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DISSERTATION

Cadmium, copper, zinc and iron accumulation in aquatic food chains: Initial survey of trout and carp ponds in Austria

Verfasserin

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Samar Kamel

To be dedicated;

with love & passion

to my dear husband,

my son,

my parents,

my parents in law

& family Mack

Abbreviations

AAS	Atomic spectrophotometer
(A)	Austrian fish
BAF	Bioaccumulation Factor
B+N	Benthos and nekton
Carp1	Carp Site1
Carp2	Carp Site2
Cd	Cadmium
Chl.-a	Chlorophyll a
Cu	Copper
Det	Detritivores
DIC	Dissolved inorganic carbon
DOC	Dissolved organic carbon
DW	Dry weight
ET-AAS	Electrothermic AAS
F.W	Freshwater
Fe	Iron
GF-ASS	Graphite furnace
Graz	Grazer
Gut	Gut contents
Imp	Imported samples
LOD	Limit of Detection
MAX	Maximum
MED	Median
MF	Muscle flesh
MIN	Minimum
MV	Mean value
PE	Polyethylene
Pheo	Pheophytine
POM	Particular organic matter
Ppb	Parts per billion (µg/kg)
Ppm	Parts per million (mg/kg)
Pred	Predator
SD	Standard deviation
Shred	Shredder
SM	Suspended matter
S.W	Salt water
Temp	Temperature
TIC	Total inorganic carbon
Trout1	Trout Site1
Trout2	Trout Site2
TOC	Total organic carbon
WF	Wild fish
WW	Wet weight
Zn	Zinc
Zoo	Zooplankton

Contents

ZUSAMMENFASSUNG

Daten zu Cadmium- Kupfer- Zink- und Eisengehalt in Karpfen und Forellen aus österreichischen Zuchtbetrieben waren bisher nicht verfügbar. Von März 2007 bis Oktober 2008 wurden insgesamt 608 Proben genommen. Vier Fischfarmen wurden mindestens zweimal besucht. Bei jeder Probenentnahme wurden sowohl abiotische Wasserparameter verzeichnet als auch Wasserproben entnommen sowie Fische unterschiedlichen Alters. Weiters wurden Sedimentproben, Wasserpflanzen, Macroinvertebraten und Zooplankton soweit möglich beprobt. Als Eränzung wurden Fische aus dem Maktverkauf sowie Wildfänge dem Untersuchungsmaterial zugefügt. Alle Proben wurden mittels Absorptionsphotospectrometrie untersucht. Der Cadmiumanteil wurde mittels GF-ASS (Graphite furnace atomic absorption spectrometer) analysiert, die Elemente Kupfer, Zink und Eisen mittels ET-ASS (Flame atomic absorption spectrometry). Die Karpfenstandorte zeigten grundsätzlich höhere Primärproduktion (bezogen auf den Chlrorphyll a – Wert), Trübe und Zooplanktondichte als Forellenstandorte. Cadmiumkonzentration in Muskelgewebe von Forellen und Karpfen unterschieden sich nur unwesentlich mit Ausnahme von Forellenstandort zwei, deren durchschnittliche Konzentration dreimal höhere Werte aufwies. Im Vergleich einzelner Organe zeigten Darm und Leber grundsätzlich höhere Werte als andere Organe. Ein Antagonismus von Cadmium und Eisen konnte für Kiemen und Hirn der Zuchtforellen festgestellt werden. Die wesentlichen Faktoren für Aufnahme und Akkumulation von Cadmium setzen sich aus Spezies, Standort, Gewicht, Alter, Länge, Gewicht und Jahreszeit zusammen. Bei beiden Forellenzuchtbetrieben konnte eine zunehmende Cadmiumkonzentration in Muskel mit zunehmendem Alter festgestellt werden. Bei allen untersuchten Proben aus österreichischen Fischfarmen lagen die gefundenen Werte für Cadmium unter dem zulässigen Grenzwert (EC European community regulation No.104/2000 and 2001/22/EC). Anders verhielt es sich bei Betrachtung der Marktproben – Eine Schwertfischprobe lag mit 59,4µg/kg deutlich über dem erlaubten Grenzwert von 50µg/kg. Die Konzentrationen für Kupfer und Eisen lagen sowohl für Forellen als auch Karpfen unter den zulässigen Grenzwerten im Muskelgewebe. Auch die Werte für Zink bewegten sich innerhalb zulässiger Parameter (40mg/kg), sowohl in Forelle als auch im Karpfen ließen sich bei Jungfischen weit höhere Konzentrationen feststellen als bei Adulttieren. Generell läßt sich feststellen, dass Wildtiere für alle vier untersuchten Schwermetalle höhere Konzentrationen aufwiesen als Tiere aus Zuchtbetrieben. Die erlaubten Grenzwerte in Muskelgewebe wurden allerdings in keinem Fall überschritten. Es wurden keine Hinweise für Bioakkumulation innherhalb der Nahrungskette gefunden. Die Tiere in den Forellenzuchtbetrieben wiesen durch reine Pelletfütterung eine kürzere Nahrungskette als die Tiere in den Karpfenzuchtbetrieben auf, die auch auf Zooplankton und Macroinvertebraten angewiesen waren. Grundsätzlich zeigte das in allen Zuchtbetrieben verwendete Pelletfutter mit 488 bzw 218 µg/kg hohe Cadmiumkonzentrationen. Erhöhte Cadmiumwerte wurden auch in Teilen der Benthos- und Nektonproben gefunden. Weitere Untersuchungen zur Thematik Cadmium in Fischfutter und Einflussnahme auf die Nahrungskette von Zuchtfischen scheint angebracht. Abschließend läßt sich sagen, dass nach den derzeit gültigen Umweltqualitätsnormen sich alle vier untersuchten Schwermetalle (Cu, Zn, Cd, Fe) in Muskelfleisch von von Zucht- und Wildfischen innerhalb zulässiger Konzentrationen befinden.

Aim of the work

This study was designed to evaluate whether cadmium, copper, zinc and iron concentrations in fish exceeded quality standard. The main focus is on fish from fish farms in Austria and their food chain. Other samples include fish from natural ecosystems and fish from Austrian markets.

ABSTRACT

No data on the cadmium, copper, zinc and iron contents of cultured trout and carp or of wild and market fish in Austria are available. A total of 608 samples were collected between March 2007 and October 2008. Four fish farms were visited at least twice. At carp sampling site 2, samples were collected eighteen times in the period between April and November 2007. At each sampling date abiotic water parameters were recorded. Water samples, fish, sediment, hydrophytes, macroinvertebrates and zooplankton were collected. Other sampling included market fish and wild fish. Cadmium analysis was carried out by GF-ASS (Graphite furnace atomic absorption spectrometer), whereas copper, zinc and iron were analysed by ET-ASS (Flame atomic absorption spectrometry). Carp sampling sites showed higher primary production (represented by chlorophyll-a), turbidity and zooplankton density than trout sampling sites. Cadmium levels in muscle of cultured trout and carp were similar, with the exception of one site where concentrations were three-fold higher in trout. The gut and liver had higher cadmium contents than other organs. Antagonism between iron/cadmium occurred in farmed trout gills and brains. The major factors modifying cadmium accumulation in the fish were sampling site, species, age, weight, length and season. Cadmium in both trout sites accumulated in the muscle with growth. Nonetheless, the concentrations in cultured trout and carp were below the permissible level 50 µg/kg (EC European community regulation No.104/2000 and 2001/22/EC). One swordfish muscle sample exceeded the regulatory guideline (59.4 µg/kg). Copper and iron concentrations in cultured trout and carp were non-detected in muscle. Zinc in muscle was within permissible levels (40 mg/kg). Zinc in cultured young trout and carp muscle showed significantly higher concentrations than in older fish. The organs of wild fish generally showed higher levels of the four metals than those of cultured fish. No evidence was found for cadmium, copper, zinc and iron bioaccumulation. The farmed trout food chain was shorter than the farmed carp food chain because the former fed on commercial pellets, the latter also on zooplankton and macroinvertebrates. The food supplements (pellets) fed to both trout and carp showed high cadmium concentrations ((488 and 218 µg/kg, respectively); some components of the benthos and nekton also showed elevated cadmium levels. Cadmium, copper, zinc and iron levels in the muscle of cultured and wild fish in Austria did not exceed the environmental quality standard.

1. Introduction

The Republic of Austria, the most mountainous state in Europe after Switzerland, lies in central Europe and surrounded by seven other countries. Dominated by the Eastern Alps and the Danube basin, this forested country is threaded by swift mountain streams and has a large complement of Alpine lakes. Good care of forests and water has a long tradition in Austria, but effluents from ever-increasing industry and more intensive agricultural practices have created severe pollution problems in some areas. Recently, there has been a moderate growth in aquaculture, especially of trout, but a decrease in commercial fishing – now almost completely confined to lakes. Sport fishing for cold-water species continues to be the dominant aspect of Austria's inland fisheries (Austria/EIFAC 1980). Austria has the most abundant water resources in Europe and has been called the “water castle of Europe”. All usable water resources combined are estimated to amount to appr. 84 bn m³ a year, about one third of which is groundwater. Total annual water demand in Austria is appr. 2.6 bn m³ and thus corresponds to only about 3 % of available water resources. 99 % of the population is supplied with spring water and groundwater, a situation which is unparalleled worldwide. All Austrian lakes have a water quality that makes them suitable for bathing. Eighty seven percent of flowing waters feature quality category II levels (2001) or even better; in 1998, only 81 % had reached this quality level (Lebensministerium VII/1, 2005)

1.1. Fish nutrition

Fish is an excellent source of protein and omega-3 fatty acids known as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). Diets rich in fish oil may help reduce inflammation and reduce the risk of cardiovascular disease. The omega-3 fatty acids found in fish are also essential for brain and eye development. The American Heart Association suggests consuming oily fish a minimum of twice a week to maintain a healthy heart. DHA supplementation may be the most beneficial for babies. The developing brain accumulates large amounts of DHA during the third trimester of pregnancy through the first three months of infancy. Women can take DHA supplements during their pregnancy and in the initial months of breastfeeding to ensure that their babies receive sufficient DHA for normal cognitive development (Cetin et al. 2008). DHA also has an important effect in disease prevention and health promotion. Finally, certain trace elements may have therapeutic effects in preventing particular diseases (Alasalvar et al. 2002, Celik et al. 2008, Tuzen & Soylak 2007).

The highly unsaturated fatty acids found in salmon, carp and other oily fish have also been associated with a wide variety of other health benefits. The consumption of fish oils has been reported to relieve the symptoms of manic depression, protect against relapse of Crohn's disease, decrease the severity of strokes, and help prevent cancer. Increasing the concentrations of HDL (the “useful” cholesterol) in salmon helps lower the level of LDL (the “harmful” cholesterol), triglycerides and overall cholesterol (Barter et al. 2007) While eating fish would help prevent heart disease, the mercury, pesticides, PCBs and DDT in the environment significantly impact human health and are present in nearly all fish. The heavy metal mercury is a case in point. It has contaminated by water bodies, and some fish species are prone to accumulating mercury. The amount of mercury in fish varies according to the mercury content of the water. Generally, mercury contents ratio is higher in predatory, long-lived fish at the top of the aquatic food chain because they consume other mercury-contaminated fish (Burger et al. 2003).

Besides tuna, species most prone to mercury contamination include cod, halibut, mackerel, mahi-mahi, marlin, pike, sea bass, shark, swordfish and tilefish – sometimes referred to by names such as golden bass or golden snapper. As all fish are potentially contaminated, the risks of contamination seem to outweigh the health benefits for humans (FDA 2005).

One often-cited strategy to avoid this risk is aqua- or mari-culture. Unfortunately, farm-raised fish do not represent a clear-cut solution – they often still contain mercury and are high in less beneficial omega-6. Such fish are fed grain products, especially corn, so the beneficial omega-3 fatty acids (EPA and DHA) are distorted. Thus, the contaminant levels in farmed salmon from certain regions of the world increased the risk of cancer enough to outweigh the heart health benefits of salmon. The toxicant levels were so high in some farmed salmon from Europe a recommendation was given to eat only a single serving once every five months (Foran et al. 2005).

1.2. Fish consumption in Austria

The average per-capita consumption of fish in Austria is about 6.3 kg/year. This is equivalent to about three fish a month. Nutritionists recommend eating fish twice a week.

Table 1. Fish consumption in Austria

Balance sheet item	2004	2005	2006	2007	2008	2009	Change on pervious Year (%) (2008-2009)
Production in ton	2900	3100	3200	3300	2900	3000	3.4
Imported	55449	62113	61724	68509	64908	62956	-3.0
exported	968	1449	2003	3074	2725	3253	19.4
Human consumption	57381	63764	62922	68734	65083	62702	-3.7
Per capita in Kg	7	7.7	7.6	8.3	7.8	7.5	-4.0
Self-sufficiency %	5	5	5	5	4	5	

Source: Statistic Austria 2010

In Austria, carp are raised in around 190 ponds (approx. 2.000 ha of pond surface) and (approx. 232 ha) facilities raise trout. In total, around 3.300 tons of fish are produced per year, including 2.400 tons of edible fish and about 900 tons of eel fish. The main contributors are trout (approx. 2.000 tons) and carp (770 tons). In recent years there has been a stronger ecological orientation of fisheries and of their legal basis, with the prime goal being to maintaining the natural diversity of species and the genetic variability of the fish fauna (e.g. by restoring endangered fish populations) (Lebensministerium III/2, 2009).

Traditional aquaculture species in Germany include common carp and rainbow trout, which are farmed in earthen ponds, raceways and other modern indoor and outdoor facilities (Rosenthal et al. 2000). Carp farming in freshwater ponds is the second major type of aquaculture practiced in Germany and has a long tradition. In 2005, 16.711 tons were harvested, producing total revenue of more than € 52 million. Pond culture of fish and carp in particular has a long tradition in Germany. The first records of common carp (*Cyprinus carpio*) culture in Bavarian ponds date back to the eleventh century (Geldhauser & Gerstner 2003).

1.3. Effect of cadmium, copper, zinc and iron on the human health

1.3.1. Cadmium

Cadmium (Cd), a by-product of zinc production, is one of the most toxic elements for humans at the workplace or in the environment. Once absorbed, Cd is efficiently retained in the human body, in which it accumulates throughout life. Cd is primarily toxic to the kidney, especially to the proximal tubular cells, the main site of accumulation. Cd can also cause bone demineralization, either through direct bone damage or indirectly as a result of renal dysfunction. In industry, excessive exposures to airborne Cd may impair lung function and increase the risk of lung cancer. All these effects have been described in populations with relatively high exposures to Cd in industrial or other heavily polluted environments. Recent studies also suggest that the chronic low environmental exposure to Cd now prevailing in industrialized countries can adversely affect the kidneys and bones of the general population. These studies show consistent associations between various renal and bone biomarkers and the urinary excretion of Cd used to assess Cd body burden (Araya et al. 2007). Cd dispersed in the environment can persist in soils and sediments for decades. When taken up by plants, Cd concentrates along the food chain and ultimately accumulates in the body of people eating contaminated foods. Cd is also present in tobacco smoke, further contributing to human exposure. By far, the most salient toxicological property of Cd is its exceptionally long half-life in the human body. Once absorbed, Cd irreversibly accumulates in the human body, in particularly in the kidneys and other vital organs such the lungs or the liver. In addition to its extraordinary cumulative properties, Cd is also a highly toxic metal that can disrupt a number of biological systems, usually at doses that are much lower than most toxic metals (Nordberg et al. 2007, Bernard et al. 2004).

The Cd body burden, negligible at birth, increases continuously during life until approximately the age of about 60-70 yr, at which body burden levels off and can even decrease. Cd does not easily cross the placental or the haemato-encephalic barriers, explaining its very low toxicity to the foetus and the central nervous system as compared with other heavy metals (Bernard et al. 2008).

1.3.2. Copper

Copper is an essential mineral for human health and at the same time can be toxic, depending upon the amounts ingested. Copper is associated with bone health, immune function and increased frequency of infections, cardiovascular risk and alterations in cholesterol metabolism. Its metabolism is tightly intertwined with other microminerals and its deficiency is known to impair iron mobilisation, resulting in secondary iron deficiency (Araya et al. 2007). Copper is a major component of haemoglobin, the protein responsible for oxygen transport in blood cells. As an antioxidant, copper plays a strong dual role. First, it is a central component of both the superoxide dismutase molecule, which helps prevent cellular free-radical damage (Johnson et al. 1992). Second, it helps form the protein ceruloplasmin, which helps prevent free-radical damage caused by iron. Copper is also required by the central nervous system as a component in the production of noradrenaline, the brain's version of adrenaline and the neurotransmitter that keeps humans alert. Copper is also involved in the production of prostaglandins, hormone-like chemicals that regulate blood pressure, pulse and healing. Current research is examining deeper aspects of copper’s role in human health, from protecting against cancer and heart disease, to boosting the immune system (Babu & Failla 1990).

Mutations in ATP7A or ATP7B disrupt the homeostatic copper balance, resulting in copper deficiency (Menkes Disease) or copper overload (Wilson's Disease) (De Bie 2007). The antagonism between zinc and copper has been utilized as a treatment of Wilson's disease by lowering copper, with 50 mg of zinc taken with each meal being effective in lowering the abnormal accumulation of copper in people afflicted with this genetic disease of copper metabolism (Fischer et al. 1984). The adult human body contains between 80 and150 mg of copper (Cartwright &Wintrobe, 1964). The liver is the major location of stored copper, containing about 10 percent of the total-body content (Iyenger 1978).

1.3.3. Zinc

Zinc is important for normal growth and survival of plants and animals. Doubts on a potential deficiency in humans were raised because of the element's ubiquitous distribution in the environment. The evidence of such a deficiency began to emerge during the 1960s, when cases of zinc-responsive dwarfism and delayed sexual maturation were first reported in Egyptian adolescents (Prasad 1991).

Zinc is the most ubiquitous of all trace elements involved in human metabolism. More than one hundred specific enzymes require zinc for their catalytic function (Cousins 1996).

If zinc is removed from the catalytic site, activity is lost; replacing zinc restores the activity. Zinc participates in all major biochemical pathways and plays multiple roles in the perpetuation of genetic material, including transcription of DNA, translation of RNA, and ultimately cell division. When the supply of dietary zinc is insufficient to support these functions, biochemical abnormalities and clinical signs may develop. Studies in individuals with acrodermatitis enteropathica, a genetic disorder with zinc malabsorption resulting in severe deficiency, shows impairments of dermal, gastrointestinal, neurologic and immunologic systems (Van Wouwe 1989).

The dietary components that substantially impact zinc absorption are phytate and calcium, which inhibit zinc absorption, and protein, which enhances the absorption. (Lonnerdal 2000).

Table2. Estimated physiological requirements for absorbed zinc by age group and sex

Age	Reference Wt. (Kg)	Requirement (mg/day)
6-12 mo	9	0.84
1-3 yr	12	0.83
3-6 yr	17	0.97
6-10 yr	25	1.12
10-12 yr	35	1.4
12-15 yr	48	1.82
15-18 yr M	64	1.97
15-18 yr F	55	1.54
Pregnancy		2.27
Lactation		2.89

Source: WHO 2002

Zinc is toxic in humans exposed to high intake levels, either through supplemental zinc or by contact with environmental zinc. Symptoms include nausea, vomiting, epigastric pain, diarrhea and lethargy (Fosmire 1990). Approximately 200-400 mg zinc is known to produce immediate vomiting in adults. Short-term exposure to high levels (>300 ppm) from improper storage of food or beverages in galvanized vessels has caused acute gastroenteritis.

Chronic over dosage of zinc, in the range of 100-300 mg zinc/day for adults, may induce copper deficiency (Prasad et al. 1978) and alter the immune response (Chandra 1984). Intakes as low as 50 mg supplemental zinc/day affected copper metabolism as measured by a decrease in erythrocyte copper-zinc SOD activity (Yadrick et al. 1989). A study in female subjects receiving 100 mg zinc/day showed a significant reduction in high density lipoprotein cholesterol levels after four weeks (Freeland-Graves et al. 1982). These levels, however, returned to normal after eight weeks, suggesting a transient effect.

1.3.4. Iron

Dietary iron exists in two different forms. Haem iron is present only in animal tissues, whereas in plant foods, iron is present as non-haem iron. In a mixed omnivore diet, around 25% of dietary iron is non-haem iron. Non-haem iron is less easily absorbed by the body than is haem iron. The amount of iron absorbed from various foods ranges from around 1 to 10% from plant foods and 10 to 20% from animal foods. Iron is an essential component of haemoglobin, transporting oxygen in the blood to all parts of the body. It also plays a vital role in many metabolic reactions. A deficiency can cause anaemia due to low levels of haemoglobin in the blood. Iron deficiency is the most widespread mineral nutritional deficiency. The body contains between 3.5 and 4.5 g of iron, 2/3 of which is present in haemoglobin. The remainder is stored in the liver, spleen and bone-marrow. A small amount is present as myoglobin, which acts as an oxygen store in muscle tissue (Anderson et al. 1981).

Table 3. Estimated physiological requirements for absorbed iron by age group and sex

Dietary Reference Intakes (DRI) for Iron.

Age	Fe (mg)	Age	Fe (mg)
Infants and Children			
0-6 months	0.27		
7-12 months	11		
1-3 years	7		
4-8 years	10		
Males		**Females**	
9-13 years	8	9-13 years	8
14-18 years	11	14-18 years	15
19+ years	8	19-50 years	18
		51+ years	8
Pregnancy		**Lactation**	
≤18 years	27	≤18 years	10
18+ years	27	18+ years	9

Source: National Academy of Sciences, 2001

Iron stores in the body become depleted and haemoglobin synthesis is inhibited. Symptoms of anaemia include tiredness, lack of stamina, breathlessness, headaches, insomnia, loss of appetite and pallor. All these symptoms are associated with a decreased oxygen supply to tissues and organs. Iron also plays an important role in the immune system: people with low iron levels having a lowered resistance to infection. A deficiency can also be associated with impaired brain function, and in infants can impair learning ability and cause behavioural problems (Draper & Wheeler, 1989).

Iron overdose has been one of the leading causes of death involving toxicological agents in children younger than 6 years. Iron is used as a paediatric or prenatal vitamin supplement and to treat anaemia. Patients with anemias that require frequent blood transfusions also are at risk for developing chronic iron toxicity (Morse et al. 1997).

Iron overload may develop chronically as well, especially in patients requiring multiple transfusions of red blood cells. This condition develops in patients with Sickle cell disease, thalassemia or myelodyplastic syndrome (Carlsson et al. 2008).

Iron toxicity can be classified as corrosive or cellular:

Corrosive toxicity: Iron is an extremely corrosive substance to the GI tract.

Cellular toxicity: The absorption of excessive quantities of ingested iron results in systemic iron toxicity (Telfer et al, 2000).

Adequate copper nutritional status appears to be necessary for iron metabolism (Vulpe et al. 1999). Iron accumulates in the liver of copper-deficient animals, indicating that copper is required for iron transport to the bone marrow for red blood cell formation (Turnlund et al. 2006).

1.4. Cadmium, copper, zinc and iron in the aquatic ecosystem

1.4.1 Cadmium

In freshwater and marine systems, the phytoplankton and periphyton (biofilm) as well as macrophytes and macroalgae are reported to accumulate Cd to a high extent from the surrounding medium. The bioconcentration factors, BCF, can stretch across 4 orders of magnitude. However, substantial amounts (up to about 60%) were found to be adsorbed by the outer surface of the plants. An additional source of cadmium at the base of the food chain is the sediment, to which Cd adsorbs readily and which is consumed by detritivorous organisms. Herbivores and detritus feeders (e.g. zooplankton, molluscs) are known to accumulate Cd in similar amounts (BCFs up to 4 orders of magnitude). As detritus feeders ingest sediment particles containing microalgae, bacteria and fungi, one cannot distinguish between uptakes from abiotic or biotic sources. In herbivorous and detrivorous arthropods, the uptake of Cd from food plays a minor role. As in plants, adsorption and subsequent binding to the exoskeleton plays an important role in the accumulation of non-essential heavy metals. Although temporary accumulation of Cd occurs in arthropods, the relatively strong binding to the exoskeleton and its regular loss due to molting prevents marked bioaccumulation in subsequent trophic levels. The zooplankton thus is seen as a breakpoint in the pelagic food chain transfer and biomagnification of heavy metals. The transfer of cadmium across trophic levels (from zooplankton to fish) has been experimentally proven by radiotracer methods. Its biomagnification in fish is controversial because values are generally lower than in their prey organisms. Uptake from ingested food is thus generally not considered as a significant source for metal accumulation in fish, although many previous studies have indicated that food is a major source of metal accumulation. Nonetheless, bioconcentration and food chain transfer in some marine fish may lead to concentrations exceeding the critical body burden, so that toxicity is likely to occur. Contaminated fish is the main source of heavy metals for predatory birds and mammals, whose accumulation especially in the liver may result in a considerable body burden. Cd biomagnification in fish-eating birds and mammals may result in concentrations 20-30 fold higher than in the fish consumed. Thus, the concentration in marine fish may cause indirect poisoning of predatory organisms (Schäffer et al. 1998). It bioaccumulates at all trophic levels, e.g. in the livers and kidneys of fish (Sindayigaya et al. 1994). Crustaceans appear to be more sensitive to cadmium than fish and mollusks (Sadiq, 1992).

1.4.2. Copper

Copper is a micronutrient and toxicant. It strongly adsorbs to organic matter, carbonates and clay, which reduces its bioavailability. Copper is highly toxic in aquatic environments and has effects in fish, invertebrates and amphibians, with all three groups equally sensitive to chronic toxicity (U.S.EPA 1993). Copper is highly toxic to amphibians (including mortality and sodium loss), with adverse effects in tadpoles and embryos (Horne & Dunson 1995). Copper can bioconcentrate in many different organs in fish and mollusks (Owen 1981), whereby the potential is know in fish, but high in mollusks. Copper sulfate and other copper compounds are effective algaecides (free copper ions are the lethal agent). Single-cell and filamentous algae and cyanobacteria are particularly susceptible to the acute effects, which include reduced photosynthesis and growth, loss of photosynthetic pigments, disruption of potassium regulation, and mortality. Sensitive algae may be affected by free copper at low (parts per billion – ppb) concentrations in freshwater.

There is a moderate potential for bioaccumulation in plants and no biomagnification. Toxic effects in birds include reduced growth rates, lowered egg production and developmental abnormalities. While mammals are not as sensitive to copper toxicity as aquatic organisms, toxicity has been reported in a wide range of animals and results in liver cirrhosis, necrosis in kidneys and the brain, gastrointestinal distress, lesions, low blood pressure and fetal mortality. (ATSDR 1990c, Kabata-Pendias & Pendias 1992, Ware 1983, Vymazal 1995). Residues in marine fish are generally higher than those in freshwater species, again reflecting the increased bioavailability of CuCl2. In polluted freshwaters, maximum concentrations in muscle tissue seldom exceed 1 mg/kg wet weight. In fish collected from polluted marine waters, contamination may lead to muscle concentrations of 3-6 mg/kg. Because muscle residues are generally low, copper does not pose a threat to most fisheries, even those in polluted waters (Roth & Hornung 1977).

1.4.3. Zinc

In many types of aquatic plants and animals, growth, survival and reproduction can all be adversely affected by elevated zinc levels. Zinc in aquatic systems tends to be partitioned into sediment and less frequently dissolved as hydrated zinc ions and organic and inorganic complexes (MacDonald 1994). Zinc is toxic to plants at elevated levels, adversely affecting growth, survival and reproduction (Eisler 1993). Terrestrial invertebrates are sensitive to elevated zinc levels, with reduced survival, growth and reproduction. Elevated levels can cause mortality, pancreatic degradation, reduced growth and decreased weight gain in birds (NAS 1980) they can also cause a wide range of problems in mammals including: cardiovascular, developmental, immunological, liver and kidney problems, neurological, hematological, pancreatic, and reproductive problems (Domingo 1994).

1.4.4. Iron

The effects of iron on aquatic animals and their habitats are mainly indirect, although the direct toxic effects of Fe2+ are also important in some lotic habitats receiving Fe-enriched effluents, especially in cold seasons. Ferric hydroxide and Fe-humus precipitates on both biological and other surface, indirectly affecting lotic organisms by disturbing and by changing the structure and quality of benthic communities. The combined direct and indirect effects of iron contamination decrease the species diversity and abundance of periphyton, benthic invertebrates and fishes. Sorption and co-precipitation of metals by Fe-oxides decrease the bioavailability and toxicity of waterborne metals, but may increase the dietary supply of metals and lead to toxic effects along the food chain. Formation of iron precipitates on biological surfaces has frequently been reported to effect the survival, reproduction and behaviour of aquatic animals (Walter 1996, Smith et al. 1973, Smith & Sykora 1976, Amelung 1982). The toxic effect of iron on rainbow trout, at neutral pH, was due to the precipitation of ferric hydroxide on the eggs and gill surfaces. Field observation showed the clogging of fish gills by Fe-hydroxide (Anderson & Nyberg 1984, Weatherly et al. 1991, Steffens et al. 1992). Staining of whole gill arches indicated that at least some of the Fe was bound to the gills even in the presence of humic material. Gill damage, consisting of fusion of the lamellae and hypertrophy of epithelial cells, reduced oxygen uptake and impaired the iron regulation of the Fe-exposed brown trout. The gill damage was more adverse at pH5 than at pH6 (Peuranen et al. 1994).

2. Materials and Methods

2.1. Examined species

2.1.1. Carps (*Cyprinus carpio)*

The major species sampled were the common carp (*Cyprinus carpio* LINNAEUS 1758) and mirror carp (*Cyprinus carpio morpha noblis*) originating from warmer climatic regions of Asia. In the Middle Ages, monasteries started the first breeding efforts under the rather cold climatic conditions of the Waldviertel. Since then, several carp varieties have been developed which vary in size, form and body surface. Today's farmed carps usually have high backs, which increases the meat content. Carps prefer slowly running water or stagnant water which does not necessarily have to be rich in oxygen or be clear. Under the specific climatic conditions of the Waldviertel, carps need 3-4 years to reach 2-3 kg and to be ready for consumption. During the long, harsh winter period the carps rest in the deepest spots of the ponds without feeding and growing. Carp are omnivorous and feed mainly on living organisms in the ponds.

As the stocking rate in the water bodies is low, only a limited amount of supplementary feed, which contains cereal but no fish meal or industrial feed, is necessary. In autumn, fish harvesting takes place using centuries-old techniques. First, most of the water is drained out of the fish ponds. Then the fishermen, , round up the fish with hand nets near the pond bank.

Finally, the fish are removed with scoops. After having being graded manually, the carp have to spend some time in tanks to eliminate the muddy taste of their meat. Yields are comparatively low due to the nature-oriented, species-adapted way of fish farming, but this ensures highest standards regarding the health and resistance of the fish and the meat quality. The meat is white to pale pink, firm and low in fat.

Today, carps are raised in more than 1.000 ponds covering over 1.650 hectares. An annual production of about 500 tons of carp ensures substantial income for about 400 agricultural enterprises. Common carp (*Cyprinus carpio* LINNAEUS 1758) production is centred in the north (Lower Austria) and southeast (Styria and Burgenland). In 1970 there were about 2.000 ha of carp ponds in Austria and in 1978 about 2.550 ha. The total area of ponds in Austria at about 3.500 ha. There are only a few producers with 50-ha pond areas and more. Earthen ponds are used, with water added only to offset evaporation. The climatic conditions in northern Austria require a longer growing period (three to four years); in south Austria, market sizes are attained in only two to three years (1.600-2.000 g). The average yield in the growing ponds is 550 kg/ha/year. Most carp are sold alive. About 500 tons are imported from Czechoslovakia, former Yugoslavia and other eastern European countries.

In 2007, production of common carp, one of the major species in the Austrian aquaculture production, was about 377 tons live weight. This represented 23% of the total aquaculture production.

Fig 1. Carp (*Cyprinus carpio*)

2.1.2. Trout (*Oncorhynchus mykiss*)

The large family of Salmonidae is represented by only 3 indigenous species in Austria. These are the trout (*Salmo trutta fario* LINNEAUS 1758, the Danube salmon *(Hucho hucho* LINNEAUS 1758) and Arctic charr (Salvelinus *alpinus* LINNEAUS 1758) Umweltbundesamt/Federal Environment Agency – Austria. M-087 (1997). Since 1870, trout and salmon have been bred in America. A significant milestone in the history of trout farming in Austria was the import of the first rainbow trout (*Oncorhynchus mykiss* WALBAUM 1792*)* from 1880 to 1890 from California. Between 1907 and 1926, more species and subspecies of rainbow trout from the Pacific Northwest of America were introduced, grown and mixed. They were not kept separately, but mixed as a "rainbow trout breed" and sold.

Trout in Austria is mainly produced by the small family farms. The production of a few larger companies ranges from 400 to 800 tons per year. The majority of the remaining farms produce less than 50 tons per year and sell mostly self-produced trout directly to consumers or through retailers. Moreover, there are still a number of small direct marketers in operation. The typical trout farm in Austria consists of natural ponds and a good water supply with spring water, and a crucial factor for the quality and the quantity of fish produced. Thus, successful cultivation of trout requires clean, cold and oxygen-rich water, which should be exchanged at least 4 to 5 times per day.

Dry food pellet technology has revolutionized the quality of feed since the 1960s. Trout are produced primarily in central and western Austria in raceways and in ponds. Net-cage culture is not significant in Austria. Market size is between 250 and 300 g, which requires between 14 and 20 months (average 17 months) of growth. Most trout are sold alive, but the market for smoked trout is increasing. A large trout farm produces 100 tons or more. In 1988 about 450 tons of trout and salmon were imported, and also most of the fertilized eggs are imported.

Rainbow trout (*Oncorhynchus mykiss* WALBAUM 1792*)* is one of the major species of the total production in Austria. In 2007, with 1.633 tons live weight it was around 64% of the aquaculture production in Austria.

Fig 2. Rainbow trout (*Oncorhynchus mykiss*)

2.1.3. Chub, perch, pike, catfish, roach, Danube bleak, reinanken and brown trout.

Some other species were sampled and studied such as chub (*Leuciscus Cephalus* LINNAEUS 1758, Cyprinidae), perch (*Perca fluviatilis* LINNAEUS 1758*,* Percidae), pike (*Esox lucius* LINNAEUS 1758*,* Esocidae), catfish (*Silurus glanis* LINNAEUS 1758*,* Siluridae), roach *(Rutilus rutilus* LINNAEUS 1758, Cyprinidae), Danube Bleak (*Chalcaburnus chalcoides*, Güldenstaedt, 1772, Cyprinidae), reinanke (*Coregonus sp* LINNAEUS 1758*,* Salmonidae) and brown trout (*Salmo trutta fario* LINNAEUS 1758, Salmonidae).

Chubs (*Leuciscus Cephalus*, LINNAEUS 1758, Cyprinidae) live small stretches of water such as drainage ditches. It favours low-flow areas behind rocks or in bays. It feeds on insects and other small animals, sometimes even on plants. Larger individuals also eat smaller fish and amphibians. The spawning season is in April-June. At this time, each female sets about 100.000 eggs on plants or in gravel. In some German-speaking regions the fish is known as "chub" (Austria, Bavaria), or "Alet" (Alemannisch, such as Lake Constance and Switzerland). Another name is "pipe carp". The chubs examined here were sampled from Fuschlsee.

Fig 3. Chub (*Leuciscus Cephalus*)

Perch (*Perca fluviatilis* LINNAEUS 1758*,* Percidae) are opportunistic predators that will attack anything that swims, including their own kin. After hatching, perch larvae feed on rotifers and other microscopic zooplankton before switching to bottom-dwelling insect larvae, pond snails, leeches and other invertebrates. The perch diet also includes small fish such as minnows, roach and other perch. In many waters a few perch grow exceptionally large by cannibalising the others. Feeding: Redfin perch are carnivorous and feed on a wide variety of foods ranging from small invertebrates (such as crustaceans, worms, molluscs and insect larvae) to fish.
Reproduction: Redfin spawn in late winter and spring, when each female lays several hundred thousand eggs in gelatinous ribbons amongst aquatic vegetation, submerged logs or other sheltered areas. The egg mass is unpalatable to most other fish and is hence generally protected from predation. The eggs develop and hatch in

about a week, and the young fish school to help avoid predation. Redfin usually take 2-6 years to reach sexual maturity, but some have been found to be reproductively mature at 1 year of age. The examined fish were obtained from Fuschlsee.

Fig 4. Perch (*Perca fluviatilis*)

Pike (*Esox lucius* LINNAEUS 1758*,* Esocidae) are members of a genus of spindle-shaped fish. Five predatory species live in Europe, North America and northern Asia. The pike (*Esox lucius*) has the widest distribution. Pike are voracious predators and feed on other fish, frogs, newts, mice, rats and young ducks, occasionally even crabs. The young, free-swimming pike feed on small invertebrates starting with daphnia, and quickly move on to bigger prey such as isopods or the amphipod. When the body length is 4 to 8 cm they start feeding on small fish. The present samples were obtained from Neusiedlersee.

Fig 5. Pike (*Esox lucius*)

Wels catfish (*Silurus glanis* LINNAEUS 1758*,* Siluridae) lives in large, warm lakes and deep, slow-flowing rivers. It prefers sheltered locations such as holes in the riverbed or sunken trees. It consumes its food in the open water or on the bottom. The wels catfish lives on annelid worms, gastropods, insects, crustaceans and fish. The larger ones also eat frogs, mice, rats and aquatic birds such as ducks. The investigated fish from the Neusiedlersee.

Fig 6. Wels catfish (*Silurus glanis*)

Roach (*Rutilus rutilus* LINNAEUS 1758, Cyprinidae) juveniles feed on zooplankton and other small invertebrates, and larger fish typically feed on larger invertebrates and filamentous algae. Roaches are shoal fish that prefer shallow shore areas and a rich flora. They eat mostly small animals (worms, crabs, mussels, snails, insect larvae) , but also aquatic plants. Size: 25-30 cm, max. 50 cm. Weight: approx 1kg. Depending on water and food supply, the body shape and colouring can vary widley. Roach from Fuschlsee were investigated.

Fig 7. Roach (*Rutilus rutilus*)

Danube Bleak (*Chalcaburnus chalcoides*, Güldenstaedt 1772, Cyprinidae). This species inhabits Europe from Austria to Kazakhstan. Its length ranges between 20-40 cm in males and typically 28 cm in females. Body weight: 300g.

Adults feed on plankton and insects, less frequently on small benthic animals. Eastern populations migrate upstream for spawning. Larvae and young juveniles feed on zooplankton, algae and insect larvae. They have been classified as benthopelagic, potamodromous, and freshwater fish. The individuals included in this study are from the Fuschlsee.

Fig 8. Danube Bleak (*Chalcaburnus chalcoides*)

Rinanken or Renken (*Coregonus* sp. LINNAEUS 1758, Salmonidae) is the common name for fish of the genus *Coregonus*, family Salmonidea, in Austria. Numerous other names for this species are found in Europe, such as Felche, Maräne or Schnäpel. Reinanken are cold-water fish which require abundant oxygen and live in swarms in cold, deep lakes of the Alps. Salzkammergut Reinanken are 35 - 40 cm in length. The fishing season is from April to October. The whitefish feed mostly on small water animals which they find on the bottom or in the open water area. There is no supplementary feeding. The samples were obtained from Fuschlsee.

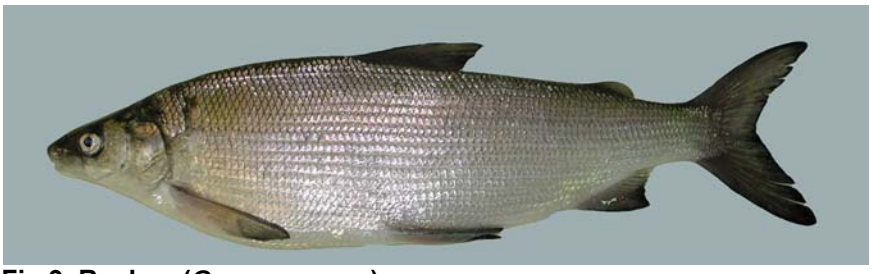


Fig 9. Renken (*Coregonus sp*)

Brown trout ((*Salmo trutta fario* LINNAEUS 1758, Salmonidae) this species is native to Europe, with a population still existing on the island of Corsica in the Mediterranean Sea. Often found in fast-flowing streams of mountain and sub-mountainous regions and sometimes even valleys. Their food ranges from benthic invertebrates, insect larvae, aerial insects (in rivers) and molluscs, with adults also consuming fish and frogs. Max length: 100 cm, common length 20 cm, max. Published weight: 20 kg and max. reported age: 8 years. The obtained samples were from Gossenköllesee.

Fig 10. Brown Trout (*Salmo trutta Fario*)

2.2. Sampling sites

2.2.1. Trout Site 1

This site is located in Lower Austria, close to the village of Traismauer. This site located on the lower reaches of Traisen and the Danube in the long and it has 3 fishing spots. It covers an area of more than 1.5 ha water surface. The farm is situated close to a mixed forest and is surrounded by fields. A natural channel is used as well as natural pools for keeping the fish. The natural channel has gravel bedrock; with an average width of around 4 m and a depth of 70 cm. Fish of different size are separated by mesh grid. The water supply is approx. 150 liter inflow per second. Commercial pellet feed is provided manually. This site is one of the largest fish farming in Lower Austria, supplying the top restaurants in Austria with fish dishes.

Fig 11. Trout Site 1

2.2.2. Trout Site 2

This site is located close to the village of Pottenbrunn. It covers an area of around 6-5 ha water surface. The fish grow either in artificial pools made completely of concrete or in natural pools. Artificial ponds show patches of algae on the edges. Water inflow is via a source which originates near the farm. The animals are fed with commercial pellet food. For harvesting, electric shocking is used.

Fig 12. Trout Site 2

2.2.3. Carp Site 1

This site is located in Lower Austria, in the northern part of the Waldviertel (Forest Quarter), which derives its name from its abundance of forests. The farm consists of 36 pools with a total water surface of around 200 ha. The ponds differ in size and depth; most of the investigated fish came from the largest pool. This pond is encircled by trees, bordered on one side by a highway. The water is 3 m deep at its deepest point and has a muddy substrate. The shore area shows a dense reed belt, and over a length of 20m from a concrete wall. Each pool has a part reserved for raising the offspring. This area is named 'Dubitschpools'. The ponds are fully harvested in spring and autumn with towed nets, using a procedure where the water is drained at intervals. The emptied ponds are refilled with water from adjoining carp ponds located at a higher level and from a nearby creek (2.6.3. Food supplements).

Fig 13. Carp Site 1

2.2.4. Carp Site 2

This bio-farm is located close to the city of Gföhl in the Waldviertel in Lower Austria. Accordingly, only cereals from controlled fields and regional crops are fed to the carp. All analyzed fish are from the largest pond of the farm. This pond measure 4.5 ha water surface and is surrounded by mixed forest on three sides. One side the pond is bordered by a small and little-used street. In three places, direct access to the water surface is possible through meadows; all other shore areas are covered with reeds.
The sediment is coarse-grained; in the deeper, more centrally located area, the sediment is muddy.

Fig 14. Carp Site 2

Fig 15. Carp fish caught by net

2.2.5. Wild fish sampling sites

Lake Fuschl

The maximum lake depth is 67.3 m. The lake has an area about 2.65 km² and its volume is approximately 97.43 million m². The lake extends to a maximum length of 4.1 km from the village of Fuschl. Lake Fuschl is located at the beginning of the Salzkammergut region, about 20 km east of the city of Salzburg at about 662 meters above sea level. Its scenic location in the Kalkvoralpen, the presence of a loop trail and the proximity to the capital make it a popular tourist destination. The water visibility often exceeds 6 m, and the lake reaches a relatively high water temperature in summer. This relatively deep alpine lake is rich in nutrients and oxygen, and the main fish are white fish (Rankin) and Arctic char, followed by lake trout, pike, carp, tench, burbot, eel, perch, chubs, roach, rudd and pavilions.

Fig 16. Lake Fuschl (Salzburg)

Lake Neusiedl

This is the second largest steppe lake in Central Europe, situated in North Burgenland and straddling the Austrian-Hungarian border. The lake covers 315 km², of which 240 km² are on the Austrian side, with a maximum depth of 1.8 m. The lake is drained by only one artificial sluice (Einser-Kanal). After common eel was introduced into Lake Neusiedl in 1958, it became a problem as a predator of spawn. Today, eels have been replaced mainly by pike, catfish, carp, and tench. The prediction is that eels will be rare in Lake Neusiedl in about ten years. Lake Neusiedl has more than 30 fish species. The highest fish biodiversity is in the reed zone surrounding the lake. The most common fish are eel, carp, pike-perch, pike and catfish.

Fig 17. Lake Neusiedler (Burgenland)

Gossenköllesee

It is a typical high-mountain lake. Located in the alpine region of the Tyrolean Stubbier Alpen at 47°14'N; 11°01'E, it is a typical high-mountain lake at an altitude of about 2.400 meters above sea level. It measures ca.1.6 ha. Gossenköllesee is a nutrient–poor, high-mountain lake ecosystem (mean depth 4.8m) with nanoplanctic, epilithic and epipelic algae. The lake is inhabited by Brown trout (*Salmo trutta fario*, Linnaeus, 1758), which was introduced in the 15th or 16th century. The fish population is of special research interest. To secure the long-term scientific investigations, the catchment of the lake was declared a UNESCO Biosphere Reserve. The governor signed the international declaration in 1977. The lake and 500m surrounding it are under the nature protection law of Tyrol.

Fig 18. Gossenköllesee (Tyrol)

Thaya River

The Thaya, in Lower Austria, is a strongly sinuous flowing tributary of the Morava, Thaya is 235 km long. The Austrian sections of the Thaya have a relatively poor water quality (levels 2-3). In Hardegg the average flow is 10.8 m3/s. The upper (Waldviertel) Thaya valley is a deep and narrow valley. In Drosendorf-city (423 m) the Thaya leaves Austrian territory and flows (except for short sections near Hardegg (309 m) and Laa an der Thaya 183 m), through Czech territory. The common fish species in Thaya are carp, pike, perch and eels.

Fig 19. Thaya (Lower Austria)

Untertalbach (Steiermark)

Mountain stream, about 7.5 km long and 12 m wide. Fish: Brown trout (*Salmo trutta fario*) and Brook trout (*Salvelinus fontinalis*). In the upper reaches it has the character of a mountain stream, the lower 4 km show creek character. There are many meanders, surrounded by a bog landscape. The upper limit is the outflow from the Riesachsee, the lower limit is located close to the restaurant Tetter".

Fig 20. Untertalbach (Steiermark)

Kühwörther Wasser

Fig 21. Kühwörtherwater (north-western part of Lobau)

The Kühwörtherwater is part of the lower Lobau, an area which covers the northwestern section of the Danube River National Park. Around 1875, the Danube River in Vienna was regulated, so most parts of the backwater system lost connection to the main stream. The only direct connection to flowing water is currently through the so-called Schönauer slot, a superficial link to flowing water. The position of this connection is also important for the dominance of the fish species and the presence of specific ecological groups. With the distance to the Danube, the proportion of stagnophilic species increases; no rheophilic species were caught in the area of Kühwörtherwasser.

The north-western part of Lobau is characterized by dense reed zones, followed by trees. The bottom is muddy, and water depth measures 1-5 m. Typical fish species in the Kühwörterwasser are several kinds of cyprinids, perch, pike, catfish and carp.

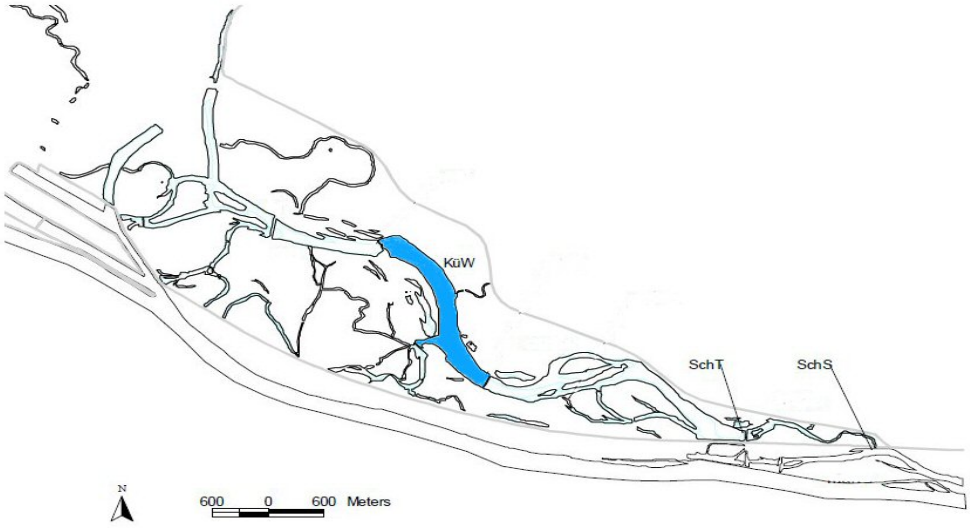


Fig 22. Küw = Kühwörtherwasser (north-western part of Lobau)
Schs = Schönauer slot

2.3. Market samples

Market samples were taken in supermarkets, fish stores and market stands in Vienna. Salmon, butterfish, crab, shrimp and some of the tuna samples were taken from sushi restaurants. The source of the fish was not known in all cases. The data written on the respective packages was used.

2.4. Sampling times

Both trout sites and Carp Site 1 were sampled between March and October 2007 at least three times. Carp Site 2 was sampled between August and November 2007 at intervals between 10-15 days for a total of eighteen times. At each sampling date, water, sediment, hydrophytes, benthic organisms, zooplankton and nekton were sampled. Abiotic water parameters were recorded where possibe.

The collected trout and carp were from different age groups. In addition, the time of year, weather (temperature, precipitation, solar radiation or cloud cover, presence and thickness of the ice on the water) and fish food were also recorded.

Table 4. Sampling times of farmed and wild-caught fish

Trout Site1	Trout Site2	Carp Site1	Carp Site2	Wild fish Lobau	Other Wild fish
Mar 07	Mar 07	Mar 07	Aug 07	Jun 07	
Aug 07	Aug 07	Jul 07	Nov 07	Oct 07	
Nov 07	Nov 07	Oct 07		May 08	Aug 08
				Jun 08	Oct 08

2.5. Abiotic water parameters

At each study site, abiotic water parameters (temperature, conductivity and pH) were determined by manual meter (Multiline P4, WTW). Water hardness was measured with test strips (Merck). The oxygen values were recorded by the Winkler method (iodometric determination by Winkler, Merck). Other factors determined in water samples (unfiltered and filtered water) were the content of TOC / TIC (total organic carbon, total inorganic carbon) and DOC/DIC (dissolved organic carbon, dissolved inorganic carbon).

2.6. Sampling and sampling storage

2.6.1. Water, sediment and turbidity

From each waterbody, a 2-l sample was taken in the middle, at a depth between 20 and 100 cm, depending on its total depth. Samples were kept in sealed 1-l glass bottles. Other parameters such as inorganic and organic turbidity, TOC/TIC, DOC/DIC, trophic level, cadmium, copper, and zinc and iron contents were analysed in the filtrate suspended matter. Sediment samples were taken where possible. It was for example not possible to collect sediment from trout sites (artificial channels). The filtered water samples were divided in the laboratory and filtered through the corresponding filters for turbidity, chlorophyll (GF/C glassfiber filter, Whatman) and heavy metal determination (<0.45 microns, Millipore) and then deep frozen (-20°C). Sediment samples were divided wet into two fractions (<1mm, and <50 µm sieve), freeze-dried (-48°C) for more than 48 h and stored in a desiccator.

2.6.2. Zooplankton, benthos and nekton

On all sampling locations (trout and carp), benthos and nekton organisms were collected by hand or by net. All samples were transported in cooling boxes to the laboratory. On four sites, at a water depth at ca 50 cm, zooplankton was collected by using a “Schindler-Schöpfer” along a linear transects across the water. The 4x5 l samples were combined to 20 l, homogenized and again divided for the respective analyses. The rest was deep frozen (-20°C) and stored for possible follow-up studies. The macroinvertebrates were kept in plastic bags for 24 h and then deep frozen.

The plankton samples were homogenized by shaking. A 2000 ml sample adequate aliquot was brought to ethanol to determine the taxonomic status. The remaining material was deep frozen. The plankton was filtered through a net (50 microns) and then transferred into a diluted solution of 80% propylenglycol. Cladocerans, ostracods, insects, and Acari were determined in a volume of 2000 ml, copepods were determined in at least 300 ml (later extrapolated to 2000 ml). Rotifers were determined in 150 ml and also extrapolated to 2000 ml.

2.6.3. Food supplements

At both trout farms, fish were fed with commercial pellet feed (DAN-EX). At Carp Site 1, animals were fed on demand; fish at Carp Site 2 were not fed at all. Young fish were fed with pellets, larger fish occasionally with cereals (barley, rye, wheat) or legumes (peas). On demand, pellets were also fed (DAN-EX), depending on the age of the animals. Feeding with such pellets was rarely practiced according to the fish farmer. From each brand of feed, 200 g were taken as a sample out of the original bag, transferred into plastic bags, transported in to the laboratory and stored in a dry place until analysis.

2.6.4. Fish

Wild samples: Fish were killed immediately and transferred to the laboratory in an ice box. Carp from the Thaya and Lobau were caught by hook and net, using grains of maize as bait. From the Neusiedlersee, carp were caught with a net, as were pike and catfish. From the Fuschelsee, perch and reinanke were captured. At the lake of Gossenkölle, fly fishing (dry-flies) was used.

Market samples*:* Samples were bought fresh or deep frozen. All samples were deep frozen (-20°C) until analyses.

Aquaculture: Trout was always caught by net. From Carp Site 2, eight fish were caught with hooks (maize grains as bait), but most animals were caught by net. The fish were killed immediately and transferred to the laboratory in an ice box.

2.7. Sample preparation

No equipment made of metal was used; the instruments were all either plastic or ceramic. All used materials and containers were cleaned with washing acid (HNO3 65%, aliquot to distilled water at a ratio of 1:10) to avoid contamination.

After washing and cleaning the fish in distilled water, the animals were dried, weighed and measured. Gender could be determined only in mature carp.

2.8. Determination of turbidity, chlorophyll, TOC / TIC and DOC / DIC

Turbidity and chlorophyll

0.5 l of water filtered through GF/C glassfiber filters by a vacuum pump was used to determine the turbidity. The filters were ashed in a muffle furnace at 550°C, filter

weight recorded, and turbidity as a whole, annealing loss and inorganic turbidity calculated. To determine chlorophyll-a and pheophytin in the remaining solution, the method of Eder (1979) was used, as modified by Marker and Jings (1982). Measurements were made using a spectrophotometer (ULTROSPEC, 4054 UV / visible spectrophotometer, LKB BIOCHROM) at wavelengths of 630, 647, 663, 665 and 750 nm.

TOC / TIC, DOC / TIC

TOC/TIC and DOC/DIC content was determined using a TOC analyzer (Elementar High TOC) in unfiltered water samples and filtrated samples.
Two stock solutions (500 ppm TOC: 1260.7 Tris and 500 ppm TIC: 4412.2 mg Na2CO3 produced anhydrous) were prepared. Calibration was made in range of 0.5 mg / l - 500 mg / l, three blank values were measured.

2.9. Heavy metal analysis

The metal concentrations were measured with a HITACHI Z 8200 Polarized Zeeman atomic absorption spectrophotometer. Cadmium analysis was conducted with a GF-ASS (Graphite furnace atomic absorption spectrometer (GF-AAS) and a specially adapted autosampler. Copper, zinc and iron were analyzed by ET-ASS (Flame atomic absorption spectrometry). For quality assurance, blanks (2ml 65% HNO3 and 0.5 ml H2O2 pA) and reference standards (Reference Materials, Commission of the European Communities. Bureau of References: Mussel Tissue ERM-CE278, Lake Sediment, reference material No.142R and Whole blood seronorm MR 4206).

Muscle tissue % recovery: showed for each heavy metal the following values: Cd =98%, Cu=86% and Zn=98%. Lake sediment recovery: Cd =97%, Cu = 94%. While the lake sediment reference materials were significantly lower than the certified value (recovery =50%). Whole blood seronorm was used as reference material for iron and showed a recovery of 88%. For muscle tissue and lake sediment, the reference material was not certified for iron.

The cadmium concentrations of the Reference samples (N =12-15) and for Cu, Zn and Fe (10-30) were in the range of certified values. The limit of detection (LOD) was determined by the concentration equivalent to the threefold standard deviation of the signal of the blank solution (10 duplicate measurements). LODs were Cd =0.0223 ppb, Cu=0.0218 ppm, Zn=0.0178 and Fe=0.204 ppm.

2.10. Statistical analysis

The heavy metals data were not normally distributed; therefore, non-parametric tests (Kruskal-Wallis test for three-group unpaired variables, Mann-Whitney *U* test for two-group unpaired variables, Spearman's rank correlation coefficient) were performed. SPSS statistics software 17 was used for statistical calculation. The end of the Whisker represented the minimum values at the 5^{th} percentile and the maximum values at the 95^{th} percentile.

Subgroups consisting of N<5 have mentioned in the results section were not respected for group comparison. Imputation methods were used, with the missing value of ½ LOD replaced. Mean±3SD, cadmium= 0.00335±0.0137, copper= -0.0297±0.0735, zinc= -0.2545±0.140 and iron= -0.4086±0.2044.

Bioaccumulation (BAF) was calculated by using average values, by dividing the muscle content by the content of different organs for each of the following: sediment, suspended matter, zooplankton, macro-invertebrates and artificial fish food.

3. Results.

3.1. Sample number

The samples were collected in this study from spring 2007 to autumn 2008 (total number of samples: 562). These included four fish sampling sites, wild fish samples, market fish samples, and in addition some marine fish (see Tables 4-5), Water samples were taken from the four fish sampling sites as well as sediment, zooplankton, nekton and benthos. In some cases the number of samples for certain parameters was lower than the total number of fish samples. Examples include gut contents and gonads, which were sometimes absent. This also refers to certain organs such as the brain, liver and gills which were often very small, or the liver and brain, which were occasionally destroyed by freezing.

Table 5. Number of fish from four fish sites

Sampling Site	Trout Site1	Trout Site2	Carp Site1	Carp Site2
Total Fish N	**48**	**48**	**55**	**21**
Cd Muscle	45(16)	48	54(19)	20
Cd Gut	36	38(1)	36(11)	18
Cd Liver	24	23(5)	21	7
Cd Gonad	8	24(8)	16	_
Cd Brain	23	24	22	_
Cd Gill	5	24(8)	31	8
Cu Muscle	32(32)	16(16)	54(47)	20(13)
Cu Gut	41(9)	17(1)	43(1)	21(18)
Cu Liver	30	37	24(5)	8(8)
Cu Gonad	8(5)	23(19)	19(16)	_
Cu Brain	20(20)	40(8)	32(16)	7(4)
Cu Gill	14(14)	24(24)	16(28)	8(7)
Zn Muscle	14	21	23	3
Zn Gut	20	6	22	_
Zn Liver	21	20	6	5
Zn Gonad	_	2	5	_
Zn Brain	22	8	22	2
Zn Gill	18(1)	18	30	5
Fe Muscle	24(16)	23(15)	24(15)	_
Fe Gut	22(2)	11(3)	28(4)	_
Fe Liver	23(1)	20(1)	14	8
Fe Gonad	8(1)	21(3)	4	_
Fe Brain	24(3)	23(1)	23	5
Fe Gill	13(0)	24	30	8

Sampling Site	Trout Site1	Trout Site2	Carp Site1	Carp Site2
Filtered Water	**6(6)**	**4(4)**	**6(6)**	**18(8)**
<LOD				
Turbidity	5	4	6	18
Chylorophyll	5	4	6	18
Filter				
Suspended Matter	**5**	**3**	**4**	**18**
Cd	3	3	3	17
Cu	_	_	4	4
Zn	_	_	1	4
Fe	_	_	2	5(1)
Sediment <50µm	**1**	**0**	**4**	**9**
Cd	1	0	3	8
Cu	1	0	4	7
Zn	1	0	2	5
Fe	1	0	2	7
Zooplankton	**0**	**0**	**2**	**16**
Cd	0	0	2	14
Cu	0	0	1	4(12)
Zn	0	0	1	9(1)
Fe	0	0	2	9
Food Supplement	**6**	**6**	**4**	**0**
Cd	6	6	4	0
Cu	6	6	4	0
Zn	6(1)	4	0	0
Fe	5(1)	4	3	0

*Numbers in brackets: N of samples below limits of detection

Table 6. Number of marine fish and crustaceans

	Tuna Fish	Swordfish	Butterfish	Shark	Lachs	Pangasius	Carp	Shrimp
Total N	**6**	**2**	**3**	**2**	**3**	**5**	**2**	**2**
Cd Muscle	5(0)	2(0)	3(0)	2(0)	2(0)	5(4)	2(0)	2(0)
Cu Muscle	5(2)	2(1)	3(0)	2(0)	2(0)	5(5)	1(0)	2(0)
Zn Muscle	4(0)	2(0)	3(0)	_	_	4(0)	1(0)	_
Fe Muscle	5(0)	1(1)	3(3)	2(0)	_	3(0)	2(0)	___

*Numbers in brackets: N of samples below limits of detection

Table 7. Number of market and wild fish samples

	Market fish		Wild Fish								
	Carp	Trout	Carp	Trout	Perch	Chub	Danube Bleak	Roach	Reinanke	Pike	Catfish
Total N	**28**	**10**	**16**	**5**	**3**	**3**	**3**	**7**	**4**	**1**	**1**
Cd Muscle	21(8)	9(3)	16(1)	2(0)	_	3(0)	3(0)	5(0)	4(0)	1(0)	1(0)
Cd Gut	3(0)	1(0)	13(0)	3(0)	_	3(0)	2(0)	7(0)	4(0)	_	_
Cd Liver	2(0)	_	2(0)	4(0)	_	3(0)	1(0)	7(0)	_	_	_
Cd Gonad	2(0)	_	2(0)	3(0)	_	3(0)	2(0)	7(0)	_	_	_
Cd Brain	3(0)	_	3(0)	4(0)	_	3(0)	3(0)	6(0)	_	_	_
Cd Gill	13(0)	2(0)	2(0)	2(0)	_	3(0)	_	7(0)	_	_	_
Cu Muscle	23(10)	6(4)	14(0)	2(0)	3(0)	3(0)	3(0)	7(0)	4(0)	_	1(0)
Cu Gut	5(1)	_	14(0)	2(0)	1(0)	3(0)	_	7(0)	4(0)	_	1(0)
Cu Liver	10(0)	_	_	5(0)	2(0)	2(0)	_	6(0)	_	_	_
Cu Gonad	10(10)	_	4(3)	2(0)	1(0)	3(0)	2(0)	6(0)	_	_	_
Cu Brain	9(9)	_	_	2(0)	_	3(0)	3(0)	7(0)	_	_	_
Cu Gill	12(2)	3(3)	4(3)	2(0)	1(0)	3(0)	3(0)	7(0)	_	_	_
Zn Muscle	22(0)	2(0)	7(0)	4(0)	2(0)	3(0)	3(0)	7(0)	3(0)	1(0)	1(0)
Zn Gut	4(0)	_	8(0)	2(0)	1(0)	3(0)	_	7(0)	4(0)	_	_
Zn Liver	5(0)	_	2(0)	2(0)	_	3(0)	1(0)	6(0)	_	_	_
Zn Gonad	_	_	2(0)	1(0)	1(0)	_	_	7(0)	_	_	_
Zn Brain	6(0)	_	7(0)	2(0)	_	3(0)	2(0)	7(0)	_	_	_
Zn Gill	10(0)	5(0)	5(0)	2(0)	1(0)	2(0)	_	6(0)	_	_	_
Fe Muscle	22(6)	_	12(1)	3(0)	3(0)	3(0)	_	7(0)	4(0)	1(0)	1(0)
Fe Gut	3(0)	_	10(0)	5(0)	2(0)	3(0)	3(0)	7(0)	4(0)	_	1(0)
Fe Liver	3(0)	_	3(0)	5(0)	2(0)	3(0)	1(0)	7(0)	_	_	_
Fe Gonad	2(2)	_	3(0)	5(0)	2(0)	2(0)	3(0)	7(0)	_	_	_
Fe Brain	3(0)	_	3(0)	4(0)	_	3(0)	3(0)	7(0)	_	_	_
Fe Gill	16(0)	2(0)	_	4(0)	1(0)	2(0)	_	7(0)	_	_	_

*Numbers in brackets: N of samples below limits of detection

Tabel 8. Number of benthos and nekton samples of the four sites.

Trout Site1	**Mar 07**	**May 07**						**N total**
Dragonfly larvae, Odonata, *Lestes* sp. (Fam. Lestidae)	**1**	**_**						**1**
Freshwater amphipod, Amphipoda, *Gammarus roeseli* (Fam. Gammaridae)	**5**	**_**						**5**
Common midges, Nematocera (Fam. Chironomidae)	**12**	**_**						**12**
Flatworms, Turbellaria, *Polycelis felina (*Fam. Planariidae)	**_**	**4**						**4**
Wandering snail, *Lymnaea peregra f. ovata* (Fam.Lymnaeidae)	**_**	**2**						**2**
Trout Site2	**Feb 07**	**Mar 07**	**Aug 07**					
Wandering snail, *Lymnaea peregra f. ovata* (Fam.Lymnaeidae)	**1**	**1**	**_**					**2**
Freshwater amphipod, Amphipoda, *Gammarus roeseli*) GERVAIS (Fam. Gammaridae)	**_**	**4**	**_**					**4**
Freshwater amphipod, Amphipoda, *Gammarus pulex* L. (Fam. Gammaridae).	**_**	**12**	**10**					**22**
Carp Site1	**Mar 07**	**May 07**	**Jun 07**	**Jul 07**				
Caddisflies, Trichoptera, L. v. Anabolia sp.	**1**	**_**	**_**	**_**				**1**
Dragonfly larvae, Odonata, *Lestes* sp. (Fam. Lestidae)	**1**	**1**	**_**	**_**				**2**
Mayfly larvae, Ephemoptera, *Baetis* sp. (Fam. Baetidae)	**5**	**_**	**_**	**_**				**5**
Water boatmen, *Corixidae gen* sp. (Fam. Corixidae)	**_**	**2**	**8**	**7**				**17**
Creeping water bugs, *Ilyocoris cimicoides* (L.) (Fam. Naucoridae)	**_**	**7**	**_**	**_**				**7**
Carp Site2	**Apr 07**	**May 07**	**Jun 07**	**Jul 07**	**Aug 07**	**Sep 07**	**Oct 07**	
Water boatmen, *Corixidae* gen sp. (Fam. Corixidae)	**12**	**5**	**14**	**_**	**8**	**10**	**6**	**55**
Creeping water bugs, *Ilyocoris cimicoides* (L.) (Fam Naucoridae)	**1**	**_**	**_**	**_**	**_**	**_**	**_**	**1**
Creeping water bugs, *Notonecta* sp. (Fam. Notonectidae)	**_**	**1**	**_**	**_**	**1**	**_**	**_**	**2**
Creeping water bugs, *Ilyocoris cimicoides* (L.) (Fam. Naucoridae)	**_**	**1**	**1**	**_**	**_**	**_**	**_**	**2**
Dragonfly larvae, Odonata, *Lestes* sp. (Fam. Lestidae)	**_**	**1**	**1**	**_**	**_**	**_**	**_**	**2**
Water flea, *Chydorus sphaericus* (O.F.M.) (Fam. Chydoridae)	**_**	**8**	**_**	**_**	**_**	**_**	**_**	**8**
Frog larvae	**_**	**1**	**_**	**_**	**_**	**_**	**_**	**1**
Flatworm, *Dugesia lugubris* (Fam. Planariidae)	**_**	**_**	**2**	**_**	**_**	**_**	**_**	**2**
Mayfly larvae, Ephemoptera, *Baetis* sp. (Fam. Baetidae)	**_**	**_**	**_**	**8**	**8**	**_**	**_**	**16**

3.2. Abiotic parameters of water from the sampling sites

3.2.1. Trout sampling sites

Table 9. Abiotic parameters of water from Trout Site 1

Date	Total Turb µg/L	Org. Turb µg/L	Inorg.Turb µg/L	TIC mg/L	TOC mg/L	DIC mg/L	DOC mg/L
20.03.2007	0.49	0.49	0	-	-	-	-
25.05.2007	0.52	0.52	0	-	-	41.3	13.2
04.08.2007	0.49	0.49	0	43.2	13.9	37.2	6.9
19.11.2007	0.52	0.52	0	53.6	17.9	45	13
Date	**Hardness°dH**	**pH**	**Conductivity µS/cm**	**O2 mg/L**	**Temp.°C**	**Chl.-a µg/L**	**Phaeo.µg/kg**
20.03.2007	10	6	647	7.8	8.7	0.6	0
25.05.2007	10	7.3	570	6.7	15	5.3	0
04.08.2007	10	7.7	609	5.4	13.5	3.6	0
19.11.2007	10	7.5	661	5.3	12.6	2.4	0

Table 10. Abiotic parameters of water from Trout Site 2

Date	Total Turb µg/L	Org. Turb µg/L	Inorg.Turb µg/L	TIC mg/L	TOC mg/L	DIC mg/L	DOC mg/L
15.03.2007	0.43	0.43	0	-	-	33.2	6.8
20.03.2007	0.48	0.48	0	48.2	14.9	-	-
28.08.2007	0.44	0.44	0	46.1	13.2	39.7	11.1
12.11.2007	0.51	0.52	0	44.6	17.2	47.8	12.4
Date	**Hardness°dH**	**pH**	**Conductivity µS/cm**	**O2 mg/L**	**Temp.°C**	**Chl.-a µg/L**	**Phaeo.µg/kg**
15.03.2007	12	6.1	733	7.9	10.9	3.6	0
20.03.2007	12	7	701	7.7	10.7	3	0
28.08.2007	12	7.5	709	8.4	15.9	1.2	0
12.11.2007	12	7.4	578	7.8	11.5	2.4	0

3.2.2. Carp sampling site

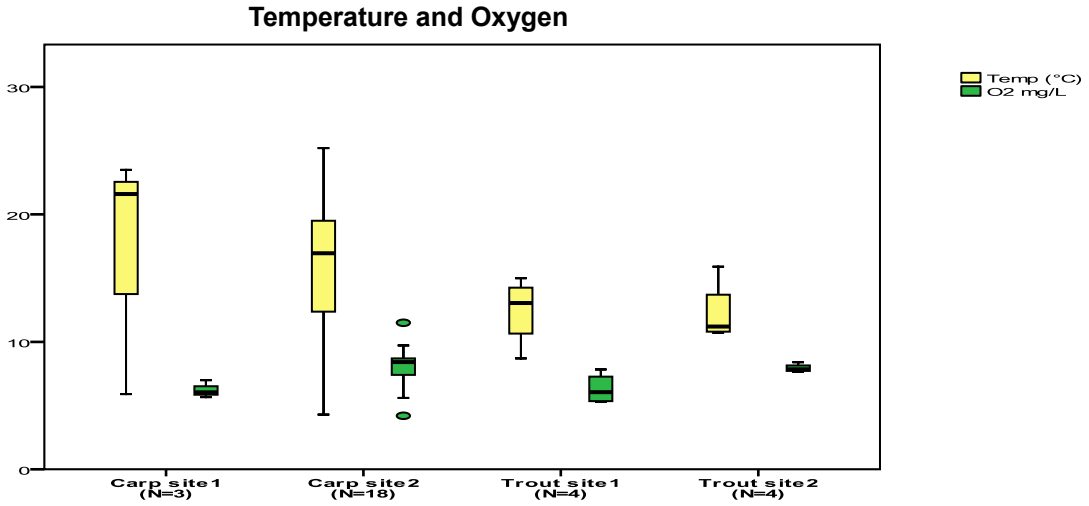
Table 11. Abiotic parameters of water from Carp Site 1

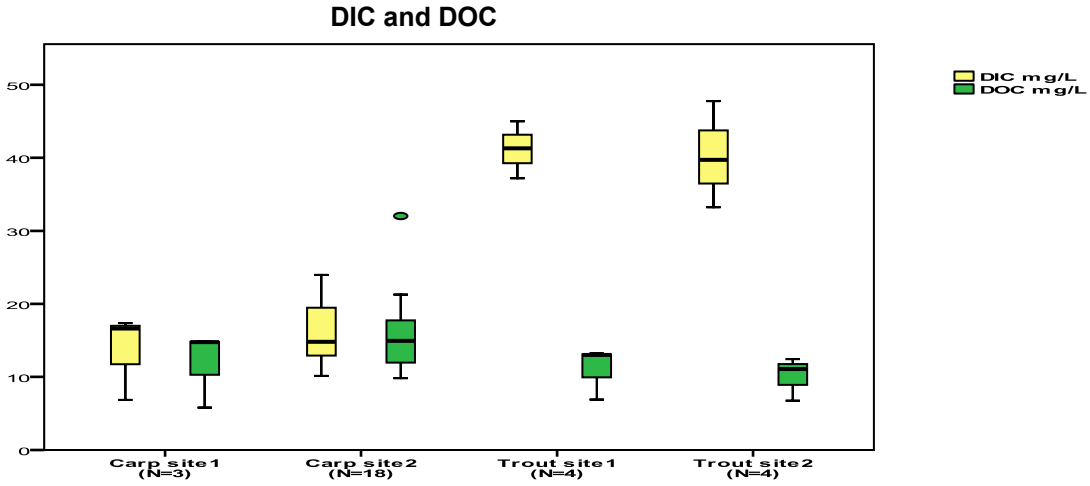
Date	Total Turb µg/L	Org. Turb µg/L	Inorg.Turb µg/L	TIC mg/L	TOC mg/L	DIC mg/L	DOC mg/L
29.03.2007	1.53	1.52	0.01	-	-	6.7	5.8
22.05.2007	0.46	0.46	0	-	-	16.6	14.9
23.07.2007	1.32	1.31	0.01	16.1	20.9	17.4	14.8
Date	**Hardness°dH**	**pH**	**Conductivity µS/cm**	**O2 mg/L**	**Temp.°C**	**Chl.-a µg/L**	**Phaeo.µg/kg**
29.03.2007	8	3.5	128	5.7	5.9	8.3	0
22.05.2007	9	7.3	284	6	21.6	0.6	0
23.07.2007	9	8.3	277	7	23.5	33.2	0

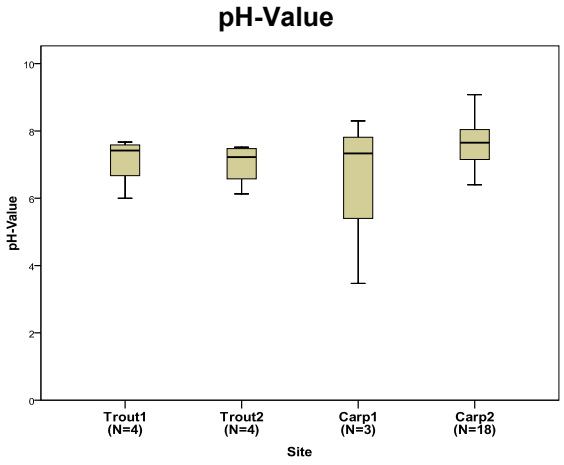
Table 12. Abiotic parameters of water from Carp Site2

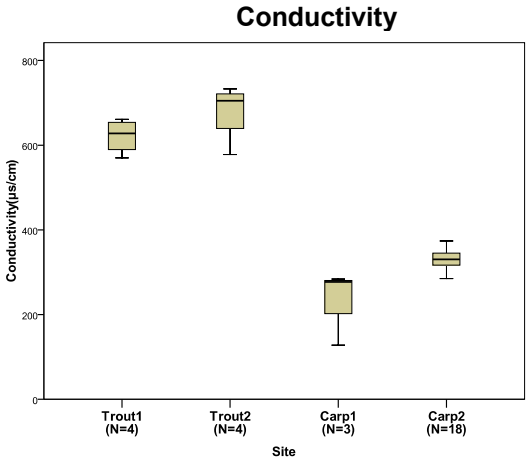
Date	Total Turb µg/L	Org. Turb µg/L	Inorg. Turb µg/L	TIC mg/L	TOC mg/L	DIC mg/L	DOC mg/L
16.04.2007	0.61	0.61	0.01	-	-	10.9	12
10.05.2007	0.61	0.6	0.01	-	-	12.9	9.8
21.05.2007	0.71	0.71	0	-	-	12.9	14.4
01.06.2007	1.28	1.27	0.01	-	-	14.5	15.8
14.06.2007	0.72	0.72	0	-	-	15.1	12.3
26.06.2007	1.37	1.36	0.01	-	-	20.4	18.1
07.07.2007	1.4	1.39	0.01	-	-	17.5	14.5
19.07.2007	1.25	1.25	0	18.1	29.9	19.5	15.4
01.08.2007	1.34	1.33	0.01	20.8	15.9	24	32
12.08.2007	0.77	0.77	0.01	23.5	29.6	18.7	19.8
24.08.2007	1.14	1.13	0.01	20.2	29.4	20.4	21.3
06.09.2007	0.97	0.96	0.01	23	31.6	21.9	15.7
18.09.2007	0.41	0.41	0	13.6	24.5	14.4	14
28.09.2007	0.59	0.59	0	15.5	20.8	15.6	17.7
11.10.2007	0.52	0.52	0	16.5	19.2	12.9	15.7
24.10.2007	0.59	0.59	0	-	-	14.4	11
05.11.2007	0.5	0.5	0	14	17.6	11.9	11.7
21.11.2007	0.52	0.52	0	10.7	12.2	10.1	11.3

Date	Hardness°dH	pH	Conductivity µS/cm	O2 mg/L	Temp.°C	Chl.-a µg/L	Phaeo.µg/kg
16.04.2007	8	8.3	332	9.3	16.2	13.6	0
10.05.2007	8	7.3	297	7.4	13.9	21.3	0
21.05.2007	8	8	297	9.7	18.6	15.4	0
01.06.2007	8	6.8	345	8.7	19.5	13	0
14.06.2007	8	7.5	339	8.4	21.6	3.6	0
26.06.2007	8	9.1	353	8	25.2	9.5	0
07.07.2007	8	8.2	334	8	17.8	16	0
19.07.2007	8	9	327	4.2	24.7	24.9	0
01.08.2007	8	8	347	7.3	18.2	10.7	0
12.08.2007	8	7.8	339	5.6	17.7	18.9	0
24.08.2007	8	7.4	326	8.7	21	-	-
06.09.2007	8	6.4	317	8.5	12.2	4.7	0
18.09.2007	8	7.3	285	11.5	15.6	27.2	0
28.09.2007	8	7.8	306	6.8	13	9.5	0
11.10.2007	8	7.9	321	7.7	12.4	8.9	0
24.10.2007	8	7.2	329	8.8	4.3	3.6	0
05.11.2007	8	7	358	8.7	6.6	7.1	0
21.11.2007	8	6.8	374	8.4	5.9	2.4	0


Temperature and Oxygen
Temp (°C)
O2 mg/L
30
20
10
0
Carp site1 (N=3)
Carp site2 (N=18)
Trout site1 (N=4)
Trout site2 (N=4)


DIC and DOC
DIC mg/L
DOC mg/L
50
40
30
20
10
0
Carp site1 (N=3)
Carp site2 (N=18)
Trout site1 (N=4)
Trout site2 (N=4)


pH-Value
pH-Value
10
8
6
4
2
0
Trout1 (N=4)
Trout2 (N=4)
Carp1 (N=3)
Carp2 (N=18)
Site


Conductivity
Conductivity(µs/cm)
800
600
400
200
0
Trout1 (N=4)
Trout2 (N=4)
Carp1 (N=3)
Carp2 (N=18)
Site

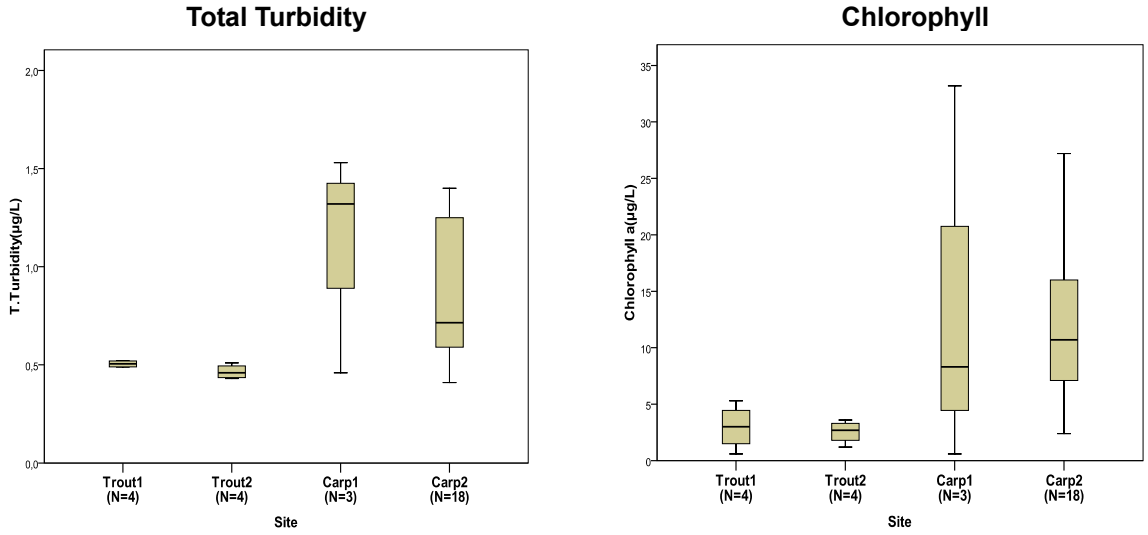


Fig 23. Temperature and oxygen, DIC and DOC, pH, conductivity, total turbidity and chlorophyll from four sampling sites (farmed fish): trout 1, trout 2, carp 1 and carp 2.

Similarity between sites

Abiotic water parameters in trout and carp sites were significantly different from one another: oxygen, conductivity, TIC, DIC, total turbidity, and chlorophyll were different at the $P<0.05$ levels, hardness differed at $P=0.000$ (highly significant). There was no significant difference between the sites in the following parameters: temperature, pH, TOC and DOC ($P>0.05$).

Water abiotic parameters: correlation of the two trout sites

Total turbidity and organic turbidity were positively correlated with TOC, DOC but negatively correlated to conductivity. Temperature was positively correlated with pH. The differences for all correlations shown above were significant at $P<0.05$.
Salinity in Trout Site 1 and 2 were 0.02 and 0.03 mg/L respectively (Table 13).

Table 13. Correlation of abiotic water parameters from both trout sampling sites

Trout		Total Turbidity	Org. Turbidity	TOC	DIC	DOC	Hardness	pH	Conductivity	Temp
Org. Turb	r	**1,000****								
	P	**.**								
	N	**8**								
TOC	r	**0.900**	**0.900**							
	P	**0.037***	**0.037***							
	N	**5**	**5**							
DIC	r	0.754	0.754	0.6						
	P	0.084	0.084	0.4						
	N	6	6	4						
DOC	r	**0.928**	**0.928**	0.8	0.771					
	P	**0.008****	**0.008****	0.2	0.072					
	N	**6**	**6**	4	6					
Hardness	r	-0.663	-0.663	-0.289	-0.098	-0.49				
	P	0.073	0.073	0.638	0.854	0.326				
	N	8	8	5	6	6				
pH_Values	r	0.205	0.205	-0.5	0.029	-0.09	-0.109			
	P	0.627	0.627	0.391	0.957	0.872	0.797			
	N	8	8	5	6	6	8			
Conductivity	r	**-0.807**	**-0.807***	-0.5	-0,6	-0.71	0.546	-0.167		
	P	**0.015***	**0.015***	0.391	0.208	0.111	0.162	0.693		
	N	**8**	**8**	5	6	6	8	8		
Temp	r	0.205	0.205	-0.6	0.029	0.371	-0.109	**0.786**	-0.19	
	P	0.627	0.627	0.285	0.957	0,468	0.797	**0.021***	0.651	
	N	8	8	5	6	6	8	**8**	8	
Chlorophyll	r	0.169	0.169	0.1	-0.314	0.143	-0.218	0.167	-0.262	0.31
	P	0.690	0.690	0.873	0.544	0.787	0.604	0.693	0.531	0.456
	N	8	8	5	6	6	8	8	8	8

****Correlation significant at P≤0.01 level (2-tailed)**

*** Correlation significant at P≤0.05 level (2-tailed)**

Water abiotic parameters: correlation of the two carp sites

Total turbidity, organic turbidity with inorganic turbidity and DIC were positively correlated (P<0.05). DOC and DIC are positively correlated. Temperature was correlated positively to DIC, DOC and pH. Chlorophyll and hardness were positively correlated, while conductivity correlated negatively to hardness. Salinity in the two carp sites were 0.01mg/L (Table 14).

Table 14. Correlation of abiotic water parameters from both carp sampling sites

Carp		Total Turbidity	Org. Turbidity	Inorg. Turbidity	TOC	DIC	DOC	Hardness	pH	Conductivity	Temp
Org.Turb	r	**1.000****									
	P	**0.000**									
	N	**21**									
Inorg.Turb	r	**0.725**	**0.725**								
	P	**0.000****	**0.000****								
	N	**21**	**21**								
TOC	r	0.241	0.241	0.346							
	P	0.474	0.474	0.297							
	N	11	11	11							
DIC	r	**0.465**	**0.463**	0.346	0.536						
	P	**0.034***	**0.035***	0.124	0.089						
	N	**21**	**21**	21	11						
DOC	r	0.293	0.296	0.268	0.209	**0.774**					
	P	0.197	0.193	0.241	0.537	**0.000****					
	N	21	21	21	11	**21**					
Hardness	r	-0.080	-0.080	-0.015	0.000	0.134	0.027				
	P	0.729	0.729	0.947	1	0.563	0.908				
	N	21	21	21	11	21	21				
pH	r	0.294	0.301	0.102	0.064	0.405	0.405	0.161			
	P	0.195	0.185	0.659	0.853	0.068	0.068	0.486			
	N	21	21	21	11	21	21	21			
Conductivity	r	0.074	0.079	0.032	-0.436	0.121	0.198	**-0.456**	0.082		
	P	0.748	0.733	0.892	0.18	0.602	0.389	**0.038***	0.725		
	N	21	21	21	11	21	21	**21**	21		
Temp	r	0.391	0.392	0.181	0.436	**0.579**	**0.506**	0.389	**0.656**	0.006	
	P	0.08	0.079	0.432	0.18	**0.006****	**0.019***	0.082	**0.001****	0.981	
	N	21	21	21	11	**21**	**21**	21	**21**	21	
Chlorophyll	r	0.442	0.439	**0.506**	0.564	0.315	0.086	0.000	0.322	-0.125	0.185
	P	0.051	0.053	**0.023***	0.090	0.176	0.719	1	0.166	0.598	0.435
	N	20	20	**20**	10	20	20	20	20	20	20

****Correlation significant at P≤0.01 level (2-tailed)**

*** Correlation significant at P≤0.05 level (2-tailed)**

3.3. Heavy metal concentrations in water, suspended matter and sediment

Cadmium, copper, zinc and iron concentrations in water samples (filtrate <0.45) were <LOD at all four sampling sites (Trout Site 1, Trout Site 2, Carp Site 1 and Carp Site 2). Cadmium concentrations in suspended matter showed significant site-specific differences (P=0.019). Values <LOD were omitted from the calculations. (Fig 24).

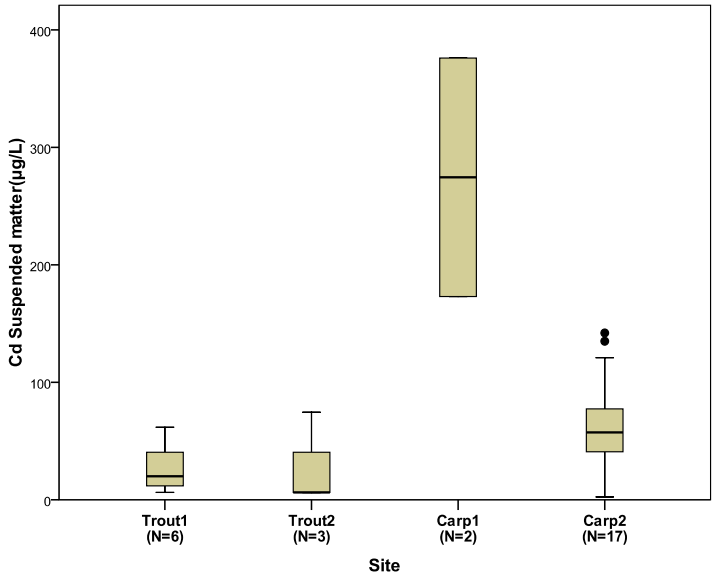


Fig 24. Cadmium concentration in the suspended matter from four fish sampling sites (farmed fish)

Table15. Cadmium, copper, zinc and iron concentrations in the sediments collected from farmed and wild fish sites (sediment size ≥ 1mm)

Sampling Site	Date		Cd (µg/kg DW)	Cu (mg/kg DW)	Zn (mg/kg DW)	Fe (mg/kg DW)
Trout Site 1	Mar 07		878	42	634	17006
Carp Site 1	Mar 07		2019	9	456	26480
	Jul 07		104	3.7	6.55	8051
	Oct 07		451	15.8	_	_
		Median	**451**	**9**	**231**	**17266**
Carp Site 2	Jun 07		9	0.5	30	8928
	Jul 07		29.4	12	3.33	_
	Jul 07		174	_	_	4599
	Aug 07		26.9	4.3	_	4760
	Aug 07		14.7	4	69.5	8038
	Jul 08		851	33.8	_	35734
	Jul 08		834	21.2	324	40624
		Median	**29.4**	**8.15**	**49.8**	**8483**
Lobau	Jun 08		_	20.3		
	Jun 08		668	25.1	198	19116
	Jun 08		633	23.7	202	23658
		Median	**650**	**24**	**200**	**21387**
Neusiedlersee	Aug 08		281	10	78.4	11898
	Aug 08		368	8	71.3	7813
		Median	**325**	**9**	**75**	**9856**
Untertalbach	Aug 08		203	5	143	20595

Sediment samples from natural pools for trout were difficult to collect because they have a coarse bottom surface; artificial pools were made of concrete and lacked sediment. Sediment collected from three fish sites (Trout Site 1, Carp Site 1, Carp Site 2) showed relatively high concentrations of Cd, Cu, Zn and Fe in the following sequence: Trout Site 1 › Carp Site 1 › Carp Site 2. No significant differences, however, were found between these three sites for these metals (P>0.05). The same result was found between sediments from wild fish sites, where no significant difference between sites for the four heavy metals was detected (P>0.05) (Table 15).

3.4. Zooplankton

Table 16. Cadmium, copper, zinc and iron in zooplankton from two carp sites

Site	Date	Cd(µg/kg)	Cu(mg/kg)	Zn(mg/kg)	Fe(mg/kg)
Carp Site 1	22.05.2007	44	1.3	_	1309
	23.07.2007	108	_	39.2	931
Carp Site 2		**Cd(µg/kg)**	**Cu(mg/kg)**	**Zn(mg/kg)**	**Fe(mg/kg)**
	10.05.2007	56.2	8.5	328	_
	21.05.2007	87.9	8.6	<LOD	_
	01.06.2007	64.3	<LOD	28.2	611
	14.06.2007	88	1.02	_	1053
	26.06.2007	60.5	<LOD	_	
	07.07.2007	77.1	<LOD	_	1310
	19.07.2007	79.6	<LOD	_	989
	01.08.2007	639	<LOD	34	
	12.08.2007	100	<LOD	_	3019
	24.08.2007	_	<LOD	_	_
	06.09.2007	_	<LOD	48.5	_
	18.09.2007	167	0.48	38.6	635
	28.09.2007	145	<LOD	20.3	594
	11.10.2007	53.8	<LOD	38.6	531
	24.10.2007	149	<LOD	20.4	_
	05.11.2007	33.1	<LOD	12.8	459

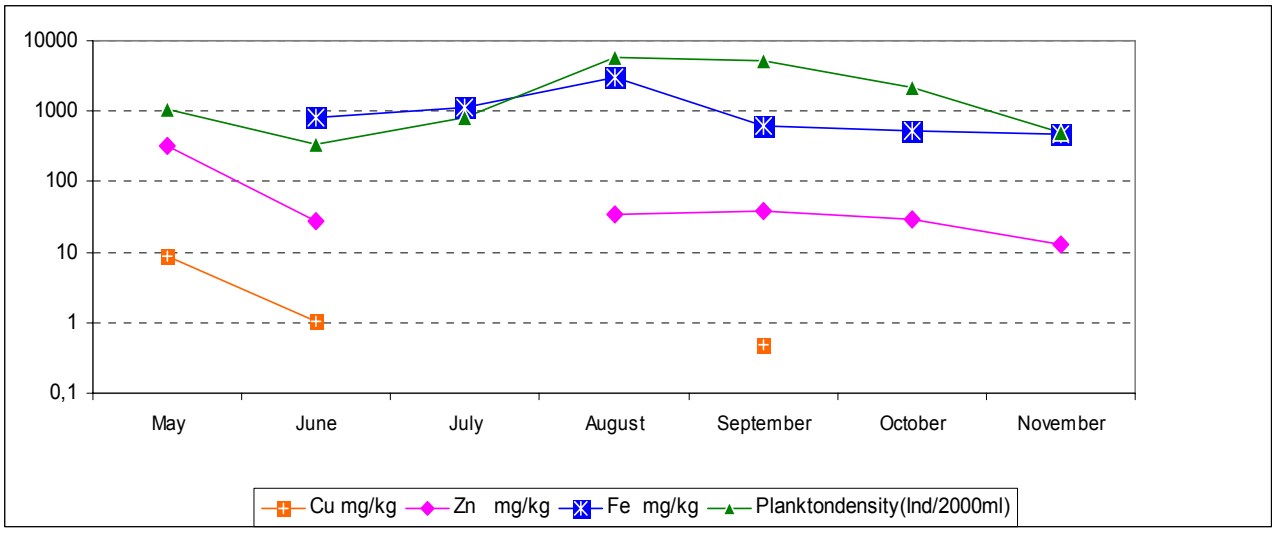


Fig 25. Copper, zinc, iron concentrations and planktonic density variation over different at months 2007 in Carp Site 2 water body.

The following parameters were recorded over 2007. Planktonic density from Carp Site 2 was observed monthly from May until November. Cadmium, copper, zinc and iron content were analysed. Cadmium and iron in zooplankton followed the planktonic density changes (Fig 26). Zinc also followed the planktonic density change in general.

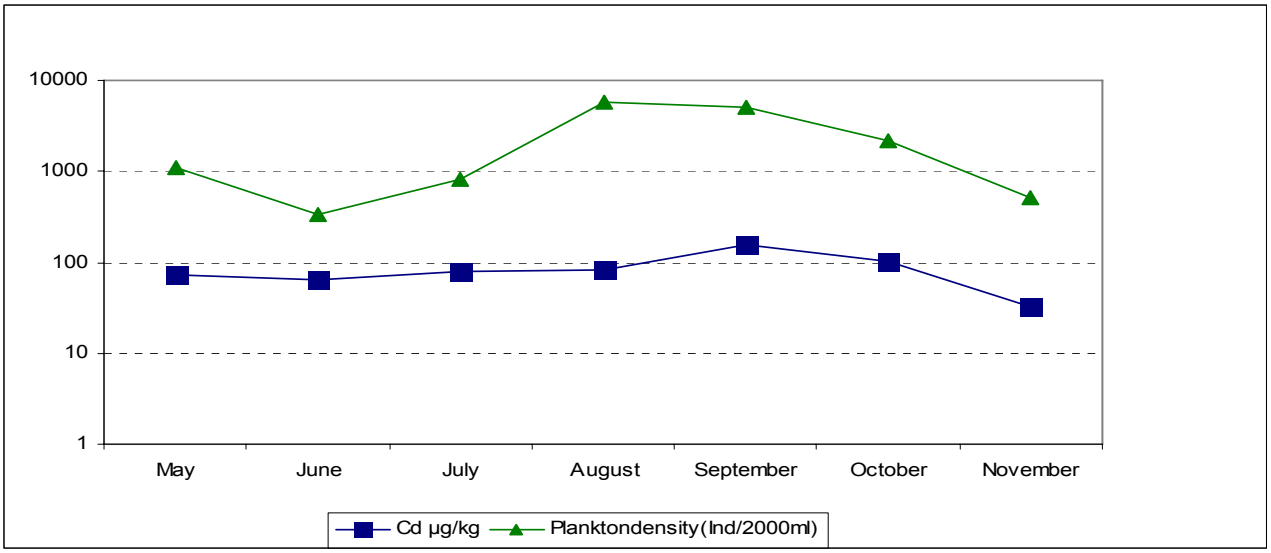


Fig 26. Cadmium concentrations and planktonic density variation over different months at 2007 in Carp Site 2 water body.

The cadmium concentration in zooplankton in Carp Site 2 reached its maximum (167 µg/L) in September 2007. The zinc content peaked at 48.5 mg/L in September as well, whereas the maximum iron content was measured in August 2007. Copper in most sampling dates was <LOD except in May (8.5 mg/L) (Table16).

Copepod density was positively correlated with total turbidity, organic, inorganic turbidity and temperature (r=0.886, P=0.019, N=6). Cladocera density was negatively correlated to pH (r= -0.090, P=0.037, N=5), while rotifer density showed no significant correlations to any abiotic parameter.

The zinc concentration in zooplankton correlated positively to total organic carbon (TOC) (r=0.975, P=0.005, N=5) and negatively to conductivity (r= -0.757, P=0.049, N=7).

The iron concentration in the zooplankton was positively correlated to total turbidity and organic turbidity (r=0.667, P=0.050, N=9) along with inorganic turbidity (P=0.767, r=0.016, N=9). It was also positively correlated with (TIC), (TOC) (r=0.900, P= 0.037, N=5) and DIC (r=0.850, P=0.004, N=9).

The cadmium concentration in suspended matter showed positive correlations with pH (r=0.565, P=0.023, N=16) and temperature (r=0.576, P=0.019, N=16).
Zinc and iron concentrations in suspended matter showed no significant correlation to any chemical parameter or to the metal concentrations in zooplankton.

3.5. Benthos and nekton

Table 17. Cadmium, copper, zinc and iron concentration in benthos and nekton organisms (on a wet weight basis) from four sampling sites (farmed fish)

Site	Feeding position	N Total	Date	N	Length (mm)	Weight (g)	Cd µg/kg	Cu mg/kg	Zn mg/kg	Fe mg/kg
Trout Site 1										
Lestes sp.	Pred	1	15.03.2007	1	15	0.034	_	_	29.3	-
Gammarus roeseli	Shred/Det	5	15.03.2007	5	8.2±2.3	0.077	_	_	30.8	<LOD
Nematocera	Pred	12	15.03.2007	12	12.5±12	0.033	95.7	_	80	2053
			15.03.2007	1	17	0.076	_	_	26	-
Polycelis felina	Pred	4	15.03.2007	3	17±0.7	0.17	_	_	81.2	26.13
			15.03.2007	1	14	0.207	6.7	5.63	30.9	<LOD
Lymnaea peregra f. ovata	Shred/Graz	**2**	28.08.2007	1	12	0.21	693	275	39.1	371.2
Trout Site 2										
Lymnaea peregra f. ovata	Shred/Graz	2	20.03.2007	1	12	0.221	33.3	2.13	0.28	-
			20.03.2007	1	15	0.463	_	_	49.6	<LOD
Gammarus roeseli	Shred/Det	4	20.03.2007	1	10	0.016	58.9	2.4	66.3	<LOD
			25.05.2007	3	14.7±0.4	0.048	32.2	_	45.7	<LOD
Gammarus pulex	Shred	22	25.05.2007	6	8.5±1.4	0.044	352	<LOD	0.2	-
			25.05.2007	6	9.2±2.3	0.035	59.7	_	55.1	52.7
			18.09.2007	10	9.2±1.9	0.159	_	_	26.2	9.66
				52						
Carp Site 1										
Larvae *Anabolia* sp.	Shred/Graz	1	29.03.2007	1	19	0.113	95.3	_	38.7	81
Lestes sp.	Pred	2	29.03.2007	1	14	0.019	338	10.7	13.1	86.9
			22.05.2007	1	11	0.015	883	7.36	23.8	<LOD
Larvae *Baetis* sp.	Graz	5	29.03.2007	5	10,4±0.9	0.027	58.3	<LOD	57	1064
Corixidae sp.	Det	17	23.10.1007	1	9	0.012	17.8	9.8	12.7	<LOD
			22.05.2007	2	5±1	0.017	304	12.1	107	<LOD
			10.05.2007	6	6±0,8	0.065	8.3	9.5	35.4	<LOD
			23.07.2007	8	6.9±1.1	0.076	_	_	<LOD	<LOD
Ilyocoris cimicoides	Pred	7	22.05.2007	7	2.6±0.5	0.03	32.1	<LOD	11.8	<LOD
Carp Site 2										
Corixidae sp.	Det	54	21.05.2007	5	5.8±0.7	0.055	-	-	47	78.8
			11.10.2007	6	8±0.8	0.052	-	<LOD	48.3	17.6
			14.06.2007	6	7.2±1.3	0.075	52.1	6.61	26.1	<LOD
			24.08.2007	8	6.5±1.1	0.077	31.3	6	26.6	20.8
			01.06.2007	8	6.4±1.3	0.08	70.5	<LOD	43	52.2
			18.09.2007	9	8±2.3	0.141	25.4	5.3	22.6	47.9
			16.04.2007	12	6±2.3	0.201	_	_	29.5	14.5
Ilyocoris cimicoides larvae *Ilyocoris cimicoides*	Pred	1	21.05.2007	1	12	0.152	_	_	9.1	581
	Pred	2	10.05.2007	1	5	0.003	1241	<LOD	159	<LOD
			26.06.2007	1	4	0.005	_	<LOD	91.6	<LOD
Notonecta sp.	Pred	2	21.05.2007	1	16	0.135	67.4	_	5.82	1762
			12.08.2007	1	15	0.129	_	<LOD	51.4	<LOD
Lestes sp.	Pred	2	10.05.2007	1	15	0.043	115	12.2	550	<LOD
			14.06.2007	1	12	0.021	61.2	2.13	2.31	<LOD
Chydorus sphaericus	Graz/Det	8	21.05.2007	8	0.5±0.1	0.005	63.5	<LOD	<LOD	<LOD
Dugesia lugubris	Pred	2	01.06.2007	2	19.5±0.5	0.575	0.8	<LOD	21.1	75
Larve *Baetis* sp.	Graz/Det	16	07.07.2007	8	6.5±1.1	0,025	_	<LOD	91	_
			12.08.2007	8	8±1.7	0.06	1009	2.5	207	646
Frog larvae		1	21.05.2007	1	20	0.135	1.4	0.82	55	_
		119								

3.6. Food Supplements

The comparison of Cadmium, copper, zinc and iron between trout 1, trout 2 and carp 1 food supplement (DAN-EX) and between carp 1 cereals. All four metals showed significant difference between sites ($P<0.05$).

Table 18. Cadmium, copper, zinc and iron concentrations in food supplements

Trout Site 1

Date	Food Supplement	Cd µg/kg	Cu mg/kg	Zn mg/kg	Fe mg/kg
20.03.2007	DANEX 1640	970	13.3	<LOD	_
	DANEX f.young F.	846	8.8	137	<LOD
	DANEX Feed A/S	829	14.3	285	136
04.08.2007	DANEX std.small.	615	22	226	72
	DANEX std.Large.	613	15.7	141	28.6
19.11.2007	DANEX 1640	622	9.3	183	126
	Mean	776	12.3	186	91
	Median	726	14	183	99
	SD	153	4.8	96.9	61

Trout Site 2

Date	Food Supplement	Cd µg/kg	Cu mg/kg	Zn mg/kg	Fe mg/kg
20.03.2007	TROCO	251	11.6	33	28
	DANEX 1640	286	2.78	_	_
	DANEX 2242	206	2.4	29.3	80.7
	DANEX 1640	409	16.4	_	_
27.08.2007	DANEX 1640	228	6.3	22	63.8
	DANEX f.young F.	251	13.3	27.7	30.9
	Mean	272	8.4	31.6	50.9
	Median	251	9	28.5	47.4
	SD	72.3	5.8	14.9	32.9

Carp Site 1

Date	Food Supplement	Cd µg/kg	Cu mg/kg	Zn mg/kg	Fe mg/kg
27.05.2007	DAN-EX	297	6.2	37.8	_
28.05.2007	DAN-EX	139	5.7	156	1.8
	Mean	218	6	97	1.8
	Median	218	5.95	97	1.8
	SD	111	0	84	1
22.05.2007	Cereal	50.7	6.94	53.3	66.5
23.05.2007	Cereal	49.9	3.17	183	31.8
	Mean	50.3	10.1	118	49.2
	Median	50	5	118	49
	SD	0.6	2.7	92	24.5

3.7. Fish

3.7.1. Trout

Forty-eight trout samples were collected from Trout Site 1 in August and November of 2007. Also, forty-eight trout were sampled from Trout Site 2 at the same time as site 1. Different ages and sizes were collected. Size and weight at age ≥ 11 months differed in the two sites; in site 2 the average size and weights were higher than at site 1. Average size (14±2cm versus 22 ±1.3cm) and average weight (35 ±11g versus 122±28g) showed a highly significant difference between the two sites (P=0.000). Also, trout age group 2 (11-23 months) showed a significant difference (P<0.05) in average size (28±2cm versus 30±3cm) and average weight (261±47g versus 310±36 g) between the two sites. This was not the case for age group 3 (≤24 months): 33±2cm versus 33±1cm and 413±55 g versus 409±33 g.

Table 19.Cadmium concentrations (µg/kg WW) in trout sites

Cd	Trout1						Trout2					
(µg/kg)	Muscle	Gut	Liver	Gonad	Gills	Brain	Muscle	Gut	Liver	Gonad	Gills	Brain
TOTAL N	45	36	24	8	5	23	48	38	23	24	24	24
Median	0.23	53.3	6.2	2.1	0.7	1.14	0.9	31.2	9	1.2	0.9	1.1
Mean	0.3	73	6.1	2.2	0.8	1.3	1.5	35.5	9	3.5	0.9	1.5
Max	1	285	13	4	1	4	7.6	92	28	16.1	3.1	3.3
Min>LOD	0.05	20	0.34	0.65	0.5	0.12	0.1	1.1	0.3	0.8	0.7	0.5
SD	0.3	61.4	2.8	1.13	0.31	1	1.5	22	7.8	4.1	0.6	1
N>LOD	29	36	24	8	5	23	48	37	18	16	16	24
N<LOD	16							1	5	8	8	

Table 20. Correlation of cadmium concentrations in trout organs from both sites

		Cd Muscle (µg/kg)	Cd Gut (µg/kg)	Cd Liver (µg/kg)	Cd Gonad (µg/kg)	Cd Gills (µg/kg)	Cd Brain (µg/kg)
Total length (cm)	r	**0.403**	**-0.252**	-0.151	**-0.462**	-0.297	**-0.812**
	P	**0.000****	**0.026***	0.291	**0.005***	0.083	**0.000****
	N	104	78	51	35	35	49
Weight (g)	r	**0.332**	-0.145	-0.146	**-0.483**	-0.283	**-0.812**
	P	**0.001****	0.206	0.308	**0.003***	0.1	**0.000****
	N	104	78	51	35	35	49
Cd Muscle (µg/kg)	r		**-0.313**	0.308	**-0.533**	-0.256	-0.226
	P		**0.007***	**0.037***	**0.002***	0.15	0.131
	N		73	46	30	33	46
Cd Gut ((µg/kg)	r			-0.193	-0.342	-0.145	-0.053
	P			0.178	0.048	0.421	0.722
	N			50	34	33	48
Cd Liver (µg/kg)	r				0	0.139	0.081
	P				0.999	0.448	0.585
	N				34	32	48
Cd Gonad (µg/kg)	r					0.758	**0.464**
	P					0	**0.007***
	N					27	33
Cd Gills (µg/kg)	r						**0.531**
	P						**0.002***
	N						31

****Correlation significant at P≤0.01 level (2-tailed)**

***Correlation significant at P≤0.05 level (2-tailed)**

Total length correlated with weight highly positively in trout. Cadmium concentrations in the muscle showed a significant positive correlation to body size, while gonads and brain were negatively correlated with body size. Cadmium concentrations in gonads correlated positively with concentrations in the brain and gills. In addition, the muscle values showed a negative correlation with those of the gut contents. Also, the values in muscle and liver tissue were significantly positively correlated.

Table 21. Copper concentrations (mg/kg WW) in trout sites

Cu (mg/kg)	Trout1						Trout2					
	Muscle	Gut	Liver	Gonad	Gills	Brain	Muscle	Gut	Liver	Gonad	Gills	Brain
TOTAL N	32	41	30	8	14	20	16	15	37	23	24	40
Median	0.31	2.9	71.7	1.15	0.22	0.22	0.3	3.7	68	0.2	0.2	1.1
Mean	0.3	3.5	84.2	0.6	0.2	0.2	0.3	3.6	72	0.4	0.2	1.1
Max	0.37	38	290	1	_	_	0.4	11	178	3.5	0.2	2.5
Min>LOD	0.24	0.2	7.8	1.2	_	_	_	0.32	14.8	0.17	0.2	0.6
SD	0.03	5.9	70.6	0.53	_	_	_	2.9	42.5	0.7	0	0.7
N>LOD	9	32	30	5	_	_	_	1	37	19	_	32
N<LOD	23	9		3	14	20	16	14		4	24	8

Correlation of copper concentrations in trout organs from both sites

Copper concentrations in the liver correlated positively with body size ($P<0.05$). Values in other organs such as muscle and gills (making up 50% of the total number of samples) were below the detection limit.

Table 22. Zinc concentrations (mg/kg WW) in trout sites

Zn (mg/kg)	Trout1						Trout2					
	Muscle	Gut	Liver	Gonad	Gills	Brain	Muscle	Gut	Liver	Gonad	Gills	Brain
Total N	14	20	21	_	13	22	21	6	20	2	18	22
Max	9	450	43	_	374	34	3.6	45.3	23.4	28.5	189	8.3
Min	0.6	4.9	13.4	_	4.5	2	3.6	43.8	24.5	28.5	187	8.4
Median	4.3	44	27.7	_	161	8.6	4.7	63.1	34.3	31	485	11.1
Mean	4.3	77.6	29.3	_	160	10.5	2.5	27.7	16.7	26.1	23.4	5.7
SD	1.9	96	7.3	_	101	6.4	0.7	13.3	4.4	3.4	121	1.4

Correlation of zinc concentrations in trout organs from both sites

The correlation between zinc concentrations in trout organs in general from both sites showed no significant differences between organs.

Table 23. Iron concentration (mg/kg WW) in trout sites

Fe (mg/kg)	Trout1						Trout2					
	Muscle	Gut	Liver	Gonad	Gill	Brain	Muscle	Gut	Liver	Gonad	Gill	Brain
TOTAL N	24	22	23	8	13	24	23	11	19	21	24	23
Median	2.42	77	57.6	46.4	30	21	2.4	130	81.4	33.5	28	12
Mean	21.7	72	56.5	36.8	29.7	20.2	18.6	148	85.5	38.7	29.5	13.5
Max	118	170	101	59	48	71	80.4	297	128	91.5	62.3	22.4
Min>LOD	2.4	12.7	27.6	19	16.2	3	10.5	75	30		12.3	1.4
SD	38	51.4	19	18.8	9.1	18.2	25.4	117	26	31.7	13.2	6.2
N>LOD	12	22	23	7	13	21	8	8	19	18	24	22
N<LOD	12			1			15	3		3		

Table 24. Correlation of iron in trout organs from both sites.

		Fe Gut (mg/kg)	Fe Liver (mg/kg)	Fe Gonad (mg/kg)	Fe Gills (mg/kg)	Fe Brain (mg/kg)
Total length (cm)	r	0.136	-0.047	**0.563**	**0.379**	**0.379**
	P	0.417	0.751	**0.001****	**0.012***	**0.006****
	N	38	48	33	43	51
weight (g)	r	0.1	-0.104	**0.523**	**0.379***	**0.393**
	P	0.551	0.483	**0.002****	**0.012**	**0.004****
	N	38	48	33	43	51
Fe Gut (mg/kg)	r		**0.404**	0.198	**0.654**	0.221
	P		**0.013***	0.39	**0.000****	0.188
	N		37	21	26	37
Fe Liver (mg/kg)	r			-0.14	0.322	0.03
	P			0.479	0.055	0.843
	N			28	36	47
Fe Gonad (mg/kg)	r				0.28	0.259
	P				0.128	0.153
	N				31	32
Fe Gills (mg/kg)	r					**0.448**
	P					**0.004****
	N					40

****Correlation is significant at P≤0.01 level (2-tailed)**

***** Correlation is significant at P≤0.05 level (2-tailed)**

The situation of iron accumulation in muscle could not be evaluated because more than 50% of the muscle samples of trout were below the detection limit. All other organs were within detection limit. Body size was positively correlated with gonad, gill and brain iron content. Values in the gut were correlated significantly with those in liver tissues and highly significantly with gills. A highly significant positive correlation was detected between gill and brain tissues.

Heavy metals concentrations in trout organs and variation with age and season.

Cadmium concentrations in muscle tissue did not show significant differences between age groups in August 2007 (P=0.054). Cadmium concentrations in gonadal, gill and brain tissue in November 2007 were highly significantly different between three age categories (P=0.000); values in the muscle and liver also differed significantly with age (P<0.05). Copper concentrations in gut content and liver in August 2007 showed significant difference from one another (P<0.05).

Iron concentration in gut content and brain showed a significant difference from one another. In November 2007 different age groups showed highly significant difference (P<0.003) as well as iron concentrations in gonad (P=0.027) and gills (P=0.000) showed high significant difference. Iron concentrations in gills and brain increased significantly with age (P=0.000). Cadmium concentrations in muscle increased with age: November values were significantly higher than in August (P=0.019).

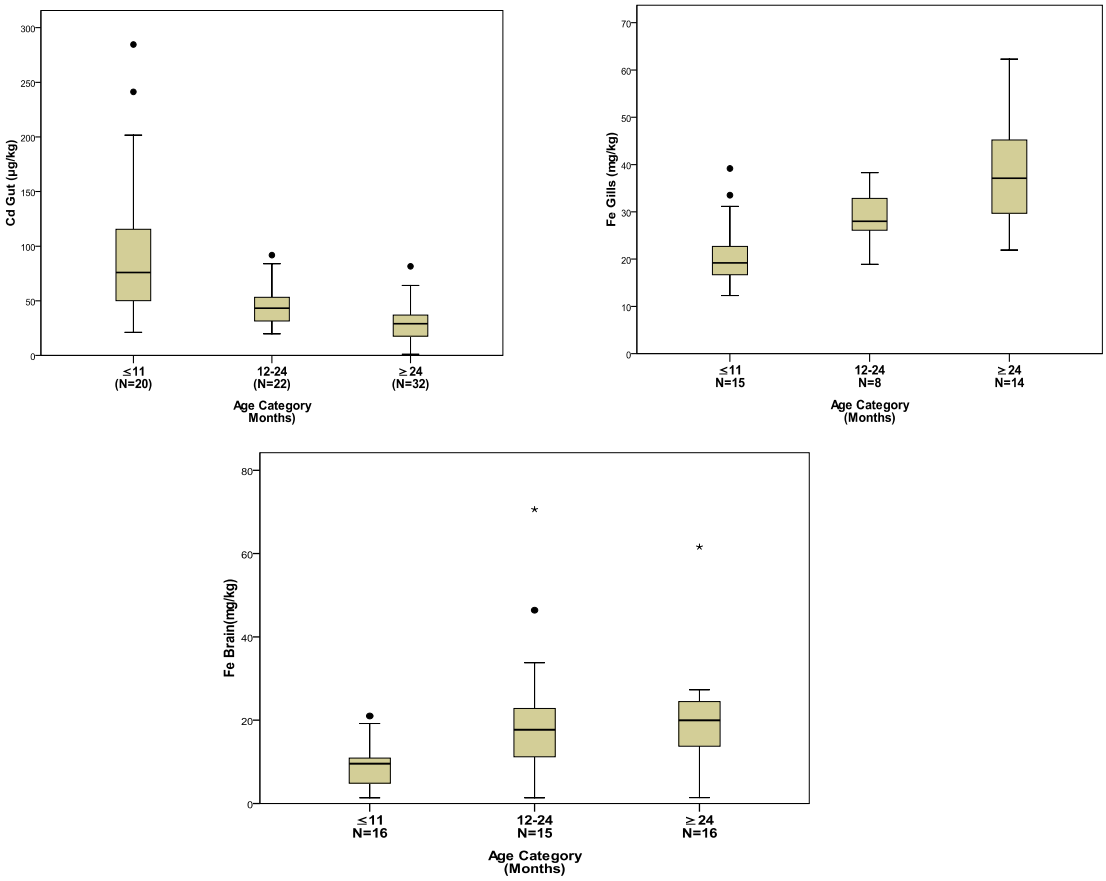


Fig 27. Comparison of cadmium, zinc and iron concentrations in trout organs and age categories.

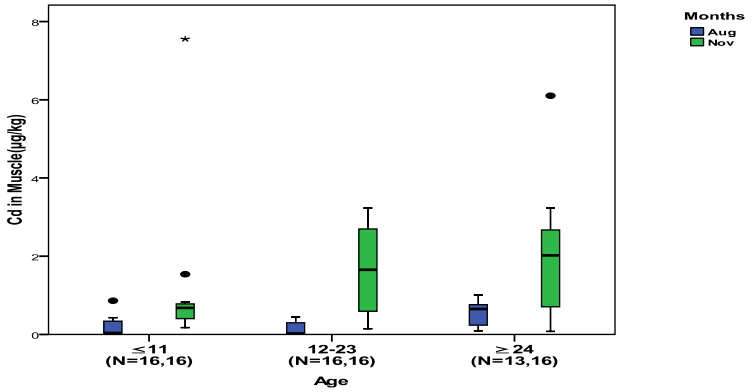


Fig 28. Seasonal variation effect on cadmium concentrations in trout muscle in different age categories with during 2007.

3.7.2. Carp

A total number of fifty-five specimens were collected from carp site 1, once in March, July and October 2007. A total of 21 fish were collected from carp site 2 in August and November 2007. Size and weight of one year old carp were not different between the 2 sites (12±6 cm versus 14±2.1 cm; 34±125 g versus 48±14.1 g) (P>0.05). Two years fish were collected from site 2 only: length (30.8±10 cm) and weight (910±582 g). Three years carp showed a slight difference between the two sites in length (37±7 cm versus 50±2 cm) (P=0.056), whereas weight (1322±440 versus 1873±4.3 g) did not differ (P>0.05). Some organs such as gut contents and gonads were not available.

Table 25. Cd concentrations (µg/kg WW) in carp sites

Cd (µg/kg)	Carp1						Carp2					
	Muscle	Gut	Liver	Gonad	Gills	Brain	Muscle	Gut	Liver	Gonad	Gill	Brain
Total N	40	36	21	16	31	22	19	18	7	_	8	6
Median	0.3	7.7	3.7	1.2	1.64	0.7	0.3	11.2	3.8	_	4.1	0.7
Mean	1	12.3	5.5	1.2	6.2	0.7	0.3	67	8.7	_	2.2	4.6
Max	4.8	47	13.2	3.6	26	1.5	0.7	255	24.3	_	8.13	12
Min>LOD	0.1	0.6	2	0.31	0.6	0.2	0.05	10	2	_	0.1	1.2
SD	0.1	15	3.5	0.75	4.1	0.4	0.13	69	8.2	_	2.6	4
N>LOD	20	25	21	16	31	22	11	18	7	_	8	6
N<LOD	20	11					8					

Correlation of cadmium concentrations (µg/kg) in carp organs from both sites

Cadmium contents in muscle correlated negatively with weight ($P<0.05$).

Gill values were highly positively correlated with gonads ($r= -0.584$, $P=0.009$, $N=19$).

Table 26. Cu concentrations (mg/kg WW) in carp sites

Cu (mg/kg)	Carp1						Carp2					
	Muscle	Gut	Liver	Gonad	Gills	Brain	Muscle	Gut	Liver	Gonad	Gill	Brain
Total N	54	42	24	19	16	32	21	21	8	_	8	7
Median	0.3	1.3	1	0.3	0.06	0.04	0.2	1.32	1.3	_	0.06	0.06
Mean	0.27	1.7	1.62	0.6	0.06	0.1	0.1	1	0.4	_	0.1	0.1
Max	0.42	6.8	5	3	0.06	0.6	0.31	4.2	0.43	_	0.13	0.08
Min>LOD	0.01	0.01	0.43	0.2	_	0.08	0.02	0.3		_	0.12	
SD	0.1	1	1.5	0.8	0	0	0.1	0.72	0.43	_	0.12	0.08
N>LOD	7	42	19	16		28	8	13	_	_	4	_
N<LOD	47		5	3	16	4	13	8	8		4	7

Correlation of copper concentrations in carp organs from both sites

Copper concentrations in the gut contents showed a significant positive increase with total length and weight ($r=0.291$, $P=0.088$, $N=82$). Copper concentrations in liver tissue showed a significant positive increase with weight ($r=0.424$, $P=0.004$, $N=44$), while copper concentrations in the other organs did not.

Table 27. Zinc concentrations (mg/kg WW) in carp sites

Zn (mg/kg)	Carp1						Carp2					
	Muscle	Gut	Liver	Gonad	Gills	Brain	Muscle	Gut	Liver	Gonad	Gills	Brain
Total N	23	22	6	5	30	22	3	_	5	_	5	2
Median	8	106	77	123	30	18	8.2	_	88.5	_	71.6	29.2
Mean	8.2	118	99	121	52.9	19.8	10.2	_	73.8	_	98.4	29.2
Max	13	300	184	148	179	37	19.9	_	101	_	193	29
Min>LOD	4.4	31	41.2	69.7	10.9	8.57	2.6	_	33.1	_	61	29
SD	2	74	60	32	53	9	8.8		32		54.7	0.29

Table 28. Correlation of zinc concentration in carp organs from both sites

		Zn Muscle (mg/kg)	Zn Gut (mg/kg)	Zn Liver (mg/kg)	Zn Gonad (mg/kg)	Zn Gills (mg/kg)	Zn Brain (mg/kg)
Total length (cm)	r	**- 0.299**	0.157	0.061	0.872	**0.623**	- 0.046
	P	**0.028***	0.382	0.824	0.054	**0.000****	0.801
	N	54	33	16	5	50	33
weight (g)	r	**- 0.381**	0.205	- 0.021	**0.900**	**0.497**	- 0.216
	P	**0.004****	0.252	0.94	**0.037***	**0.000****	0.227
	N	54	33	16	5	50	33
Zn Muscle (mg/kg)	r		0.09	- 0.252	-0.3	- 0.213	**0.485**
	P		0.691	0.43	0.624	0.225	**0.012***
	N		22	12	5	34	26
Zn Gut (mg/kg)	r			**0.714**	**0.900**	0.027	0.246
	P			**0.047***	**0.037***	0.9	0.271
	N			8	5	24	22
Zn Liver (mg/kg)	r				.	- 0.198	0.503
	P				.	0.517	0.095
	N					13	12
Zn Gonad (mg/kg)	r					0.7	**0.900**
	P					0.188	**0.037***
	N					5	5
Zn Gills (mg/kg)	r						0.243
	P						0.213
	N						28

****Correlation is significant at P≤0.01 level (2-tailed)**

***** Correlation is significant at P≤0.05 level (2-tailed)**

Zinc in muscle was negatively correlated with weight and length. Gill tissue showed a positive correlation with body size. Zinc concentrations in gut content correlated positively with liver and gills, as did zinc in brain with muscle and gonads.

Table 29. Fe concentrations (mg/kg WW) in carp sites

Fe	Carp1						Carp2					
(mg/kg)	Muscle	Gut	Liver	Gonad	Gill	Brain	Muscle	Gut	Liver	Gonad	Gill	Brain
Total N	16	20	14	_	22	15	_	_	8	_	8	7
Median	0.05	95.4	47.8	_	56	27.2	_	_	36.02	_	51	7.4
Mean	0.46	103	77.4	_	61.8	29.6	_	_	37.5	_	136	9.3
Max	3.5	243	309	_	139	72.2	_	_	51.5	_	426	22.8
Min>LOD	0.1	4.5	24	_	17	8.9	_	_	29	_	21.4	7.4
SD	0.98	84.5	85.5	_	29	15	_	_	7.26	_	161	6.8
N>LOD	12	17	14	_	22	15	_	_	8	_	8	5
N<LOD	4	3										2

Correlation of iron concentrations in carp organs from both sites

Fish length and weight were positively correlated iron concentrations in liver, gills and brain. Organs such as Muscle, gut contents, gonads were not available).

Cadmium, copper, zinc and iron in different age groups of both carp sites

Cadmium concentrations in muscle samples differed highly significantly between different age groups (P=0.000). The values in gonads, gills and brain showed significant differences between different age groups (P<0.05). The copper concentration in the gut differed significantly between different age groups (P=0.003).

Zinc concentrations in the gills and brain showed highly significant differences as age increased (P=0.000). Iron in the gills of both carp increased with age and showed significant differences (P<0.05).

Table 30. Cadmium concentrations (µg/kg WW) from both carp sites at different ages and seasons

Age		Cd Muscle	Cd Gut	Cd Gills	Cd Liver	Cd Gonad	Cd Brain
≤1 year	**N**	**29**	**26**	**23**	**15**		**17**
	Median	0.4	17.9	8.1	3.7		1.8
	Minimum	0.05	9.7	0.07	2.01		0.1
	Maximum	4.8	255	26	24		13.4
≤2 years	**N**	**8**	**8**	**8**	**6**	**8**	**5**
	Median	0.29	26	1	3.3	0.8	0.69
	Minimum	0.1	0.60	0.76	2.03	0.31	0.41
	Maximum	0.38	47.1	3	8	1.4	1.04
≤3 years	**N**	**23**	19	**8**	**7**	**8**	**8**
	Median	0.3	15.2	1.3	6.4	1.3	0.58
	Minimum	0.14	2.7	0.6	2.5	0.8	0.15
	Maximum	0.71	45	2.4	13.2	3.6	1.5

Table 31. Zinc concentrations (mg/kg WW) from both carp sites at different ages and seasons

Age		Zn Muscle	Zn Gut	Zn Gills	Zn Liver	Zn Gonad	Zn Brain
≤1 year	**N**	**11**	**3**	**19**	**5**		**8**
	Median	9.2	18	40.2	88,5		28.3
	Minimum	2.6	32	10.9	33.1		18
	Maximum	20	54	193	101		31
≤2 years	**N**	**8**	**8**	**8**	**1**	**3**	**8**
	Median	7.7	12	20.5	51	119	11.3
	Minimum	5.1	104	14.4	51	70	8.6
	Maximum	13	95	37.6	51	123	17.4
≤3 years	**N**	**7**	**11**	**8**	**5**	**2**	**8**
	Median	7.7	11	107	88.7	147	22
	Minimum	4.4	108	75.6	41.2	146	11.8
	Maximum	8.5	97	179	184	148	37

Table 32. Iron concentrations (mg/kg WW) in both carp sites at different ages and seasons

Age		Fe Muscle	Fe Gut	Fe Liver	Fe Gill	Fe Brain
≤1 year	**N**	**8**	**11**	**15**	**16**	**7**
	Median	0.05	6	34	12,5	7.4
	Minimum	0.05	1	23.8	111	4.4
	Maximum	1.99	85	54	63	23
≤2 years	**N**	**8**	**6**	**7**	**7**	**7**
	Median	0.05	15	57.3	23	27.2
	Minimum	0.05	17	42	103	8.9
	Maximum	3.52	85	309	87	72.2
≤3 years	**N**		**3**		**7**	**8**
	Median		10		22	25
	Minimum		130		120	17
	Maximum		207		90	43.3

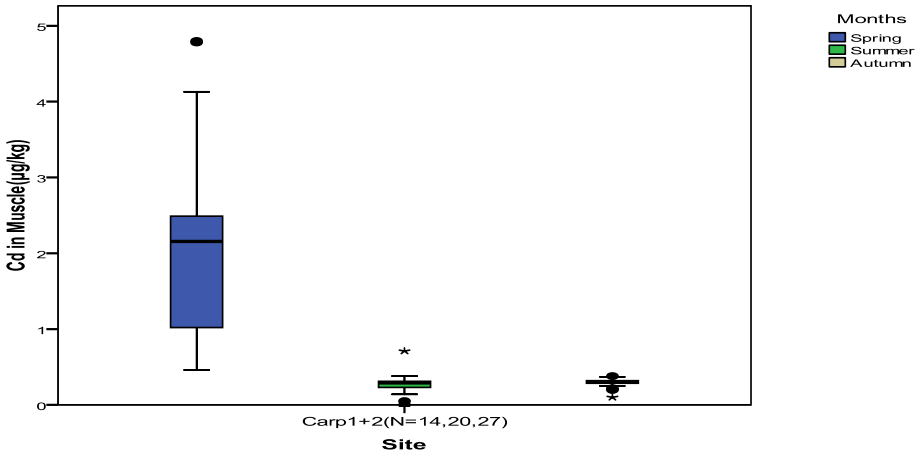


Fig 29. Cadmium concentrations in carp muscle in different seasons during 2007

The cadmium concentration in muscle showed the highest value in spring versus summer and autumn (P=0.000). Values in the brain decreased significantly with age (P=0.022).

The zinc concentration in gills increased significantly with age (P=0.001). Values in the brain differed significantly between different age groups (P=0.001). Iron concentrations in the gills increased significantly with age (P=0.019).

3.7.3. Wild and market trout and carp

Wild trout (N=5): Two trout specimens came from Gossenköllesee in Tyrol, three from Untertalbach in Styria.
Market specimens (N=9): Four samples came from aquaculture in Italy, two from aquaculture in Spain, and the remaining three from Austrian Aquaculture.

Wild carp (N=16): Thirteen carp for the sample came from Kühwörtherwasser (Lobau), two were from Waidhofen/Thaya (Waldviertel), and the last one came from Neusiedlersee.

Market fish (N=28): There were 28 samples from different fish shops and supermarkets in Vienna.

Table 33. Cadmium, copper, zinc and iron contents in trout (wild fish)

		Muscle	Gut	Liver	Gonad	Gill	Brain
Cd (µg/kg WW)	Median	2.2	3.8	828	110	156	7.4
	MAX	2.5	288	1269	115	185	10.3
	MIN	1.8	1.1	304	19.4	119	4.5
	N	2	3	4	3	4	2
Cu (mg/kg WW)	Median	1.3	3.4	34	1	0.8	1
	MAX	1.5	6	345	1.8	0.8	1.4
	MIN	0.9	1.2	11	0.2	0.7	0.5
	N	3	5	5	2	2	2
Zn (mg/kg WW)	Median	3	97.1	38	18.2	11.4	10.0
	MAX	4.7	125	38	18.2	22	12
	MIN	2.8	69.6	37.9	18.2	0.8	8.0
	N	4	2	2	1	2	2
Fe (mg/kg WW)	Median	4.6	818	507	16.6	42.4	22
	MAX	9.4	1632	781	56.2	571	43.5
	MIN	2.4	542	149	12.7	29	13.5
	N	3	5	5	4	4	4

Table 34. Cadmium, copper, zinc and iron contents in trout (market fish)

		Muscle
Cd (µg/kg WW)	Median	2.2
	MAX	15.2
	MIN	0.1
	N	9
Cu (mg/kgWW)	Median	0.3
	MAX	0.4
	MIN	0.2
	N	6
Zn (mg/kgWW)	Median	4
	MAX	4.8
	MIN	3.2
	N	2

Table 35. Cadmium, copper, zinc and iron concentrations in carp (wild fish)

		Muscle	Gut	Liver	Gonad	Gill	Brain
Cd (µg/kgWW)	Median	0.4	20.1	11.3	5.5	11.2	2.0
	MAX	14.2	39.0	14.9	6.6	11.2	33.3
	MIN	0.1	10.7	7.6	1.2	11.2	1.4
	N	16	11	2	3	1	3
Cu (mg/kgWW)	Median	0.7	2.9	0.3	0.2	0.3	
	MAX	1.2	5.3	0.3	15.3	9.0	
	MIN	0.6	1.2	0.3	0.2	0.2	
	N	14	14	2	4	4	
Zn (mg/kgWW)	Median	8.5	60.6			113	42.9
	MAX	20.7	128			202	136
	MIN	4.5	3.4			7	5
	N	9	12			9	9
Fe (mg/kgWW)	Median	1.6	368	332	5		26.7
	MAX	5.4	2594	352	5.8		29.2
	MIN	0.1	2.7	327	2.5		21.8
	N	14	10	3	3		3

Table 36. Cadmium, copper, zinc and iron contents in carp (market fish)

		Muscle	Gut	Liver	Gonad	Gill	Brain
Cd (µg/kg WW)	Median	0.62	6.2	13.4	0.40	12.6	12
	MAX	12.5	57.8	18.4	0.56	15.2	12.9
	MIN	0.18	0.55	8.4	0.24	0.41	11.3
	N	21	3	2	2	13	3
Cu (mg/kgWW)	Median	0.31	1.14	3.2	0.30	0.49	0.31
	MAX	1.03	1.67	6.14	0.32	1.26	0.36
	MIN	0.14	0.28	2	0.29	0.15	0.28
	N	23	5	10	10	10	9
Zn (mg/kgWW)	Median	5	35.1	44		120	14.3
	MAX	23.1	47.6	72.9		161	34.7
	MIN	2.9	5.1	26.9		40	1.1
	N	22	4	5		10	6
Fe (mg/kgWW)	Median	1.52	168	41.5	19.7	57	58
	MAX	117	249	75.8	35.7	90	68
	MIN	0.1	137	23.7	3.8	29.6	18
	N	22	3	3	2	16	3

3.8. Accumulation of heavy metals cadmium, copper, zinc and iron in the food chain

3.8.1. Cadmium

High cadmium concentrations were recorded in benthos and nekton (*Lymnaea peregra f. ovata, Gammarus roeselli, Lestes,* Corixidae*, Larvae Ilyocoris, Larvae Baties sp* (Table17). The cadmium concentration in fish organs, muscle, gonad and brain showed no significant difference between trout and carp P>0.05. Interestingly, the cadmium concentrations in food supplement (DAN-EX) for trout and carp were highly significantly different (488 versus 218 µg/kg, P=0.009). Gill tissues showed highly significant differences between carp and trout sites: carp sites showed half the Cd concentration of trout sites (0.8 versus 1.64 µg/kg, P=0.000).

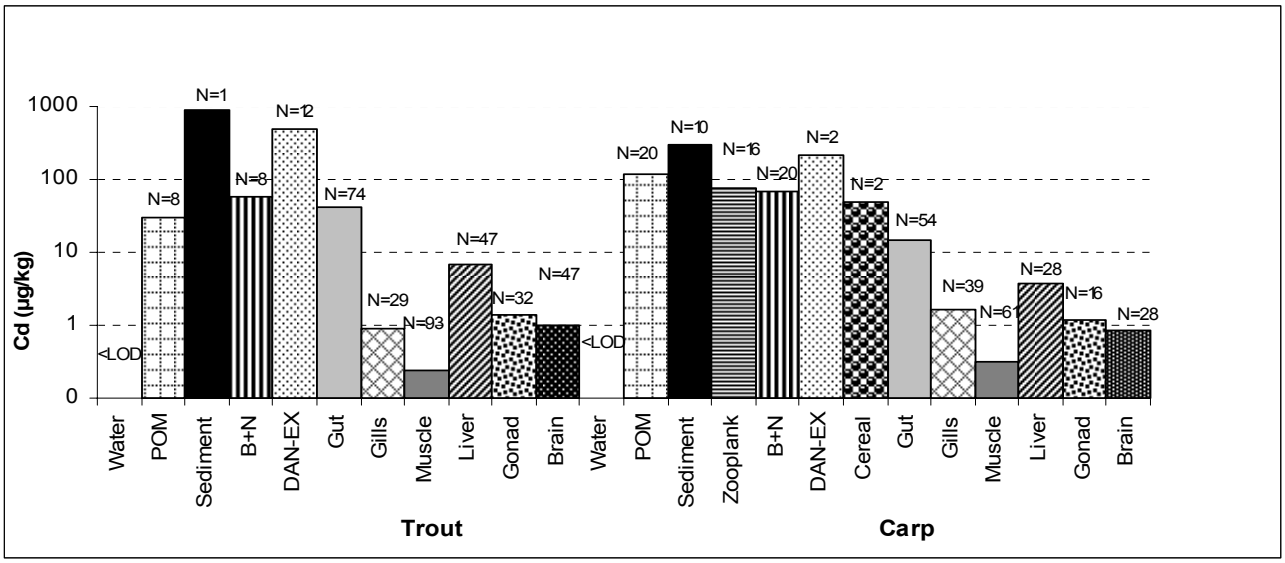


Fig 30. Median cadmium concentrations in fish and the semi-natural aquatic food chain.

3.8.2. Copper

Copper contents in benthos and nekton (*Lymnaea peregra*, *Lestes* sp., *Corixidae* were higher than in zooplankton, DAN-EX (food pellet), cereal and fish organs. Values from the surrounding living conditions for the trout sites such as sediments, benthos nekton and DAN-EX showed no difference in copper concentration (P >0.05) as well as cereals for carp. Muscle, gills, gonads and brain were <LOD at carp sites. Gut content and liver from both trout and carp sites showed highly significant differences (P=0.000).

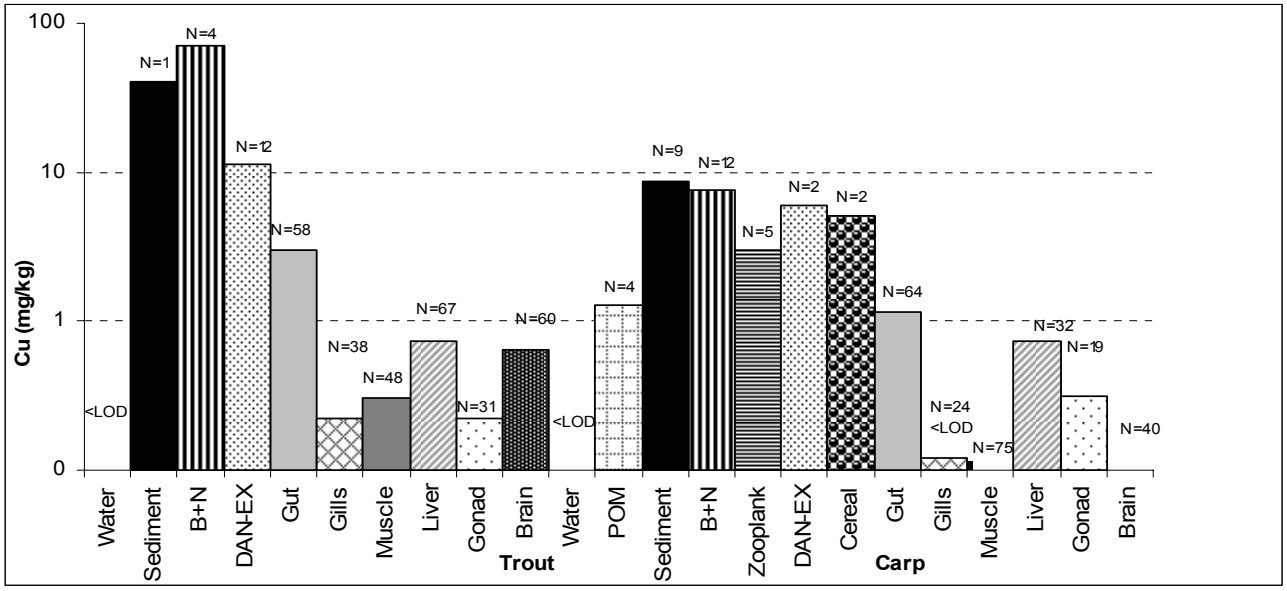


Fig 31. Median copper concentrations in fish and semi-natural aquatic food chain.

3.8.3. Zinc

High zinc concentrations in the food web were shown in DAN-EX and cereals, gut, zooplankton, benthos and nekton. Zinc content in DAN-EX used for both, trout and carp sites was similar. High significance between trout and carp were recorded in muscle, brain, benthos and nekton (B+N): 8.1 versus 4 mg/kg, 19.5 versus 8.4 mg/kg and 37.3 versus 16 mg/kg, respectively (P=0.000).

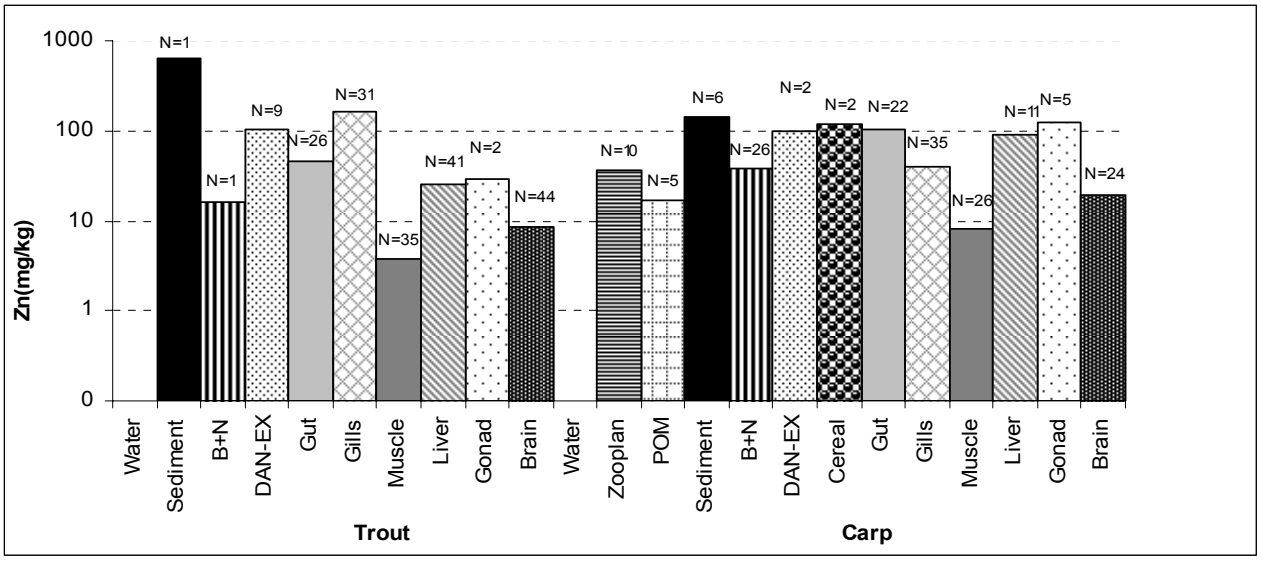


Fig 32. Median zinc concentrations in fish and semi-natural aquatic food chain.

3.8.4. Iron

Iron concentrations in zooplankton were consistently higher than in benthos and nekton, gut, DAN-EX, cereals and fish organs. The concentration in muscle from both sites showed differences (2.4 versus <LOD, P=0.000). The values in carp were twice as high as in trout with respect to gills (64 versus 28.4 mg/kg, P=0.000) and brain (25.7 versus 14 mg/kg, P=0.011). At the same time, liver from carp showed half the concentration of trout livers (67 versus 41.4 mg/kg, P=0.001). Trout gonads had three times higher concentrations than those from carp (35.4 versus11.2 mg/kg, P=0.036). Trout and carp sites did not differ with respect to gut, DAN-EX and cereal values (P>0.05).

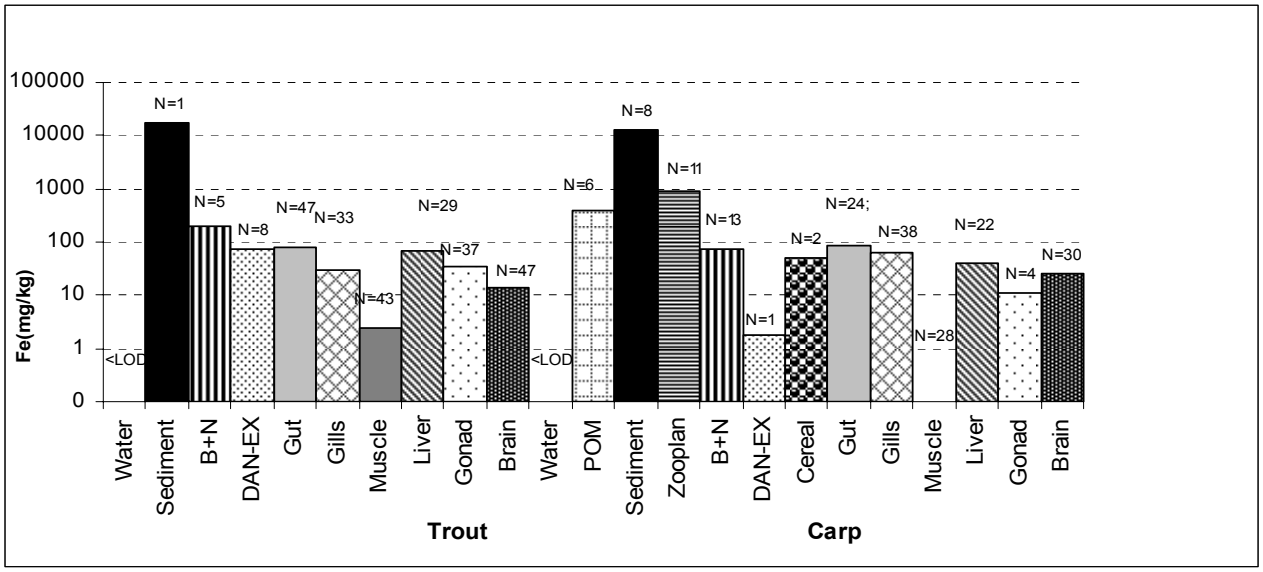


Fig 33. Median iron concentrations in fish and semi-natural aquatic food chain.

3.9. Comparison of cadmium, copper, zinc and iron between different fresh water fish, marine fish and crustaceans

3.9.1. Cadmium

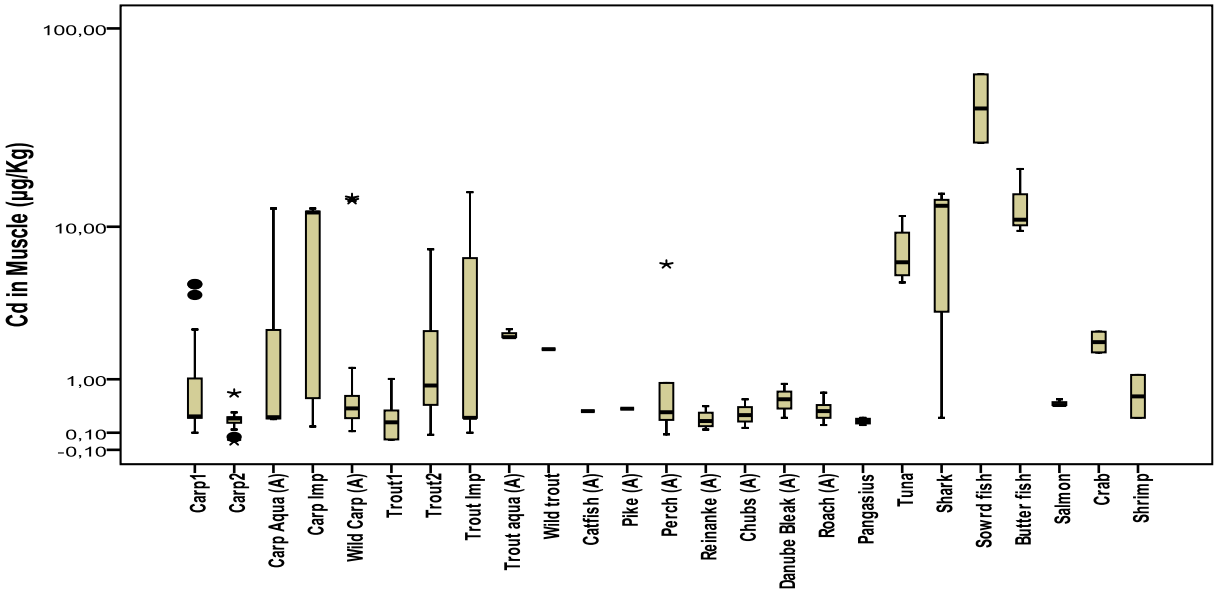


Fig 34. Cadmium concentrations in carp, trout, wild fish and marine fish (N= 40, 20, 12, 9, 16, 45, 48, 7, 13, 1, 1, 1, 6, 4, 3, 3, 5, 4, 5, 3, 2, 3, 3, 2, 2 respectively).

Marine fish such as swordfish, butterfish, shark and tuna show high concentrations of cadmium in their muscle.

Cadmium concentrations in carp muscle, gut and gills differed significantly between the two investigated sites ($p<0.05$). Concentrations in the carp muscle from different sources (import, Austrian aquaculture) were the highest in imported carp, followed by carp aquaculture in Austria ($P=0.006$). Gut values were the highest in Carp Site 2, followed by wild carp ($P=0.000$). Gills in carp from Austrian aquaculture showed the highest concentration, with a highly significance difference between the two sites ($P=0.000$).

3.9.2. Copper

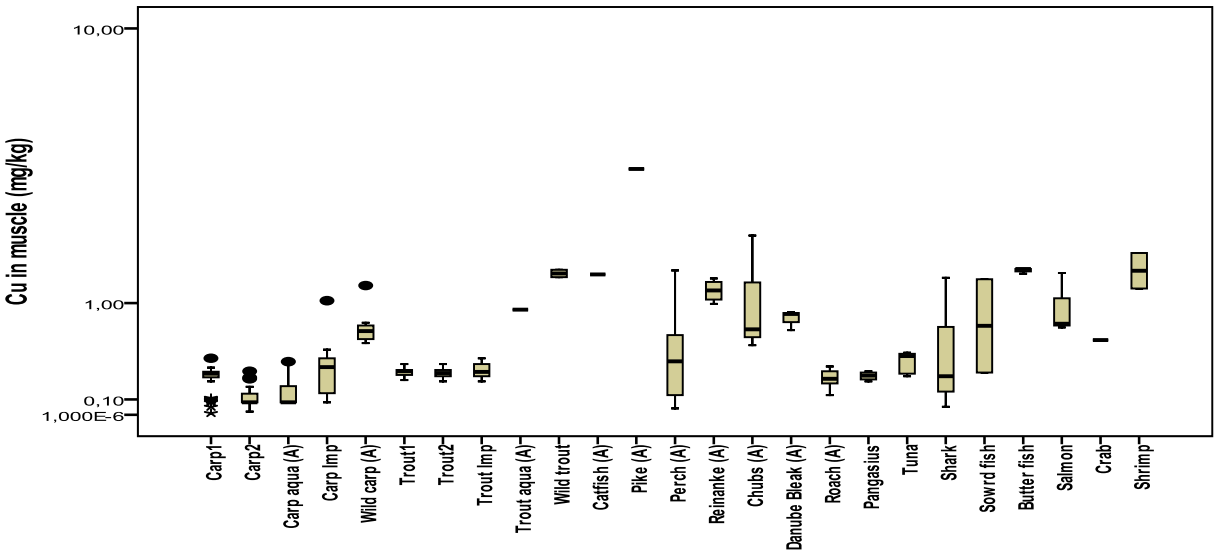


Fig 35. Copper concentrations in carp, trout, wild fish and marine fish.
(N= 54, 21, 11, 12, 14, 32, 16, 6, 1, 2, 1, 1, 6, 4, 3, 3, 7, 4, 5, 3, 2, 3, 3, 1, 2 respectively)

Copper showed elevated concentrations in the muscle of most of the wild fish samples such as pike, catfish (one of each from Neusiedlersee), followed by chubs, reinanke, Danube bleak, perch (all four from Fuschlsee), and wild carp from Austria.

3.9.3. Zinc

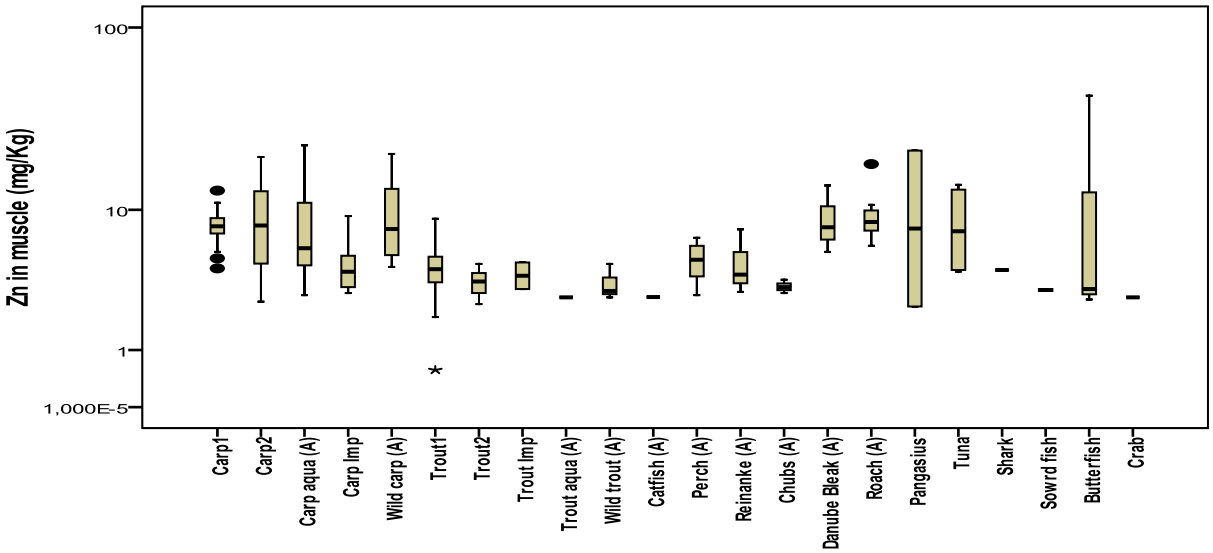


Fig 36. Zinc concentrations in carp, trout, wild fish and marine fish
(N= 23, 3, 13, 9, 7, 14, 21, 2, 1, 3, 1, 5, 3, 3, 3, 7, 2, 4, 1, 2, 3, 1 respectively)

Zinc in the following fish – Carp Site 1, Carp Site 2, wild carp Austria, Danube bleak, roach, pangasius and tuna – showed almost the same concentrations levels and were higher than in the remaining species ($P < 0.001$).

3.9.4. Iron

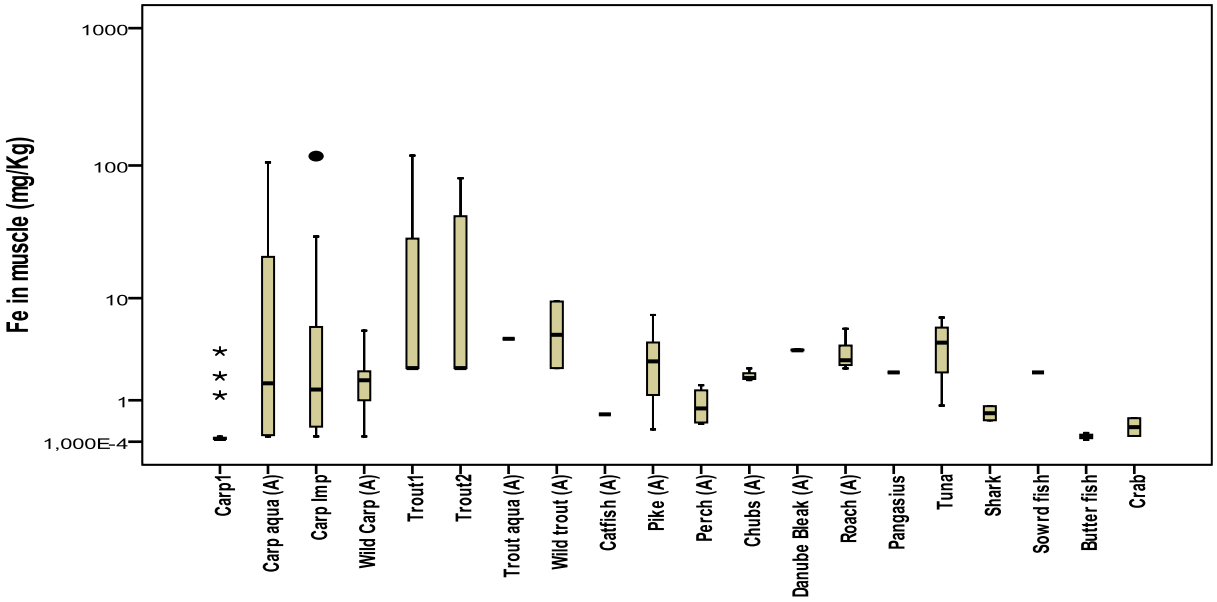


Fig 37. Iron concentrations in carp, trout, wild fish and marine fish (N = 16, 11, 11, 14, 24, 23, 11, 2, 1, 6, 4, 3, 3, 7, 1, 6, 2, 1, 3, 2 respectively)

Iron concentrations in the muscle of wild trout, tuna, Danube bleak, roach and carp from aquaculture (4.6, 4.2, 3.6, 2.9, 2.42 mg/kg) showed highly significant differences (P=0.000).

3.10. Wild and market fish samples

Table 37. Cadmium median concentrations (µg/kg WW) in wild fish from Austria

			Cd (mg/kg)					
Species	Site	N	Muscle	Gut	Liver	Gonad	Gill	Brain
Trout	Gössenköllesee Untertallbach	5	2.2	3.8	828	110	156	7.4
Carp	Lobau,Thaya, Neussiedlersee	16	0.4	17.2	11.3	5.5	11.2	2
Perch	Fuschlsee, Neusiedlersee	6	0.33	0.5	159	2.7	_	_
Chub	Fuschlsee	3	0.34	19.2	26.5	5.34	1.4	1.2
Roach	Fuschlsee	7	0.4	86	21.8	1.8	1.8	2.4
Danube Bleak	Fuschlsee	2	0.6	2.5	1.6	9.9	2.1	_
Pike	Neusiedlersee	1	<(LOD)	_	_	_	_	_
Catfish	Neusiedlersee	1	<(LOD)	_	_	_	_	_
Reinanke	Fuschlsee	4	0.36	4	_	_	_	_

Trout from Gössenköllesee and Untertalbach showed the highest average cadmium concentration in muscle, liver, gonads, gills and brain. The highest value in the gut was recorded in roach. Values in muscle of pike and catfish from Neusiedlersee were below the detection limit. Copper average concentrations in the muscle of catfish, trout, pike and reinanke were in the same range (1.2-1.4 mg/kg).

Table 38. Copper median concentrations (mg/kg WW) in wild fish from Austria

			Cu (mg/kg)					
Species	Site	N	Muscle	Gut	Liver	Gonad	Gill	Brain
Trout	Gössenköllesee	5	1.3	3.4	34	1	0.8	1
Carp	Untertalbach Lobau,Thaya, Neussiedlersee	16	0.7	2.9	0.3	0.3	0.3	0.3
Perch	Fuschlsee, Neusiedlersee	6	0.22	1.13	4.3	0.6	0.97	1.6
Chub	Fuschlsee	3	0.7	1.7	7.2	1.82	0.58	0.6
Roach	Fuschlsee	7	0.3	1.8	7.8	3	0.6	0.4
Danube Bleak	Fuschlsee	2	0.9	_	_	2.4	1.1	1.1
Pike	Neusiedlersee	1	1.2	3.6	_	15.3	9	_
Catfish	Neusiedlersee	1	1.4	1	_	_	_	_
Reinanke	Fuschlsee	4	1.2	1.9	_	_	_	_

Table 39. Zinc median concentrations (mg/kg WW) in wild fish from Austria

			Zn(mg/kg)					
Species	Site	N	Muscle	Gut	Liver	Gonad	Gill	Brain
Trout	Gössenköllesee	5	3	97	38	18.2	11.4	10
Carp	Untertalbach Lobau,Thaya, Neussiedlersee	16	7.7	55.5	_	_	128	23.6
Perch	Fuschlsee, Neusiedlersee	6	4.47	42.1	26.6	16.6	10.1	28
Chub	Fuschlsee	3	3.25	65.6	26.1	_	36.1	12.2
Roach	Fuschlsee	7	8.2	122	40.1	7.2	63.4	23.4
Danube Bleak	Fuschlsee	2	7.9	_	33.7	_	29.2	_
Catfish	Neusiedlersee	1	15	_	_	_	_	_
Reinanke	Fuschlsee	4	4	30.7	_	_	_	_

Table 40. Iron median concentrations (mg/kg WW) in wild fish from Austria

			Fe(mg/kg)					
Species	Site	N	Muscle	Gut	Liver	Gonad	Gill	Brain
Trout	Gössenköllesee	5	4.6	818	507	16.6	42.4	22.1
Carp	Untertalbach Lobau,Thaya, Neussiedlersee	16	1.57	435	332	4.96	_	26.7
Perch	Fuschlsee, Neusiedlersee	6	3.9	7	30.5	16.3	_	20.3
Chub	Fuschlsee	3	2	61	113	18,7	24.1	10.2
Roach	Fuschlsee	7	3	67	70	15	42.7	18.3
Danube Bleak	Fuschlsee	2	3.6	_	3.4	51	32.2	_
Catfish	Neusiedlersee	1	0.58	2.9	_	_	_	_
Reinanke	Fuschlsee	4	0.8	25	_	_	_	_

The highest zinc average concentration was found in catfish muscle from Neusiedlersee (15 mg/kg), almost double that found in the muscle of carp, roach and Danube bleak. Trout, perch, Danube bleak and roach showed average concentrations of iron in muscle ranging from 4.6-2.9 mg/kg. Iron concentrations in trout gut content, liver and gonads were almost double the concentrations in carp.

3.10. Gender differences

Gender was determined by mature gonad investigation. Significantly higher cadmium levels were found in the gut contents of male than in female carp at carp site 1 and wild carp from Austria (P=0.054). The remaining carp organs showed no significant difference in cadmium concentration

4. Discussion:

4.1. Characteristic of trout and carp sampling sites

4.1.1. Physico-chemical parameter

Water quality in fish ponds is affected by the interactions of many chemical components. Carbon dioxide, pH, alkalinity and hardness are interrelated and can have profound effects on pond productivity, the level of stress and fish health, oxygen availability and the toxicity of ammonia as well as that of certain metals. Most features of water quality are not constant. Carbon dioxide and pH concentrations fluctuate or cycle daily. Alkalinity and hardness are relatively stable but can change over time, usually weeks to months, depending on the pH or mineral content of the watershed and bottom soils (Tucker, 1984). In the present study the physico-chemical parameters such as temperature (°C), pH, turbidity, hardness, conductivity, DIC, TIC, DOC, TOC and chlorophyll as indicator of primary production were measured from the two trout sites (Tables 9,10) and two carp sites (Tables 11,12). Trout sites showed higher conductivity, hardness, DIC and TIC than carp sites, while carp sites showed higher temperature (°C), turbidity, chlorophyll and pH-values than trout sites. Oxygen values were higher in site 2 for both carp and trout, as shown in Fig. 23. The highest temperatures of the four sites were as follows: Trout Site 1 showed the highest in May (15°C), Trout Site 2 in August (15.9°C), Carp Site 1 in late July (23.5°C) and Carp Site 2 in late June (25.2°C). With increasing temperature also DOC, DIC and pH-value increased (Table 14). Primary production in carp sites was higher than in trout sites (corresponded to chlorophyll-a-content). It has to be considered, that in times of high primary production the amount of heavy metals is reduced in water because of sorption accumulation processes.

pH is a key parameter in water as it affects many chemical and biological processes, hence influencing aquatic life, both directly and indirectly. Unpolluted surface waters should have a pH of between 6.5 and 8.5, which is ideal for the growth and well being of most aquatic organisms (Radojevic & Bashkin, 2006).

The solubility of many metal compounds also changes considerably with pH-value. Generally, a reduction in pH (more acidic) increases the solubility of heavy metals. When more metals are dissolved in the water, aquatic animals may absorb them faster. Therefore, a lower pH (more acidic) may increase the toxicity of these metals to aquatic life. pH from both trout sites ranged between 6 and 7.5, which was within normal range. An exception was found at Carp Site 1, which showed a minimum pH of 3.5 in March. Other values were within range with a peak in summer. (July 8.3). Also Carp site 2 showed in summer the highest values with a maximum value of 9.1 (Table 12). This could explain that in the spring cadmium concentrations in carp muscles from both sites showed highest concentrations in comparison to the other seasons, when the pH-values for both sites were lower than in summer (high

temperature and pH) (Fig 29).

Total hardness in water refers to the amount of dissolved calcium and magnesium, as well as other elements in smaller quantities, e.g. iron. The major source of hardness is from the erosion of soil and rocks, with rainwater constituting a minor source. The amount of calcium carbonate in a water body has also indirect influence of the mobility of heavy metals as the buffer capacity regulates the pH-value. Pascoe et al. (1986) recorded that cadmium in hard water has less toxic effects on fish than in soft water. As metal- carbonate complexes are less bioavilable.

Hardness is usually measured in terms of mg/L of calcium carbonate ($CaCO_3$) and presented in °dH (deutsche Härte). Calcium hardness of 8 - 12 °dH (140 – 210 mg/L) is ideal for egg and larval development. Freshwater fish survive under stress when the calcium hardness is above 25-50mg/L CaCO3 (Boyd &Tucker, 1998).

In the present study Trout Sites 1 and 2 showed 10°dH and 12°dH. Carp Sites 1 and 2, had the following hardness: 9°dH and 8°dH. Total hardness at trout sites was slightly higher than at carp sites, but all four were found within range.

Conductivity is an indication of the ionic concentration in the water. The values are predominantly derived from the erosion of soil and rocks (Radojevic & Bashkin, 2006). Freshwater organisms osmo-regulate to maintain a steady ionic concentration in their bodies. Since the external aquatic environment has a lower ionic concentration compared to within their bodies, they need to expend energy to excrete water and retain ions. Different freshwater species have different requirements for conductivity. The conductivity at Trout site 1 and 2 were around 600 µS/cm and 700 µS/cm. Carp Site 1 showed 280 µS/cm, Carp Site 2 was 340 µS/cm respectively. All values were within normal range (freshwater streams: 100 to 2000 mS/cm).

4.1.2. Age, length and weight of fish

Comparison of age groups especially in trout sites showed clear differences: The first age group in Trout Site 2 was significantly larger than that group in site 1 (122±28 versus 35 ±11g). Trout Site 2 in all the age groups showed higher cadmium concentration than site 1. In general Trout Site 2 and Carp Site 2 had larger and heavier fish. The growth rate could not be determined for several reasons. First, the age determination was not precise (The age was done by the breeder), Second, continuous sampling of the same age groups of fish it was in this study not possible.

4.1.3. Cadmium, copper, zinc and iron concentrations in trout and carp

Cadmium and copper concentrations were higher in trout than in carp in the gut and liver. Zinc showed higher concentrations in carp organs than in trout in muscle, liver, gonad and brain. Except zinc values were higher in trout in gills. In comparison, the corresponding iron values of the two species showed different values and trout showed higher values in most of the organs. Except in carp gills and brain were higher than trout.

Table 41. Age structure of farmed fish

Carp	N	%	Trout	N	%
≥1 year	37	49	<11 months	32	35
≥2 years	15	20	12-23 months	32	31
≥3 years	24	31	≥24 months	32	34
Total	76	100	Total	96	100

Chi-Square Tests P=0.090

Trout

The cadmium concentrations in the gut and gonad from Trout Site 1 are higher than Trout Site 2, while values in trout 2 are higher in muscle, liver and brain. The copper concentration in gonads from site 1 was higher than site 2. For copper, trout site 2 showed higher concentrations in gut and brain, but the remaining organs did not differ. This means that Cd and Cu showed some similarity (for example both higher in gonads at site 1). Zinc in most organs showed no difference except in muscle (site1 > site 2). Zinc in gills is lower in trout 2. Iron values in the gut and liver from site 1 are lower than from site 2 (Tables 19, 21, 22, 23). Ciardullo et al. (2008) studied the distribution and potential bioaccumulation of dietary arsenic, cadmium, lead, mercury and selenium in organs and tissues of rainbow trout, a major aquaculture species, in relation to fish growth over a period of >3 years. Fish were reared under normal farming conditions, i.e., fed a standard fish food and exposed to negligible levels of waterborne trace elements. Some age-related variations in the content of each trace element in gills, kidney, liver, muscle, and skin were recorded: the concentrations did not increase. Exceptions included cadmium and mercury in the liver and kidney. In muscle tissue, the concentrations of mercury, lead, and selenium did not change significantly with growth, whereas cadmium increased but remained at very low levels.

Carp

Cadmium concentrations in the gut, liver and brain from Carp Site 2 were higher than at Carp Site 1, but cadmium concentrations in muscle and gills showed no significant difference between sites. Copper in Carp Site 1 showed higher values in muscle, gut and liver, but not in gills. Zinc concentrations in both sites did not differ (exception: gills and brain in site 2 > site 1). The iron content in gills and brain were higher at site 2, while iron showed no significant difference (exception: values in brain from site 1 were higher – Tables (25, 26, 27, 29). Al-Weher (2008) found in common carp (Cyprinus carpio) with average total length (20.8 ±1.1cm) and weight (203±12.8 g) the following cadmium, copper and zinc concentrations in muscle: Cd 0.14±0.07, Cu 2.48±1.00, Zn 30.3± 4.16 mg/kg. In gills: Cd 0.70± 0.16, Cu 9.92 ±2.38, Zn 27.85±3.93 mg/kg. Generally, cadmium did not accumulate in muscle but did accumulate in the gut, liver and gills in both trout and carp sites. This agrees with Hogstrand and Haux (1991), who showed that cadmium can be incorporated into aquatic organisms by two main routes: ingestion and movement through the gills. In fish, cadmium usually accumulates in the liver, gills, kidney and gastrointestinal tract. The presence of zinc may lead to increased cadmium accumulation in the liver and kidney (Wicklund et al. 1988). When the cadmium concentration increases in the liver, this induces the synthesis of metallothionein (Nicoholson et al. 1983). The protective effects in zinc-pre-induced hepatocytes are not due to alterations in the level of total cellular cadmium, but could be accounted for by the redistribution of intracellular cadmium in the presence of high levels of zinc-metallothionein. Metallothionein exerts its protective effect by a kinetic detoxification mechanism, i.e. a decrease in reactive intracellular cadmium (Din& Frazier. 1985).

4.2. Factors influencing cadmium, copper, zinc and iron concentration in carp

4.2.1. Abiotic water parameters

Analysis of water parameters showed in trout sites no significant correlation to chlorophyll-a, while in carp sites chlorophyll-a correlated to total turbidity, organic turbidity and total turbidity. Zooplankton is part of the fish diet in the food chain. Plankton densities in the carp sites were affected by physico-chemical water parameters. For example, copepod density was positively correlated with total turbidity, organic, inorganic turbidity and temperature. Cladocerans were negatively correlated to pH, while rotifer density showed no correlation to pH.

Zinc concentrations in zooplankton correlated positively to TOC and negatively to conductivity. Iron values were positively correlated to total turbidity, organic turbidity and inorganic turbidity. Iron correlated positively to TIC, TOC and DIC.

Cadmium concentrations in suspended matter correlated positively to pH and temperature. Cadmium in suspended matter showed a positive correlation to total turbidity, organic turbidity, TIC and TOC.

Chaun et al. (1996) reported a low solubility of Pb, Zn, Cd, and Cu at pH 6 to 6.5 and an increase by several orders of magnitude at pH 2. Similarly, Pb, Cd, and Zn exhibited weak solubility at slightly alkaline condition pH 8, while at pH 3.3 the solubility was higher for cadmium.

4.2.2. Age, size and weight of fish

Cadmium

Cadmium concentrations in the muscle of trout showed a significant positive correlation to body size. While, cadmium concentrations in the muscle of carp were negatively correlated with body weight. Cadmium content in gonad and brain decreased as fish age increased. Thus, in the present study, the positive correlation between analysed heavy metals and fish species age groups may be due to loss of homeostatic ability of fish under chronic metal exposure leading to bioaccumulation (Evans 1993).

In Carp Site 1 cadmium concentrations in the muscle and gills declined significantly with growth. Carp site 2 showed a decrease in cadmium in the gut with growth. The data from both sites showed a significant decrease in the cadmium concentration in muscle with an increase in gut values.

Muscle and gonads demonstrated an increased cadmium accumulation as the concentration increased in the gills. Moreover, the accumulation in gonads increased with growth. Cadmium in trout brain and gut was higher in young than older fish. Cadmium concentrations in carp muscle and gills were higher in young fish. The present results showed that there were positive relationships between fish sizes and cadmium concentrations. In most cases such correlations are determined by the variation of feeding rate with age of individuals, the dilution by growth and by the food speciation of certain age classes (Farkas et al. 2000).

Copper

In Trout Site 1, copper concentration in gut, liver and gonad increased as length and weight increase. Copper at both carp sites increased significantly in the gut as the fish grew larger.

Zinc

The zinc concentration in Trout Site 1 decreased significantly in muscle with growth. In Trout Site 2, the concentration in the gut increased significantly with growth. An increase in the concentration in the gonads was accompanied by increased values in the brain and gut.

Carp Site 2 differed from site 1, where zinc in the gut decreased with growth. Data from both sites showed decreasing zinc values in muscle as the fish grew, while gill values increased as age increase. Increasing values in muscle are reflected by increases in the gonads and brain.

The negative relation between the heavy metal concentration of zinc and fish growth of both species suggests a relative dilution effect by the lipid content of tissue. This assumption is supported in that lipids, as a percent of body weight, are usually lower in younger fish, decrease during winter and spawning, and peak at the end of the main feeding period (Weatherly & Gill 1987). Another explanation for the negative relationships found in this study may be the difference in metabolic activity between younger and older fish (Canli & Atli 2003).

Iron

Iron concentration in Trout Site 1 in the brain also increased with growth.

Trout Site 2 showed that, as fish grow, iron accumulates in the gut and gonads. Iron concentrations increase in the gills and brain as fish grow as well.

The iron concentration in the gut at Carp Site 1 decreased as the fish grew. In site 2, increasing concentrations in the liver were positively correlated with brain values. Data from both sites showed that iron accumulated in the liver, gills and brain with growth. At both sites, iron is below the detection limit in muscle.

Cadmium interaction with zinc and iron in trout and carp organs

Cadmium is often present together with zinc in metal-polluted aquatic environment. Zinc has an antagonistic and protective action in the uptake and toxic effects of cadmium. This is because the zinc-induced synthesis of metallothionein detoxifies Cd by firmly binding this metal to heavy metals such as selenium and mercury. These results agree with Karigin and Cogun (1999), who found similar results of antagonism between zinc and cadmium in the muscle, gills and liver in *Tilapia niloticus.*

In this study, zinc and iron accumulated in brain as fish age increase, while cadmium in the gills and brain decreased with age for both trout and carp. This result agrees also with Gomaa et al. (1995), who analysed the distributions of some heavy metals in different fish organs. Maximum concentrations (ppm) of cadmium (0.82), zinc (10.9), copper (2.26) and iron (1.41) were found in the brain, whereby iron was almost double the amount of cadmium. Significant differences were found for cadmium in the brain as well as for iron in the gills and brain as the trout grew.

In Carp Site 1, zinc concentrations in the gills and brain, and iron in the gills, showed significant differences as age increases, while no such differences were evident for cadmium and iron in the brain. Cadmium and iron concentrations in the organs of the fish from both trout and carp sites show decreasing concentrations in the order: gut > liver > gills. The same is valid for zinc from the carp sites (trout sites showed higher zinc accumulation in gills than in the gut).

In that study, copper also accumulated in liver more than in the gut. Copper accumulated in trout in the liver more than in gut. In carp, however, copper accumulated in organs in the order: gut ≥ liver > gills. Which agrees with Giguère et al. (2004), who studied concentrations of Cd, Cu and Zn in various organs of eighty-one juvenile yellow perch (*Perca flavescens*) collected from eight lakes: the cadmium concentrations were significantly higher in the gastrointestinal tract than in the gills, suggesting that uptake of this metal from food is more important than uptake from water. Cadmium concentrations in both the trout and carp sample series were highest in DAN-EX, followed by benthos and nekton then suspended organic matter. The cadmium concentration in zooplankton was in Carp Site 2 the highest. Zinc showed the same performance as cadmium. For copper and iron, concentrations in benthos and nekton were high, followed by DAN-EX, then suspended matter and zooplankton for Carp Site 2. The iron concentration in cereals was as high as in benthos and nekton (Fig 33).

A comparison of different brands of DAN-EX for young and adult fish showed no significant difference between heavy metals, the concentrations of the four elements were in the same range.

4.2.3. Gender

Significantly higher cadmium levels were found in the gut of male carp than in females from Carp Site 1 and wild carp Austria the muscle and remaining carp organs showed no significant difference. This agree with Canpolat and Çalta (2003) who detected that the fish male showed higher accumulation level of heavy metals in all tissue and organs than females. Shakweer and Abbas (2005) found that sex of fish (*Oreochromis niloticus)* can affect the concentration of trace elements, whereby higher concentrations were found in muscle of females than in males. The nature of hormones and the available number of active sites in the protein and cytochrome P-450 in female and male fish may account for this behaviour (AL-Yousuf 2000).

4.2.4. Season

Cadmium concentrations in trout muscle were higher in November than in August (Fig 28). The cadmium concentration in carp muscle showed the highest value in spring compared to summer and autumn (Fig 29).

The cadmium concentrations reported here in the gut of both, trout and carp match the results of Bahnasawy (2009), who found seasonal variations in the concentrations of four heavy metals - zinc, copper, lead and cadmium - in gills, skin and muscles of two fish species (*Mugil cephalus* and *Liza ramada*) in Lake Manzala. The statistical analysis revealed a significant effect of seasons. The highest values of the metals were recorded in hot seasons (summer and spring). The highest heavy metal concentrations were found in gill tissue of both fish species, the lowest concentrations in muscle tissue. Also, Canpolat and Çalta (2003) found the higher accumulation in spring and summer could be due to an increase of physiological activity caused by increase in water temperature.

4.3. Comparison of cadmium, copper, zinc and iron contents in trout, carp (farmed, Austrian wild catch and aquaculture market fish) and marine fish

4.3.1. Cadmium

As expected, high cadmium contamination was found in marine fish muscle such as swordfish, butterfish, shark and tuna. Freshwater fish had lower values, with the exception of imported carp (Czech Republic), which had comparatively high concentrations of cadmium in the muscle.

Two of four samples exceeded permissible levels: these pertained to swordfish regarding EC European community regulation No.104/2000. Cadmium in muscle should not exceed 50 µg/kg (Fig 34). This agreed with a WHO study which carried out on ninety-three samples of fresh and canned fish (edible parts) collected from Lower Austria, Burgenland and from Viennese retail operations. The cadmium contents ranged from <0.01 (limit of detection) to 0.12 mg/kg, with a mean value of 0.014 mg/kg in comparison, high cadmium contents were measured in swordfish with a mean value of 0.046 mg/kg (FAO/WHO 1972).

The muscle of carp from different sources showed the highest cadmium concentration in imports from the Czech Republic, while gut values were highest in carp site 2. The highest cadmium values in gills were in Austrian carp aquaculture followed by Austrian wild carp from Kühwörtherwasser (Lower Lobau). These wild carp from Lobau were larger (54±5 cm), heavier (1890±325 g) and older than farmed fish. Wild trout from (Untertalbach, Gossenköllesee) showed significant contamination, with highest concentrations in muscle and then, in decreasing order, in liver, gonads, gills and brain. The highest value of cadmium in gut was recorded in roach. The levels in the muscle of pike and catfish from Neusiedlersee were below the detection limit.

4.3.2. Copper

Copper in most marine fish such as butterfish, swordfish and salmon showed elevated concentrations in muscle (Fig 35). This was also found in shrimp. Elevated values were also recorded in wild fish such as pike, catfish (one of each from Neusiedlersee), wild trout (Untertalbach, Gossenköllesee), chubs, reinanke, Danube bleak, perch from Fuschlsee and wild carp Austria (Lobau). Copper concentrations in wild fish were higher than in farmed fish. Most of wild fish had values below the detection limit in their muscle. In farmed trout (trout sites), values were higher in liver than gut. In farmed carp it accumulated in the following order: gut > liver > gills.

Copper concentrations in both saltwater and freshwater fish were about the same, i.e. approximately 2.5 mg/kg. Lindow et al. (1929) found the following average copper contents for sixteen species including yellow perch (2.6), pike (1.6), salmon (2) and trout lake (4 mg/kg wet weight), which agrees with the results we found.

4.3.3. Zinc

Zinc showed high concentrations in tuna (10.2 mg/kg), while the values in freshwater fish as Danube bleak, carp 1, carp 2, chubs, wild carp and carp aquaculture Austria were 8.5, 8.1, 8.1, 7.9, 7.7 and 5.9 mg/kg, respectively. Zinc concentrations in gills showed the highest value in wild carp Austria (Fig 36).

4.3.4. Iron

Elevated iron concentrations in muscle were found in wild trout, tuna, Danube bleak, roach and carp from aquaculture Austria. Iron in gills showed a high average concentration in wild carp Austria (Fig 37).

Peterson and Elvehjem, (1928) found the following iron concentrations in fresh muscle: yellow perch 5.6, pike 4.5, salmon 8.6 and trout 7.2 mg/kg. Interestingly, yellow perch 5.6, pike 4.5, salmon 8.6 and trout 7.2 mg/kg. Interestingly, marine fish contained about 40% more iron than freshwater species. Fish with dark-coloured tissue contained about 75% more iron than those having light-coloured tissue.

4.3.5. Wild fish

A comparison among wild-caught piscivorous fish, (pike and catfish) showed values below the detection limits for cadmium in muscle flesh. Copper in the muscle of pike and catfish (piscivorous) and fish from Fuschlsee (omnivorous) and wild carp Austria (Lobau) showed higher concentrations than farmed fish. Both carp sites, followed by wild carp (Kühwörtherwasser, Lobau) showed higher concentrations than other sites; both carp sites, followed by wild carp, showed higher concentrations than imported carp and those from aquaculture.

Iron in most wild fish and carp from aquaculture Austria also showed higher concentrations in their muscle than farmed fish (trout and carp); whereby most of the latter were lower than the detection limit. These results suggest dependence on the atmospheric input, geological background and water chemistry. This would agree with Ünlü and Gümgüm, (1993), who reported that the major source of high heavy metal concentrations in the sediment and fish in Lake Egirdir were due to direct contamination of the water by metals or the geo-chemical structure of the region.

Such differences may also reflect species-specific factors (e.g. feeding behaviour). According to Geldiay and Balik (2000), different accumulation rates of heavy metals in fish species might be due to different feeding behaviours. Chub, carp and tench are omnivorous species and have a similar feeding behaviour, whereas pike is a carnivorous species with an entirely different feeding behaviour.

4.4. Accumulation of cadmium and copper, zinc and iron in aquatic semi-natural food chains

4.4.1. Cadmium

The BAF for cadmium accumulation values were ≤1. Our results did not indicate any cadmium accumulation in farmed fish. This result matching with the findings on fish organs, cadmium appeared to accumulate from micro- to macrozooplankton but diminish from them to fish.) Ayas and Kolonkaya (1996) found that in Göksu delta, that no Cd, Pb and Cr accumulated in plankton samples. In contrast, significant concentrations of Cd, Pb and Cr were measured in planktonic organisms.

DAN-EX food pellets (Table 18) from both trout and carp aquaculture (488 versus 218 µg/kg) showed higher cadmium levels zooplankton, benthos or nekton. All these cadmium levels, however, were higher than in water or in fish organs. Analysis of data from both sites showed a lower value in muscle while the concentration in gut increased. Muscle and gonad showed a higher concentration of cadmium, as well as concentration increased in gills. Accumulation in gonads increased with growth. These data coincide with Matsuo et al. (2005), who reported that the bioaccumulation of metals in fish can occur at significant rates through the dietary route, without necessarily resulting in death of the organism. The goal of that work was to expose an economically relevant species from the Amazon basin to dietary cadmium (Cd) at

concentrations of 0, 50, 100, 200, and 400 µg/g dry foods. Cadmium accumulation in the tissues occurred in the following order: kidney > liver > gills > muscle. Relative to other freshwater fish (e.g., rainbow trout, tilapia), these fish accumulated remarkably high levels of Cd in their tissues. Although Cd is known to affect Ca^{2+} homeostasis, no mortality or growth impairment occurred during feeding trials.

The cadmium concentrations in benthos and nekton showed a positive correlation with gut values. Cadmium in the gut was negatively correlated to muscle content, while gill values were positively correlated to muscle contents. No correlation was found between fish organs and sediment (Fig 30).

Cadmium in benthos and nekton such *as Lymnaea peregra f. ovata, Gammarus roeselli, Lestes sp., Corixidae, larve* from *Ilyocoris sp., larvae* from *Baties sp.* showed high concentration in lower trophic levels, as also found by Gupta (1996). The concentrations of all the metals were higher in fine detritus than in *C. ramosus*, although Cd and Zn concentrations were elevated in *Baetis* sp. compared with values in periphytonic algae and fine detritus, indicating possible bioconcentration of these metals by mayfly (Table 17).

Cadmium concentration decreased from plankton samples to fish in a study conducted by Farkas et al. (2001). Chen et al. (2000a) reported that Cd biomagnified from micro- to macrozooplankton but diminished from them to fish. The fish is the indicator organism for heavy metal pollution and for the possible risk for human consumption.

Trace elements, like Zn, Cu and Fe, are essential for the physiological metabolism of fish, and metal deficiency or excess can cause biochemical, physiological and pathological changes depending on the metal and fish species (Clearwater et al. 2000). Many essential metals, such as Zn and Cu, are not taken up from water in sufficientamounts to satisfy physiological requirements and must therefore be supplied by the diet to prevent deficiency. In particular, Zn is a cofactor of over 300 enzymes and is involved in several metabolic pathways (Vallee et al. 1993).
In this study, copper and iron were below the detection values in more than 50% of trout and carp muscle samples. Zinc showed expected values, but its concentration decreased with growth instead of biomagnifying. The present zinc results were in contrast to Chen et al. (2000a), who stated that Hg and Zn concentrations in fish were both biomagnified.

4.4.2. Copper

The BAF of copper accumulation values were ≤1. Concentrations in benthos and nekton showed positive correlation to the gut values and negative correlated to liver content (Fig 31).

No accumulation was shown in the following organs: muscle, gonad, gills and brain in carp. More than 50% of all samples were below the detection value in trout as well (except brain). Only the gut and liver of both species showed high copper values, whereby the brain of trout also had higher values.
Copper contents in benthos and nekton (*Lymnaea peregra*, *Lestes* sp. *Corixidae*.) were higher than in zooplankton, DAN-EX, cereals and fish organs. Copper concentrations in muscle of both fish species were lower than the detection limits.

Lymnaea ovata and *Gammarus pulex* tend to bioaccumulate and store copper at levels proportionate to its exposure concentration (Van Hattum et al. 1991). Much of

this cadmium and copper accumulation could probably be attributed to adsorption or accumulation of metal within the exoskeleton, because odonate larvae (*Lestes* sp.) are known to sequester metals into this material. The results were generally consistent with previous observations indicating that odonates are tolerant to metal exposures, even in comparison with other aquatic invertebrates. Odonate larvae can be useful toxicological model organisms (Tollett et al. 2009) (Table 17).

4.4.3. Zinc

The BAF of zinc accumulation also was ≤1. The highest zinc content in the food web was shown, in decreasing order, in DAN-EX, cereals, gut, zooplankton, benthos and nekton. Values in DAN-EX from both trout and carp sites were similar. Liver values were negatively correlated to muscle values. Zinc in the gut and brain was positively correlated with gonad values; gut values correlated positively with gills; and gut contents were positively correlated with the brain content. Lanno et al. (1985) reported that zinc levels in the liver and whole body increased with dietary copper supplementation. Johnston et al. (2000) suggested that a diet containing 120 mg Zn/kg feed should satisfy the metal requirement in young gilthead sea bream, maintaining an optimal muscular Zn level, which together with muscle fiber density will contribute to the nutritional characteristics of the flesh for human nutrition. This agrees with our food analysis (Table 18), where the average concentration of zinc in Trout Site 1 was 183 mg/kg and Carp Site 2 was 118 mg/kg. In Trout Site 2, however, values were low (28.5 mg/kg) (Fig 32).

Papagiannis et al. (2004) reported that *C. carpio* and *R. ylikiensis* in general showed the highest metal content. Tissue analysis revealed that liver and gonads accumulated the highest levels of Cu and Zn. In this case, metal concentrations in the edible part of the examined fish (muscle) were within the safety-permissible levels for human consumption.

4.4.4. Iron

As was the case with the other three elements above, the BAF of iron accumulation in the surroundings and in different fish organs also was less 1. Iron concentrations in organs showed no significant correlation to the surroundings, i.e. sediment, zooplankton, benthos and nekton. Iron concentrations in zooplankton were one order of magnitude higher than benthos and nekton, gut, DAN-EX, cereals and other fish organs. Muscle values were below the detection limit in both fish species (Fig 33). So, the edible parts of both fish species in Austria do not represent hazard for human health.

The results on the trout and carp food chain and especially food supplement (pellets) show elevated concentrations of cadmium. Given high toxicant of cadmium, this issue should be further investigated.

5. Summary and conclusions

This is the first study on the concentrations of Cd, Cu, Zn and Fe in in Austria, focusing mainly on Austrian aquaculture. Wild fish and freshwater and marine fish from different markets were also collected from spring 2007 to autumn 2008, with a total of 608 samples.

Austrian aquaculture

The study focused mainly on trout and carp from Austrian aquaculture (four main sampling sites, two for each species). The concentrations of Cd, Cu, Zn and Fe from these four sampling sites were analysed in various fish organs (muscle tissue, gut content, liver, gonads, gills, brain) and compared to the concentrations in filtered water, sediment, zooplankton, food supplements, nekton and benthos.

Wild fish samples

The following fish were obtained: Kühwörtherwasser, Lobau (13 carp); Neusiedlersee (one each of carp, pike and catfish); Untertal, Styria and Gossenköllessee, Tyrol (5 trout), Fuschlsee, Salzburg (6 perch, 7 roach, 4 reinanke, 3 chub, and 3 Danube bleak).

Market fish samples

These specimens were obtained from supermarkets, fish stores and market stands in Vienna. The edible part only was available from 5 pangasius from supermarkets. Marine samples (3 salmon, 3 butterfish, 6 tuna, 2 crab, and 2 shrimp) were taken from sushi restaurants. Some aquaculture fishes were purchased from market stands: 9 trout from Austria and 6 imported trout from southern Europe) and 16 carp from Austria and 12 carp imported from the Czech Republic. The source of the fish was not always known.

Cadmium

Cadmium concentrations in the muscle tissue of both carp sites showed concentrations below the limit of detection in 50% of the investigated individuals. In Carp Site 1, cadmium concentrations in the muscle had a median value of 0.3 and a maximum value of 4.8 µg/kg. Carp Site 2 had a median value of 0.3 and a maximum of 0.7 µg/kg. In comparison, wild carp in Austria showed a median value of 0.4 µg/kg and maximum of 14.2. The highest median value of cadmium in carp muscle was found in the carp imported from the Czech Republic: median value 11.9 µg/kg, maximum value12.5 µg/kg. Although these values are high, they were all within the permissible level (< 50µg/kg).

Comparing the cadmium concentrations in the gut content from different carp sites revealed the highest values in Carp Site 2 (35.5 µg/kg), followed by wild carp from Austria (14 µg/kg). Carp from site 1 and aquaculture in Austria showed 6.0 µg/kg. Carp Site 2 is a bio-farm and the fish fed on bio-cereal. The cadmium concentration in the cereal was 50 µg/kg, i.e. lower than in DAN-EX (food pellets; 218 µg/kg), the food at Carp Site 1. A possible explanation for high cadmium concentrations in Carp Site 2 is that carp are benthivores and that the cadmium concentrations in some benthic and nektonic organisms were very high.

Gut contents of trout from both trout sites had twice the concentrations of carp from both carp sites (42 versus 15.1 µg/kg). This could be explained by the cadmium concentrations in the food supplement DAN-EX (488 versus 218 µg/kg).

Cadmium concentrations in muscle tissues of wild trout in Austria (Untertalbach and Gossenköllesee) showed higher values (2.2 µg/kg) compared to all the other wild fish species were the maximum did not exceed 0.6 µg/kg. Liver, gonad, gills and brain from wild fish in Austria were 828, 110, 156 and 7.4 µg/kg, respectively. These values were higher than in other wild fish organs.

In the marine fishes (swordfish, shark, butterfish and tuna), muscle concentrations were elevated: 43.3, 13.9, 10.9 and 6.4 µg/kg, respectively.

Copper

Copper concentrations in muscle in most of the individuals from both carp sites, both trout sites and trout aquaculture in Austria were below the detection limit. On the other hand, all of the wild fish samples were above this value. The highest value in the muscle was in pike (3.6), followed by catfish 1.4), reinanke (1.2), chubs (0.7) and wild carp from Lobau (0.7 mg/kg). Imported carp (Czech Republic) showed 0.4 mg/kg, while imported trout was below the detection limit.

The highest copper values at both trout sites averaged 70 mg/kg (liver), followed by gut content (< 38 mg/kg). In carp sites, higher concentrations were found in gut content: site 1 (6.3 mg/kg), site 2 (4.2 mg/kg). Liver values were < 0.5 mg/kg at both sites.

Most of the marine fish and crustaceans showed muscle concentrations above the detection limits. The median value was 1.5 mg/kg in muscle of butter fish and shrimp. This value was the highest in all marine fish but still within permissible levels of copper (5.0 mg/kg in fish muscle).

Zinc

Zinc concentrations in the muscle from both trout sites showed the same median values (~ 4.0 mg/kg), with the maximum at 9.0 mg/kg. Both carp sites and wild carp in Austria showed almost same median value of (7.96 mg/kg), (~ 8.0 mg/kg), with a maximum in both carp sites of 20 mg/kg. In Austrian wild carp, the maximum was 40 mg/kg. The muscles of young fish from both trout and carp sites showed significantly higher zinc concentrations than older fish ($P>0.05$). The fish muscle values mentioned above were within permissible levels of zinc (40 mg/kg); only wild carp were close to the upper border of the permissible level.

Zinc concentrations in wild fish muscle showed the highest median value in catfish (15 mg/kg). The other wild fish ranged between 3.0 and 8.0 mg/kg. Of all studied species, pangasius fish showed the highest median (12 mg/kg), followed by tuna (10.5 mg/kg).

Comparing the zinc concentrations among the different organs revealed equal values between the same organ in both trout sites (valid for all organs). Gills in both showed the highest concentration (median: 180 mg/kg). In Carp Site 1 the ranking between the organs was as follows: gonad > gut > liver > gill > brain > muscle. In gonads the median value was 123 mg/kg. For Carp Site 2: liver > gill > brain > muscle. The median value in the liver was 90 mg/kg. Conducting the same comparison between the organs of all the wild fish, the gut content values were mostly higher than in the other organs, ranging from 30-122 mg/kg. The maximum (122 mg/kg in gut content) was recorded in roach. The highest value for all organs of all wild fish was found in wild carp gills from Austria (128 mg/kg).

Iron

Elevated concentrations were found in the muscles of Austrian wild trout, Danube bleak, roach from Fuschlsee and carp from Austrian aquaculture (4.6, 3.6, 2.9, 2.4 mg/kg, respectively). Tuna showed 4.2 mg/kg. Trout Site 1 and 2 showed a median value of 2.4 mg/kg for each site; this concentration was higher than at Carp Site 1 (0.05 mg/kg). The permissible level in fish muscle should not exceed 5 mg/kg according to FAO/WHO standards.

The comparison of different organs in farmed trout and carp showed that muscle tissues of both species had the lowest cadmium, zinc and iron concentrations of all organs. Both these fish groups showed the highest concentrations of cadmium, copper and iron in the gut contents. The liver – a detoxifying organ – showed elevated concentrations of these four heavy metals in most of the investigated fish.

Age, length and weight of fish

Cadmium

In this study age, body size and season were the major factors modifying metal accumulation in fish.

Cadmium concentrations in trout muscle showed a significant positive correlation to body size. Cadmium content in the gonad and brain decreased with size. The data from both carp sites showed a significant decrease in the cadmium concentration in muscle with size, but an increase in gut values. Cadmium in the trout brain and gut was higher in young than older fish. Cadmium concentrations in carp muscle and gills were higher in young fish.

The concentration in the gills was highest in Austrian carp aquaculture and wild carp from Lobau (13.5 µg/kg), followed by Carp Site 1 and Site 2 (almost the same value 1.5 µg/kg). The Lobau specimens were larger (54±5 cm), heavier (1890±325 g) and older than 3 years, which is reflected in a more long-term exposure to cadmium.

Copper

In Trout Site 1, the copper concentration in gut, liver and gonad increased with length and weight. Values at both carp sites increased significantly in the gut contents as the fish grew larger.

Zinc

The zinc concentration in Trout Site 1 decreased significantly in muscle with growth. In Trout Site 2, the gut concentration increased significantly with growth. Data from both carp sites showed decreasing zinc values in muscle as the fish grew, whereas gill values increased with age.

Iron

The iron concentration in both trout sites dropped in gills, gut and liver with growth. Data from both sites showed that iron accumulated in the liver, gills and brain with growth. At both sites, iron was below the detection limit in muscle.

The present results showed positive relationships between fish size and cadmium concentrations. In most cases such correlations are determined by the different feeding rate with age, the dilution by growth and by the food preferences of certain age classes. The explanation for the negative relationships between metal concentrations and growth may be a difference in metabolic activity between younger

and older fish. Moreover, lipids, as a percent of body weight, are usually lower in younger fish, decrease during winter and spawning, and peak at the end of the main feeding period.

Season

Cadmium concentrations in trout muscle were higher in November than in August. The values in carp muscle were highest in spring compared to summer and autumn.

Gender

Carp from site 1 and Austrian wild carp showed higher cadmium levels in the gut of males than in females. There were no significant differences in muscle values or other tested carp organs.

Heavy metal interactions

In this study, iron antagonizes cadmium concentrations in the trout gills and brains. Iron accumulated in the gills and brain as trout body size increased, while cadmium in the gills and brain decreased as trout body size increased.

In selected organs, one metal can affect the accumulation of another metal, either increasing or decreasing the toxic effect. In fish, toxic levels of metals depend on the type of compound (organic or inorganic), the presence of other metals, temperature, dissolved oxygen, salinity, pH, feeding behaviour, mating season, sex and ontogenetic period of the fish .

No evidence was found for cadmium, copper, zinc and iron bioaccumulation along the food chains. The BAF value for each of cadmium, copper, zinc and iron was ≤1.

The cadmium, copper, zinc and iron content in the edible part (muscle) of fish collected in Austria is unlikely to constitute a significant health hazard. In contrast, imported fish such as carp (from the Czech Republic) and some marine fish (swordfish, shark, butterfish, tuna), contained elevated levels (although within permissible levels), especially of cadmium. Elevated metal concentrations in the other organs (not muscle)- as this not human health issue, but is an issue for ecological risk due to predation by fish eating birds and mammals.

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Nationality: Egyptian, born in Doha Katar

Education: I attended primary school in Doha, Katar, then the Egyptian governmental school in 1982

June 1994 Completed secondary school .

Sept 1994 I attended the faculty of science at the University of Alexandria, Egypt .

1994-1998 BSc in Chemistry & Oceanography from Alexandria University, Egypt, with the general grade of Good.

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1999-2004 MSc in Enviromental studies on 26 May 2004.
I worked during my MSc as a research student in a link between the Royal Holloway University in the UK & the Institute of Graduate studies in Egypt on the Lake Maryut Project, for studying the possibilities to improve the condition of the lake. (I performed the water and sediment toxicity test by using *Ceriodaphnia dubia* as a biomarker).

Sept 2003 I attended the training course in Fish Parasitology at the University of Vienna, Department of Limnology through UNESCO Training.

Sept 2007 Beginning of the Dissertation