

Supplementary material for

Preparation and characterization of innovative olive leave extract@layered double hydroxide antioxidant nanohybrids

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Materials and Methods

Enzyme-Assisted Extraction

The following commercial enzyme preparations were used: pectinolytic preparation Pectinex XXL, and Viscozyme L. (Cellulase, Hemicellulase, Xylanase) (all from Novozymes A/S, Bagsvaerd, Denmark). Finely grounded (particle size < 700 µm) olive leaves were mixed with water (10:1, v/w), acidified (pH 4.0) with HCl, and left for 1 h for rehydration at 25 °C. After pH adjustment (pH 4.0), the suspension (100.0 g) was placed in a 50 °C water bath (Mettler Schuetzart DIN 40,050-IP 20, Germany) for 20 min before enzymes (Pectinex XXL, and Viscozyme L.) were added. After incubation at 50 °C, the sample was placed in a boiling water bath for 10 min to inactivate the enzymes, then immediately cooled and finally filtered through a paper filter under vacuum and weighed to determine the extract yield [1]. The same process was followed for the control sample and, instead of enzymes, distilled water was used at the same amount as the enzymes in its sample.

Quantitative Analysis of Hydroxytyrosol, Luteolin-7-O-glucoside, Apigenin-4-O-glucoside and Oleuropein of OLE with HPLC-DAD method

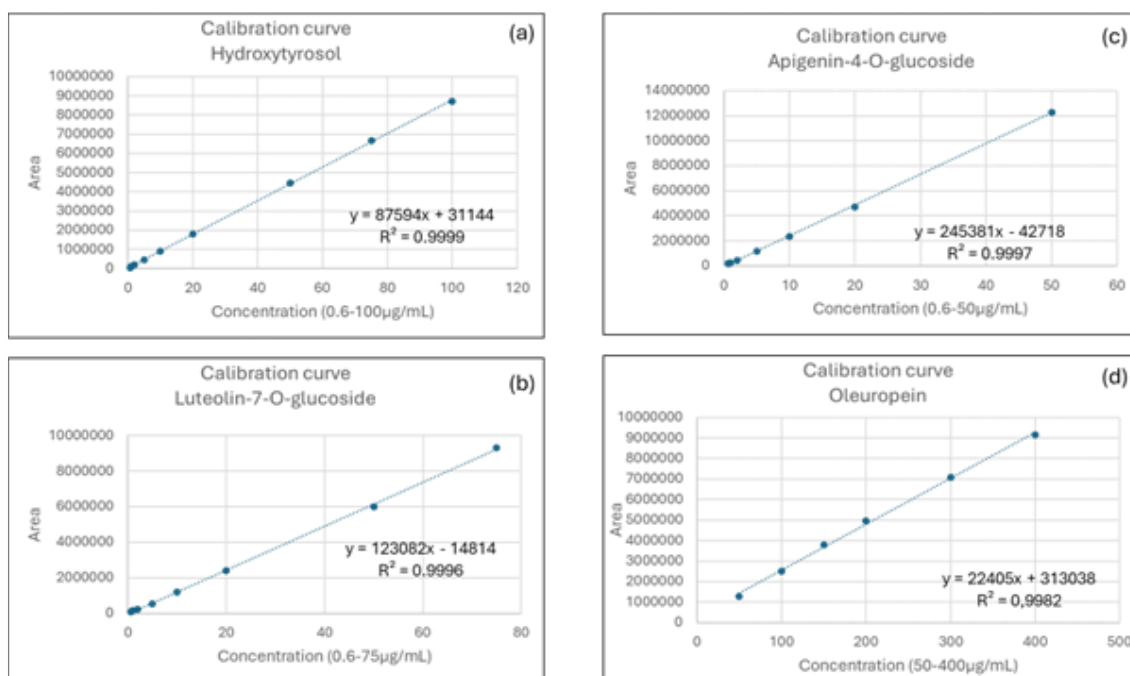


Figure S1. Obtained calibration curves of (a) Hydroxytyrosol, (b) Luteolin-7-O-glucoside, (c) Apigenin-4-O-glucoside and (d) Oleuropein.

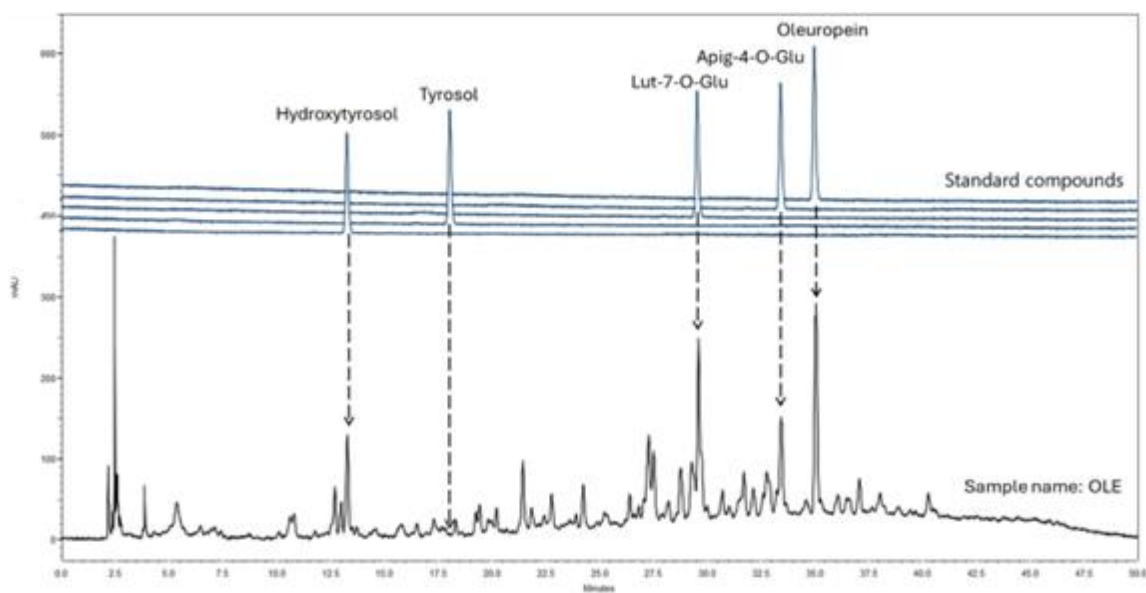


Figure S2. HPLC-DAD chromatographic plot at 280 nm for pure OLE

Physicochemical characterization of OLE@LDH_Zn/Al_x/1 nanohybrids

X-Ray Diffraction (XRD) studies

Obtained OLE@LDH_Zn/Al_x/1 nanohybrids were characterized with X-Ray Diffraction (XRD) analysis using a Brüker XRD D8 Advance diffractometer (Brüker, Analytical Instruments, S.A., Athens, Greece) equipped with a LINXEYE XE High Resolution Energy Dispersive detector. Diffractometer was thermostated at 20 °C, and the beam monochromator was operated at a voltage of 40 kV and a beam current of 40 mA. CuK α radiator worked in 1-D mode with a wave length λ = 1.541874 Å. Scanning parameters were set as follows: two theta range 0.5–40°, increment 0.03°, PSD 0.764 counting time 1022 s and slit width 0.6 mm. Each sample was oriented onto glass samplers and was carefully adjusted so that its surface was at the same level as the reference plane of the instrument

Fourier Transform Infrared Spectroscopy (FTIR) studies

FTIR spectra of pure OLE and all obtained OLE@LDH_Zn/Al_x/1 nanohybrids were recorded using an FT/IR-6000 JASCO Fourier-transform spectrometer (JASCO, Interlab, S.A., Athens, Greece). Measurements were carried out using the KBr (0.5 %wt. to 1 %wt.) tablet technique. The spectra recorded over the wavenumber range from 4000 to 400 cm⁻¹ at a resolution of 4 cm⁻¹ and 64 scans were averaged to reduce noise.

Scanning Electron Microscopy (SEM) studies

High Resolution Scanning electron microscopy (HR-SEM) images of pure OLE and all obtained OLE@LDH_Zn/Al_x/1 nanohybrids acquired using a Zeiss Gemini 500 SEM at a low accelerating voltage of 3 kV to reduce the excitation volume and enhance resolution.

In Vitro Antioxidant Activity Determination of pure OLE and obtained OLE@LDH_Zn/Al_x/1 nanohybrids

For all obtained OLE@LDH_Zn/Al_x/1 nanohybrids as well as pure OLE the concentration required to obtain a 50% antioxidant effect (EC₅₀) was calculated according to the methodology described recently [3], [4].

For the preparation of 2,2-diphenyl-1-picrylhydrazyl free radical [DPPH•] standard solutions, 0.0212 g of [DPPH•] free radical was dissolved in 250 mL of methanol to obtain a 2.16 mM (mmol/L) methanolic solution. Next, the flask was vortexed under dark conditions, and its pH (Milwaukee MW102-FOOD PRO+ 2-in-1 pH and Temperature Meter) was measured to ensure its neutrality (7.02 $\pm$ 0.01). Finally, the solution was placed in a refrigerator at 4 $\pm$ 1 °C under dark conditions for stabilization.

For the preparation of a [DPPH•] free radical calibration curve, 2.16 mM (mmol/L) methanolic solution of [DPPH•] free radical was diluted by adding appropriate volumes of methanol to obtain concentrations of 10, 20, 30, 40, and 50 mg/L, and their absorbance was measured with a SHIMADZU UV-1900 UV/VIS Spectrometer at 517 nm. The calibration curve of absorbance (y) versus the concentration (x) of [DPPH•] free radical was expressed by the following equation:

$$y = 0.0388x + 0.015; R^2 = 0.9994 \quad (1)$$

For the determination of the concentration required to obtain a 50% or 60% antioxidant effect (EC₅₀) from all obtained OLE@LDH_Zn/Al_x/1 nanohybrids, as well as pure OLE 5, 10, 20, 30, and 40 mg of granule nanohybrids or 5, 10, 20, 30, and 50 μ L of pure OLE were placed in dark vials and three replicates were performed for each sample. Thereafter, 3 mL of [DPPH•] free radical methanolic solution and 2 mL of acetate buffer 100 mM (pH = 7.10) were added to each vial, and the absorbance of the reaction mixture was measured at 517 nm after 24 h. For a blank sample, we

used a vial containing 3 mL of [DPPH•] free radical methanolic solution and 2 mL of acetate buffer without the addition of any granule film. The % inhibition of [DPPH•] was calculated using the following equation:

$$\% \text{ scavenged DPPH}^{\bullet} \text{ at steady state} = \frac{A_0^{517} - A_{\text{sample}}^{517}}{A_0^{517}} \times 100 \quad (2)$$

Table S1. Experimental data used for the calculation of obtained average EC₅₀ values

Χρόνος Αντίδρασης: 15 min		Απορρόφηση			Μέσος Όρος										
mg Δείγματος		1	2	3										% AA	%inhibition
DPPH		2.635													
A0		1.627	1.616	1.621	1.621333333										
		1	2	3											
LDH-OLE_Zn-Al_1-1		10	1.519	1.516	1.5	1.511666667	6.63798402	6.622372465	7.865777565	93.36201598	93.17762754	92.1942225	6.763980263	93.23601974	83.64480928
		20	1.409	1.41	1.412	1.410333333	13.39889367	13.33743085	13.21450522	86.60110633	86.66256915	86.78549478	13.01398026	86.98601974	81.16237366
		30	1.368	1.34	1.308	1.338666667	15.91886908	17.6398279	19.60663798	84.08113092	82.3601721	80.30336202	17.43421053	82.56578947	89.89707476
		40	1.2	1.239	1.205	1.2195	26.244622	23.84757222	25.93730793	73.755378	76.15242778	74.06269207	24.78412829	75.21587171	79.87497944
		1	2	3											
LDH-OLE_Zn-Al_2-1		10	1.511	1.532	1.544	1.529	7.12968654	5.838967425	5.101413645	92.87031346	94.16103258	94.89859636	5.694901316	94.30509868	100.4487756
		20	1.409	1.439	1.427	1.425	13.39889367	11.55500922	12.292563	86.60110633	88.44498078	87.707437	12.108375	87.898625	123.1756757
		30	1.377	1.391	1.392	1.386666667	15.36570375	14.50522434	14.44376152	84.63629625	85.49477566	85.56623848	14.4788421	85.52631579	75.6675063
		40	1.321	1.312	1.297	1.31	18.80762139	19.36078672	20.28272895	81.19237861	80.63821328	79.71727105	19.20230263	80.79769737	102.5031447
		1	2	3											
LDH-OLE_Zn-Al_3-1		10	1.542	1.571	1.572	1.561666667	5.224339275	3.44191784	3.380454825	95.8956	96.55808236	96.61954518	3.680006684	96.31990132	207.2003567
		20	1.537	1.514	1.525	1.525333333	5.53165335	6.945298095	6.26920713	92.354	93.05470191	93.73070287	5.921052632	94.07894737	173.7701975
		30	1.478	1.536	1.476	1.496666667	9.157959435	5.593116165	9.280885065	90.125	91.25	90.7191484	7.689144737	92.31865526	211.528021
		40	1.401	1.444	1.478	1.441	13.89059619	11.24789614	9.157959435	87.356	89.5454	90.84204057	11.12253289	88.87746711	236.3028515
		1	2	3											
A0		1.647	1.629	1.636	1.637333333										
OLE		10	1.561	1.556	1.55	1.555666667	4.05654579	4.363859865	4.732636755	95.94345421	95.63614014	95.26736325	4.987785016	95.01221498	81.67455007
		20	1.452	1.478	1.467	1.465666667	10.75599262	9.157959435	9.8340504	89.24400738	90.84204057	90.1659496	10.48452769	89.51547231	90.02454418
		30	1.379	1.387	1.383	1.383	15.24277812	14.7510756	14.99602686	84.75722188	85.2489244	85.00307314	15.53338762	84.46661238	92.31181834
		40	1.276	1.283	1.253	1.270666667	21.57344806	21.14320836	22.98709281	78.42655194	78.85679164	77.01290719	22.39413681	77.60598319	92.68276768

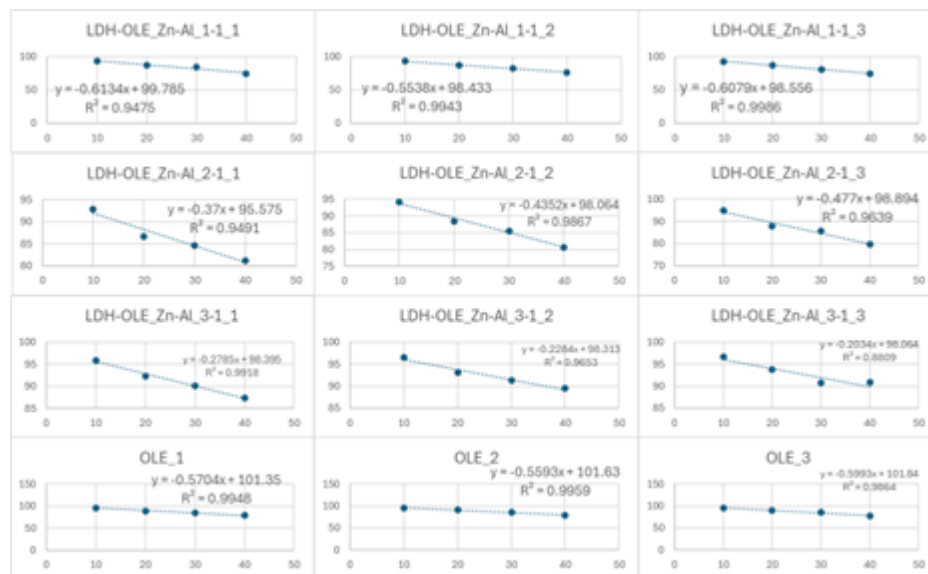
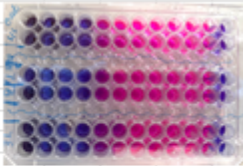


Figure S3. Linear plots used for the calculation of average values of EC₅₀

E. coli													
		LDH-OLE (mg/mL)											
		1	2	3	4	5	6	7	8	9	10	11	12
1/1	A	25.0	12.50	6.25	3.13	1.56	0.78	0.39	0.20	0.10	0.05	Culture	Media
	B	25.0	12.50	6.25	3.13	1.56	0.78	0.39	0.20	0.10	0.05	Culture	Media
	C	25.0	12.50	6.25	3.13	1.56	0.78	0.39	0.20	0.10	0.05	Culture	Media
2/1	D	25.0	12.50	6.25	3.13	1.56	0.78	0.39	0.20	0.10	0.05	Culture	Media
	E	25.0	12.50	6.25	3.13	1.56	0.78	0.39	0.20	0.10	0.05	Culture	Media
	F	25.0	12.50	6.25	3.13	1.56	0.78	0.39	0.20	0.10	0.05	Culture	Media
3/1	G	25.0	12.50	6.25	3.13	1.56	0.78	0.39	0.20	0.10	0.05	Culture	Media
	H	25.0	12.50	6.25	3.13	1.56	0.78	0.39	0.20	0.10	0.05	Culture	Media
	I	25.0	12.50	6.25	3.13	1.56	0.78	0.39	0.20	0.10	0.05	Culture	Media



S. aureus													
		LDH-OLE (mg/mL)											
		1	2	3	4	5	6	7	8	9	10	11	12
1/1	A	25.0	12.50	6.25	3.13	1.56	0.78	0.39	0.20	0.10	0.05	Culture	Media
	B	25.0	12.50	6.25	3.13	1.56	0.78	0.39	0.20	0.10	0.05	Culture	Media
	C	25.0	12.50	6.25	3.13	1.56	0.78	0.39	0.20	0.10	0.05	Culture	Media
2/1	D	25.0	12.50	6.25	3.13	1.56	0.78	0.39	0.20	0.10	0.05	Culture	Media
	E	25.0	12.50	6.25	3.13	1.56	0.78	0.39	0.20	0.10	0.05	Culture	Media
	F	25.0	12.50	6.25	3.13	1.56	0.78	0.39	0.20	0.10	0.05	Culture	Media
3/1	G	25.0	12.50	6.25	3.13	1.56	0.78	0.39	0.20	0.10	0.05	Culture	Media
	H	25.0	12.50	6.25	3.13	1.56	0.78	0.39	0.20	0.10	0.05	Culture	Media
	I	25.0	12.50	6.25	3.13	1.56	0.78	0.39	0.20	0.10	0.05	Culture	Media

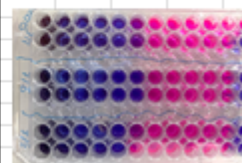
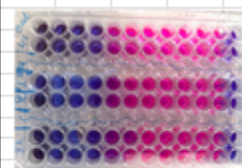
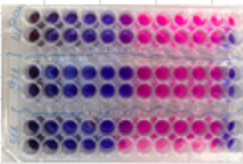


Figure S4. Results (three repetitions) to determine the minimum bactericidal concentration (MBC) of all OLE@LDH_Zn/Al_x/1 nanohybrids against *E. Coli* and *S. Aureus*. Representative images of resazurin-based 96-well plate microdilution method used to determine the minimum bactericidal concentration (MBC) of all OLE@LDH_Zn/Al_x/1 nanohybrids against *E. Coli* and *S. Aureus*.

Table S2. Statistical result of EC50 values

Descriptives

sample_code		Statistic		Std. Error
EC50	OLE@LDH_Zn/AI_1/1	Mean	83,6448	3,14815
		95% Confidence Interval for Mean	Lower Bound	70,0994
			Upper Bound	97,1902
		5% Trimmed Mean	.	.
		Median	81,1624	.
		Variance	29,732	.
		Std. Deviation	5,45275	.
		Minimum	79,87	.
		Maximum	89,90	.
		Range	10,02	.
		Interquartile Range	.	.
		Skewness	1,624	1,225
		Kurtosis	.	.
	OLE@LDH_Zn/AI_2/1	Mean	107,0456	8,31754
		95% Confidence Interval for Mean	Lower Bound	71,2581
			Upper Bound	142,8330
		5% Trimmed Mean	.	.
		Median	102,5031	.
		Variance	207,544	.
		Std. Deviation	14,40640	.
		Minimum	95,46	.
		Maximum	123,18	.
		Range	27,72	.
		Interquartile Range	.	.
		Skewness	1,278	1,225
		Kurtosis	.	.
	OLE@LDH_Zn/AI_3/1	Mean	207,2004	18,18085
		95% Confidence Interval for Mean	Lower Bound	128,9745
			Upper Bound	285,4262
		5% Trimmed Mean	.	.
		Median	211,5280	.
		Variance	991,630	.
		Std. Deviation	31,49015	.
		Minimum	173,77	.
		Maximum	236,30	.
		Range	62,53	.
		Interquartile Range	.	.
		Skewness	-,607	1,225
		Kurtosis	.	.
	OLE	Mean	91,6746	,83209
		95% Confidence Interval for Mean	Lower Bound	88,0943
			Upper Bound	95,2548
		5% Trimmed Mean	.	.
		Median	92,3118	.
		Variance	2,077	.
		Std. Deviation	1,44123	.
		Minimum	90,02	.
		Maximum	92,69	.
		Range	2,66	.
		Interquartile Range	.	.
		Skewness	-1,601	1,225
		Kurtosis	.	.

Multiple Comparisons

Dependent Variable: EC50

Tukey HSD

(I) sample_code	(J) sample_code	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval Lower Bound
OLE@LDH_Zn/AI_1/1	OLE@LDH_Zn/AI_2/1	-23,40075	14,32355	,414	-69,2698
	OLE@LDH_Zn/AI_3/1	-123,55555*	14,32355	<.001	-169,4246
	OLE	-8,02974	14,32355	,941	-53,8988
OLE@LDH_Zn/AI_2/1	OLE@LDH_Zn/AI_1/1	23,40075	14,32355	,414	-22,4683
	OLE@LDH_Zn/AI_3/1	-100,15479*	14,32355	<.001	-146,0238
	OLE	15,37101	14,32355	,714	-30,4980
OLE@LDH_Zn/AI_3/1	OLE@LDH_Zn/AI_1/1	123,55555*	14,32355	<.001	77,6865
	OLE@LDH_Zn/AI_2/1	100,15479*	14,32355	<.001	54,2858
	OLE	115,52581*	14,32355	<.001	69,6568
OLE	OLE@LDH_Zn/AI_1/1	8,02974	14,32355	,941	-37,8393
	OLE@LDH_Zn/AI_2/1	-15,37101	14,32355	,714	-61,2400
	OLE@LDH_Zn/AI_3/1	-115,52581*	14,32355	<.001	-161,3948

Table S3. Statistical result of TPC values

Descriptives

sample_code		Statistic		Std. Error
TPC_pH7	OLE@LDH_Zn/AI_1/1	Mean	21,6296	3,12766
		95% Confidence Interval for Mean	Lower Bound	8,1724
			Upper Bound	35,0868
		5% Trimmed Mean	.	
		Median	19,2222	
		Variance	29,347	
		Std. Deviation	5,41726	
		Minimum	17,83	
		Maximum	27,83	
		Range	10,00	
		Interquartile Range	.	
		Skewness	1,605	1,225
		Kurtosis	.	.
	OLE@LDH_Zn/AI_2/1	Mean	23,8519	2,82521
		95% Confidence Interval for Mean	Lower Bound	11,6959
			Upper Bound	36,0078
		5% Trimmed Mean	.	
		Median	21,1667	

		Variance	23,945	
		Std. Deviation	4,89341	
		Minimum	20,89	
		Maximum	29,50	
		Range	8,61	
		Interquartile Range	.	
		Skewness	1,726	1,225
		Kurtosis	.	.
OLE@LDH_Zn/Al_3/1	Mean		28,5741	4,78534
	95% Confidence Interval for Mean	Lower Bound	7,9844	
		Upper Bound	49,1637	
	5% Trimmed Mean		.	
	Median		24,5000	
	Variance		68,699	
	Std. Deviation		8,28846	
	Minimum		23,11	
	Maximum		38,11	
	Range		15,00	
	Interquartile Range		.	
	Skewness		1,677	1,225
	Kurtosis		.	.
	Mean		646,4444	1,60375
	95% Confidence Interval for Mean	Lower Bound	639,5441	
		Upper Bound	653,3448	
	5% Trimmed Mean		.	
	Median		646,4444	
	Variance		7,716	
	Std. Deviation		2,77778	
	Minimum		643,67	
	Maximum		649,22	
	Range		5,56	
	Interquartile Range		.	
	Skewness		,000	1,225
	Kurtosis		.	.
OLE	Mean		646,4444	1,60375
	95% Confidence Interval for Mean	Lower Bound	639,5441	
		Upper Bound	653,3448	
	5% Trimmed Mean		.	
	Median		646,4444	
	Variance		7,716	
	Std. Deviation		2,77778	
	Minimum		643,67	
	Maximum		649,22	
	Range		5,56	
	Interquartile Range		.	
	Skewness		,000	1,225
	Kurtosis		.	.
TPC_pH3.6	OLE@LDH_Zn/Al_1/1	Mean	25,0556	,57824
		95% Confidence Interval for Mean	Lower Bound	22,5676
			Upper Bound	27,5435
		5% Trimmed Mean	.	

	Median		24,7778	
	Variance		1,003	
	Std. Deviation		1,00154	
	Minimum		24,22	
	Maximum		26,17	
	Range		1,94	
	Interquartile Range		.	
	Skewness		1,152	1,225
	Kurtosis		.	.
OLE@LDH_Zn/Al_2/1	Mean		40,1481	1,06783
	95% Confidence Interval for Mean	Lower Bound	35,5536	
		Upper Bound	44,7426	
	5% Trimmed Mean		.	
	Median		40,6111	
	Variance		3,421	
	Std. Deviation		1,84954	
	Minimum		38,11	
	Maximum		41,72	
	Range		3,61	
	Interquartile Range		.	
	Skewness		-1,056	1,225
	Kurtosis		.	.
	Mean		22,7407	,37037
OLE@LDH_Zn/Al_3/1	95% Confidence Interval for Mean	Lower Bound	21,1472	
		Upper Bound	24,3343	
	5% Trimmed Mean		.	
	Median		23,1111	
	Variance		,412	
	Std. Deviation		,64150	
	Minimum		22,00	
	Maximum		23,11	
	Range		1,11	
	Interquartile Range		.	
	Skewness		-1,732	1,225
	Kurtosis		.	.
	Mean		643,6667	1,60375
	95% Confidence Interval for Mean	Lower Bound	636,7663	
		Upper Bound	650,5670	
OLE				
	Mean		643,6667	1,60375
	95% Confidence Interval for Mean	Lower Bound	636,7663	
		Upper Bound	650,5670	

		5% Trimmed Mean	.	
		Median	643,6667	
		Variance	7,716	
		Std. Deviation	2,77778	
		Minimum	640,89	
		Maximum	646,44	
		Range	5,56	
		Interquartile Range	.	
		Skewness	,000	1,225
		Kurtosis	.	.
TPC_pH1	OLE@LDH_Zn/Al_1/1	Mean	606,6296	4,03602
		95% Confidence Interval for Mean	Lower Bound	589,2640
			Upper Bound	623,9952
		5% Trimmed Mean	.	
		Median	607,5556	
		Variance	48,868	
		Std. Deviation	6,99059	
		Minimum	599,22	
		Maximum	613,11	
		Range	13,89	
		Interquartile Range	.	
		Skewness	-,586	1,225
		Kurtosis	.	.
	OLE@LDH_Zn/Al_2/1	Mean	232,0926	4,34594
		95% Confidence Interval for Mean	Lower Bound	213,3935
			Upper Bound	250,7917
		5% Trimmed Mean	.	
		Median	232,8333	
		Variance	56,662	
		Std. Deviation	7,52738	
		Minimum	224,22	
		Maximum	239,22	
		Range	15,00	
		Interquartile Range	.	
		Skewness	-,439	1,225
		Kurtosis	.	.
	OLE@LDH_Zn/Al_3/1	Mean	186,9074	8,81966
		95% Confidence Interval for Mean	Lower Bound	148,9595

		Upper Bound	224,8553	
		5% Trimmed Mean	.	
		Median	181,1667	
		Variance	233,359	
		Std. Deviation	15,27609	
		Minimum	175,33	
		Maximum	204,22	
		Range	28,89	
		Interquartile Range	.	
		Skewness	1,452	1,225
		Kurtosis	.	.
	OLE	Mean	634,4074	2,44977
		95% Confidence Interval for Mean	Lower Bound	623,8669
			Upper Bound	644,9479
		5% Trimmed Mean	.	
		Median	635,3333	
		Variance	18,004	
		Std. Deviation	4,24313	
		Minimum	629,78	
		Maximum	638,11	
		Range	8,33	
		Interquartile Range	.	
		Skewness	-,935	1,225
		Kurtosis	.	.

Multiple Comparisons

Tukey HSD

Dependent Variable	(I) sample code	(J) sample code	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
TPC_pH1	OLE@LDH_Zn/Al_1/1	OLE@LDH_Zn/Al_2/1	374,53704*	7,71247	<.001	349,8390	399,2351
		OLE@LDH_Zn/Al_3/1	419,72222*	7,71247	<.001	395,0242	444,4203
		OLE	-27,77778*	7,71247	,029	-52,4758	-3,0797
	OLE@LDH_Zn/Al_2/1	OLE@LDH_Zn/Al_1/1	-374,53704*	7,71247	<.001	-399,2351	-349,8390
		OLE@LDH_Zn/Al_3/1	45,18519*	7,71247	,002	20,4871	69,8832
		OLE	-402,31481*	7,71247	<.001	-427,0129	-377,6168

	OLE@LDH_Zn/Al_3/1	OLE@LDH_Zn/Al_1/1	-419,72222*	7,71247	<.001	-444,4203	-395,0242
		OLE@LDH_Zn/Al_2/1	-45,18519*	7,71247	,002	-69,8832	-20,4871
		OLE	-447,50000*	7,71247	<.001	-472,1980	-422,8020
	OLE	OLE@LDH_Zn/Al_1/1	27,77778*	7,71247	,029	3,0797	52,4758
		OLE@LDH_Zn/Al_2/1	402,31481*	7,71247	<.001	377,6168	427,0129
		OLE@LDH_Zn/Al_3/1	447,50000*	7,71247	<.001	422,8020	472,1980
	TPC_pH3.6	OLE@LDH_Zn/Al_1/1	OLE@LDH_Zn/Al_2/1	-15,09259*	1,44634	<.001	-19,7243
			OLE@LDH_Zn/Al_3/1	2,31481	1,44634	,430	-2,3169
			OLE	-618,61111*	1,44634	<.001	-623,2428
		OLE@LDH_Zn/Al_2/1	OLE@LDH_Zn/Al_1/1	15,09259*	1,44634	<.001	10,4609
			OLE@LDH_Zn/Al_3/1	17,40741*	1,44634	<.001	12,7757
			OLE	-603,51852*	1,44634	<.001	-608,1502
		OLE@LDH_Zn/Al_3/1	OLE@LDH_Zn/Al_1/1	-2,31481	1,44634	,430	-6,9465
			OLE@LDH_Zn/Al_2/1	-17,40741*	1,44634	<.001	-22,0391
			OLE	-620,92593*	1,44634	<.001	-625,5576
TPC_pH7	OLE	OLE@LDH_Zn/Al_1/1	618,61111*	1,44634	<.001	613,9794	623,2428
		OLE@LDH_Zn/Al_2/1	603,51852*	1,44634	<.001	598,8868	608,1502
		OLE@LDH_Zn/Al_3/1	620,92593*	1,44634	<.001	616,2942	625,5576
	OLE@LDH_Zn/Al_1/1	OLE@LDH_Zn/Al_2/1	-2,22222	4,64949	,962	-17,1115	12,6671
		OLE@LDH_Zn/Al_3/1	-6,94444	4,64949	,483	-21,8338	7,9449
		OLE	-624,81481*	4,64949	<.001	-639,7041	-609,9255
	OLE@LDH_Zn/Al_2/1	OLE@LDH_Zn/Al_1/1	2,22222	4,64949	,962	-12,6671	17,1115
		OLE@LDH_Zn/Al_3/1	-4,72222	4,64949	,745	-19,6115	10,1671
		OLE	-622,59259*	4,64949	<.001	-637,4819	-607,7033
	OLE@LDH_Zn/Al_3/1	OLE@LDH_Zn/Al_1/1	6,94444	4,64949	,483	-7,9449	21,8338
		OLE@LDH_Zn/Al_2/1	4,72222	4,64949	,745	-10,1671	19,6115
		OLE	-617,87037*	4,64949	<.001	-632,7597	-602,9811
	OLE	OLE@LDH_Zn/Al_1/1	624,81481*	4,64949	<.001	609,9255	639,7041
		OLE@LDH_Zn/Al_2/1	622,59259*	4,64949	<.001	607,7033	637,4819
		OLE@LDH_Zn/Al_3/1	617,87037*	4,64949	<.001	602,9811	632,7597

*. The mean difference is significant at the 0.05 level.

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