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**Trophic transfer of CuO NPs from sediment to worms (*Tubifex tubifex*)
to fish (*Gasterosteus aculeatus*): A comparative study of dissolved Cu
and NPs enriched with a stable isotope tracer (⁶⁵Cu)**

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Abstract

Metal nanoparticles (NPs) released into the aquatic environment will likely accumulate in sediment and be available for sediment-dwelling invertebrates that serve as food for other organisms, such as fish. The aim of the present study was to investigate trophic transfer of copper oxide (CuO) NPs and dissolved Cu (CuCl₂) from natural sediment to the sediment-dwelling worm *Tubifex tubifex*, and then to the predatory fish *Gasterosteus aculeatus* (three-spined stickleback). Cu enriched in the stable isotope ⁶⁵Cu was used to increase detection and discriminate newly added/assimilated Cu from background Cu levels. Worms were exposed to sediment spiked with ⁶⁵CuCl₂ or ⁶⁵CuO NPs (~20 nm) at environmentally relevant concentrations for 7 days and subsequently fed to fish for 7 days. Worms accumulated ⁶⁵Cu during sediment exposure to both ⁶⁵CuCl₂ and ⁶⁵CuO NPs (0.7 and 1.1 µg ⁶⁵Cu g⁻¹ dw tissue, respectively), resulting in ⁶⁵Cu body burdens significantly different from control. Furthermore, significantly more ⁶⁵Cu was released from the sediment into the overlying water in the ⁶⁵CuO NP exposures compared to the ⁶⁵CuCl₂ exposures. ⁶⁵Cu accumulation in fish feeding on ⁶⁵CuCl₂ and ⁶⁵CuO NP-exposed worms was limited (intestinal tissue: 80 and 65 ng g⁻¹ dw; fish carcass: 7 and 10 ng g⁻¹ dw, and liver: 50 and 10 ng g⁻¹ dw, respectively). Glutathione peroxidase (*gpx*) mRNA expression was significantly higher in fish feeding on ⁶⁵CuCl₂-exposed worms compared to ⁶⁵CuO NP-exposed worms (though ⁶⁵Cu tissue concentrations were similar). No significant effects were detected for the other investigated genes (*ctr1*, *mta*, *gcl*, *gr*, *sod*, *cat*, *zo-1*). Our results show that NP-derived Cu can enter freshwater food webs, but bioaccumulation and trophic transfer under environmentally realistic exposures is low (detectable with a tracer) and not different from that of dissolved Cu.

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Environmental significance

Engineered metal nanoparticles (NPs) released into the aquatic environment will likely accumulate in sediment, where they may be available for uptake by sediment-dwelling invertebrates such as *T. tubifex* that serve as food for higher trophic level organisms, such as fish. Through this dietary uptake NPs may be transferred in aquatic food webs with risk of biomagnification. In the present study, we compared transfer of ⁶⁵CuO NPs with dissolved copper (added as ⁶⁵CuCl₂) in an experimental food chain involving sediment, sediment-dwelling worms, and predatory fish. Isotopically enriched ⁶⁵Cu was used to enhance detection and discriminate between newly accumulated and background Cu, making it possible to use environmentally realistic Cu concentrations in sediment (100 µg Cu g⁻¹ dw sediment). Our findings suggest that dietary transfer of metal NPs may occur to a limited extent in freshwater environments.

Keywords: Bioaccumulation, Metal, Dietary uptake, Oxidative stress, Environmental fate, Isotopically labeled nanomaterials



1. Introduction

Over the past few decades, there has been a dramatic increase in the industrial production and use of copper (Cu)-bearing nanoparticles (NPs), such as Cu, CuO, Cu₂O and Cu(OH)₂ NPs¹. For CuO NPs, the total production amount was estimated to be 20 tons in Europe in 2016, of which less than 1% was predicted to end up in the aquatic environment². Possible entry routes of these NPs into the aquatic environment include urban stormwater, untreated wastewater, wastewater treatment plants effluents²⁻⁴, runoff from agricultural land treated with NP-containing biosolids and pesticides^{3, 5, 6}, and release from weathered surfaces treated with NP-containing wood coatings or antifouling paints^{2, 7}. After release into natural water bodies, Cu-bearing NPs are likely to undergo a series of transformations that may affect their fate, bioavailability, and toxicity. For instance, they are likely to aggregate, settle out and accumulate in sediments⁸⁻¹². Furthermore, dissolution may occur in both water and sediment. CuO NPs tend to dissolve less than other Cu-bearing NPs and may thus prevail in the environment^{8, 12-17}. Using a multimedia fate and transport model simulating ten years of release of manufactured nanomaterials including CuO NPs in the San Francisco Bay area, Garner et al. (2017) predicted that CuO NPs will accumulate in freshwater and marine sediment beds potentially reaching concentrations in the ng kg⁻¹ to µg kg⁻¹ range⁴. Similarly, Caballero-Guzman and Nowack, estimated that alone the use of CuO NP-containing wood coatings in Europe could result in an increase of their concentration in sediments by about 400 ng kg⁻¹ annually². The increasing occurrence of CuO NPs in sediment beds augments the likelihood for exposure of benthic deposit-feeders. Previous research has established that sediment-associated CuO NPs are available for endobenthic worms such as *Nereis (Hediste) diversicolor* and *Lumbriculus variegatus* living in estuarine and freshwater systems^{9, 18, 19}. Since benthic invertebrates represent a major food source for fish, it is hypothesized that CuO NPs may be further transferred along the food chain.

Today a range of studies have focused on the fate of manufactured nanomaterials in aquatic food webs (for reviews see^{20, 21}) but only a few on their transfer along food chains including predatory

fish as a secondary consumer^{22-32, 33}. Furthermore, most studies focused on pelagic food chains using, for instance, artemia^{22, 25, 27, 29, 32}, or daphnia^{23, 24, 28, 31} as the model prey, and zebrafish (*Danio rerio*) as the model predator^{23-28, 31, 32}. An exception is published by Wang et al. (2016), who studied the trophic transfer of TiO₂ NPs in a marine benthic food chain from the clamworm (*Perinereis aibuhitensis*) to juvenile turbot (*Scophthalmus maximus*)³⁰. The authors reported trophic transfer, but no biomagnification of TiO₂ NPs between trophic levels. Even though metal(oxide) NPs constitute the biggest group of manufactured nanomaterials that has been tested in food chains including fish^{22, 23, 25, 28-32}, CuO NPs appear to have been used in only one study so far²². The authors found that dietary exposure was a likely uptake route of CuO NPs and that accumulation primarily occurred in the intestine. There are only a few studies assessing dietary uptake of nanoparticulate Cu in fish, and findings concerning the intestinal absorption efficiency and accumulation in inner tissues compared to dissolved Cu species vary³⁴⁻³⁶. Some information on NP transfer from invertebrate prey organisms to fish can be inferred from studies that examined intestinal uptake and accumulation of metal (oxide) NPs from spiked artificial diets³⁷⁻⁴⁵. Altogether, the data from the few studies published on dietary exposure of fish to Cu(O) NPs suggest that Cu from NP-spiked diet is available, but uptake is limited compared to dissolved Cu^{34, 38,40,41}. The present study is a continuation of such a study where we examined Cu uptake from Cu salt and CuO NPs in spiked artificial diets (worm homogenates)⁴². Such test design provides considerable logistical advantages, as well as the possibility of strict control of the exposure dose etc. However, it is important to bear in mind that these studies ignore physical, chemical, and biological processes occurring in the environment and the influence that the prey organisms may play in altering the availability of NPs to predators.

In a review, Tangaa et al. defined four processes that influence trophic transfer of metal NPs: environmental transformations of metal NPs, uptake and accumulation in the prey organism, internal fate and localization in the prey, and the digestive physiology of the predator²⁰. Therefore, in the present study, fish were exposed *via* live worms to increase environmental realism of the exposure scenario regarding both food type (live worms), complexation with biological tissue and

realistic exposure dose (dependent on actual bioaccumulation of Cu in worms during sediment exposure).

The objective of this work was to study whether transfer of CuO NPs from sediment to worms (prey) to fish (predator) occurs at environmentally relevant exposure concentrations and conditions. In addition, the aim was to evaluate if potential changes in gene expression resulting from NP exposure could be detected. Dissolved Cu applied as CuCl₂ was used as a reference (soluble Cu counterpart). CuO NPs and CuCl₂ enriched in the stable isotope ⁶⁵Cu (⁶⁵CuO NP and ⁶⁵CuCl₂)³⁴ were used to trace the transfer of Cu along the different compartments of the simulated food chain (sediment, worms, and fish). The model prey and predator organisms chosen for this study were the sediment-dwelling freshwater oligochaete *Tubifex tubifex* and the fish three-spined stickleback (*Gasterosteus aculeatus*). *T. tubifex* is a widely used standard test organism for bioaccumulation testing⁴⁶, and is, as a deposit-feeder, at particular risk of exposure to sediment-associated metal NPs and metals. In addition, previous research has established that *T. tubifex* is relatively insensitive to Cu-induced toxicity and can accumulate high amounts of the metal before lethality occurs⁴⁷⁻⁵¹. This makes it a good model prey organism for studying trophic transfer of Cu including Cu-bearing NPs. Three-spined stickleback (*Gasterosteus aculeatus*) is a widely used fish model in ecotoxicology and as a bottom-feeder a natural predator of tubificid worms⁵²⁻⁵⁴.

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2. Methods

2.1 Synthesis and characterization of ⁶⁵CuO NPs

The synthesis of ⁶⁵CuO NPs were described previously ³⁴. In brief, the metallic precursor ⁶⁵Cu (isotope purity > 99 %, Trace Sciences International, USA) was dissolved in a mixture of HCl and H₂O₂. Solvent removal on rotatory evaporator under vacuum at 90°C yielded a powder of ⁶⁵CuCl₂. ⁶⁵Cu NPs were synthesized by thermolysis of ⁶⁵Cu-oleate obtained by reflux of a mixture of ⁶⁵CuCl₂ and sodium-oleate in water, ethanol and hexane. ⁶⁵CuO NPs were produced by oxidation of the ⁶⁵Cu NPs, and were finally dispersed in 1 mmol/L sodium citrate solution. All data on the characteristics of the ⁶⁵CuO NPs including size (20 nm; TEM; JEOL2100F transmission electron microscope), shape (spherical) and surface chemistry (mixture phase of CuO, Cu₂O, and Cu; x-ray diffraction) are shown in Lammel et al. (2020) ³⁴.

2.2 General experimental design of trophic transfer study

An overview of the overall experimental design is given in Figure 1. Trophic transfer of ⁶⁵CuO NPs and dissolved ⁶⁵Cu (⁶⁵CuCl₂) from sediment to worms to fish was examined by conducting two successive dietary exposures, each lasting 7 days. In the first exposure, *T. tubifex* were exposed to clean sediment and sediment spiked with ⁶⁵CuO NPs or ⁶⁵CuCl₂. At the end of this exposure, the worms were recovered from the exposure vessels and used as live food for three-spined sticklebacks (second exposure). In total, seven separate repetitions of the worm exposure were carried out. The repetitions were staggered by one day to ensure that, each day, fish received worms that had been exposed for the same duration (Figure 1B).

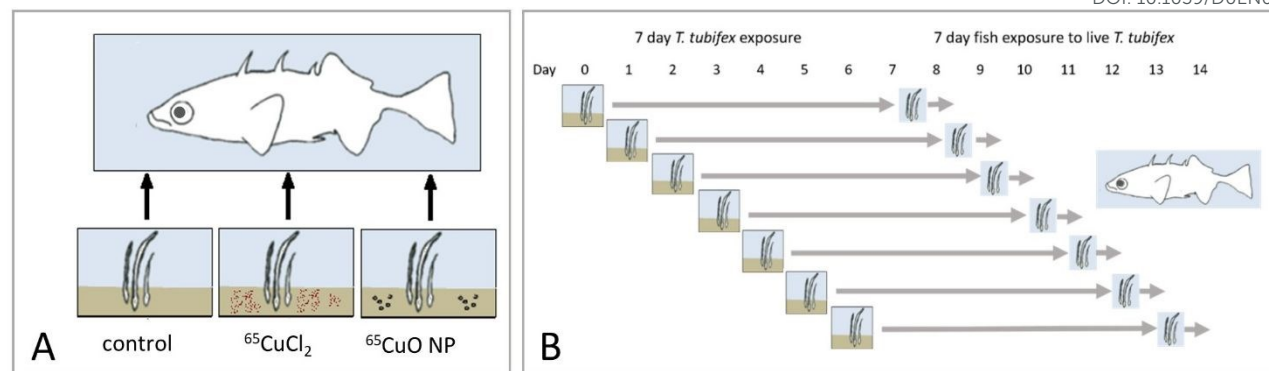


Figure 1: Overall experimental design. A. Illustration of the overall approach where worms were exposed to sediment and subsequently fed to fish. B. New *T. tubifex* exposures were initiated daily for seven days. Each worm exposure was terminated after seven days and worms were fed to fish. Thus, dietary fish exposures were initiated seven days after the first worm exposure was initiated.

2.3 Worm exposure

2.3.1 Experimental organism, *T. tubifex*

T. tubifex (EAN 4038358100154, Batch-No.: 1518413 produced by Live is Life for Zooschatz, Berlin, Germany) were purchased through ZooCenter Bäckebol (Hisings Backa, Göteborg, Sweden). The worms were reared in the laboratory in glass beakers (250 mL Schott bottles) containing 150 mL *T. tubifex* medium (described below) continuously aerated by a glass Pasteur pipette connected by silicon tubing to the in-house pressurized air system. The culture was kept at 17 °C at a 12h/12h darkness/light cycle (conditions in the aquarium facility).

2.3.2 Tubifex medium preparation

Tubifex medium (artificial freshwater) was prepared as specified in OECD Guideline 203. In brief, 25 mL of a calcium chloride stock solution (11.76 g/L $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, 10043-52-4, Merck), a magnesium sulfate stock solution (4.93 g/L $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 10034-99-8, Sigma-Aldrich), a sodium bicarbonate stock solution (2.59g, NaHCO_3 , 144-55-8, Merck) and a potassium chloride stock solution (0.23 g/L KCl, 7447-40-7, Merck) were mixed and the total volume made up to 1 L with

deionized water. The final solution was kept at 17 °C and aerated for at least 24 h before being used.

2.3.3 Sediment preparation

Sediment was collected from Roskilde Fjord, Munkholmbroen (55°40'25"N 11°48'44"E) in October 2016 by scraping of the top few centimeters of the sediment surface and subsequently sieved at the site (<0.5 mm). After settling, the overlying water was removed and the sediment was frozen at -20°C to kill macro- and meiofauna. Thereafter, the sediment was thawed, sieved to <125 µm, rinsed with fresh *T. tubifex* medium and stored at 4°C until experimental use. Dry weight (dw) to wet weight (ww) ratio determined using a Metler Toledo moisture analyzer was 0.374 ± 0.001 (mean \pm sd, $n = 2$). The background Cu concentration of the sediment (<125 µm) was 29.4 ± 3.7 µg Cu g⁻¹ dw sediment ($n = 7$; Cu concentrations inferred by either of the two naturally occurring stable Cu isotopes, ⁶³Cu or ⁶⁵Cu, as described below).

For exposures, sieved sediment (about 240 g ww sediment per treatment) was spiked with ⁶⁵CuO NPs or ⁶⁵CuCl₂ at a nominal concentration of 100 µg ⁶⁵Cu g⁻¹ dw sediment (the measured concentrations were 88 and 95 µg ⁶⁵Cu g⁻¹ dw sediment, respectively; see Table 2). In addition, control sediment was prepared. This sediment was spiked only with the solvent in which ⁶⁵CuO NPs and ⁶⁵CuCl₂ spiking solutions were prepared (2 mM citrate solution, see above). To obtain a homogenous Cu distribution, the sediment was first stirred by hand and then by automated shaking at 300 rpm and room temperature overnight (Heidolph Unimax 1010 Orbital Shaker). Thereafter, the sediment was kept at 4°C until use for exposures (one week).

2.3.4 Exposures of *T. tubifex* to ⁶⁵CuO NP and ⁶⁵CuCl₂-spiked sediment

Initiation of T. tubifex exposures: Beakers (100 mL Schott bottles, 56 mm in diameter) were loaded with 3 g of natural non-contaminated (control), ⁶⁵CuCl₂- or ⁶⁵CuO NP-spiked sediment (100 µg ⁶⁵Cu g⁻¹ dw sediment; see above). Subsequently, 100 mL of aerated tubifex medium was added to the

beakers and the sediment left to settle for 24 h. Before experimental start, the overlying water was carefully removed and replaced with fresh aerated tubifex medium. Then, ~1.2 g ww healthy and intact *T. tubifex* of similar size were selected, sub-divided into three portions of ~0.4 g ww each, and added to the exposure beakers. A random sub-sample of ten worms was taken and their ww determined to ensure that worm sizes were comparable between treatment groups and independent repetitions (**Error! Reference source not found.**). *T. tubifex* exposures lasted seven days and were carried out in a room with controlled air temperature (17 °C). The overlying water was gently aerated (~1-2 bubbles per second) throughout the experiment via a glass Pasteur pipette positioned 2 cm above the sediment surface. The temperature, dissolved oxygen content and conductivity of the water overlying the sediment was 17.0 ± 0.1 °C, 94.3 ± 1.0 % and 553.9 ± 6.2 $\mu\text{S cm}^{-1}$, respectively.

Sampling of overlying water and sediment: For three of the seven independent repetitions, sub-samples (1 mL) of overlying water of each exposure vessel were taken at experimental day 0, 1, 4, and 7 to determine ^{65}Cu remobilization from sediment into water (determined by inductively coupled plasma-mass spectrometry, ICP-MS; see below). The samples were taken from four different locations (4 x 250 μl) around the beaker's circumference (staggered by 90°) and ~1.5 cm above the sediment surface. In addition, representative samples of sediment (0.2-0.3 g ww) were taken of each exposure vessel at the end of the exposure. All samples were stored at -20 °C until analysis.

Sampling of worms: At the end of experimental day 7, worms were carefully sampled from the exposure vessels using a 1 mm sieve. The worms were collected in a beaker with fresh aerated tubifex medium and all sediment associated with the outer surface of the worms was removed by repeatedly rinsing and transferring the worms into new beakers with clean aerated tubifex medium. Following determination of the total number and weight of worms recovered from each exposure (data shown in Table S2), the cleaned worms were incubated for additional 20 h to purge the gut

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from sediment particles and unassimilated Cu, and then fed to fish (see below). A sub-sample of ten purged worms of each group was used to determine the average body ww of individual worms (data shown in Table S3) needed to calculate the number of worms corresponding to the fish' food ration (representative images of proportionated purged *T. tubifex* fish were fed during exposures are shown in Figure S2). Moreover, two purged worms of each treatment group and independent repetition were set aside for quantification of ⁶⁵Cu body burden using ICP-MS (n = 14).

2.4 Fish exposure

2.4.1 Experimental organism: Three-spined stickleback (*Gasterosteus aculeatus*)

Three-spined sticklebacks (*G. aculeatus*) were caught from a small channel (58° 13' 57.4" N, 11° 28' 22.9"E) close to the marine research station Kristineberg, Sven Lovén Centre for Marine Infrastructure, on the island of Skaftö on the Swedish west coast in September 2018. The fish were transported to the animal facility at the Department of Biological and Environmental Sciences, University of Gothenburg, where they were transferred to an acclimatization tank with environmental enrichment, aeration, and ~14 °C-cold, brackish water (~18 ppt). After one week, the fish were slowly acclimatized to freshwater by gradually replacing the brackish water with filtered, UV-treated tap water (~155 µS cm⁻¹, ~10 °C) originating from the in-house semi-recirculating system. The fish were maintained at these conditions under continuous flow-through and were fed *ad libitum* with frozen blood worms (Röda Mygglarver, Aquarietechnik) until used for experimentation in November 2018. All animal procedures were performed in accordance with national and international guidelines for care and use of laboratory animals following the policy for animal experimentation of the University of Gothenburg and were approved by the Animal Ethics Committee of the Swedish Board of Agriculture (Ethical permit 15986-2018).

2.4.2 Dietary exposure of three-spined sticklebacks to live ⁶⁵CuO NP and ⁶⁵CuCl₂-exposed *T. tubifex*

Two weeks before experimental start, 45 fish with similar weight were selected and transferred to an experimental flow-through aquaria system (fish were of male and female sex; see Table S5 for sex distribution of analyzed fish). The experimental tanks contained 1.5 L of filtered, UV-treated tap water (10 °C) supplied by the in-house semi-recirculating system. The water turnover in the experimental tanks was $\sim 0.1 \text{ L min}^{-1}$. Each tank contained one fish and was assigned to one of the three treatment groups (control, $^{65}\text{CuCl}_2$, ^{65}CuO NP). During the first seven days of the study (experimental day 1-7) all fish were fed live, uncontaminated *T. tubifex* collected from the *T. tubifex* culture (see above). On experimental day 8, feeding was suspended so that fish could empty their guts and the tanks be cleaned from feces before starting with the dietary exposure. From experimental days 9 to 15, the fish were fed live *T. tubifex*, which were previously exposed to non-contaminated (control), $^{65}\text{CuCl}_2$ -spiked or ^{65}CuO NP-spiked sediment for seven days (see above). Each fish was fed six to seven worms each day corresponding to an average daily food ration of approximately $26 \pm 0.4 \text{ mg}$ and 4% of the fish body ww ($0.627 \pm 0.114 \text{ g}$, $n = 45$; the average body ww between treatment groups was similar). More detailed information on the number, weight and ^{65}Cu body burden of worms fed to each fish on each day is shown in Table S5. In addition, feces egested by each fish were collected at the end of each experimental day (i.e., prior to feeding) and stored at -20 °C until ICP-MS analysis. Furthermore, water samples (50 mL) were taken from three experimental tanks of each treatment group on a daily basis. Water samples originating from tanks belonging to the same treatment group were pooled and analyzed by ICP-MS to determine Cu background levels and control for potential ^{65}Cu release from feces. The temperature, dissolved oxygen content and conductivity of the water in the flow-through system were monitored daily and were $10.0 \pm 0.2 \text{ °C}$, $79.3 \pm 3.3 \%$ and $159.3 \pm 4.3 \mu\text{S cm}^{-1}$, respectively. Furthermore, acidity (pH), general hardness (GH), carbonate hardness (KH), nitrite (NO_2^-), nitrate (NO_3^-) and chlorine (Cl) levels of the water in the system were controlled at the beginning of the experiment and the end of the exposure. All parameters were within the quality criteria (pH: 6.8 to 7.2, GH: $>4 \text{ °}$, KH: 3 to 6 ° , NO_2^- : 0 mg/L, NO_3^- : 0 to 10 mg/L, Cl: 0 to 0.8 mg/L). There were no significant differences among treatment groups.

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2.4.3 Sampling and tissue dissection

After the 7-day exposure period (end of experimental day 15), all fish were stunned by a sharp blow to the head and killed by transection of the spinal cord. Following determination of the body weight and standard length, liver and intestine were excised. The intestine was gently flushed with a physiological buffer solution to remove any remaining content (chyme). Liver and intestinal tissue samples of each fish were split into a small tissue sample for gene expression analysis (abscised from the proximal end of the intestine and the distinct, pointy end of the liver) and a large tissue sample for ICP-MS analysis. Tissue samples were placed in cryotubes, snap-frozen and stored in liquid nitrogen. The remainders of the fish (in the following referred to as carcass) were frozen on dry ice and stored at -80 °C. Tissue samples of 8 of the 15 fish in each treatment group were analyzed (see below).

2.5 Gene expression analysis

Liver and intestine samples were homogenized in RLT plus buffer using a TissueLyser II apparatus and stainless-steel beads (5 mm) according to the manufacturer's instructions (all stated materials and instruments were from Quiagen). Total RNA was isolated using RNeasy Mini Kit (Quiagen). RNA concentration and purity were measured using a NanoDrop 2000c spectrophotometer (ThermoFisher). cDNA was synthesized using the iScript™ cDNA synthesis kit from BioRad. Quantification of relative mRNA expression levels (cDNA copy numbers) was carried out using SsoAdvanced™ Universal SYBR Green Supermix (BioRad) and appropriate primer pairs (Eurofins Genomics, Ebersberg, DE) ⁴². The sequence and concentrations of the oligonucleotide primers, and the efficiencies of the respective qPCR (quantitative polymerase chain reaction) assays are presented in Table 1. All qPCR reactions were run in duplicate on a Bio-Rad CFX Connect™ Real-Time System (one cycle of 3 min-cycle at 95°C followed by 40 cycles of 10 s at 95°C (denaturation) and 30 s at 60°C or 62°C (annealing and extension). ΔCq values were calculated

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subtracting the mean Cq value of each target gene by the average of the mean Cq values of the two reference genes.

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Table 1. List of analyzed reference and target genes and information on primers used for qPCR including sequence, concentration, annealing temperature and efficiencies of optimized assays.

Gene name and abbreviation	Sequence (5'→3') of forward (fw) and reverse (rv) primers	Conc. [nM]	Temp. [°C]	Eff. [%]	Ref.
β-Actin (<i>β-act</i>)	fw CTGTCTTTCCCTCCATCGTC	500	60	103.4	55
	rv CTCTTGCTCTGGGCTTCATC	500			
Ubiquitin (<i>ubq</i>)	fw AGACGGGCATAGCACTTGC	500	60	93.4	56
	rv CAGGACAAGGAAGGCATCC	500			
Tight junction protein 1a (<i>zo-1</i>)	fw CTCTCTTAGGAGGCCACCA	250	62	98.6	42
	rv TCTCCCCGTGTTTCTACGC	250			
High affinity copper transporter (<i>ctr1</i>)	fw TCAACGTCCGCTACAACTCC	500	60	103.0	42
	rv ACCTGGACGATGTGCAACAG	500			
Metallothionein-A (<i>mta</i>)	fw CCCCTGCTGCCCCGACTG	500	60	102.4	57
	rv TGTTCAAACCTGCCGCCATCTC	500			
Glutamate-cystein-ligase, catalytic subunit (<i>gcl</i>)	fw CGTGTTGAAATGGGGCGATG	250	60	104.8	42
	rv TCCAAAGGTTGGGGTGATTG	250			
Glutathione reductase (<i>gr</i>)	fw GCTGCAAAACTCTGGTGTGG	500	60	98.0	42
	rv CATTTCCAAACCCATGGCGG	500			
Glutathione peroxidase (<i>gpx</i>)	fw ATCAGGAGAACTGCAAGAATGAAG	100	60	110.3	58 a
	rv GTTCACCTTCTCAAGGAGCTG	100			
Superoxide dismutase-1 (<i>sod-1</i>)	fw AGCAGGAGAGCGATAAAGCG	250	60	102.5	42
	rv TCATCATTAGGGCCTGCGTG	250			
Catalase (<i>cat</i>)	fw ACCAAGGTTTGGTCCCACAAAG	500	60	102.4	58 a
	rv TGCTCCACCTCTGCAAAGTAG	500			

^a Oligonucleotide sequence was shortened.

2.6 Concentrations of background Cu and newly accumulated ⁶⁵Cu

2.6.1 Sample analysis with ICP-MS

Cu concentrations in fish and worm tissues, sediment, water, and fish feces were measured by ICP-MS (7900, Agilent). Samples were dried in an oven at 40 °C for at least 24 h. Larger samples (fish carcass and sediment samples) were weighed into Weflon™ vials (Milestone, Germany) and dissolved with 65% ultrapure nitric acid (HNO₃) and Milli-Q water (1:1) in a volume of 6.25 mL. All smaller sized samples were weighted into Teflon™ inserts, which made it possible to dissolve samples in smaller volumes of HNO₃ and Milli-Q water (1:1) (0.625 mL). Three Teflon™ inserts were placed in each Weflon™ vial containing 10 mL Milli-Q water and 2mL 30 % H₂O₂. All samples were subsequently digested according to ISO15587-2. Briefly, samples were heated in a microwave oven (Start D microwave digestion system, Milestone, Germany). All samples were then transferred into volumetric flasks, resulting in a known volume resulting in 8 % HNO₃. Finally,

the Cu concentration in each sample was determined directly after digestion or after a short storage period (<48 h). A series of standard Cu solutions (8 % HNO₃) was used to calibrate Cu concentrations (six standards were selected from 0, 0.1, 2, 5, 10, 50, 100, and 1000 µg Cu L⁻¹ to cover the range of expected Cu concentrations in the sample batch). During each analytical run, at least one of these standards were re-analyzed (approximately for every ten samples analyzed) to check for analytical drift and were all in agreement with expected Cu concentrations. Samples were analyzed in duplicate (each analysis averaged 32 measurements) for the naturally occurring stable isotopes ⁶³Cu and ⁶⁵Cu by ICP-MS (Agilent) as described in Lammel and Thit et al. (2019)⁴². Samples were set to zero if their Cu concentrations inferred from ⁶³Cu were higher than those of ⁶⁵Cu.

Cu recovery was examined using certified mussel tissue (European reference materials, ERM®-CE278k; 5.98 µg Cu g⁻¹ dw tissue), and certified freshwater sediment (RIZA, Trace metals WD CRM-CNS301-050; 44.2 µg Cu g⁻¹ dw sediment), which were digested and analyzed together with different sample batches (at least one reference sample per batch). Results of certified tissue and sediment) were in good agreement with the certificate of analysis (5.54 ± 0.27 µg Cu g⁻¹ dw tissue, n = 4 and 41.45 ± 2.30 µg Cu g⁻¹ dw sediment, n = 4, respectively). All equipment used for sample digestions was thoroughly acid-washed before use.

2.6.2 Calculation of concentrations of newly accumulated ⁶⁵Cu

Concentrations of newly accumulated or added ⁶⁵Cu (referred to as ⁶⁵Cu in the following) were calculated based on ICP-MS measurements of the two naturally abundant stable isotopes, ⁶⁵Cu, and ⁶³Cu, as described in Croteau et al. (2004)⁵⁹. Briefly, the relative abundance of the two isotopes in natural Cu samples in the absence of a spike (p^{65}) was set to 0.309⁶⁰. Concentrations of newly accumulated ⁶⁵Cu in the experimental organisms ($[^{65}\text{Cu}]_{\text{org}}$) were calculated as the product of p^{65} and the total Cu concentrations inferred by the ICP-MS software from ⁶⁵Cu intensity ($[T^{65}\text{Cu}]$).

$$[^{65}\text{Cu}]_{\text{org}} = p^{65} \times [T^{65}\text{Cu}] \quad (\text{eq. 1})$$

The original load of tracer ($[^{65}\text{Cu}]_{\text{org}0}$) that occurred in each sample in the absence of a ^{65}Cu spike was calculated as the product of p^{65} and the total Cu concentrations inferred from the intensity of the most abundant Cu isotope, (i.e., $[T^{63}\text{Cu}]$):

$$[^{65}\text{Cu}]_{\text{org}}^0 = p^{65} \times [T^{63}\text{Cu}] \quad (\text{eq. 2})$$

The net tracer uptake ($[^{65}\text{Cu}]_{\text{org}}$) was derived from the total Cu concentration inferred from ^{65}Cu signal ($[^{65}\text{Cu}]_{\text{org}}$ minus the pre-existing concentration of tracer ($[^{65}\text{Cu}]_{\text{org}}^0$):

$$\Delta[^{65}\text{Cu}]_{\text{org}} = [^{65}\text{Cu}]_{\text{org}} - [^{65}\text{Cu}]_{\text{org}}^0 = p^{65} \times ([T^{65}\text{Cu}] - [T^{63}\text{Cu}]) \quad (\text{eq. 3})$$

2.7 Statistical analysis

In general, data are presented as mean \pm sd (standard deviation) unless otherwise stated. One-way Analysis of Variance (one-way ANOVA) was conducted when data met the test's requirements for normality and equal variances. When requirements for parametric analysis were not met, non-parametric Kruskal–Wallis Analysis on ranks was conducted. The strength of the association between pairs of variables (for instance, ^{65}Cu concentration and target gene expression level) was measured by Pearson product–moment correlation analysis. Student's t-test were conducted to test the null hypothesis that the mean ^{65}Cu concentration in the overlying water of $^{65}\text{CuCl}_2$ and ^{65}CuO NP sediment exposures are equal. Data for ^{65}Cu concentrations in control treatment were zero/close to zero and hence failed the normality test. Thus, comparison by one-way ANOVA could not be performed. Kruskal–Wallis Analysis on ranks had too little statistical power to detect significant differences between treatments because of the low sample size and a student's t-test was conducted. Statistical analysis was conducted using SigmaPlot for Windows Version 14.0 (Systat Software, Inc., San Jose, CA, USA).

3. Results and Discussion

3.1 Bioaccumulation study of sediment-associated ^{65}Cu and ^{65}CuO NP in worms

^{65}Cu concentrations in sediment and water. The nominal sediment exposure concentrations ($\sim 100 \mu\text{g } ^{65}\text{Cu g}^{-1} \text{ dw sed}$) were selected on the basis of a worst-case scenario, where CuO NP concentrations in the sediment were assumed to be as high as the concentrations of non-particulate Cu in some sediments in Europe today (often 5-50, but reaching up to $4000 \mu\text{g Cu g}^{-1} \text{ dw sed}$ at contaminated sites)^{48, 61}. There was no significant difference between the measured sediment ^{65}Cu concentrations of the two ^{65}Cu treatments, though they were slightly higher in the sediment spiked with $^{65}\text{CuCl}_2$ compared to ^{65}CuO NP (by approximately 7 %; Table 2). The bioturbation activity of *T. tubifex* resulted in resuspension of sediment particles (Figure S1), but only little ^{65}Cu was remobilized into the overlying water. Hence, it can be assumed that exposure to waterborne ^{65}Cu was negligible during the experiment (Table 2). Interestingly, the mean ^{65}Cu concentration measured in the overlying water at day 7 was significantly higher in the ^{65}CuO NP exposure than in the $^{65}\text{CuCl}_2$ exposure ($p = 0.018$, Student's *t*-test). Several processes leading to remobilization of Cu (as nanoparticulate or dissolved form) may have been affected differently by the two ^{65}Cu treatments. 1) The conveyor-belt feeding behavior of *T. tubifex*, where sediment is ingested at depth and egested as fecal matter above the sediment surface⁶², can increase the concentration of metals in top sediment layers facilitating their remobilization into the overlying water^{63, 64}. Thus, differences in remobilization between treatments may be owed to differences in feeding activity (resulting in a lower amount of fecal pellets deposited on the surface in $^{65}\text{CuCl}_2$ -treatment) or differences in gastrointestinal ^{65}Cu absorption^{18, 42} (resulting in lower ^{65}Cu concentration in fecal pellets egested in $^{65}\text{CuCl}_2$ -treatment). 2) Cu stress can induce caudal autotomy (tail loss)⁴⁹, which precludes worms from defecating until the posterior end has been regenerated and is operational again (often after more than five days)⁶⁵. We did not specifically investigate this endpoint, but if exposure to dissolved Cu had caused caudal autotomy, egestion

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would have been decreased, resulting in decrease Cu remobilization. 3) Cu exposure has been reported to reduce locomotion and bioturbation activity of tubificid worms at sublethal concentrations (down to 10 $\mu\text{g Cu g}^{-1}$ dw sediment)^{66, 67}, and may affect remobilization of Cu. Bioturbation by *T. tubifex* likely increases the transport of metals from sediment to overlying water; thus, a reduced bioturbation activity will depress the concentration in the overlying water.

Table 2. Exposure concentrations of ^{65}Cu (i.e., all forms) in sediment and overlying water in *T. tubifex* exposures, presented as mean \pm sd. Significant differences between the means of the $^{65}\text{CuCl}_2$ - and ^{65}CuO NP-exposure are indicated by an asterisk (*) (t-test, $p < 0.05$).

Sample	Sampling time	n	Control	$^{65}\text{CuCl}_2$	^{65}CuO NP	Unit
Newly accumulated ^{65}Cu						
Sediment	7 d	7	$<0.01 \pm 0.01$	94.68 ± 27.26	88.24 ± 14.68	$\mu\text{g g}^{-1}$ dw
Water	7 d	3	0.00 ± 0.00	5.44 ± 1.28	** 10.56 ± 2.48	$\mu\text{g L}^{-1}$
Background Cu						
Sediment	7 d	7	29.35 ± 3.66			$\mu\text{g g}^{-1}$ dw
Water	7 d	7	2.18 ± 2.50			$\mu\text{g L}^{-1}$

Body burden of ^{65}Cu in T. tubifex. The mean weight-specific body burden (WSBB) of ^{65}Cu in *T. tubifex* was higher after exposure to ^{65}CuO NP than after exposure to $^{65}\text{CuCl}_2$ (1.1 ± 1.0 vs. $0.6 \pm 0.4 \mu\text{g g}^{-1}$ dw tissue, respectively; $n = 14$), though not statistically significant (Figure 2). The amount of newly accumulated Cu (measured as ^{65}Cu) was relatively low compared to Cu background concentrations ($22.3 \pm 9.6 \mu\text{g Cu g}^{-1}$ dw tissue) and would likely have been indistinguishable from background without the use of the tracer, as previously reported by Misra et al (2012)⁶⁸ and Lammel and Thit et al. (2019)⁴². Our findings suggest that ^{65}Cu from both the dissolved and nanoparticulate Cu forms in the sediment was bioavailable and accumulated to a similar extent in the worms. This observation is consistent with several other studies reporting that the difference in WSBB of worms (*N. diversicolor*, *L. variegatus*, *T. tubifex*) exposed to different Cu forms is marginal^{9, 19, 42}. It should be noted that worms in the NP treatment were not only exposed to ^{65}CuO NPs but also dissolved ^{65}Cu (i.e., non-particulate ^{65}Cu). The proportion of dissolved (i.e., non-

particulate) ^{65}Cu in the ^{65}CuO NP dispersion used to prepare the dispersion spiked into sediment was $\sim 34\%$ ³⁴. The relative amount of dissolved (i.e., non-particulate) ^{65}Cu in the exposure sediment may even have been higher, since the presence of chelating organic ligands (e.g., humic acids) can increase CuO NP dissolution ^{42, 69, 70}. Hence, it is likely that accumulation of dissolved ^{65}Cu by worms in ^{65}CuO NP-spiked sediment occurred, partly concealing potential differences between treatments.

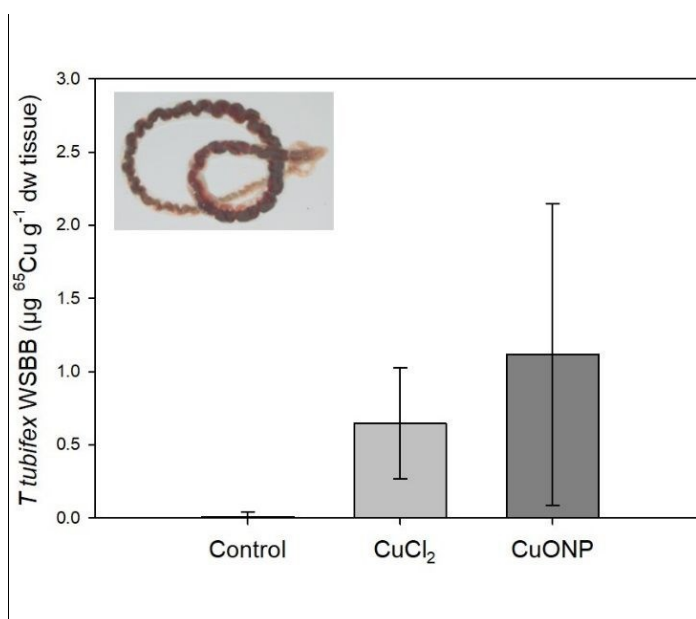


Figure 2: ^{65}Cu WSBB in *T. tubifex*. Worms were exposed for 7 days in clean sediment or sediment with $^{65}\text{CuCl}_2$ or ^{65}CuO NPs at about $100 \mu\text{g } ^{65}\text{Cu g}^{-1} \text{ dw sediment}$. Mean \pm sd ($n = 14$).

3.2. Transfer of ^{65}Cu and ^{65}CuO NPs from worms to fish

Tissue concentrations of ^{65}Cu in fish: Due to limited ^{65}Cu concentrations in worms, the dietary exposures doses of ^{65}Cu that fish received were very low ($\sim 4 \pm 1$ and $7 \pm 1 \text{ ng g}^{-1} \text{ body ww day}^{-1}$ in the $^{65}\text{CuCl}_2$ - and ^{65}CuO NP-treatment, respectively; for more details see Table S5 and Table S6). Furthermore, in both treatments, the dose of ^{65}Cu represented only a small portion ($\sim 5\%$) of the total dietary Cu dose fish received (the Cu background concentration was $\sim 22 \mu\text{g g}^{-1} \text{ dw}$, which is

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in the range of nutritional dietary Cu requirements of fish ⁷¹). At such low exposure doses, low dietary uptake is expected ^{71, 72}, and overall ⁶⁵Cu concentrations in fish tissues were low (Table 3). Generally, highest concentrations were found in intestine, followed by liver and carcass (Figure 3). No statistically significant differences in mean ⁶⁵Cu tissue concentrations were observed between treatments, though they were generally higher in fish exposed to ⁶⁵CuCl₂ than in fish exposed to ⁶⁵CuO NPs (Table 3 and Figure 3). However, it must be noted that *de facto* hepatic ⁶⁵Cu accumulation was detected in only two of the fish feeding on ⁶⁵CuCl₂-exposed worms, and in only one of the fish feeding on ⁶⁵CuO NP-exposed worms (see Table S3). Similar to our previous findings ⁴², Cu background concentrations were highest in liver tissue and Cu accumulation highest in the intestine of fish fed with CuCl₂-exposed worms or CuCl₂-spiked food packages.

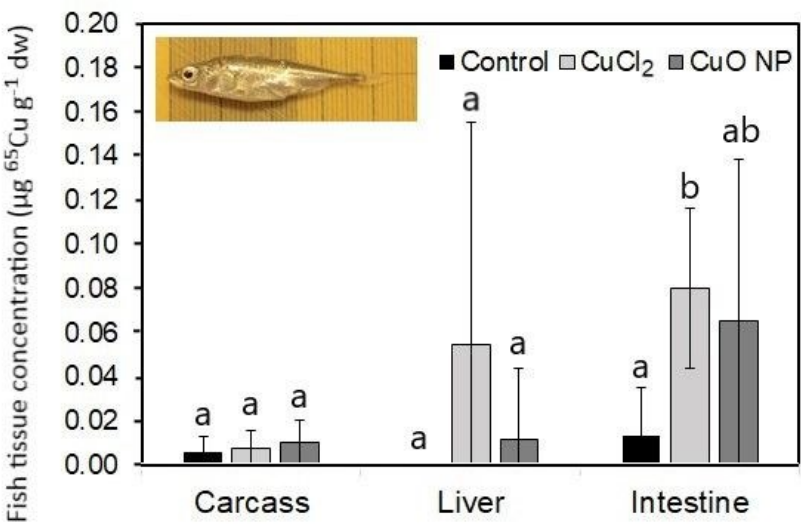


Figure 3: ⁶⁵Cu accumulation in three-spined stickleback after seven days of dietary exposure to *T. tubifex* exposed in non-contaminated sediment (control) or sediment spiked with ⁶⁵CuCl₂ or ⁶⁵CuO NPs for seven days. Bars represent the mean tissue concentrations of ⁶⁵Cu ± sd (n = 8). Different letter combinations indicate statistically significant differences (p < 0.05).

Egestion of ^{65}Cu in feces. The mean ^{65}Cu concentration in the feces of fish fed $^{65}\text{CuCl}_2$ -exposed worms was about half of that measured in feces of fish fed ^{65}CuO NP-exposed worms $^{65}\text{CuCl}_2$ and ^{65}CuO NP exposure, respectively (concentrations are shown in Table 3). This result is largely consistent with the differences in the received dietary exposure dose (*i.e.*, ^{65}Cu WSBB of $^{65}\text{CuCl}_2$ - and ^{65}CuO NP-exposed worms) and the differences in gastrointestinal ^{65}Cu assimilation between the two treatments (See Table 3 and Figure 3). Altogether, ^{65}Cu concentrations in fish tissues and feces suggest that ^{65}Cu assimilation from ^{65}CuO NP-exposed worms might be lower compared to that from $^{65}\text{CuCl}_2$ -exposed worms, albeit not significantly.

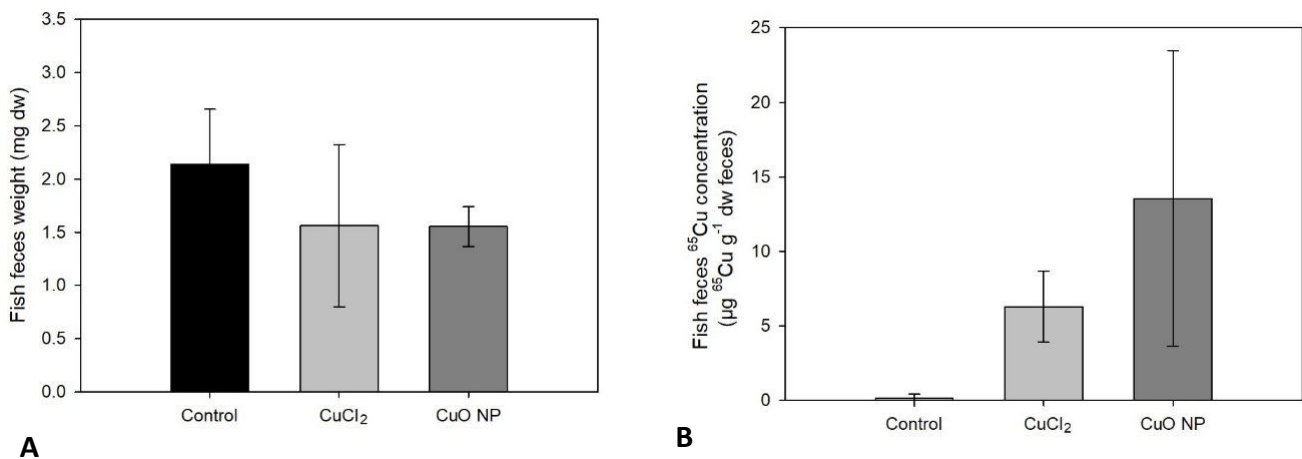


Figure 4: Fish feces collected during seven days of dietary exposure to sediment exposed *T. tubifex*. Weight (A) and ^{65}Cu concentration (B) in fish feces produced by individual fish during seven days of exposure and collected daily. Data are presented as mean \pm sd, $n = 8$.

3.3. Effects of dietary ^{65}Cu and ^{65}CuO NP exposure in fish

In accordance with the low ^{65}Cu tissue concentrations, limited alterations in the expression of investigated target genes were observed (Figure 5). Only in intestinal tissue of fish feeding on $^{65}\text{CuCl}_2$ -exposed worms, a statistically significant upregulation of one of the assessed target genes, namely *gpx*, was evident (compared to control fish and fish feeding on ^{65}CuO NP exposed worms)

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(Figure 5). Furthermore, *gpx* expression levels were found to be positively correlated with intestinal ⁶⁵Cu concentrations ($r=0.9$, $p=0.002$), showing that the effect was related to Cu exposure (Table S6). These results suggest that even minimally elevated dietary Cu levels can induce a disturbance in the cellular redox balance in intestinal tissue. This is in general agreement with previous research reporting Cu-induced oxidative stress and the induction of antioxidant defense mechanisms in intestinal cells and tissues^{73, 74}. Similarly, *gpx* appeared to be upregulated (though not significantly) in our previous study where three-spined sticklebacks were exposed to a practical diet prepared from CuCl₂-spiked worm homogenate (though at higher exposure dose)⁴².

It must be noted that in intestine of fish fed with ⁶⁵CuO NP-exposed worms, *gpx* mRNA levels were not upregulated (*i.e.*, not different from the control), although mean ⁶⁵Cu tissue concentrations were similar to those in fish fed with ⁶⁵CuCl₂-exposed worms. This may point to differences in the subcellular distribution¹⁹ and or form of ⁶⁵Cu inside intestinal cells between the exposure groups: However, this hypothesis needs further investigation.



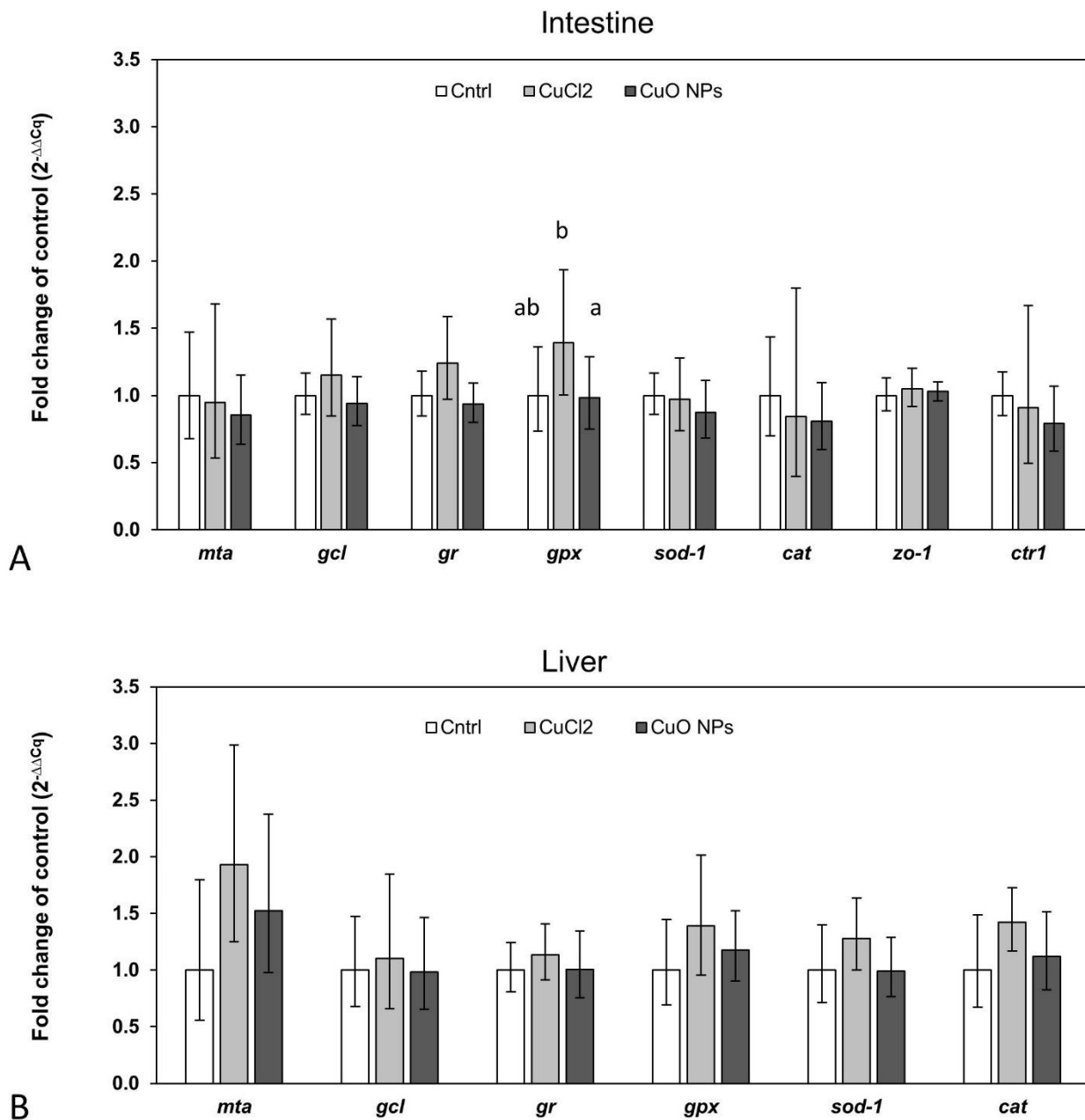


Figure 5. Relative expression levels of selected target genes in intestinal (A) and hepatic tissue (B) of three-spined stickleback fed *T. tubifex* exposed to control, $^{65}\text{CuCl}_2$ - or ^{65}CuO NP-spiked sediment (white, grey and dark grey bars, respectively). Bars and error bars represent the mean and sd, respectively. Letters above bars indicate statistically significant differences between the means. Bars that do not share the same letter are different at a significance level of 0.05 (One Way ANOVA followed by All Pairwise Multiple Comparison using Holm-Sidak method).

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Trophic transfer studies using live worms as feed for fish

Often, dietary bioaccumulation and toxicity of Cu in fish are studied using practical diets, which allows incorporation of Cu in excess (dietary exposure doses in previous studies range from ~0.4 µg to ~185 µg Cu g⁻¹ fish body ww day⁻¹ ⁷¹). This may lead to unrealistic exposure scenarios in regards to both food type, complexation with biological tissue, and exposure dose. In contrast, the use of live feed offers the possibility of environmentally realistic exposure in laboratory tests. However, there are two major challenges associated with the use of live feed in trophic transfer studies. The first challenge is to achieve similar WSBB of test compounds and reference compounds in prey organisms (and reproducible WSBBs between individuals and replicates). Here, we found high variation in ⁶⁵Cu WSBB of sediment-exposed *T. tubifex* within treatments. However, the mean ⁶⁵Cu fish exposure doses were not significantly different between the two Cu treatments.

The second challenge is to obtain a high enough WSBB of the test compound in the prey organism for it to be detectable in the predator organism. Here, we found limited ⁶⁵Cu accumulation from sediment by *T. tubifex* worms, which translated into a very low dietary exposure dose for fish. Thus, demonstrating trophic transfer was only possible with the use of a stable isotope tracer. If such tracers are unavailable in future studies, higher dietary exposure doses may potentially (depending on when steady-state body burdens are reached) be achieved by extending the time of worm exposure and subsequent fish exposure, rather than increasing exposure concentrations in sediment to unrealistic levels. Yet, it needs to be taken into consideration that this strategy will increase the experimental complexity giving rise to logistical challenges such as the requirement for more spiked sediment and more time-consuming, labor-intensive exposures of worms and fish that need to be run in parallel.

Conclusion

Isotopically labeled NPs offer unique possibilities for studying NP bioaccumulation and trophic transfer under environmentally realistic conditions. The findings from this study show that exposure to Cu at environmentally relevant concentrations in sediment, either in dissolved or nanoparticulate form, leads to increased concentrations in *T. tubifex* and can be transferred to fish (three-spined stickleback). Increases in body burdens *via* dietary exposure and trophic transfer of dissolved Cu and CuO NPs appears to be largely similar, which may be in part explained by partial dissolution of the NPs. In quantitative terms, trophic transfer of dissolved/nanoparticulate CuO across two trophic levels seems to be negligible. Based on our data, an increased risk for biomagnification of CuO NPs within the aquatic food web compared to dissolved Cu seems unlikely, which is well in accordance with the published literature. However, considering the complexity and diversity of abiotic and biotic interactions in the environment, more research is required before general conclusions can be drawn with regard to their bioaccumulation and trophic transfer potential, as well as overall environmental risk.

Conflicts of interest

There are no conflicts to declare.

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References

1. A. A. Keller, S. McFerran, A. Lazareva and S. Suh, Global life cycle releases of engineered nanomaterials, *Journal of Nanoparticle Research*, 2013, 15, 1692.
2. A. Caballero-Guzman and B. Nowack, Prospective nanomaterial mass flows to the environment by life cycle stage from five applications containing CuO, DPP, FeOx, CNT and SiO₂, *Journal of Cleaner Production*, 2018, 203, 990-1002.
3. A. A. Keller, A. S. Adeleye, J. R. Conway, K. L. Garner, L. Zhao, G. N. Cherr, J. Hong, J. L. Gardea-Torresdey, H. A. Godwin, S. Hanna, Z. Ji, C. Kaweeteerawat, S. Lin, H. S. Lenihan, R. J. Miller, A. E. Nel, J. R. Peralta-Videa, S. L. Walker, A. A. Taylor, C. Torres-Duarte, J. I. Zink and N. Zuverza-Mena, Comparative environmental fate and toxicity of copper nanomaterials, *NanoImpact*, 2017, 7, 28-40.
4. K. L. Garner, S. Suh and A. A. Keller, Assessing the Risk of Engineered Nanomaterials in the Environment: Development and Application of the nanoFate Model, *Environmental science & technology*, 2017, 51, 5541-5551.
5. A. M. Dietrich and D. L. Gallagher, Fate and Environmental Impact of Pesticides in Plastic Mulch Production Runoff: Field and Laboratory Studies, *Journal of Agricultural and Food Chemistry*, 2002, 50, 4409-4416.
6. P. Perez-Rodriguez, D. Soto-Gomez, I. De La Calle, J. E. Lopez-Periago and M. Paradelo, Rainfall-induced removal of copper-based spray residues from vines, *Ecotoxicology and Environmental Safety*, 2016, 132, 304-310.
7. A. S. Adeleye, E. A. Oranu, M. Y. Tao and A. A. Keller, Release and detection of nanosized copper from a commercial antifouling paint, *Water research*, 2016, 102, 374-382.

8. A. S. Adeleye, J. R. Conway, T. Perez, P. Rutten and A. A. Keller, Influence of Extracellular Polymeric Substances on the Long-Term Fate, Dissolution, and Speciation of Copper-Based Nanoparticles, *Environmental science & technology*, 2014, 48, 12561-12568.
9. A. Thit, A. Dybowska, C. Kobler, G. Kennaway and H. Selck, Influence of copper oxide nanoparticle shape on bioaccumulation, cellular internalization and effects in the estuarine sediment-dwelling polychaete, *Nereis diversicolor*, *Marine Environmental Research*, 2015, 111, 89-98.
10. Y. L. Xiao, M. G. Vijver and W. Peijnenburg, Impact of water chemistry on the behavior and fate of copper nanoparticles, *Environmental Pollution*, 2018, 234, 684-691.
11. L. Z. Miao, C. Wang, J. Hou, P. F. Wang, Y. H. Ao, Y. Li, B. W. Lv, Y. Y. Yang, G. X. You and Y. Xu, Enhanced stability and dissolution of CuO nanoparticles by extracellular polymeric substances in aqueous environment, *Journal of Nanoparticle Research*, 2015, 17, 12.
12. J. R. Conway, A. S. Adeleye, J. Gardea-Torresdey and A. A. Keller, Aggregation, Dissolution, and Transformation of Copper Nanoparticles in Natural Waters, *Environmental science & technology*, 2015, 49, 2749-2756.
13. I. A. Mudunkotuwa, J. M. Pettibone and V. H. Grassian, Environmental Implications of Nanoparticle Aging in the Processing and Fate of Copper-Based Nanomaterials, *Environmental science & technology*, 2012, 46, 7001-7010.
14. Z. Y. Wang, A. von dem Bussche, P. K. Kabadi, A. B. Kane and R. H. Hurt, Biological and Environmental Transformations of Copper-Based Nanomaterials, *Acs Nano*, 2013, 7, 8715-8727.

15. K. L. Garner and A. A. Keller, Emerging patterns for engineered nanomaterials in the environment: a review of fate and toxicity studies, *Journal of Nanoparticle Research*, 2014, 16, 2503.
16. Z. Liu, C. Wang, J. Hou, P. Wang, L. Miao, B. Lv, Y. Yang, G. You, Y. Xu, M. Zhang and H. Ci, Aggregation, sedimentation, and dissolution of CuO and ZnO nanoparticles in five waters, *Environ. Sci. Pollut. Res.*, 2018, 25, 31240-31249.
17. B. E. Vencalek, S. N. Laughton, E. Spielman-Sun, S. M. Rodrigues, J. M. Unrine, G. V. Lowry and K. B. Gregory, In Situ Measurement of CuO and Cu(OH)(2) Nanoparticle Dissolution Rates in Quiescent Freshwater Mesocosms, *Environ. Sci. Technol. Lett.*, 2016, 3, 375-380.
18. A. Thit, G. T. Banta and H. Selck, Bioaccumulation, subcellular distribution and toxicity of sediment-associated copper in the ragworm *Nereis diversicolor*: The relative importance of aqueous copper, copper oxide nanoparticles and microparticles, *Environmental Pollution*, 2015, 202, 50-57.
19. A. Thit, T. Ramskov, M. N. Croteau and H. Selck, Biodynamics of copper oxide nanoparticles and copper ions in an oligochaete - Part II: Subcellular distribution following sediment exposure, *Aquatic Toxicology*, 2016, 180, 25-35.
20. S. R. Tangaa, H. Selck, M. Winther-Nielsen and F. R. Khan, Trophic transfer of metal-based nanoparticles in aquatic environments: a review and recommendations for future research focus, *Environmental Science: Nano*, 2016, 3, 966-981.
21. A. Bour, F. Mouchet, J. Silvestre, L. Gauthier and E. Pinelli, Environmentally relevant approaches to assess nanoparticles ecotoxicity: a review, *Journal of hazardous materials*, 2015, 283, 764-777.

22. M. Ates, Z. Arslan, V. Demir, J. Daniels and I. O. Farah, Accumulation and toxicity of CuO and ZnO nanoparticles through waterborne and dietary exposure of goldfish (*Carassius auratus*), *Environmental toxicology*, 2015, 30, 119-128.
23. Y. Chae and Y. J. An, Toxicity and transfer of polyvinylpyrrolidone-coated silver nanowires in an aquatic food chain consisting of algae, water fleas, and zebrafish, *Aquatic toxicology (Amsterdam, Netherlands)*, 2016, 173, 94-104.
24. S. Dong, T. Xia, Y. Yang, S. Lin and L. Mao, Bioaccumulation of (14)C-Labeled Graphene in an Aquatic Food Chain through Direct Uptake or Trophic Transfer, *Environmental science & technology*, 2018, 52, 541-549.
25. J. M. Lacave, A. Fanjul, E. Bilbao, N. Gutierrez, I. Barrio, I. Arostegui, M. P. Cajaraville and A. Orbea, Acute toxicity, bioaccumulation and effects of dietary transfer of silver from brine shrimp exposed to PVP/PEI-coated silver nanoparticles to zebrafish, *Comparative biochemistry and physiology. Toxicology & pharmacology : CBP*, 2017, 199, 69-80.
26. W. M. Lee and Y. J. An, Evidence of three-level trophic transfer of quantum dots in an aquatic food chain by using bioimaging, *Nanotoxicology*, 2015, 9, 407-412.
27. N. A. Lewinski, H. Zhu, C. R. Ouyang, G. P. Conner, D. S. Wagner, V. L. Colvin and R. A. Drezek, Trophic transfer of amphiphilic polymer coated CdSe/ZnS quantum dots to *Danio rerio*, *Nanoscale*, 2011, 3, 3080-3083.
28. L. M. Skjolding, M. Winther-Nielsen and A. Baun, Trophic transfer of differently functionalized zinc oxide nanoparticles from crustaceans (*Daphnia magna*) to zebrafish (*Danio rerio*), *Aquatic toxicology (Amsterdam, Netherlands)*, 2014, 157, 101-108.

29. J. Wang and W. X. Wang, Low bioavailability of silver nanoparticles presents trophic toxicity to marine medaka (*Oryzias melastigma*), *Environmental science & technology*, 2014, 48, 8152-8161.
30. Z. Wang, L. Yin, J. Zhao and B. Xing, Trophic transfer and accumulation of TiO₂ nanoparticles from clamworm (*Perinereis aibuhitensis*) to juvenile turbot (*Scophthalmus maximus*) along a marine benthic food chain, *Water research*, 2016, 95, 250-259.
31. X. Zhu, J. Wang, X. Zhang, Y. Chang and Y. Chen, Trophic transfer of TiO₂ nanoparticles from *Daphnia* to zebrafish in a simplified freshwater food chain, *Chemosphere*, 2010, 79, 928-933.
32. C. C. Piccinetti, C. Montis, M. Bonini, R. Laura, M. C. Guerrera, G. Radaelli, F. Vianello, V. Santinelli, F. Maradonna, V. Nozzi, A. Miccoli and I. Olivotto, Transfer of Silica-Coated Magnetic (Fe₃O₄) Nanoparticles Through Food: A Molecular and Morphological Study in Zebrafish, *Zebrafish*, 2014, 11, 567-579.
33. T. Nemati, M. Sarkheil and S. A. Johari, Trophic transfer of CuO nanoparticles from brine shrimp (*Artemia salina*) nauplii to convict cichlid (*Amatitlania nigrofasciata*) larvae: uptake, accumulation and elimination, *Environ. Sci. Pollut. Res.*, 2019, 26, 9610-9618.
34. Lammel T, Thit A, Cuic X, Mouneyrac C, Baun A, Valsami-Jones E, Sturve J and Selck H, A study of the dietary uptake and effects of nanoparticulate and dissolved copper in fish (three-spined stickleback) at environmentally relevant exposure doses utilizing stable isotope-labeled ⁶⁵CuO NPs and ⁶⁵CuCl₂ (*under review*), 2020.
35. S. A. Johari, M. Sarkheil, S. Asghari, F. Haghighat, L. Dekani and S. Keyvanshokoo, Comparative toxicity of nanoparticulate and ionic copper following dietary exposure to

common carp (*Cyprinus carpio*), *Comparative Biochemistry and Physiology C-Toxicology & Pharmacology*, 2020, 229, 10.

36. D. Boyle, N. J. Clark, T. L. Botha and R. D. Handy, Comparison of the dietary bioavailability of copper sulphate and copper oxide nanomaterials in ex vivo gut sacs of rainbow trout: effects of low pH and amino acids in the lumen, *Environmental Science: Nano*, 2020.
37. C. S. Ramsden, T. J. Smith, B. J. Shaw and R. D. Handy, Dietary exposure to titanium dioxide nanoparticles in rainbow trout, (*Oncorhynchus mykiss*): no effect on growth, but subtle biochemical disturbances in the brain, *Ecotoxicology*, 2009, 18, 939-951.
38. L. Chupani, H. Niksirat, J. Velisek, A. Stara, S. Hradilova, J. Kolarik, A. Panacek and E. Zuskova, Chronic dietary toxicity of zinc oxide nanoparticles in common carp (*Cyprinus carpio* L.): Tissue accumulation and physiological responses, *Ecotoxicology and Environmental Safety*, 2018, 147, 110-116.
39. M. Connolly, M. Fernandez, E. Conde, F. Torrent, J. M. Navas and M. L. Fernandez-Cruz, Tissue distribution of zinc and subtle oxidative stress effects after dietary administration of ZnO nanoparticles to rainbow trout, *Science of the Total Environment*, 2016, 551, 334-343.
40. B. Geffroy, C. Ladhar, S. Cambier, M. Treguer-Delapierre, D. Brethes and J. P. Bourdineaud, Impact of dietary gold nanoparticles in zebrafish at very low contamination pressure: The role of size, concentration and exposure time, *Nanotoxicology*, 2012, 6, 144-160.
41. C. Ladhar, B. Geffroy, S. Cambier, M. Treguer-Delapierre, E. Durand, D. Brethes and J. P. Bourdineaud, Impact of dietary cadmium sulphide nanoparticles on *Danio rerio* zebrafish at very low contamination pressure, *Nanotoxicology*, 2014, 8, 676-685.

42. Lammel and Thit, C. Mouneyrac, A. Baun, J. Sturve and H. Selck, Trophic transfer of CuO NPs and dissolved Cu from sediment to worms to fish – a proof-of-concept study, *Environmental Science: Nano*, 2019, 6, 1140-1155
43. M. F. El Basuini, A. M. El-Hais, M. A. O. Dawood, A. E. Abou-Zeid, S. Z. El-Damrawy, M. M. E. Khalafalla, S. Koshio, M. Ishikawa and S. Dossou, Effect of different levels of dietary copper nanoparticles and copper sulfate on growth performance, blood biochemical profiles, antioxidant status and immune response of red sea bream (*Pagrus major*), *Aquaculture*, 2016, 455, 32-40.
44. H. Wang, H. Zhu, X. Wang, E. Li, Z. Du, J. Qin and L. Chen, Comparison of copper bioavailability in copper-methionine, nano-copper oxide and copper sulfate additives in the diet of Russian sturgeon *Acipenser gueldenstaedtii*, *Aquaculture*, 2018, 482, 146-154.
45. S. Lindh, P. Razmara, S. Bogart and G. Pyle, Comparative tissue distribution and depuration characteristics of copper nanoparticles and soluble copper in rainbow trout (*Oncorhynchus mykiss*), *Environmental toxicology and chemistry*, 2019, 38, 80-89.
46. OECD, *Test No. 315: Bioaccumulation in Sediment-dwelling Benthic Oligochaetes*, 2008.
47. D. Milani, T. B. Reynoldson, U. Borgmann and J. Kolasa, The relative sensitivity of four benthic invertebrates to metals in spiked-sediment exposures and application to contaminated field sediment, *Environmental toxicology and chemistry*, 2003, 22, 845-854.
48. Y. E. Roman, K. A. C. De Schamphelaere, L. T. H. Nguyen and C. R. Janssen, Chronic toxicity of copper to five benthic invertebrates in laboratory-formulated sediment: Sensitivity comparison and preliminary risk assessment, *Science of the Total Environment*, 2007, 387, 128-140.

49. M.-L. Lucan-Bouché, S. Biagianti-Risbourg, F. Arsac and G. Vernet, An original decontamination process developed by the aquatic oligochaete *Tubifex tubifex* exposed to copper and lead, *Aquatic Toxicology*, 1999, 45, 9-17.
50. Y. E. Roman, K. A. C. De Schamphelaere, L. T. H. Nguyen and C. R. Janssen, Chronic toxicity of copper to five benthic invertebrates in laboratory-formulated sediment: Sensitivity comparison and preliminary risk assessment, *Science of The Total Environment*, 2007, 387, 128-140.
51. M. Vecchi, T. B. Reynoldson, A. Pasteris and G. Bonomi, Toxicity of copper-spiked sediments to *Tubifex tubifex* (Oligochaeta, Tubificidae): Comparison of the 28-day reproductive bioassay with an early-life-stage bioassay, *Environmental toxicology and chemistry*, 1999, 18, 1173-1179.
52. R. G. Wotton, *A Functional Biology of Sticklebacks*, Springer, Boston, MA, 1984.
53. H. B. N. Hynes, THE FOOD OF FRESHWATER STICKLEBACKS (*GASTEROSTEUS-ACULEATUS* AND *PYGOSTEUS-PUNGITIUS*), WITH A REVIEW OF METHODS USED IN STUDIES OF THE FOOD OF FISHES, *Journal of Animal Ecology*, 1950, 19, 36-58.
54. I. Katsiadaki, in *Biology of the Three-Spined Stickleback*, eds. S. Ostlund-Nilsson, I. Mayer and F. A. Huntingford, CRC Press, Boca Raton, 2006, p. 408.
55. N. S. Hogan, C. A. Wartman, M. A. Finley, J. G. van der Lee and M. R. van den Heuvel, Simultaneous determination of androgenic and estrogenic endpoints in the threespine stickleback (*Gasterosteus aculeatus*) using quantitative RT-PCR, *Aquatic Toxicology*, 2008, 90, 269-276.

56. S. Hibbeler, J. P. Scharsack and S. Becker, Housekeeping genes for quantitative expression studies in the three-spined stickleback *Gasterosteus aculeatus*, *Bmc Molecular Biology*, 2008, 9.
57. E. M. Santos, J. S. Ball, T. D. Williams, H. F. Wu, F. Ortega, R. Van Aerle, I. Katsiadaki, F. Falciani, M. R. Viant, J. K. Chipman and C. R. Tyler, Identifying Health Impacts of Exposure to Copper Using Transcriptomics and Metabolomics in a Fish Model, *Environmental science & technology*, 2010, 44, 820-826.
58. P. Lubiana, J. M. Prokkola, M. Nikinmaa, T. Burmester, M. Kanerva and M. Gotting, The effects of the painkiller diclofenac and hypoxia on gene transcription and antioxidant system in the gills of three-spined stickleback, *Comparative Biochemistry and Physiology C-Toxicology & Pharmacology*, 2016, 185, 147-154.
59. M.-N. Croteau, S. N. Luoma, B. R. Topping and C. B. Lopez, Stable Metal Isotopes Reveal Copper Accumulation and Loss Dynamics in the Freshwater Bivalve Corbicula, *Environmental science & technology*, 2004, 38, 5002-5009.
60. T. B. Coplen, J. K. Bohlke, P. De Bievre, T. Ding, N. E. Holden, J. A. Hopple, H. R. Krouse, A. Lamberty, H. S. Peiser, K. Revesz, S. E. Rieder, K. J. R. Rosman, E. Roth, P. D. P. Taylor, R. D. Vocke and Y. K. Xiao, Isotope-abundance variations of selected elements - (IUPAC Technical Report), *Pure Appl. Chem.*, 2002, 74, 1987-2017.
61. B. Berthet, C. Mouneyrac, J. C. Amiard, C. Amiard-Triquet, Y. Berthelot, A. Le Hen, O. Mastain, P. S. Rainbow and B. D. Smith, Accumulation and soluble binding of cadmium, copper, and zinc in the polychaete *Hediste diversicolor* from coastal sites with different trace metal Bioavailabilities, *Archives of Environmental Contamination and Toxicology*, 2003, 45, 468-478.

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62. A. Ciutat, O. Weber, M. Gerino and A. Boudou, Stratigraphic effects of tubificids in freshwater sediments: a kinetic study based on X-ray images and grain-size analysis, *Acta Oecol.-Int. J. Ecol.*, 2006, 30, 228-237.
63. S. Lagauzere, M. Motelica-Heino, E. Viollier, G. Stora and J. M. Bonzom, Remobilisation of uranium from contaminated freshwater sediments by bioturbation, *Biogeosciences*, 2014, 11, 3381-3396.
64. Y. He, B. Men, X. Yang, Y. Li, H. Xu and D. Wang, Investigation of heavy metals release from sediment with bioturbation/bioirrigation, *Chemosphere*, 2017, 184, 235-243.
65. M. L. Bouche, F. Arnoult and G. Vernet, Caudal regeneration in *Tubifex tubifex* (Oligochaeta, Tubificidae) following copper exposure, *Invertebr. Biol.*, 2003, 122, 42-51.
66. E. R. Hunting, C. Mulder, M. H. S. Kraak, A. M. Breure and W. Admiraal, Effects of copper on invertebrate-sediment interactions, *Environmental Pollution*, 2013, 180, 131-135.
67. A. Gerhardt, Screening the Toxicity of Ni, Cd, Cu, Ivermectin, and Imidacloprid in a Short-Term Automated Behavioral Toxicity Test with *Tubifex tubifex* (Müller 1774) (Oligochaeta), *Human and Ecological Risk Assessment: An International Journal*, 2009, 15, 27-40.
68. S. K. Misra, A. Dybowska, D. Berhanu, M. N. Croteau, S. N. Luoma, A. R. Boccaccini and E. Valsami-Jones, Isotopically Modified Nanoparticles for Enhanced Detection in Bioaccumulation Studies, *Environmental science & technology*, 2012, 46, 1216-1222.
69. C. Peng, C. Shen, S. Zheng, W. Yang, H. Hu, J. Liu and J. Shi, Transformation of CuO nanoparticles in the aquatic environment: Influence of pH, electrolytes and natural organic matter, *Nanomaterials*, 2017, 7.

70. R. D. Kent and P. J. Vikesland, Dissolution and Persistence of Copper-Based Nanomaterials in Undersaturated Solutions with Respect to Cupric Solid Phases, *Environmental science & technology*, 2016, 50, 6772-6781.

71. S. J. Clearwater, A. M. Farag and J. S. Meyer, Bioavailability and toxicity of dietborne copper and zinc to fish, *Comparative Biochemistry and Physiology C-Toxicology & Pharmacology*, 2002, 132, 269-313.

72. M. Grosell, in *Fish Physiology*, eds. C. M. Wood, A. P. Farrell and C. J. Brauner, Academic Press, 2011, vol. 31, pp. 53-133.

73. W.-D. Jiang, P. Wu, S.-Y. Kuang, Y. Liu, J. Jiang, K. Hu, S.-H. Li, L. Tang, L. Feng and X.-Q. Zhou, Myo-inositol prevents copper-induced oxidative damage and changes in antioxidant capacity in various organs and the enterocytes of juvenile Jian carp (*Cyprinus carpio* var. Jian), *Aquatic Toxicology*, 2011, 105, 543-551.

74. W.-D. Jiang, Y. Liu, J. Jiang, K. Hu, S.-H. Li, L. Feng and X.-Q. Zhou, In vitro interceptive and reparative effects of myo-inositol against copper-induced oxidative damage and antioxidant system disturbance in primary cultured fish enterocytes, *Aquatic Toxicology*, 2013, 132-133, 100-110.

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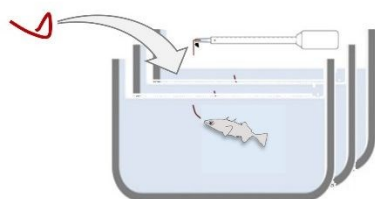
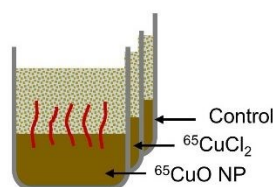
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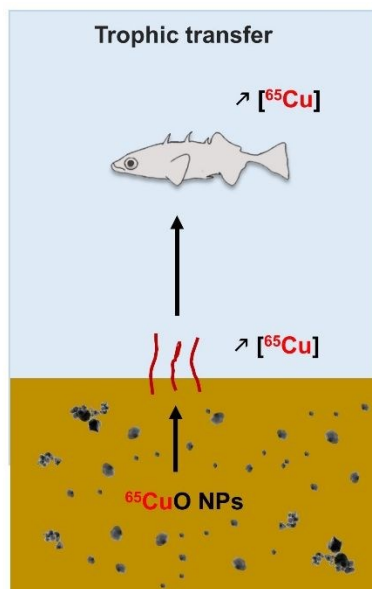
Part II: Dietary exposure of fish



Part I: Sediment exposure of worms



Trophic transfer



This study used stable isotope-labeled copper oxide nanoparticles ($^{65}\text{CuO NPs}$) to determine their trophic transfer potential from sediment to worms (*Tubifex tubifex*) to fish (three-spined stickleback, *Gasterosteus aculeatus*) at environmentally realistic exposure doses and compared to dissolved Cu ($^{65}\text{CuCl}_2$).