ELECTRONIC SUPPLEMENTARY MATERIAL

Interacting effects of graphene oxide derivatives and trace metals on freshwater brown trout (*Salmo trutta* L.) behaviour at different stages of development

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CHARACTERISATION METHODS

Experimental water characterisation

The physicochemical analysis of experimental (dilution) water used in the trials is shown in Supplementary Table 1. The discrepancy in metal ion concentrations between nominal and measured concentrations in the MIX solution was in the 5–14.3 % range. As a result, the metal ion concentrations detected in the experimental water (collected in fish-holding tanks with a water re-circulating system) used for the tests (excluding Mn^{2+}) were lower than the device detection limit. The water quality and lighting conditions under which the acclimatised fish were maintained were the same as in the experimental test. The major parameters of the water used for the experiments (pH, ORP, dissolved oxygen, and temperature) were routinely monitored with a hand-held multi-meter (HI-98196 Multi-parameter Waterproof Meter, Romania). The alkalinity, nitrates, chlorides, ammonia, and metals in the experimental water were tested in accordance with ISO standards (ISO 6332:1988; ISO 15586:2003; ISO 10304-1:2007; ISO 5814:1990; ISO 10523:2008; ISO 9963-1:1994; ISO 14911:1998). All parameters were measured with an atomic absorption spectrophotometer, Varian's SpectrAA 55, USA.

Table S1. Chemical and physical properties of experimental water and trace metals in the complex mixture

Physico-chemical characteristics							
Metals (mg L^{-1})		Cations (mg L^{-1})		Anions (mg L^{-1})		Other analytes	
Dilution water (collected in fish-holding tanks with re-circulating system)							
Mn^{2+}	6	Na^+	5.2	Cl^-	4.4	pH	For each experimental setting, pH, dissolved oxygen, and water temperature were noted separately.
Zn^{2+}	< 0.04	K^+	3.8	SO_4^{2-}	23.1	Temperature	
Cu^{2+}	< 0.001	Ca^{2+}	68	HCO_3^-	203	Dissolved oxygen (DO)	
Ni^{2+}	< 0.002	Mg^{2+}	15	CO_3^-	1.03		
Cr^{total}	< 0.001	Fe^{2+}	0.11	NO_2^-	<0.010		
Pb^{2+}	< 0.001	Fe^{3+}	<0.01	NO_3^-	<0.050		
Cd^{2+}	< 0.0003	Fe_{total}	0.12				
		NH_4^+	0.13				
Metal concentrations (μg L^{-1}) in a complex mixture solution (MIX)							
Solution	Concentration value	Zn^{2+}	Cu^{2+}	Ni^{2+}	Cr^{total}		
MIX*	Nominal	100	10	34	10		
MIX10**	Nominal	1000	100	340	100		
MIX20**	Nominal	2000	200	680	200		
MIX20**	Measured	2100	180	620	180		

< Means below the detection limit.
*MIX (lowest tested MIX solution) represents a stock solution of a metal mixture (MIX), where the concentration of Zn, Cu, Ni, and Cr in the MIX was prepared taking into account the EQC of these metals set for European surface water bodies (Directive 2013/39/EU).

**MIX10 and MIX20 represent the concentrations of each of the four metals in the prepared mixture at 10- and 20-fold increases above the maximum-allowable-concentrations (MAC) levels, respectively.

X-Ray Fluorescence Analysis (XRF)

Table S2. Results of XRF analysis of average elemental chemical composition of impurities in GO samples (in weight %).

Element	Na	Mg	Al	Si	P	S	Cl	Ca	Fe
Conc. w. (%)	0.012	0.016	0.024	0.033	0.025	0.118	0.090	0.070	0.018

Characterisation of larvae individuals in nests
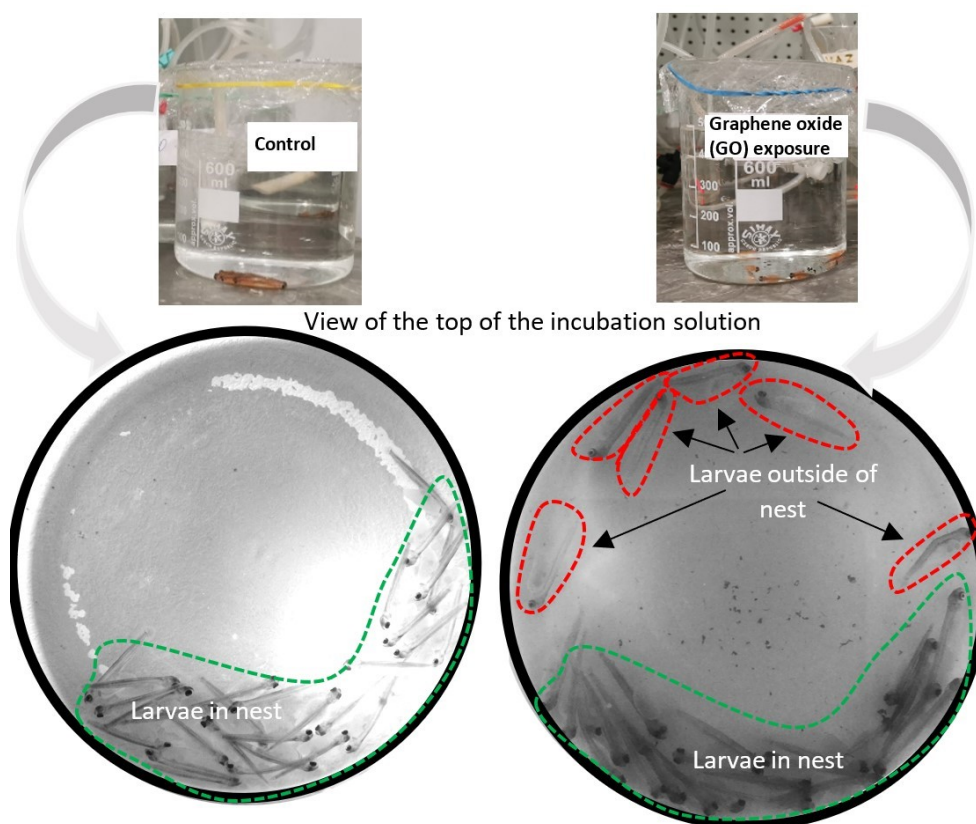
Figure S1. Differences in nesting (control) and non-nesting (treatment) behaviour of brown trout (*Salmo trutta*)

larvae exposed to GO.

Size distribution

The size distribution of synthesized GO was performed using open source programs: ImageJ (https://imagej.nih.gov/ij/index.html) and CellProfiler 4.2.5 (https://cellprofiler.org/releases).
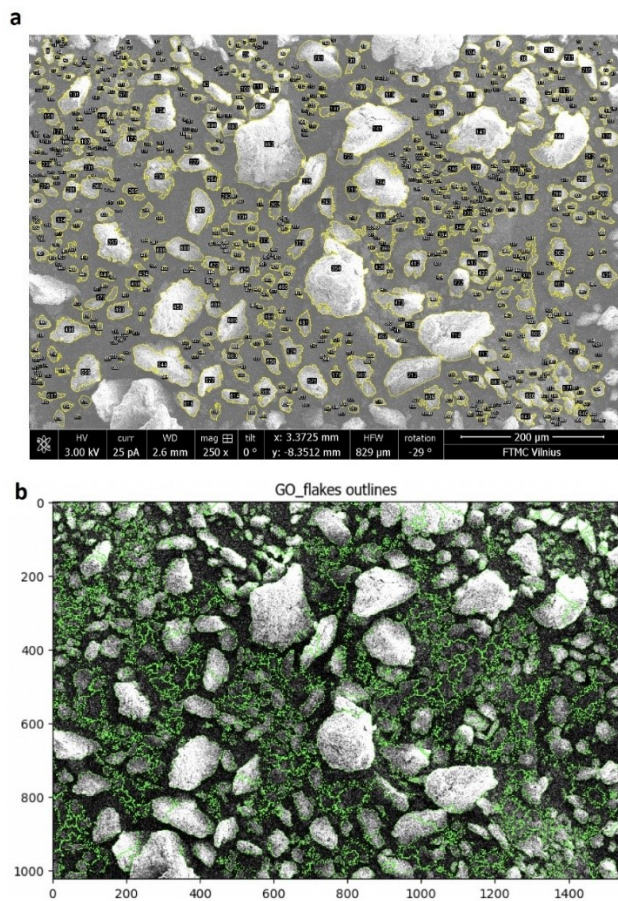
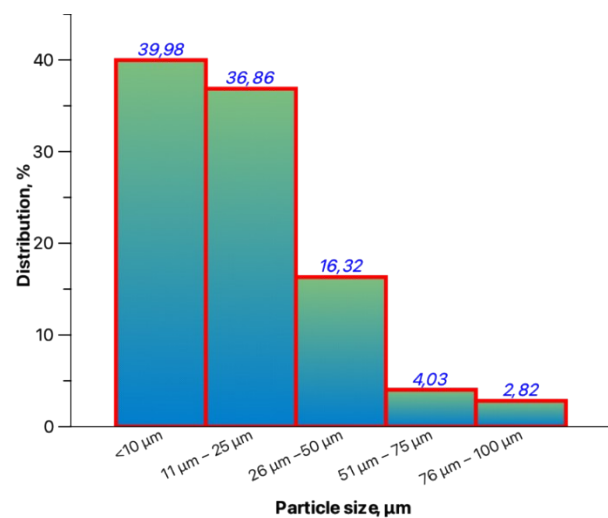Figure S2. Analysed SEM images using (a) ImageJ and (b) CellProfiler software.

Figure S3. Size distribution of synthesised GO flakes.
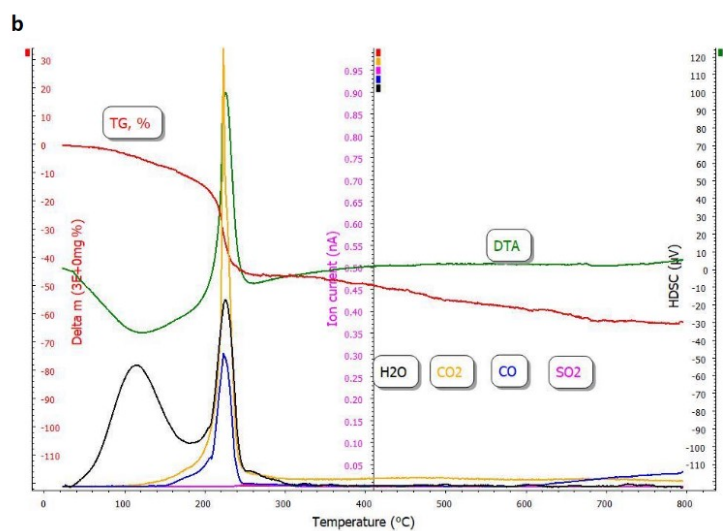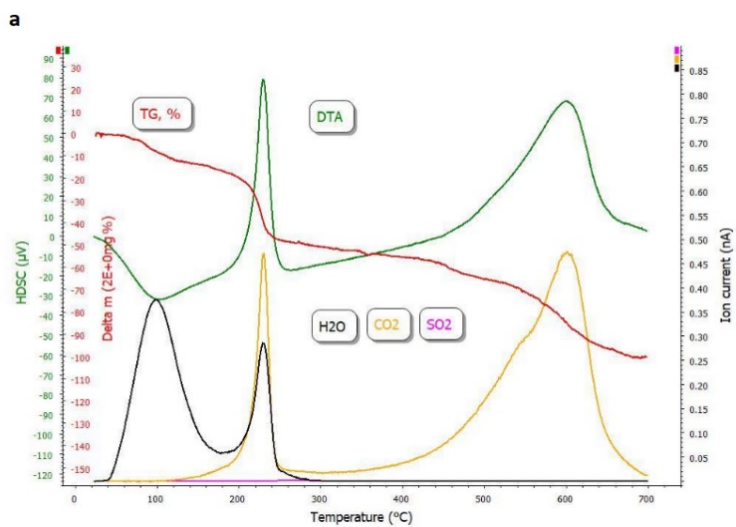
Thermogravimetric Analysis (TG/HDSC-MS)

Figure S4. TGA graphs of mass loss and detected concentrations of emitted compounds using the air (a) and argon (b) atmosphere.
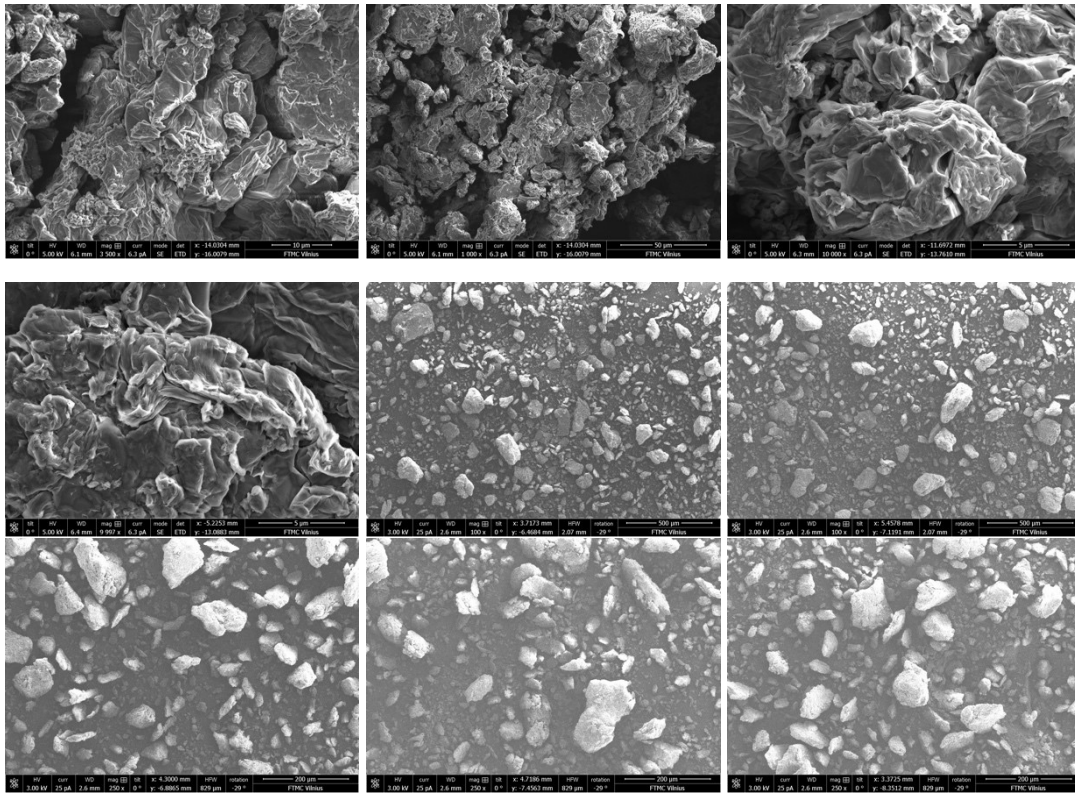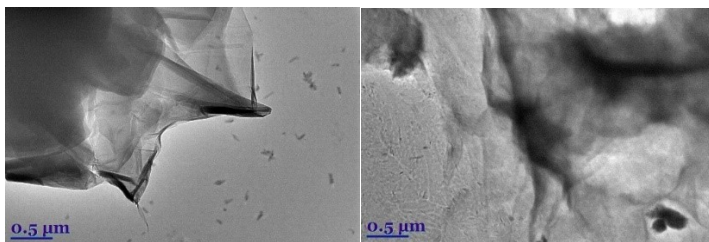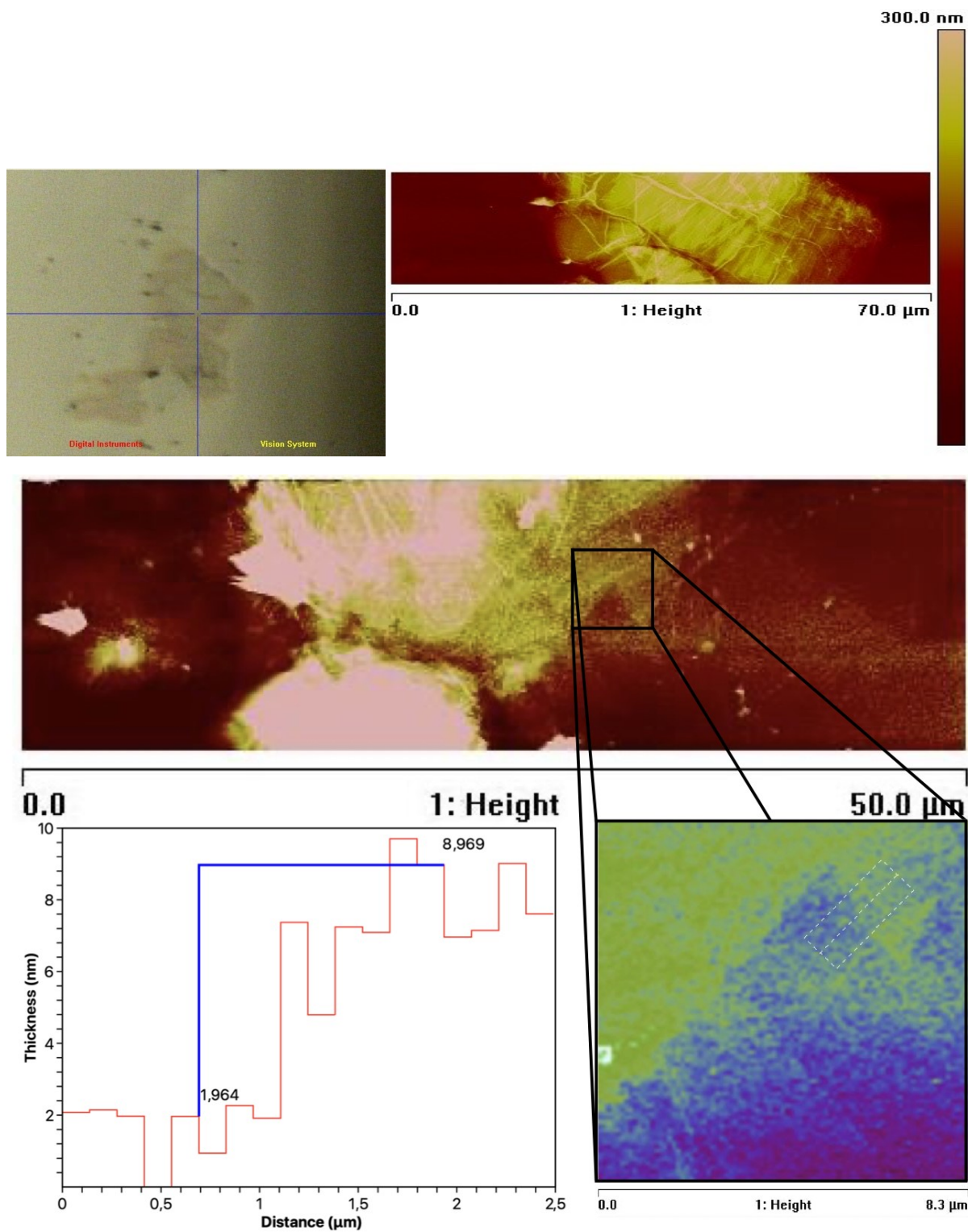
Figure S5. SEM images of GO.

Figure S6. TEM images of GO.

Figure S7. AFM thickness analysis of GO.