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Deliverable summary

This deliverable summarizes detection and characterization protocols of several emerging contaminants (ECs): silver, copper and zinc nanoparticles, and the pharmaceutically-active substance oxaliplatin in complex matrices. The complex matrices include soils and soil solutions from field sites in both Italy and Israel, and from plants (Arabidopsis and tomato). The report includes detailed sample preparation for analysis and analytical methods and parameters used for characterization, as well as results of the analysis that demonstrate our ability to detect and quantify ECs and follow their distribution in various environmental matrices. Overall, we provide here detailed protocols for the synthesis, characterization and detection of these compounds in real, environmentally-relevant matrices at expected concentrations. These methods will be used as the foundation for the next deliverables that are related to the study of transport, fate and risk assessment of these compounds in the environment.

D3.2

Application of the analytical methods of the selected ECs in complex matrices, including sample preparation

1. Introduction

This deliverable summarizes detection and characterization protocols of several emerging contaminants (ECs): silver copper and zinc nanoparticles, and the pharmaceutically active substance oxaliplatin in complex matrices. The complex matrices include soils and soil solution from field sites in both Italy and Israel, and from plants (Arabidopsis and tomato). The report includes detailed sample preparation for analysis and analytical methods and parameters used for characterization as well as results of the analysis that demonstrate our ability to detect and quantify ECs and follow their distribution in various environmental matrices. Overall, we provide here detailed protocols for the synthesis, characterization and detection of these compounds in real, environmentally-relevant matrices at expected concentrations. These methods will be used as the foundation for the next deliverables that are related to the study of transport, fate and risk assessment of these compounds in the environment.

Abbreviations appearing in the text: BTC – breakthrough curve; **CEC** – cation exchange capacity; **DDW** – double distilled water; **EC** – emerging contaminant; **ENP** – engineered nanoparticle; **ICP-MS** – inductively coupled plasma – mass spectrometer; **MW** – molecular weight; **NP** – nanoparticle; **PV** – pore volume

2. Complex matrices of this study – origins and properties

2.1 Aqueous phase matrices – synthetic groundwater composition

Synthetic groundwater based on data collected by the Italian partners was used to produce two "recipes" for synthetic groundwater that corresponds to the groundwater composition of Bologna and Cremona. The details of the aqueous concentrations of the different components are given Table 1. The "recipes" were shared with all project partners and toxicity tests are being carried out with this "synthetic groundwater" as the working solution for these experiments.

Table 1. Composition of synthetic groundwater based on chemical analysis data of Italian groundwater from Bologna and Cremona.

			Bologna		Cremona	
Substance	MW	Solubility	Concentration		Concentration	
			(mg/L)	mM	(mg/L)	mM
$MgSO_4$	120	35.1 g/100 ml	138	1.15	46.0	0.38
$NaHCO_3$	84	96 g/L	504	6	168.0	2.00
$CaCl_2$	111	74.5 g/100 mL	77.7	0.7	25.9	0.23
$Ca(NO_3)_2$	164	1212 g/L	32.8	0.2	10.9	0.07

$Ca(OH)_2$	74	1.73 g/L	259	3.5	86.3	1.17
Humic acid (Na salt)			5		5	5
Trace elements detected in the Italian groundwater; concentrations in µg/L.						
Tetrachloroethylene (PCE)			30		10	
NaF			75		25	
$(NH_4)OH$			100		33.3	
H_3BO_3			800		266.7	

2.2 Soil matrices and soil solutions - composition and properties

2.2.1 Aquifer porous media from Emilia Romagna, Italy - The aquifer material samples were collected from cores (220-S10 and 221-S6) drilled in 2016 in the Emilia Romagna, Italy (Figure 1).

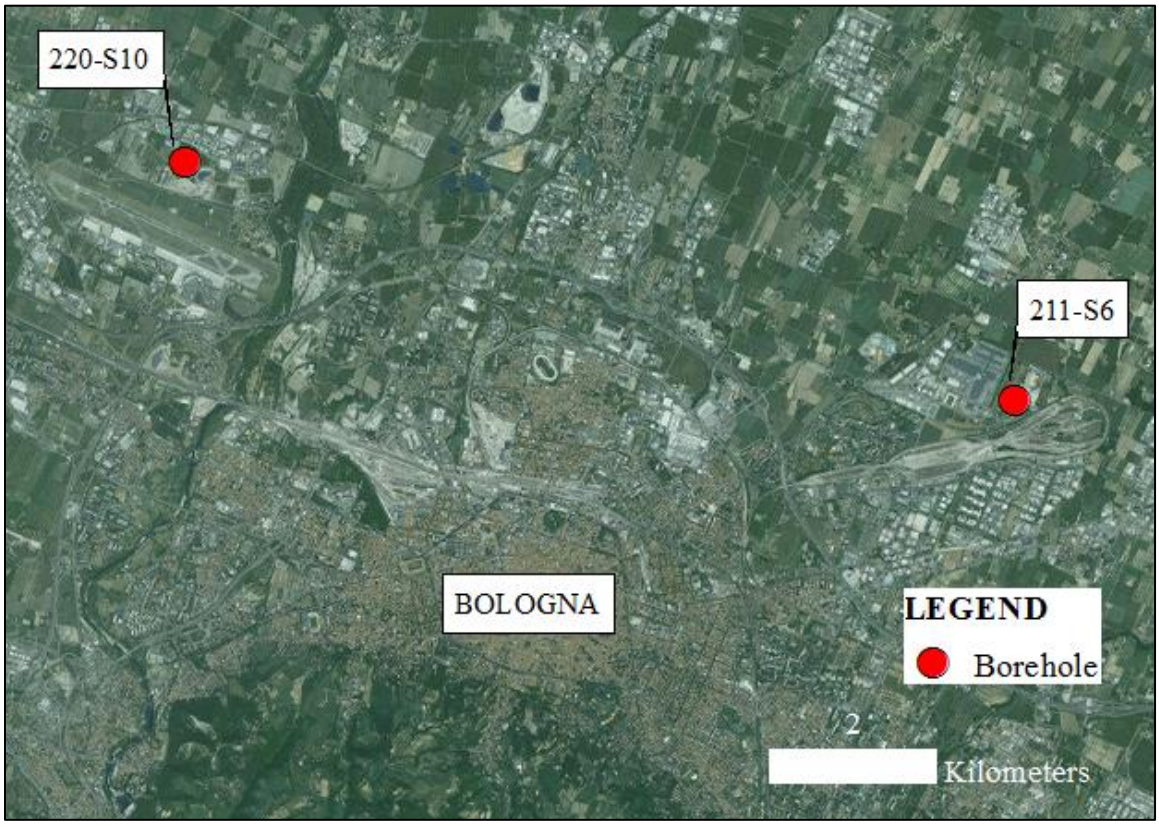
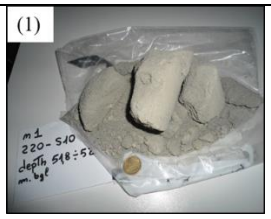


Figure 1. Borehole map: 220 S10 (UTM 32T 682136, 4934676) and 221 S6 (UTM 32T 692053, 4931815).

Core 220 S10 comes from a depth of 70 m. It was drilled starting from the bottom of a quarry (about 14 m below ground level). The core essentially contains coarse-grained

deposits (gravel and sand) characterizing aquifer A1 in this portion of the alluvial fan, ending within clayey sediments that separate A1 from the underlying aquifer A2.

Table 2. Soil sample characteristics.

Sample	Core	Depth from the ground [m]	Sample weight [g]	Geological Description	
1	220-S10	51.8-52	1129.09	The sample is mainly formed by sand (fluvial channel sands) with very few pebbles (maximum length 2 cm)	
2	220-S10	48.4-48.6	1049.64	Sandy silt with some pebbles	
3	220-S10	35.3-35.5	1486.84	Fluvial channel gravel. Heterometric gravel with grain size ranging from fine gravel (about 2 mm – the most abundant fraction) to pebbles (2-3 cm), in sandy-silt matrix	
4	220-S10	24.5-24.7	1195.89	Clay and silt of alluvial plain. Fine grained material (silt)	
5	221-S6	8.3-8.6	1184.95	reddish sand	
6	221-S6	15.6-16	1214.07	clay	

Each sample was taken from the core at different depths with the aim of characterizing the observable lithological facies. Samples n° 1 to n°4 from 220-S10 and samples n°5 and 6 come from borehole 221-S6. A more detailed characterization of each sample is given in Table 2 based on visual observation and gathering data. No information is available regarding the sand/silt/clay content, pH values, organic carbon content, organic matter content, nitrogen content, C/N ratio, or cation exchange capacity.

2.2.2 Handling and preparation of aquifer material samples from Emilia Romagna, Italy - As can be seen from the pictures in Table 2 the samples are characterized by rocky structures and occurrence of large aggregates. Usually, disaggregation should be performed with minimal force, so the original texture of the soil will be changed as little as possible. In this case, it was necessary to use anvil and hammer to process each sample to an acceptable size. The samples are sieved (mesh size 18-60 mesh) as recommended from ISO standard on soil sampling (ISO 10381-6), thus obtaining a good homogenization.

Before each experiment, the soils are air-dried at ambient temperature (preferably between 20-25 °C), weighed and then placed in the oven at 105 °C (approx. 12 hours) to enable calculation of the content of dry mass [%].

2.2.3 Characterization of soil from Weizmann Institute campus, Israel - Soil was collected from the upper 5 cm of the soil layer and for all experiments, the soil was sieved (60 mesh). The basic chemical properties of the soil are provided in Table 3. Weizmann soil is rich in sand and silt and relatively poor in clay. Thus most of the soil particles have relatively small surface area, which might decrease the retention and the number of the possible interactions of the introduced ECs.

Table 3. Weizmann Institute soil characteristics.

Properties	Methods	Weizmann Soil
Composition		89% ± 3% sand 7% ± 4% silt 3% ± 1% clay
pH	DDW	7.97 ± 0.01
	DDW+$CaCl_2$	7.41 ± 0.02
Porosity		0.39 ± 0.03
Organic matter (%)		0.5% ± 0.2%
CEC composition [meq/100g]	Na^+	0.27
	K^+	0.13
	Mg^{2+}	1.21
	Ca^{2+}	8.71
Total CEC [meq/100g]		10.32
Inorganic carbon [%]	CO_3-C, %	0.14 ± 0.02
Dissolved Organic Carbon [ppm]	UV at 254 nm	1.00 ± 0.06

The carbonate content of the soil is relatively low (which decreases soil buffering capacity), while the cation exchange capacity (CEC) is relatively high (which promotes soil redox buffering). The soil is also very poor in organic matter, which implies a lower amount of possible interactions, as well as lower redox buffering. Therefore the overall pH and redox buffering effects are difficult to predict.

2.2.4 Column experiments for fully saturated conditions - This setup was applied mostly for experiments with soil from Weizmann campus, Israel. The soil was packed into vertical polycarbonate columns, and fully saturated with water by a peristaltic pump from the bottom of the column (Figure 2). The soil was not sterilized to allow for induced development of different redox conditions in the experiment. Following saturation, the desired redox conditions were achieved in the column by flushing it with the appropriate synthetic water solution. The applied redox conditions were oxic, nitrate reducing, iron reducing, strong biologically reducing and strong chemically reducing conditions, based on the apparent buffering capacity of the soil. These conditions are the most relevant to natural prevalent redox regimes in different soil layers, and are expected to have different effects on transport and transformation of various ECs.

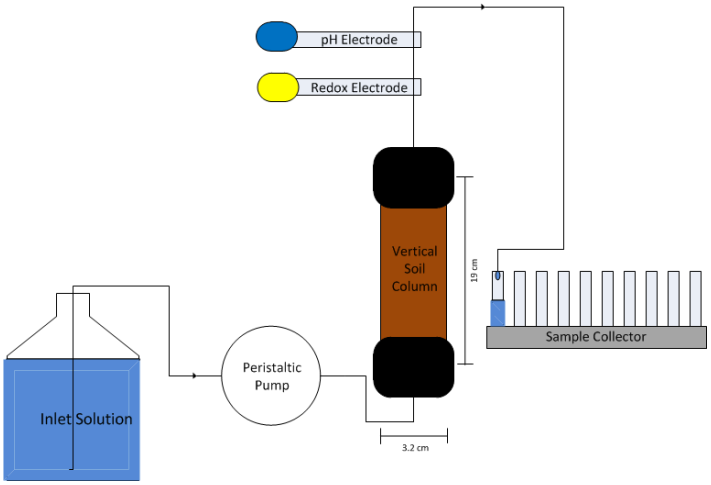


Figure 2. The experimental setup: polycarbonate vertical columns (L×D: 20 cm × 3.2 cm) with packed soil, covered in aluminum foil to prevent photochemistry. The inlet solution is fed from bottom to top. The illustration is taken from [1].

To verify that the desired redox condition was established, the redox potential Eh and pH were monitored "on-line" at the outlet of the column (according to Table 4). The synthetic water used in the experiments, to develop specific redox zones, was produced according to Table 5 (the main differences among the solutions are highlighted in green). The salt concentrations were established according to the literature [15, 16, 25-29] and several preliminary experiments.

Table 4. Experimental pe+pH regimes.

Regime	pH [avg.]	Eh [V]	p_e	p_e+pH
Oxic	9.6	414 - 668	7 - 11.3	16.6 - 20.9
Nitrate reducing	8.3	250 - 350	4.2 - 5.9	12.5 – 14.2
Iron reducing	8.5	(-120) - 120	(-2.0) - 2.0	6.5 – 10.2
Strongly reducing	9.0	(-200) - (-300)	(-3.4) - (-5.1)	5.6 - 3.9

Oxic solutions were prepared in doubly-distilled water (DDW), and the experiments were run shortly after soil saturation; thus no additional nutrients were required. Chemically reducing solutions (i.e., solutions with 60-90 ppm of the reducing agent NaBH4) were also prepared in DDW without additional nutrients, as the attempted redox regime was to be chemical rather than biological. For iron reducing conditions, Fe(III) oxide-hydroxides were incorporated into the soil as mixed ferrihydrite/goethite (1:10 by weight; other procedures were attempted unsuccessfully). Sodium acetate and methanol were used as easily degradable organic substrates, i.e., the electron donors in different redox conditions.

Table 5. Composition of redox solutions.

	Nitrate reducing		**Iron reducing**		**Strong biologically reducing**	
Salt	mmol/L	ppm	mmol/L	ppm	mmol/L	ppm
$MgSO_4*7H_2O$	0.635	157	0.635	157	0.635	157
K_2HPO_4	0.023	4	0.023	4	0.023	4
$NaHCO_3$	0.270	23	0.270	23	0.270	23
$CaCl_2$	1.144	127	1.144	127	1.144	127
NH_4Cl	0.103	6	0.103	6	0.103	6
KNO_3	6.924	**700**	0.099	10	0.099	10
KBr tracer	4.202	0.5	4.202	0.5	4.202	0.5
$NaCH_3COO$	1.097	90	1.097	90	1.097	**1000**
MeOH	986	31600	986	31600	986	31600

Upon stabilization of the desired redox conditions, a solution of the relevant synthetic water, inert tracer (500 ppb of Br^-) and the studied EC was delivered at a constant rate of 1 to 1.1 mL/min. The emerging effluent was collected into polypropylene tubes by a fraction collector and filtered through 0.45 micron filters (PVDF membrane) for further detection.

Metal-based species and bromides were detected by Inductively Coupled Plasma Mass Spectrometer (ICP-MS) (Agilent 7700s). At the end of column experiments, soil was extracted from the column and divided into slices for further detection of oxaliplatin and mass balance calculations, aimed to determine the spatial distribution (along column length).

2.2.5 Measurements of ENPs in soil and soil solution - Ag-NP concentrations were measured by inductively coupled plasma - mass spectrometry (ICP-MS, Agilent 7700), and based on a calibration curve of Ag-NP suspensions used in the same experiment and prepared by the same protocols. The samples were treated with thiosulfate-cupric-ammonia mixture to ensure Ag-NP (and possibly other silver precipitate) dissolution, based on a silver leaching method.

To determine the retention profile of nanoparticles in the soil column, the columns were dismantled at the end of each experiment and divided into 8 segments (about 1 cm each); then the wet segments were oven-dried to determine the soil moisture. Subsequently, 20 mL thiosulfate-cupric-ammonia mixture (0.05 M ammonium thiosulfate, 0.125 M ammonium sulfate and 0.5 mM cupric sulfate, the mixture pH was adjusted to ca. 9 using NaOH) was added to each dry soil sample, followed by 24 h shaking. The soil was then separated and the supernatant was analyzed. Extraction efficacy (generally >90%) was determined for each experiment separately and accounted for in yield calculations.

2.3 Organic matrices – protocol for analysis of total content of NPs in plants

2.3.1 Collection of plant samples - Plant biomass was harvested at the same physiological state. Some plants may grow faster than others, thus a particular phenological stage, such as when they began to set fruits or flowers were chosen and kept constant for all the experiments [8]. Whole plants were collected carefully to retain intact roots and shoots.

2.3.2 Processing of plant biomass - Whole intact plants were rinsed thoroughly in the laboratory with running tap water, followed by three rinses with deionized [9-10]. The specimens were then dried with Whatman filter papers and incised using acid washed knife/scissors or other accessory apparatus into root and shoot. All the containers were also soaked at least 24 h with 2% nitric acid before use to avoid any further contamination after incision of specimen [10-11]. Roots and shoots were incised into small pieces and thereafter processed separately. The samples were then oven dried at 80 ˚C/105 ˚C until a constant weight was achieved. Oven dried samples were then crushed, sieved, homogenized and weighed [9-11].

2.3.3 Acid digestion of plant biomass - Dry powdered plant biomass (0.05 g) with three replicates for each type were accurately weighed and transferred into silica crucible and 0.5 mL of concentrated HNO_3 [12] was added as an ashing aid. Digestion of plant biomass or ashing process was carried out in a muffle furnace by stepwise increase of temperature up to 500 ˚C and then left at this temperature for 5 h. The final residue was dissolved or rinsed with 1% HNO_3, filtered and final volume was made up to 2.5 mL [10,12-13].

3. Quantification of ECs in complex matrices

3.1 Quantification of ENPs in soil and soil solution

Examples of breakthrough curves of AgNPs in partially saturated Weizmann soil are shown in Figure 3a and 3b and the retention profiles in the soil column are given Figure 3c and 3d. Each panel shows three replicates of the experiment. Figures 3a and 3c show that silver nanoparticles at concentration of ~500 ppb in a solution that contains humic solution have substantial mobility through soil and uniform distribution of residual AgNPs in the soil column. When the concentration of the AgNPs is increased to almost 2000 ppb the pattern of the breakthrough curve changes and fewer particles elute from the column. The retention profile was also found to show different distribution of the AgNPs in soil with the highest concentration at the upper layer of the soil column and reducing trend with depth. Overall, in all cases, AgNPs were found to be mobile through soil and their transport behavior was found to be related to the solution composition in which they are dispersed the porous media properties (in our case Weizmann soil) and the concentration and chemical/physical properties of the particles like size coating and surface charge.

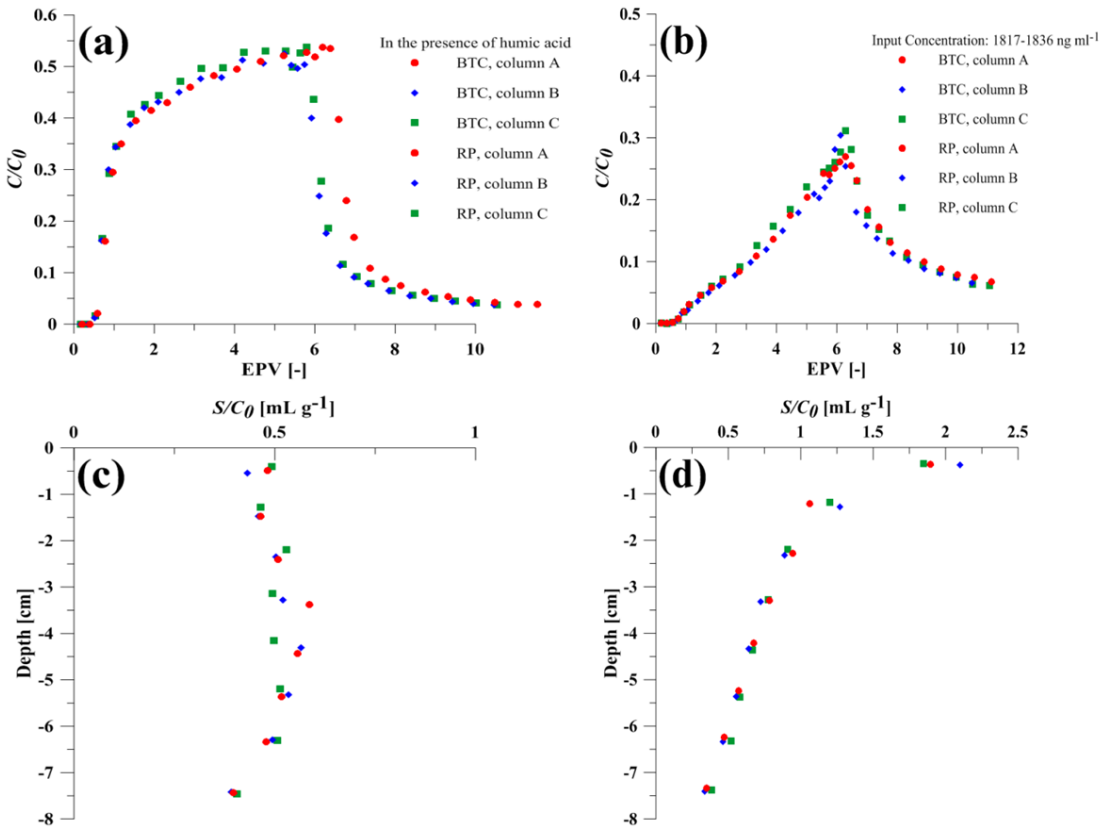


Figure 3. Breakthrough curves (a and b) and retention profiles (c and d) of AgNPs in partially saturated soil columns for two AgNP solutions; solution with 30 ppm humic acid (a and b) and high concentration ~1800 ppb of AgNPs (c and d).

3.2 Quantification of ENPs in plants

3.2.1 Processing of plant biomass - Arabidopsis samples acid digestion - The method of acid digestion allows analysis of the total metal content in plants. The concentration of metal in the acid fraction is estimated with ICP-MS, as described above. The concentrations of silver nanoparticles, copper nanoparticles and zinc nanoparticles in roots and shoots of Arabidopsis that were grown hydroponically in solution that contains nanoparticles are depicted in Figures 4-6, respectively. In all cases, a large increase in metal concentration in the roots was detected compared to control (no metal addition), or addition of similar amounts of metals in the form of slat (metal ions) or larger (bulk) particles of the metals. In the shoots the results are less dramatic and for the silver we see almost no uptake. In this case the bulk source of silver was shown to be associated with higher silver concentration while all other forms of silver show very low concentration, similar to the control values.

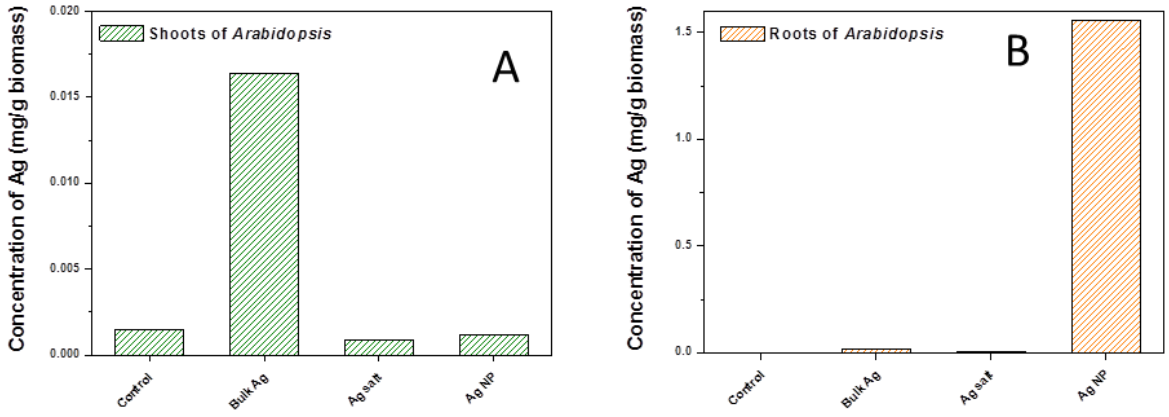


Figure 4. Silver in acid digested samples of Arabidopsis sp. (A) shoot samples and (B) root samples.

For the copper treatment shown in Figure 5, the shoot uptake of all metal sources were higher than the control and the uptake was found to be in the order salt<NPs<bulk.

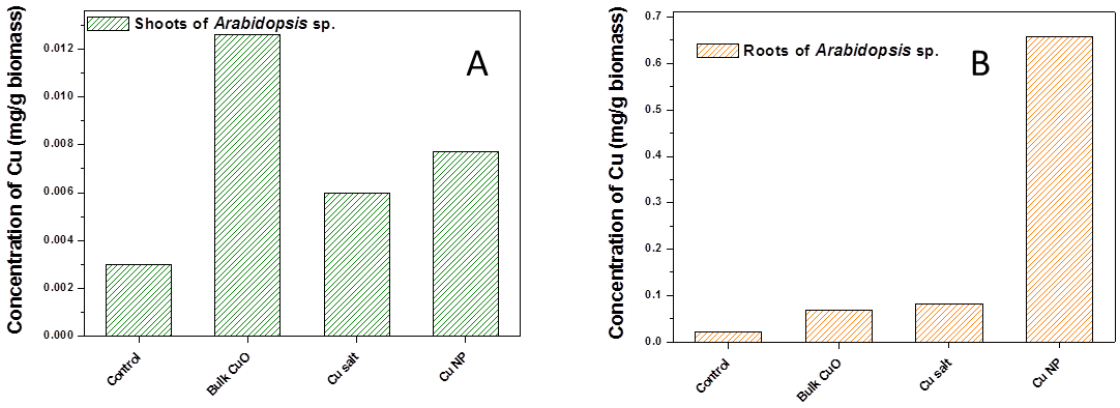


Figure 5. Copper in acid digested samples of Arabidopsis sp. (A) shoot samples and (B) root samples.

For the zinc treatment shown in Figure 6, the shoot uptake was in general very high compared to copper and silver (almost 2 orders of magnitude higher) and the addition of Zn as NP showed the highest concentration in the shoots, much higher than in the control or ionic source and ~3 times higher than the bulk zinc source.

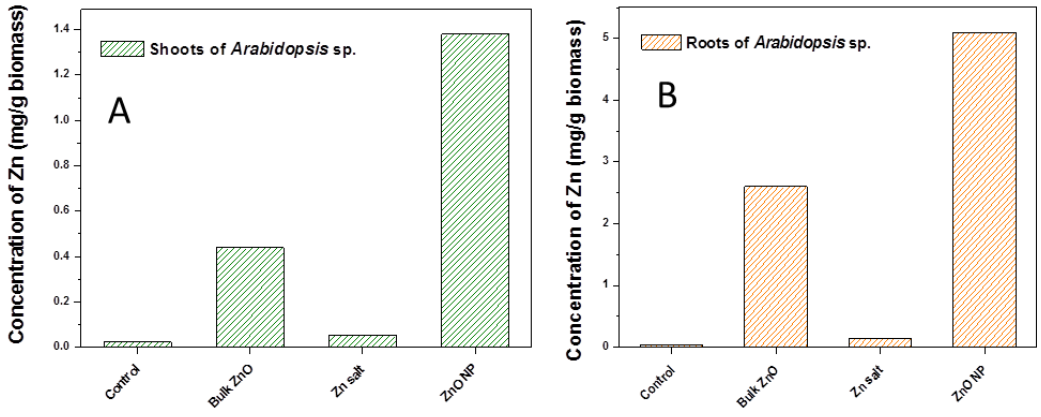


Figure 6. Zinc in acid digested samples of Arabidopsis sp. (A) shoot samples and (B) root samples.

3.2.2 Arabidopsis sample acid digestion of isotopically-labeled ENPs. To further study the source of the metal in the different plant parts, isotopically-labeled nanoparticles were synthesized and used. This allows us to differentiate between naturally-occurring metals that can be found in the plants (copper and zinc are considered micronutrients and are required for normal function of the plant cells). The concentration of the metal in the roots and shoots of Arabidopsis are depicted in Figures 7-9 for silver, copper and zinc, respectively. The same trend but much more pronounced results are shown in all cases.

For all three metals, the root analysis showed that the metals found in the roots originate almost exclusively from the nanoparticles with zero concentration in the control for silver and copper, and very low concentration in the zinc control, probably due to natural isotopic distribution. For the shoots, in the case of silver (Figure 7), it is clear that there is almost no uptake of silver and the control levels of silver in the shoots are similar to the shoots grown in the presence of AgNPs.

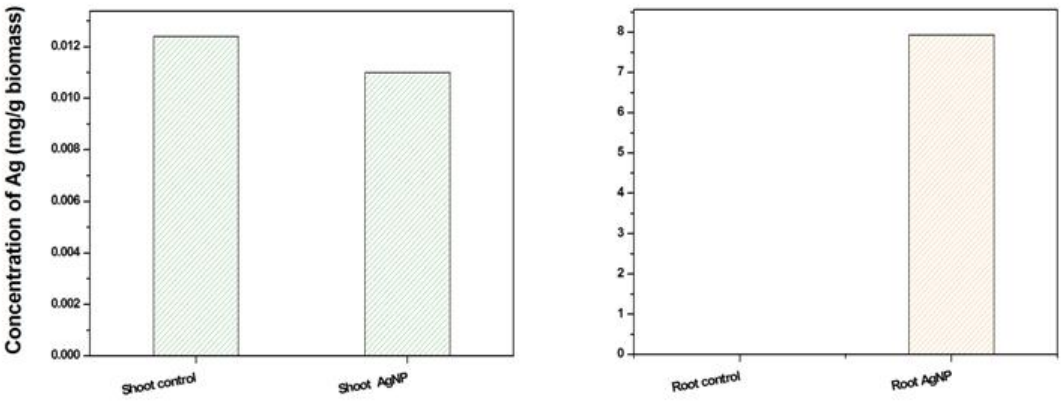


Figure 7. Silver (^{107}Ag) in acid digested samples of Arabidopsis sp. exposed to labeled AgNPs.

For the shoots in the case of copper (Figure 8), the use of isotopic labeling makes it easy to notice that the nanoparticles are the source of the copper detected in the shoots, and that the concentration is about 10 times higher than the amounts found in the control experiments.

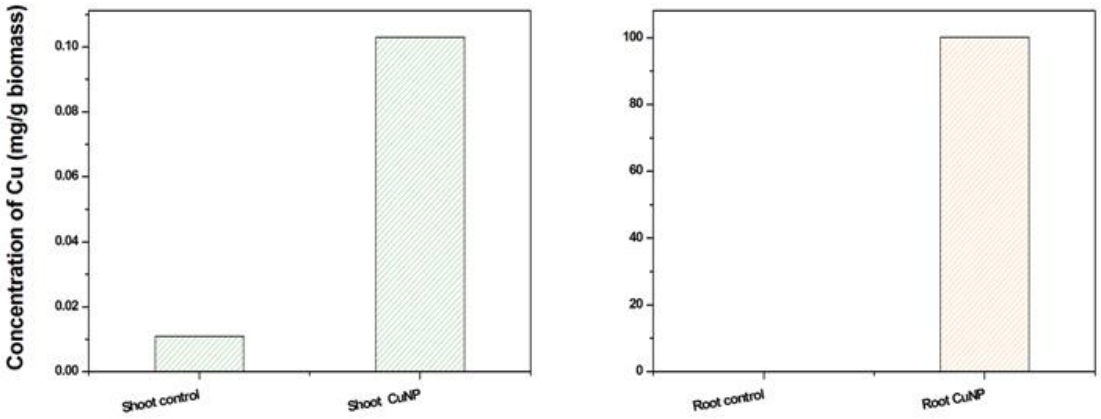


Figure 8. Copper (^{65}Cu) in acid digested samples of Arabidopsis sp. exposed to labeled CuNPs.

For the shoots in the case of zinc nanoparticles (Figure 9), the same trend was found with much higher concentrations of the zinc in the shoots (and roots) compared to the control experiments.

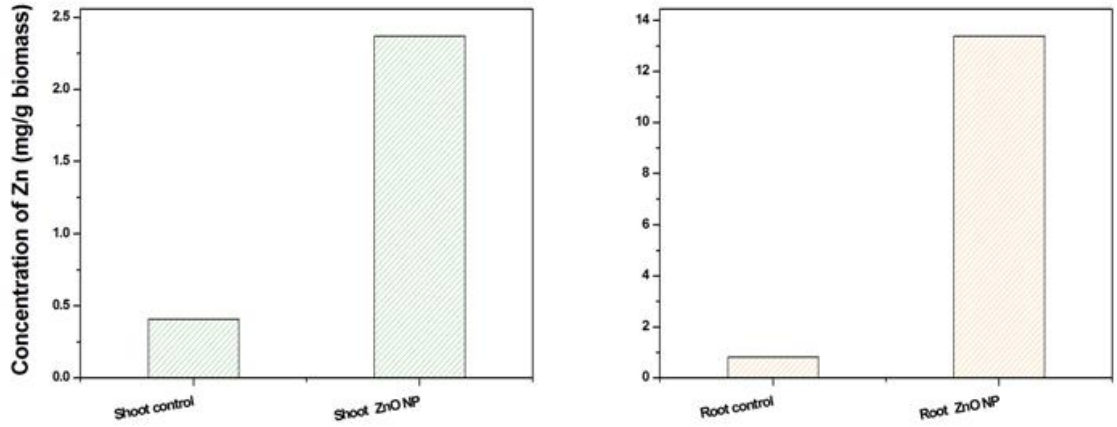


Figure 9. Zinc (^{70}Zn) in acid digested samples of Arabidopsis sp. exposed to labeled ZnO NPs.

3.2.3 Analysis of ENPs in tomato samples by acid digestion. Similar to sections 3.2.1 and 3.2.2, the concentration of silver, copper and zinc in different parts of tomato plants were measured comparing control (no addition of the element on top of the growth media) to solutions that contain nanoparticles; the results are summarized in Table 6. The

concentration of the respected elements in the roots was found to increase by more than 2 orders of magnitude for silver and copper and by more than 14 times for zinc. For the shoots, the silver and the zinc showed one order of magnitude elevated concentration while the copper concentration increased by ~60%. Finally even in the fruits, elevated concentrations compared to the control were found for the isotopically labeled silver and copper while the zinc showed concentration slightly lower than the control. In all cases the concentration of metals decreased dramatically between the roots and the shoots. In the fruits, the silver was concentrated again while the copper and zinc concentrations were further reduced.

Table 6. Concentration of silver, copper and zinc in acid digested tomato root and shoot samples treated with AgNPs, CuNPs and ZnNPs, respectively.

Samples	AgNPs	CuNPs	Zn NPs
	Average concentration (μg/g)		
Control root	0.1327	34.2	79
Root treated with NPs	18.02	4979.8	1167
Control shoot	0.0295	7.33	20.3
Shoot treated with NPs	0.2927	11.6	195
Tomato fruits control*	0	0.7	6.2
Tomato fruits treated with NPs*	1.9	2.8	6

*- For the tomato fruits isotopically labeled NPs (^{107}AgNP, ^{65}CuN, ^{70}ZnO NP) were used.

3.3 Quantification of selected pharmaceutically active substances in soil and soil solution

3.3.1 Analysis of oxalipalitin in soil column experiments – Oxaliplatin elution from soil is retarded compared to the inert tracer (Figure 10). The recovery is relatively stable, and has a relatively low value of 10-15%. It appears that oxaliplatin interacts quite strongly with soil under oxic conditions. After the water pulse, some tailing is observed, which might be attributed to a kinetic sorption process. Moreover, all replicates showed a "bump" in the breakthrough curve (BTC) of about 1 pore volume (PV) width, about 1 PV after the water pulse. This might suggest that not all the sorption sites are emptied simultaneously under stable oxic conditions. Additional replicates are needed to confirm this phenomenon (or disregard it as an analytical uncertainty).

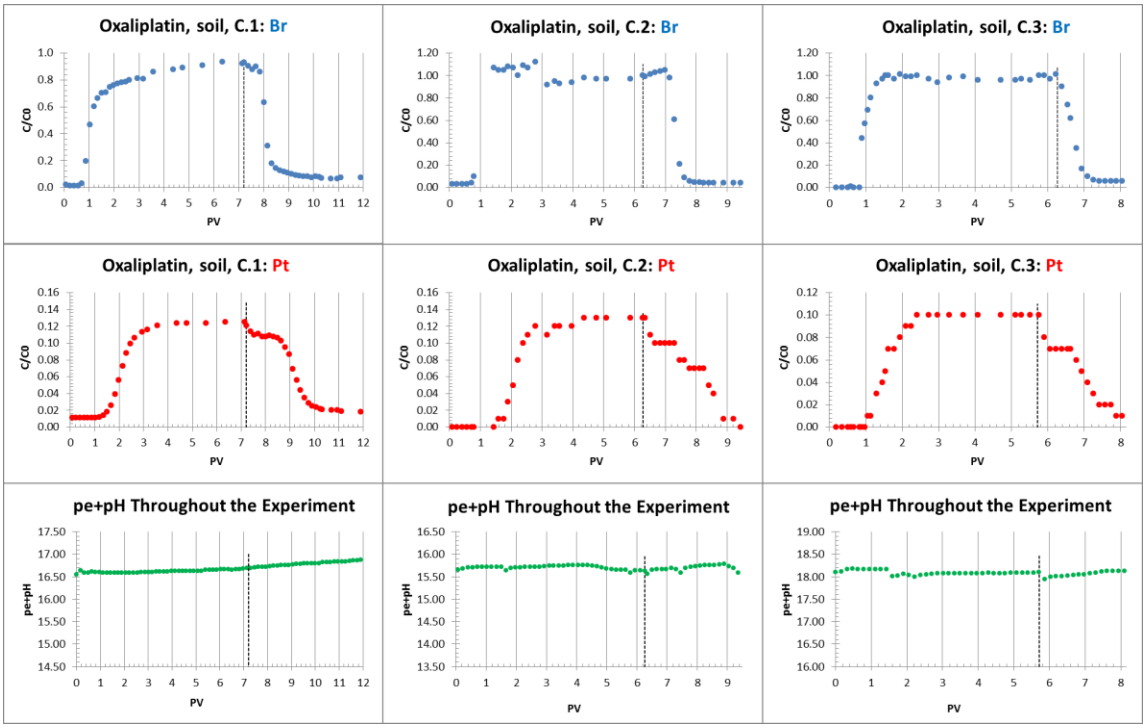


Figure 10. Three replicates of oxaliplatin breakthrough curves in soil under oxic conditions (C- "column"; 1,2,3- the number of the replicate. The dotted vertical line marks the water pulse.

Under nitrate reducing conditions, oxaliplatin transport is significantly retarded compared to the inert tracer as shown in Figure 11. Most of the oxaliplatin remains in the soil column and the eluted amount ranges between 10-20% (which is similar to oxaliplatin recovery in soil under oxic conditions).

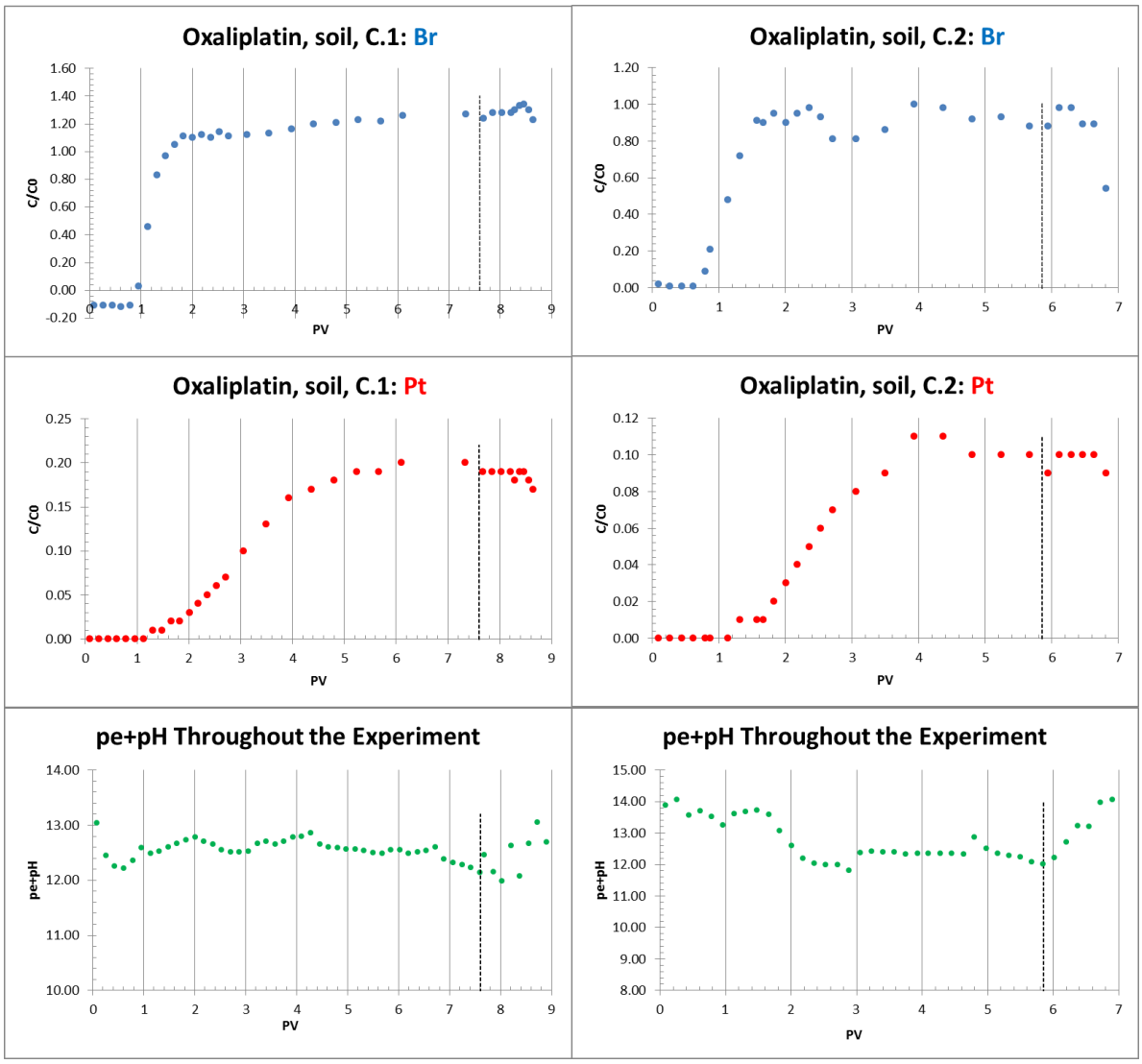


Figure 11. Two replicates of oxaliplatin breakthrough curves in soil under nitrate reducing conditions (C - "column"; 1,2,3 - the number of the replicate). The dotted vertical line marks the water pulse.

4. References

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