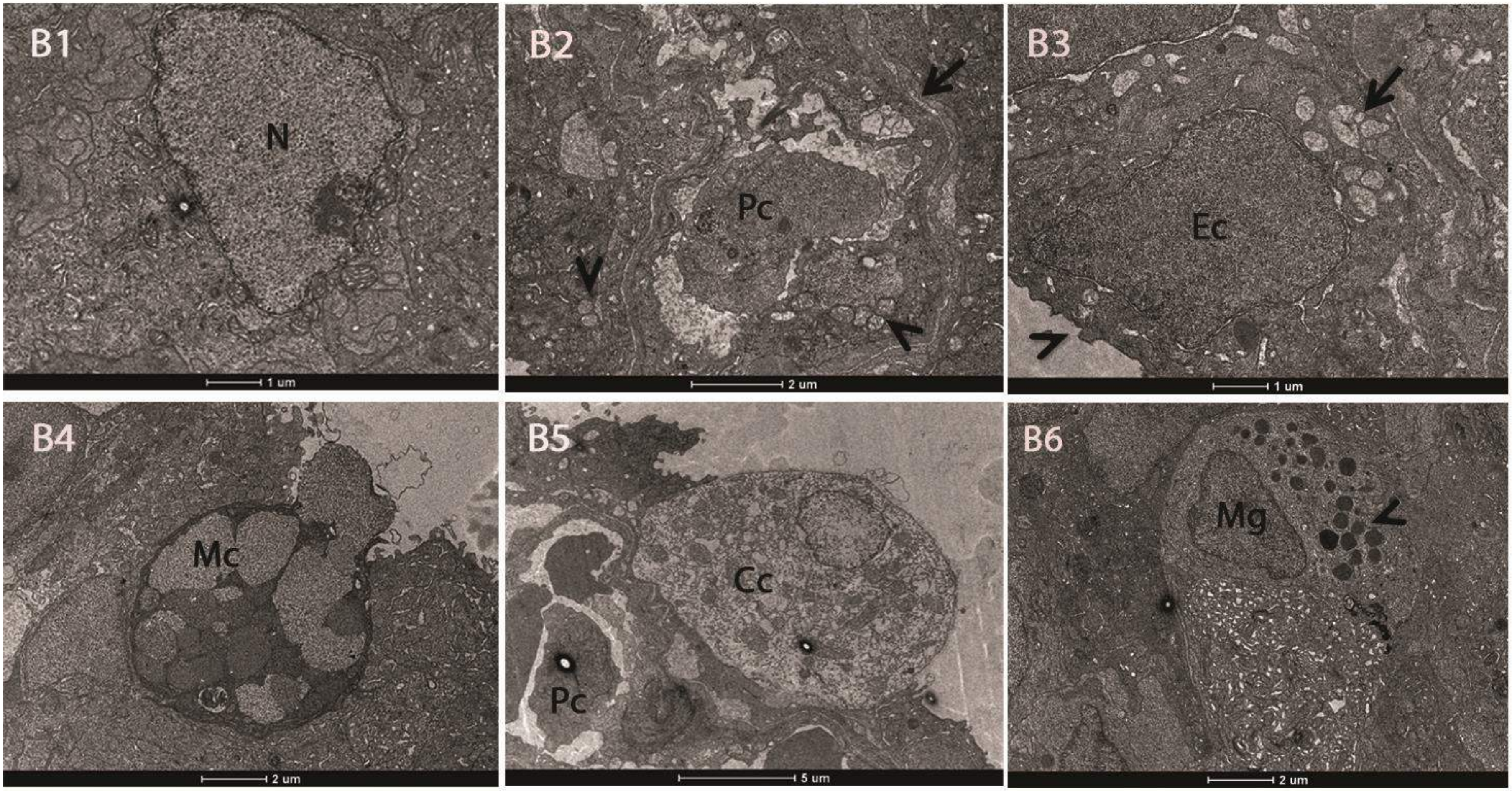
The high metabolic activity in tissues such as kidney and liver may contribute to the higher **lead** accumulation in those tissues (Kim et al, 2015) and lowered rates of filtration causing a loss of function in some nephrons (Patel at al., 2006)

The higher bioaccumulation of **Zinc** in kidney contributes substantially to injury, probably due to distal tubules deposition (Woodling et al., 2002).

The increase of zinc resorption in tubules can cause changes related to osmotic nephrosis and convoluted proximal tubules become hypertrophic and hyperplastic (Besirovic et al., 2010).

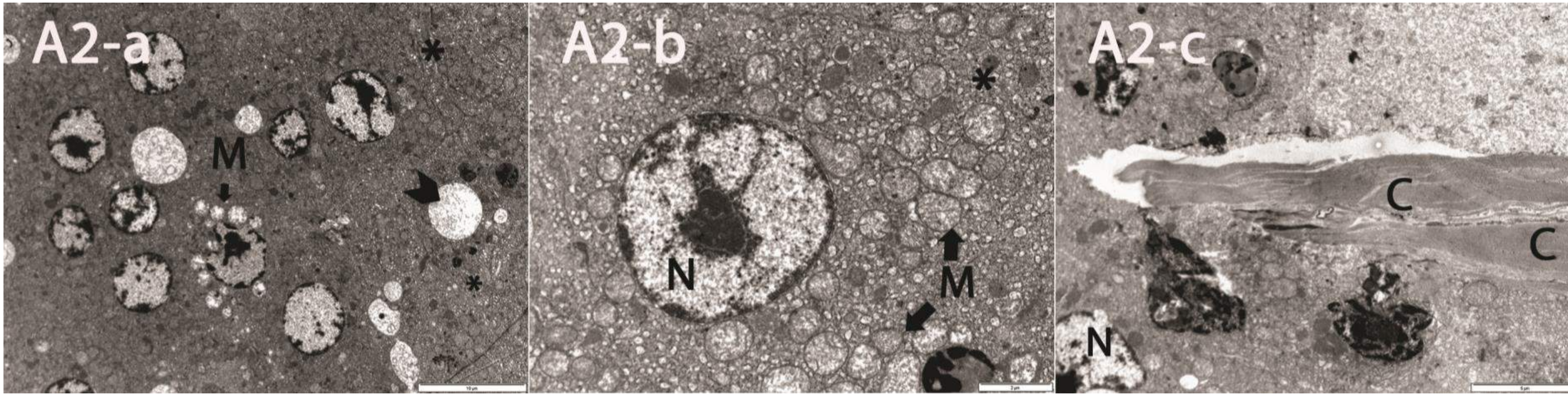
GILLS

Chondrostoma nazus (CN)

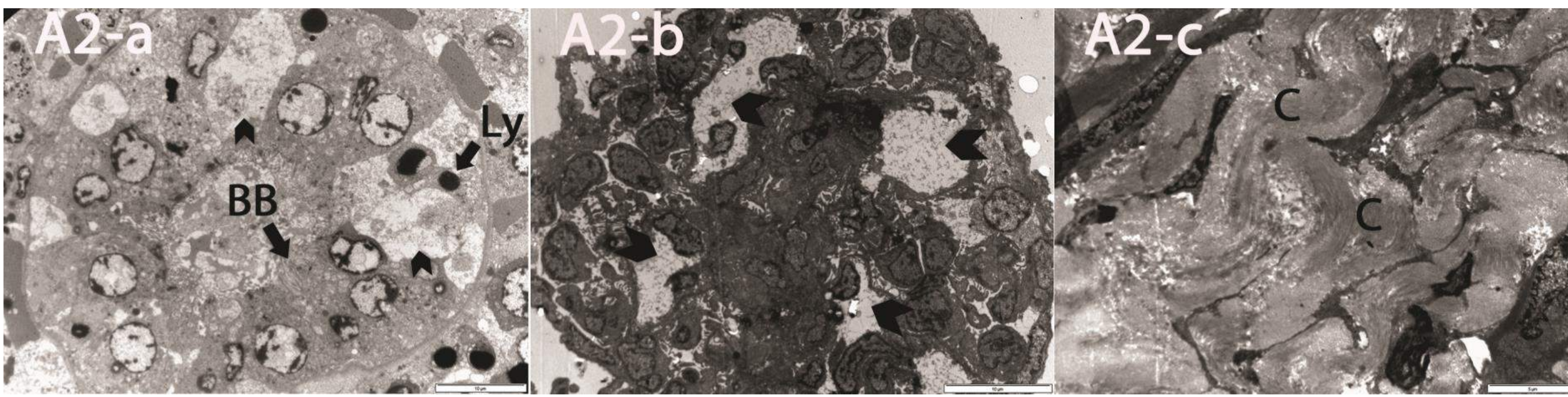


Chondrostoma nazus (CN)

LIVER



KIDNEY



CONCLUSIONS

1. Metals were differently distributed in the tissues:
   - Cr, Mn - gills > kidney≥liver
   - Cu, Fe – liver>kidney>gills
   - Cd – kidney>liver≥gills
   - Pb – kidney>liver
   - Zn – kidney>liver>gills

2. Evaluation of structural damage/organs: gills≥kidney>liver

   ! Antioxidant activity of liver > kidney (Hermenean et. al, 2015)

3. Evaluation of structural damage/species:

- inflammation – *Barbus barbus (BB)* and *Squalius cephalus (SC)* > *Chondrostoma nazus (CN)*
-necrosis - *Chondrostoma nazus (CN)* > *Barbus barbus (BB)* and *Squalius cephalus (SC)*

4. Immunopositivity for PCNA in gill, kidney and liver/species :

   Chondrostoma nazus (CN) > *Barbus barbus (BB)* and *Squalius cephalus (SC)*

   ! Levels of specific repair pathways were proportional to the level of the alterations

5. Electron microscopy analysis of gill, kidney and liver *Chondrostoma nazus (CN)* confirmed structural injuries

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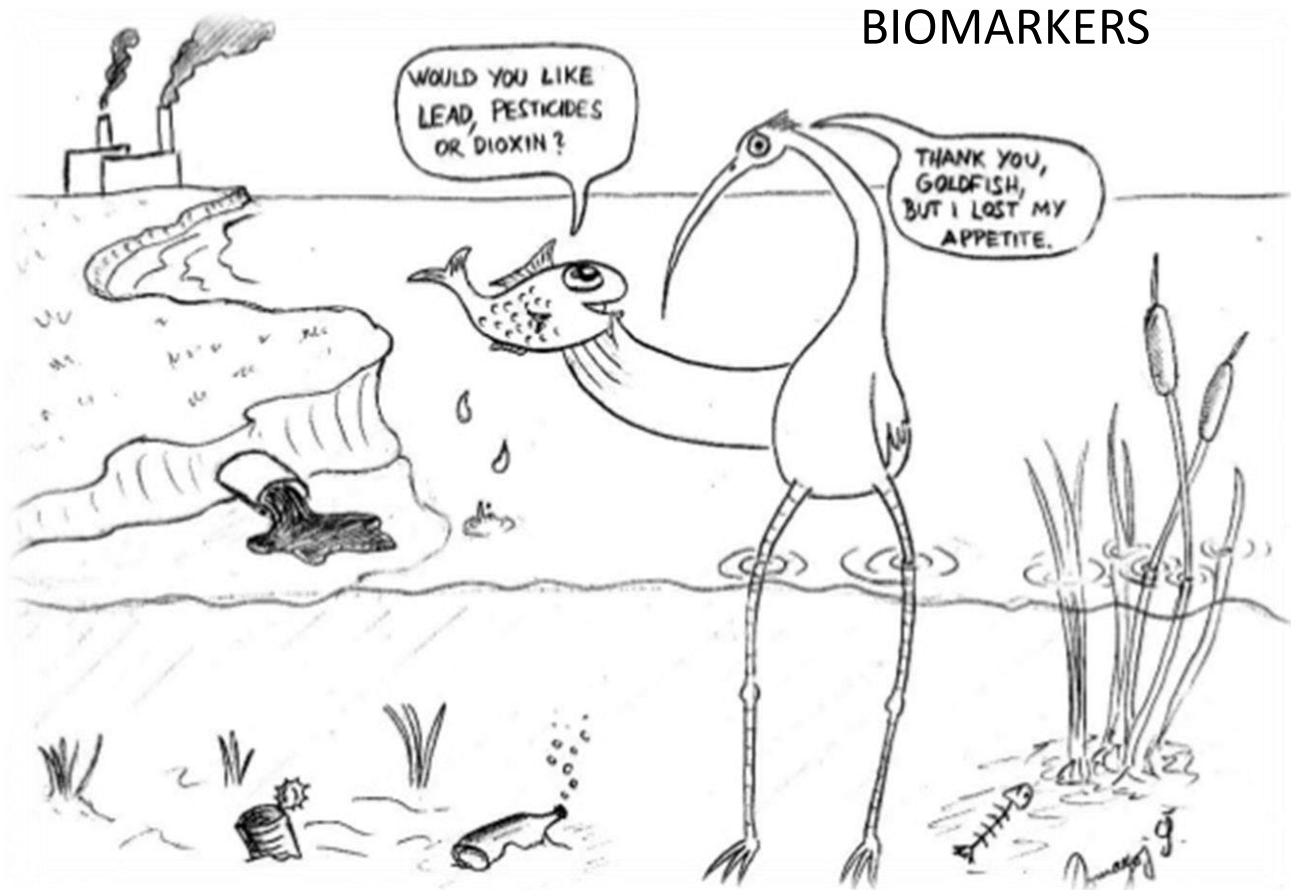
BIOMARKERS



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