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Date: April-May 2019

Location: Minoprio Analisi e Certifications, Italy.

Commissioned by: Biomedic Clinic and Research

Crops: Kagran Cabbage

Summary of Comparison Trial

Purpose: Test whether Kyminasi Plant (Harvest Harmonics) system can alter nutrient absorption in plants

The test demonstrated that by the specific programming of our Harvest Harmonics system, we can control the increase of absorption of certain nutrients, specifically: nitrogen, calcium, magnesium, manganese and zinc; while reducing the absorption of phosphorus, potassium, iron and sodium. This proves that our sonic technology works as programmed.

For full details, please see the complete report attached.

MAC
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Cap. Soc. € 96.900,00 int. vers.
Reg. Imprese Trib. di Como n. 27261
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Clinic & Research S.r.l.
Via Belvedere, 11
22079 Villa Guardia (CO)

C19012

Subject: Results of preliminary experimental activity to verify the effects on the vegetative activity of the technological application of irrigation water activation - ref. MAC offers 48/2019 + 93/2019

PREMISE

In relation to the offers indicated in the subject, Minoprio Analisi e Certificazioni S.r.l. of the Minoprio Foundation (CO) has been appointed to carry out preliminary experimental activity in order to verify the possible effects on the vegetative activity of a technological application of water activation for irrigation use that you implemented.

The experimental tests carried out have been identified following the information you provided on the technology being tested. The species chosen are those that best respond to the type of tests implemented.

The results of this preliminary experimentation may lead to the definition of tests on a larger scale.

Experimental tests were carried out at the Minoprio Foundation facilities. The experimental methodology implemented, results and conclusions are shown below.

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MATERIALS AND METHODS

The job order included the following experimental tests.

Lettuce test (ref. Lombardy Region - BU 13/05/2003 - 1st SS - DGR 16/04/2003 n. 7/12764 - All. B)

The test aims to evaluate the response of vegetation to activated water treatment in terms of aerial biomass production and content of mineral elements in the leaves.
2 types of culture media were used:
1) Mineral substrate composed of silica sand (87.5%), bentonite (9%), medium-textured earth with a neutral pH (2%) and peat (1.5%);
2) Peat-based organic substrate.

The test involved the use of *Kagran* variety cabbage lettuce, sown on 03/14/2019 and transplanted, in the different identified substrates, on 03/26/2019 in 300 ml capacity jars (3 seedlings/pot).
The pots were placed in an iron/glass greenhouse (Hortiplus glass), on pallets with small channels, with a minimum temperature of 12 °C and aeration at 18 °C.
The transplanted plants were divided according to the following experimental specimens:

- *Control on mineral substrate: irrigation with mains water;*
- *Control on organic substrate: irrigation with mains water;*
- *Treatment on mineral substrate: irrigation with activated water;*
- *Treatment on organic substrate: irrigation with activated water.*

The control specimens were placed on a pallet separated from the water treatment specimens. Irrigation was carried out manually; the first week with nebulization to facilitate rooting of the transplanted seedlings, then with a watering can, always paying maximum attention in order to exclude any possibility of contamination between the specimens irrigated with different types of water

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(normal or activated). Irrigation was carried out daily, always bringing the individual pots to maximum saturation.

For each experimental thesis, 5 replicas have been planned, each with 4 pots (total 60 plants/thesis).

On April 23, in agreement with the client, further transplanting was carried out in 16-diameter pot, using neutral mineral substrate with a low presence of limestone as a mineral substrate and the correct acid peat as an organic substrate.

The irrigations continued on a daily basis, with an average intake of 500 ml/pot.

On 15 May the aerial biomass of each individual pot was cut (except for an entire replica of each thesis) to determine the production of biomass/pot (fresh weight and dry weight at 75 °C).

The determination of the content of the main mineral elements (1 representative sample/replica) was carried out on the dried biomass: N, P, K, Ca, Mg, Na, Fe, Mn, Cu, Zn.

Irrigation was suspended for the plants of the non-harvested replicas, observing any differences in drying times.

All the results were subjected to statistical analysis for the evaluation of the presence of statistically significant differences between the average values obtained for the individual theses (ANOVA and Duncan test for $P = 0.05$).

On 10 May, the main characterization parameters of an irrigation water were determined on treated and untreated water samples.

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RESULTS AND STATISTICAL

Below are reported, for each test, summary tables of the results obtained and the outcome of their processing (analysis of variance and Duncan test: in different letters I correspond to significantly different values for P = 0.05).

Lettuce test (ref. Lombardy Region - BU 13/05/2003 - 1st SS - DGR 16/04/2003 n. 7/12764 - All. B)

The following tables (Table 1 to Table 4) show the average production data of fresh and dry aerial biomass for the different types of substrate (mineral or organic), a summary of the statistical processing (ANOVA) and the outcome of the Duncan test (95% confidence) in order to identify any statistically significant differences between the different treatments.

Table 1: Average fresh air biomass production on mineral substrate and 95% Duncan test

Thesis	*Count*	*Average*	*Standard deviation*	*Variation coeff.*	*Homogeneous*
1 - control	16	**9.4525**	0.843821	8.92696%	***a***
2 - treated	16	**8.8975**	0.983107	11.0493%	***a***

(if present, in different letters correspond statistically different averages for P = 0.05)

T

Table 2: Average dry aerial biomass production on mineral substrate and 95% Duncan test

Thesis	*Count*	*Average*	*Standard deviation*	*Variation coeff.*	*Homogeneous*
1 - control	*16*	***1.58563***	*0.212445*	*13.3982%*	***a***
2 - treated	*16*	***1.52625***	*0.199862*	*13.095%*	***a***

(if present, in different letters correspond statistically different averages for P = 0.05)

Table 3: Average fresh air biomass production on organic substrate and 95% Duncan test

Thesis	*Count*	*Average*	*Standard deviation*	*Variation coeff.*	*Homogeneous*
1- control	*16*	***29.9419***	*1.71966*	*5.74331%*	***a***
2 - treated	*16*	***29.0687***	*2.09757*	*7.21588%*	***a***

(if present, in different letters correspond statistically different averages for P = 0.05)

Table 4: Average dry aerial biomass production on organic substrate and 95% Duncan test

Thesis	*Count*	*Average*	*Standard deviation*	*Variation coeff.*	*Homogeneous*
1- control	*16*	***3.95125***	*0.248807*	*6.29692%*	***a***
2 - treated	*16*	***3.86437***	*0.439833*	*11.3817%*	***a***

(if present, in different letters correspond statistically different averages for P = 0.05)

The results of plant tissue analysis (mineral element determination) are shown in the following tables.

In Table 5 the results of the statistical analysis for the **nitrogen** parameter (N% s.s.).

The results show that the average values of the two experimental theses (control and treated), both on mineral and organic substrates, are significantly different (P = 0.05). Specifically, the plant tissues of plants irrigated with treated water have significantly higher values than those irrigated with untreated water. However, it is necessary to specify that the data obtained on both treatments, regardless of the type of substrate, are below the lower limit of sufficiency for lettuce (2.5% s.s.).

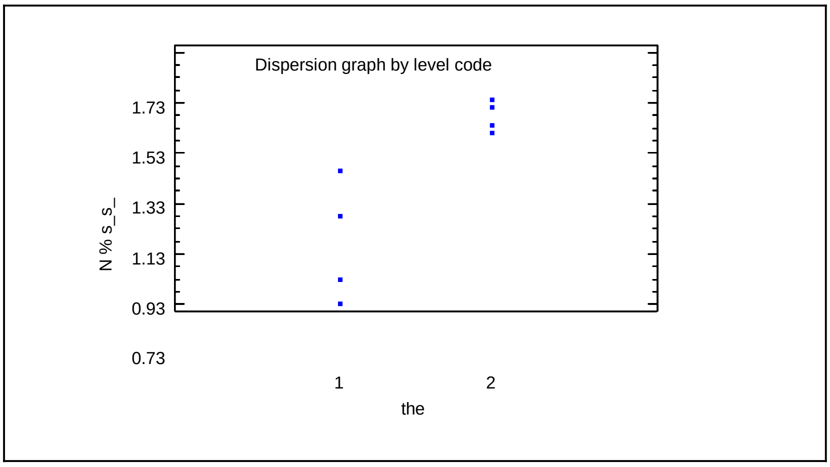
Table 5: Total nitrogen of plant tissues (% s.s.) and 95% Duncan test

Thesis - mineral	*Count*	*Avera*	*Standard deviation*	*Variation coeff.*	*Homogeneous*
1- control	*4*	***0.975***	*0.240347*	*24.651%*	***b***
2 - treated	*4*	***1.475***	*0.0602771*	*4.08659%*	***a***
Thesis - organic	*Count*	*Avera*	*Standard deviation*	*Variation coeff.*	*Homogeneous*
1- control	*4*	***1.110***	*0.116333*	*10.4804%*	***b***
2 - treated	*4*	***1.532***	*0.0906918*	*5.9179%*	***a***

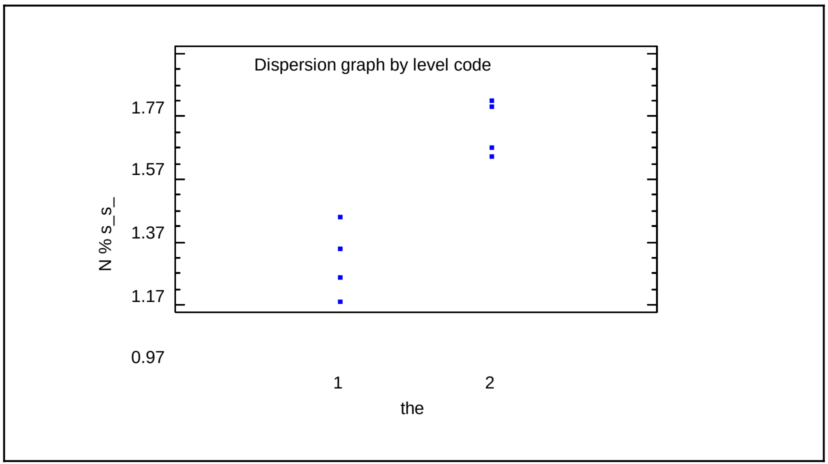
(if present, in different letters correspond statistically different averages for P = 0.05)

In the following two graphs (Graph 1 and Graph 2) the dispersions of the individual analytical values subjected to analysis of variance are visually represented: the perception of the statistically different distribution between the values of the two theses is immediate (1 = control / 2 = treated).

Graph 1: Dispersion graph by level code (% N tot on mineral substrate)



Graph 2: Dispersion graph by level code (% N tot on organic substrate)



In the following tables (from number 6 to number 14) the average results are a summary of the statistical analysis for the phosphorus, potassium, calcium, magnesium, iron, manganese, copper, zinc and sodium parameters.

The results of the **phosphorus** (Table 6) show a statistically significant difference between the two experimental theses on organic substrate (higher value in the untreated control); on the contrary, on the mineral substrate the two theses are not statistically different from each other. Also in this case the values found are well below the sufficiency limit for lettuce (0.4% ss)

Table 6: Total phosphorus of plant tissues (mg/kg s.s.) and 95% Duncan test

Thesis - mineral	*Count*	*Averag*	*Standard deviation*	*Variation coeff.*	*Homogeneous*
1- control	*4*	***878.21***	*159.57*	*18.17%*	***a***
2 - treated	*4*	***1,004.10***	*69.8515*	*6.95682%*	***a***
Thesis - organic	*Count*	*Averag*	*Standard deviation*	*Variation coeff.*	*Homogeneous*
1- control	*4*	***979.70***	*73.2274*	*7.47447%*	***a***
2 - treated	*4*	***834.23***	*80.9174*	*9.69971%*	***b***

(if present, in different letters correspond statistically different averages for P = 0.05)

The **potassium** results (Table 7) show a statistically significant difference between the two experimental theses in both types of substrate, with higher values for the untreated control. Also in this case the values found are well below the sufficiency limit for lettuce (5.0% s.s.)

Table 7: Total potassium of plant tissues (% s.s.) and 95% Duncan test

Thesis - mineral	*Count*	*Averag*	*Standard deviation*	*Variation coeff.*	*Homogeneous*
1- control	*4*	***2.393***	*0.408197*	*17.0615%*	***a***
2 - treated	*4*	***1.700***	*0.0535413*	*3.14949%*	***b***
Thesis - organic	*Count*	*Averag*	*Standard deviation*	*Variation coeff.*	*Homogeneous*
1- control	*4*	***2.258***	*0.0585235*	*2.5924%*	***a***
2 - treated	*4*	***1.545***	*0.0793725*	*5.13738%*	***b***

(if present, in different letters correspond statistically different averages for P = 0.05)

The same results are found for the **calcium** (Table 8) and **magnesium** (Table 9) parameter, where the difference is statistically significant between the two experimental theses in both types of substrate; the highest values (however below the limits of sufficiency for the crop) are found for the sample irrigated with treated water.

Table 8: Total calcium of plant tissues (% s.s.) and 95% Duncan test

Thesis - mineral	*Count*	*Avera*	*Standard deviation*	*Variation coeff.*	*Homogeneous*
1- control	*4*	***0.825***	*0.0974679*	*11.8143%*	***b***
2 - treated	*4*	***1.428***	*0.0221736*	*1.55331%*	***a***
Thesis - organic	*Count*	*Avera*	*Standard deviation*	*Variation coeff.*	*Homogeneous*
1- control	*4*	***0.753***	*0.0419325*	*5.57242%*	***b***
2 - treated	*4*	***1.548***	*0.13099*	*8.46461%*	***a***

(if present, in different letters correspond statistically different averages for P = 0.05)

Table 9: Total magnesium of plant tissues (% s.s.) and 95% Duncan test

Thesis - mineral	*Count*	*Averag*	*Standard deviation*	*Variation coeff.*	*Homogeneous*
1- control	*4*	***0.18***	*0.0173205*	*9.89743%*	***b***
2 - treated	*4*	***0.34***	*0.005*	*1.48148%*	***a***
Thesis - organic	*Count*	*Averag*	*Standard deviation*	*Variation coeff.*	*Homogeneous*
1- control	*4*	***0.16***	*0.00816497*	*5.1031%*	***b***
2 - treated	*4*	***0.36***	*0.0221736*	*6.20239%*	***a***

(if present, in different letters correspond statistically different averages for P = 0.05)

For the **iron** (Table 10) and **manganese** (Table 11) microelements, the values found are normal for the culture in question and the two experimental theses show statistically different mean values. However, it should be noted that the behavior of the two elements is strangely divergent; in fact, iron has higher values in plants irrigated with untreated water, while for manganese the outcome is the opposite.

Table 10 Total iron of plant tissues (mg/kg s.s.) and 95% Duncan test

Thesis - mineral	Count	Avera	Standard deviation	Variation coeff.	Homogeneous
1- control	4	**535.28**	80.5158	15.0419%	**a**
2 - treated	4	**75.70**	5.02517	6.63804%	**b**
Thesis - organic	Count	Avera	Standard deviation	Variation coeff.	Homogeneous
1- control	4	**391.35**	50.9596	13.0215%	**a**
2 - treated	4	**75.96**	2.6483	3.48633%	**b**

(if present, in different letters correspond statistically different averages for P = 0.05)

Table 11: Total manganese of plant tissues (mg/kg s.s.) and 95% Duncan test

Thesis - mineral	Count	Averag	Standard deviation	Variation coeff.	Homogeneous
1- control	4	**47.738**	1.53951	3.22495%	**b**
2 - treated	4	**145.93**	6.02184	4.12666%	**a**
Thesis - organic	Count	Averag	Standard deviation	Variation coeff.	Homogeneous
1- control	4	**53.168**	3.92232	7.3773%	**b**
2 - treated	4	**138.15**	10.1576	7.35258%	**a**

(if present, in different letters correspond statistically different averages for P = 0.05)

The behavior of **copper** (Table 12) and **zinc** (Table 13) microelements is similar.

For copper there is a significant difference between the treatments only on organic substrate, with a higher value for the untreated control; both theses however present levels of element sufficient for the crop. Zinc shows higher values for the thesis with treated water; for the control the result shows a lack.

Table 12: Total copper of plant tissues (mg/kg s.s.) and 95% Duncan test

Thesis - mineral	Count	Averag	Standard deviation	Variation coeff.	Homogeneous
1- control	4	**5.430**	0.0751983	1.39039%	**a**
2 - treated	4	**3.665**	1.74422	47.5912%	**a**
Thesis - organic	Count	Averag	Standard deviation	Variation coeff.	Homogeneous
1- control	4	**6.2675**	0.708725	11.3079%	**a**
2 - treated	4	**3.4475**	0.489311	14.1932%	**b**

(if present, in different letters correspond statistically different averages for P = 0.05)

Table 13: Total zinc of plant tissues (mg/kg s.s.) and 95% Duncan test

Thesis - mineral	Count	Averag	Standard deviation	Variation coeff.	Homogeneous
1- control	4	**15.188**	1.19321	7.85655%	**b**
2 - treated	4	**32.59**	1.45307	4.45862%	**a**
Thesis - organic	Count	Averag	Standard deviation	Variation coeff.	Homogeneous
1- control	4	**19.348**	0.904853	4.67685%	**b**
2 - treated	4	**36.418**	3.39024	9.30938%	**a**

(if present, in different letters correspond statistically different averages for P = 0.05)

Sodium (Table 14), which generally has high values, is higher in untreated plants, with significant difference between the theses only on organic substrate.

Table 14: Total sodium of plant tissues (mg/kg s.s.) and 95% Duncan test

Thesis - mineral	*Count*	*Averag*	*Standard deviation*	*Variation coeff.*	*Homogeneous*
1- control	*4*	***5734.2***	*2808.86*	*48.9843%*	***a***
2 - treated	*4*	***2878.8***	*239.611*	*8.32322%*	***a***
Thesis - organic	*Count*	*Averag*	*Standard deviation*	*Variation coeff.*	*Homogeneous*
1- control	*4*	***7884.8***	*927.259*	*11.7601%*	***a***
2 - treated	*4*	***1912.3***	*295.489*	*15.4519%*	***b***

(if present, in different letters correspond statistically different averages for P = 0.05)

Considering the results obtained with respect to the different type of substrate used (mineral or organic), for the investigated elements there is no difference between this factor.

In support of the above tables for the phosphorus, potassium, calcium, magnesium, iron, manganese, copper, zinc and sodium parameters, the following pages (for each of them and for the different types of substrate used) are shown, where graphs the dispersions of the individual analytical values subjected to analysis of variance are represented visually. These graphs allow a more immediate and clearer perception of the distribution of the data and, where present, of the statistically significant differences found (1 = control - 2 = treated).

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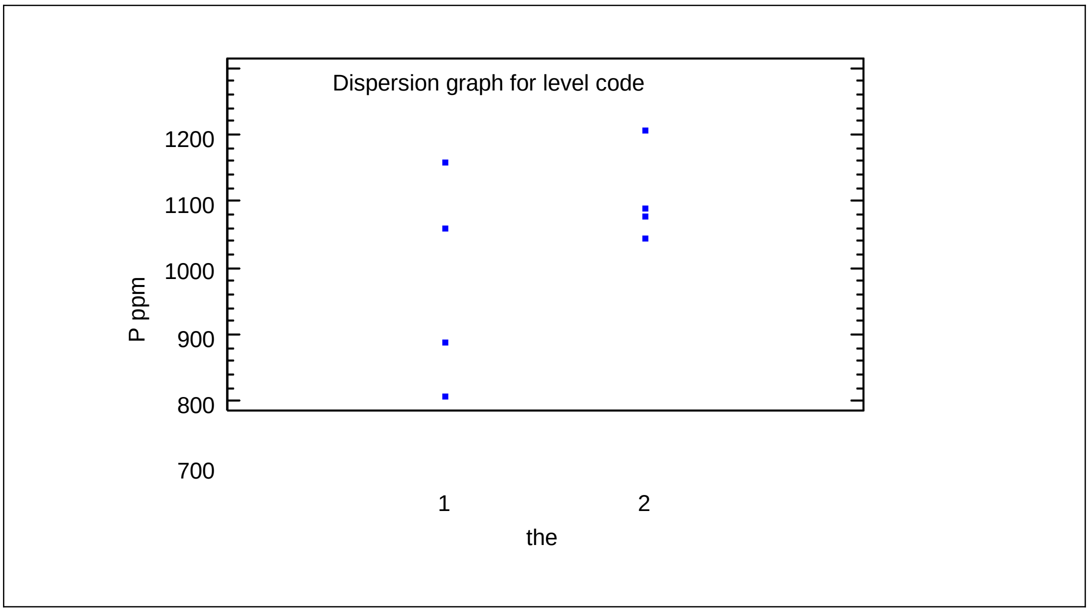
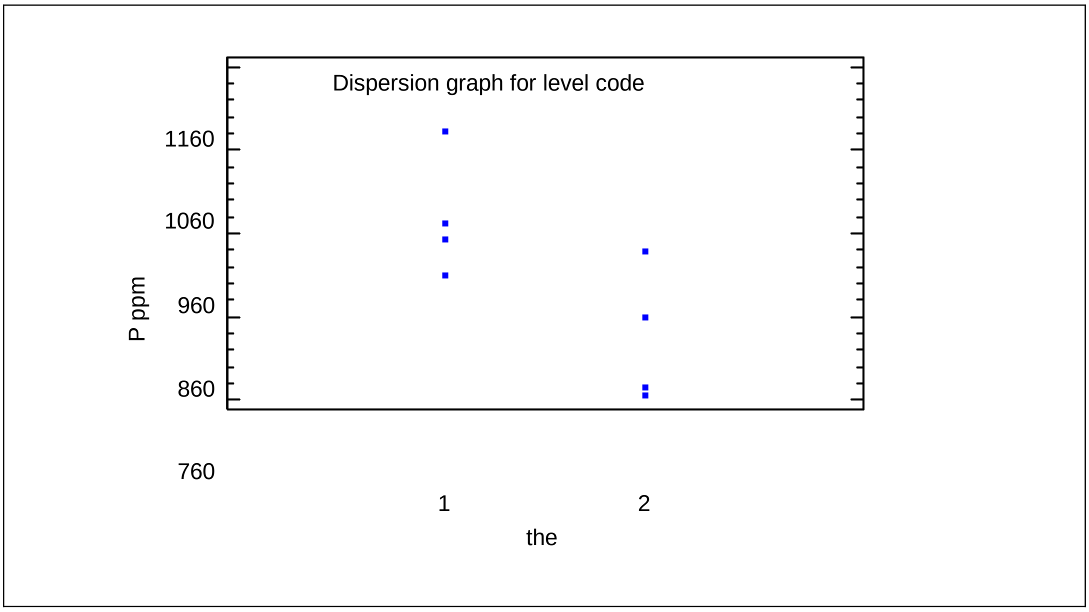
Graph 3: Dispersion graph by level code (mg/kg P tot on mineral substrate)



Absence of significance (P = 0.05)

Graph 4: Dispersion graph by level code (mg/kg P tot on organic substrate)



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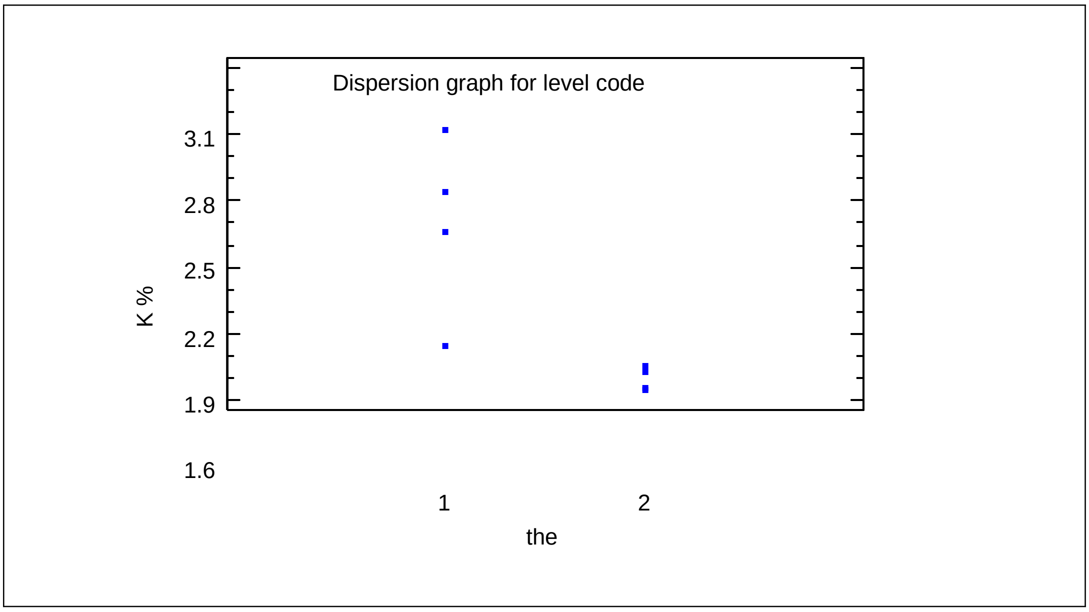
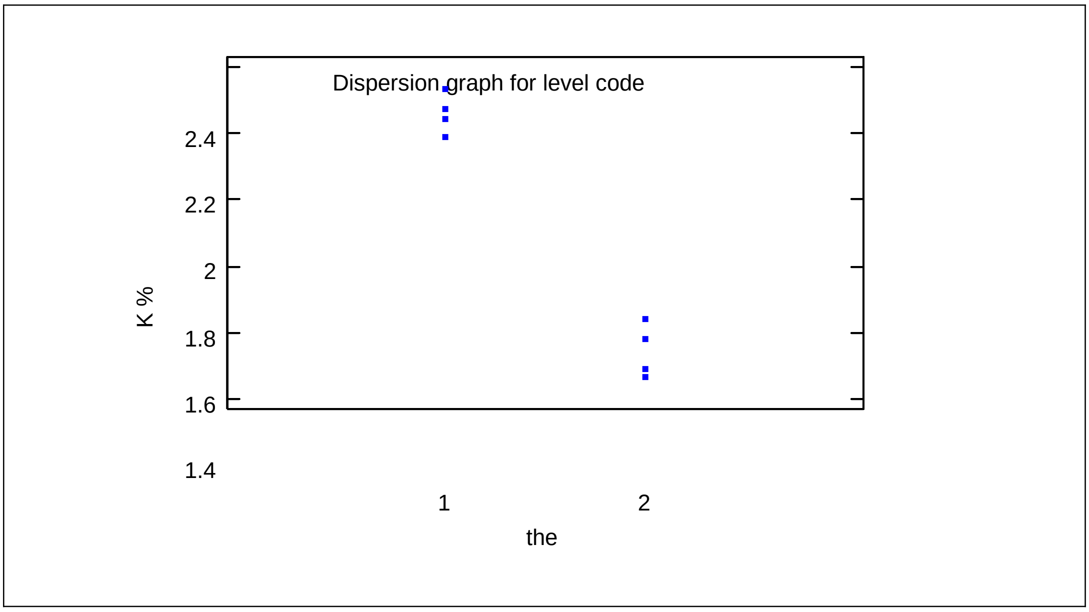
Graph 5: Dispersion graph by level code (% K tot on mineral substrate)



Graph 6: dispersion graph by level code (% K tot on organic substrate)



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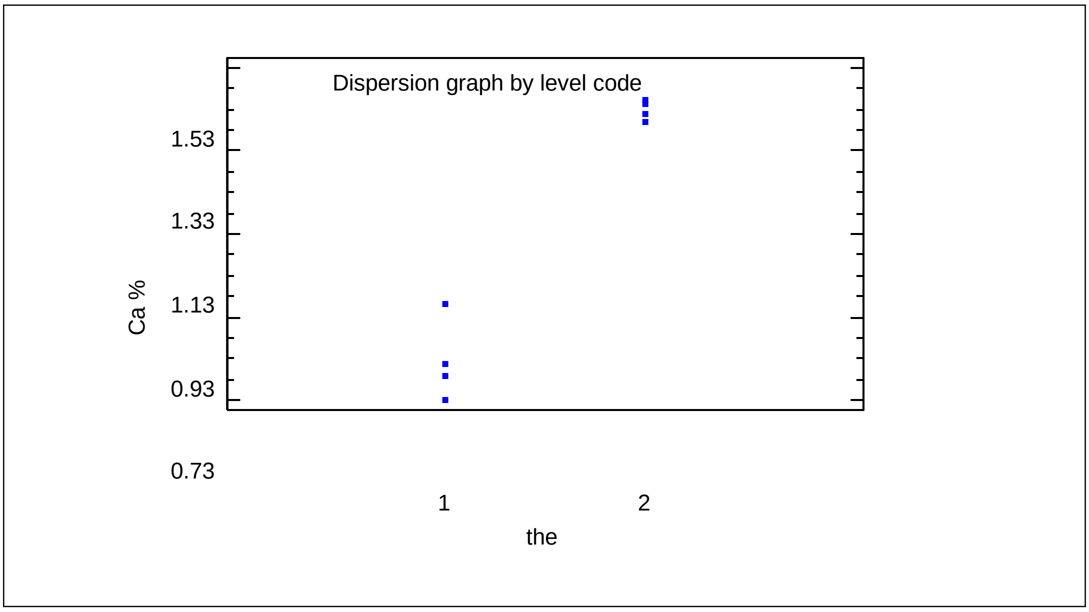
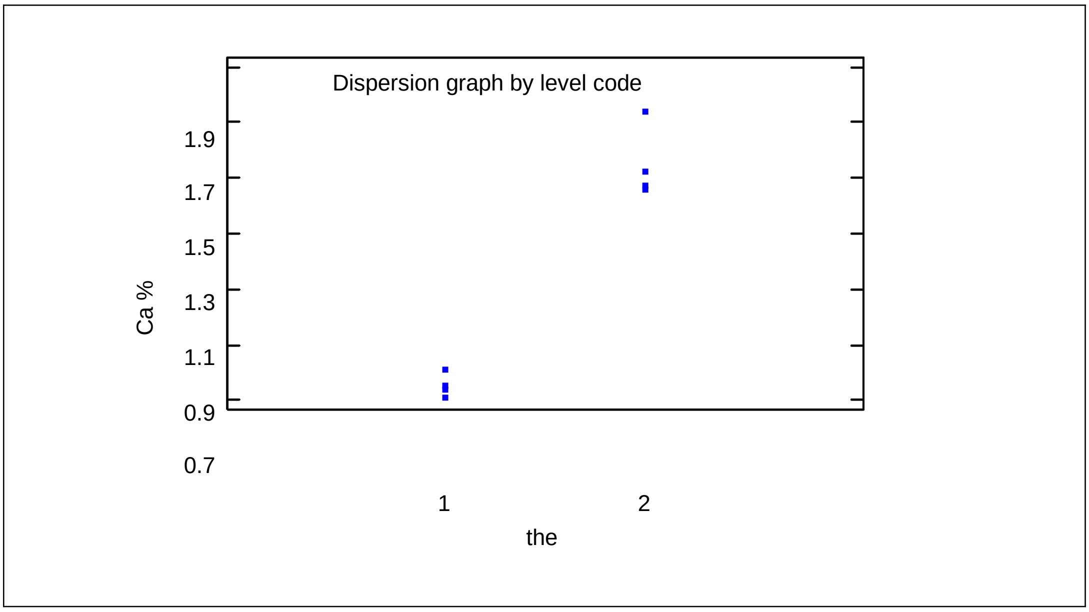
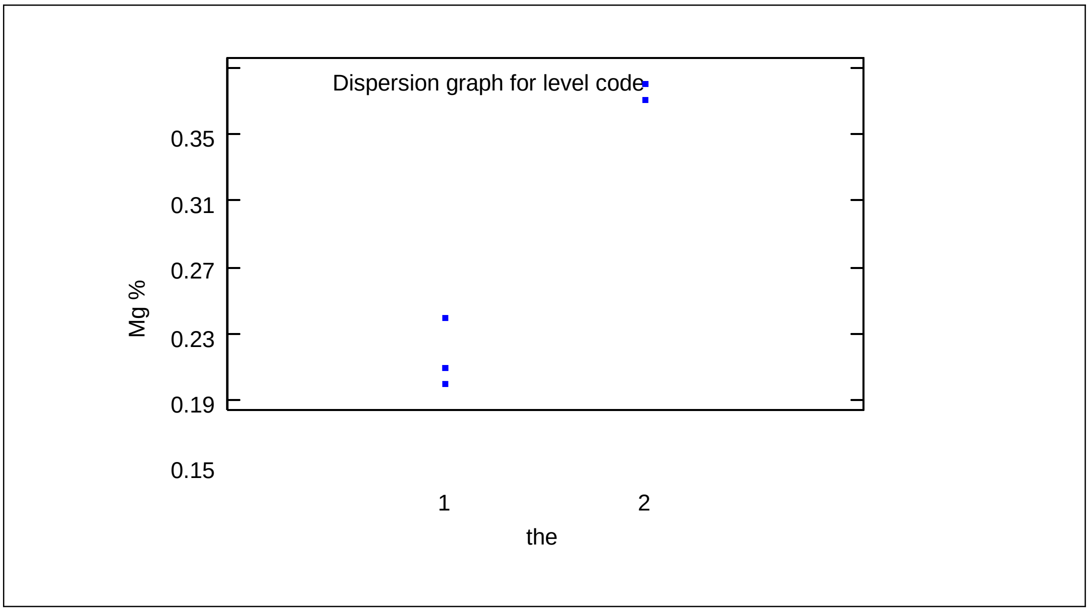
Graph 7: Dispersion graph by level code (% Ca tot on mineral substrate)



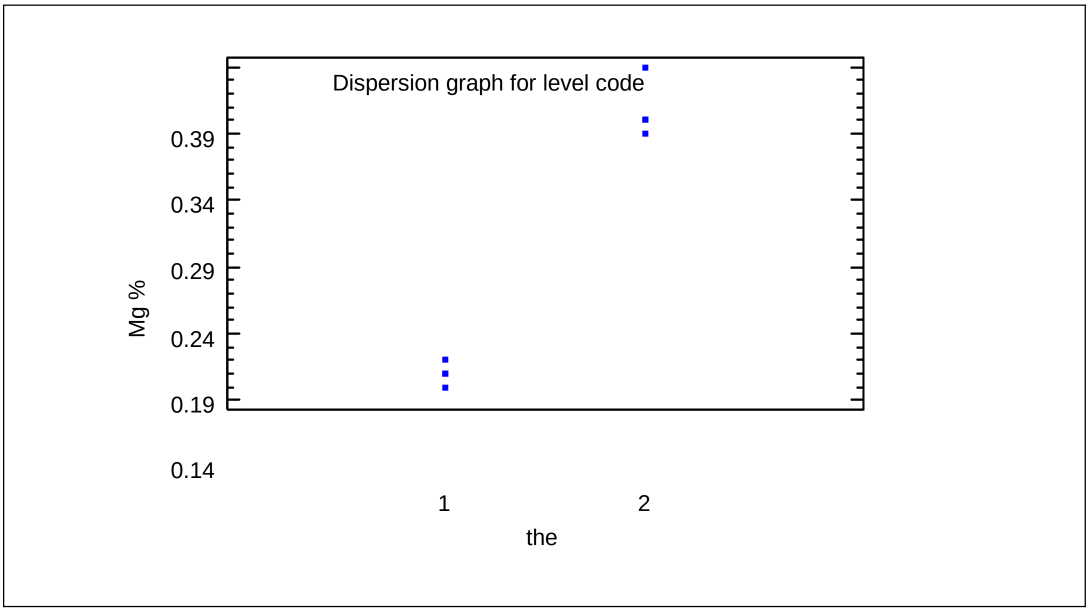
Graph 8: Dispersion graph by level code (% Ca tot on organic substrate)



Graph 9: Dispersion graph by level code (% Mg tot on mineral substrate)



Graph 10: dispersion graph by level code (% Mg tot on organic substrate)



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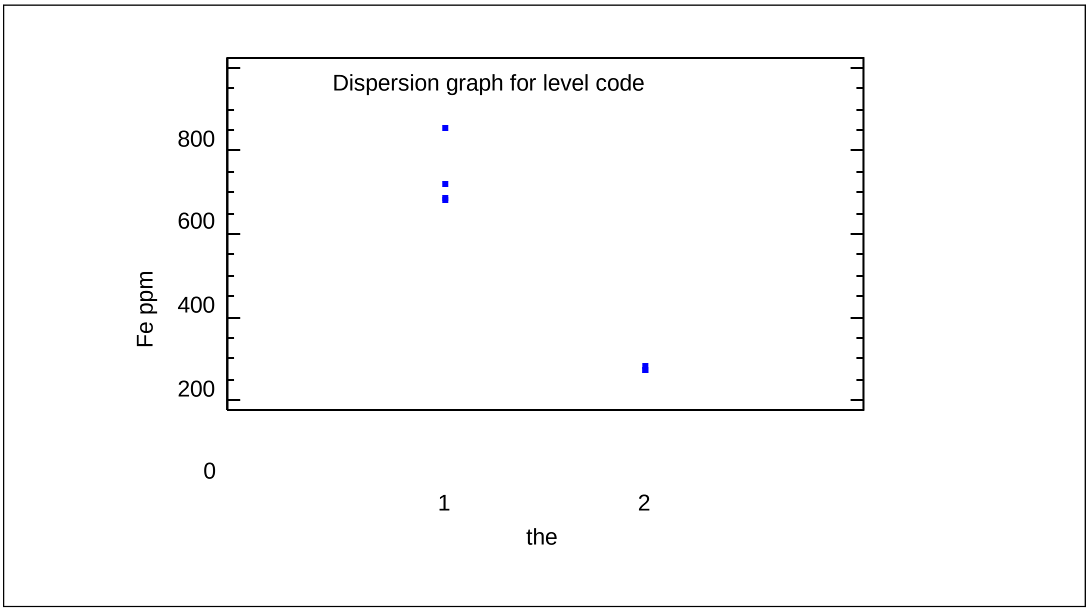
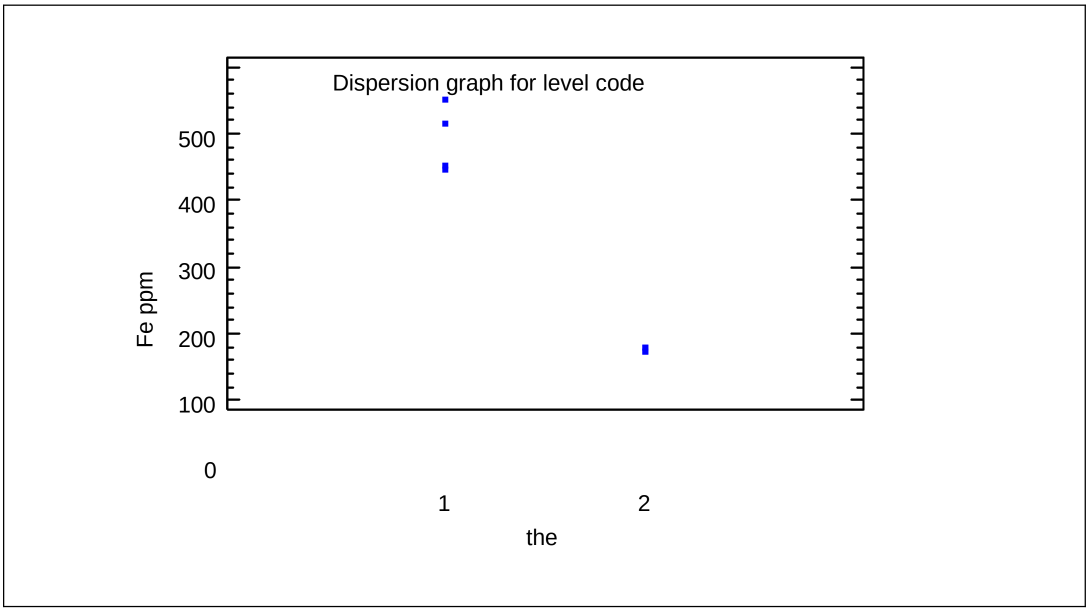
Graph 11: dispersion graph by level code (mg/kg Fe tot on mineral substrate)



Graph 12: dispersion graph by level code (mg/kg Fe tot on organic substrate)



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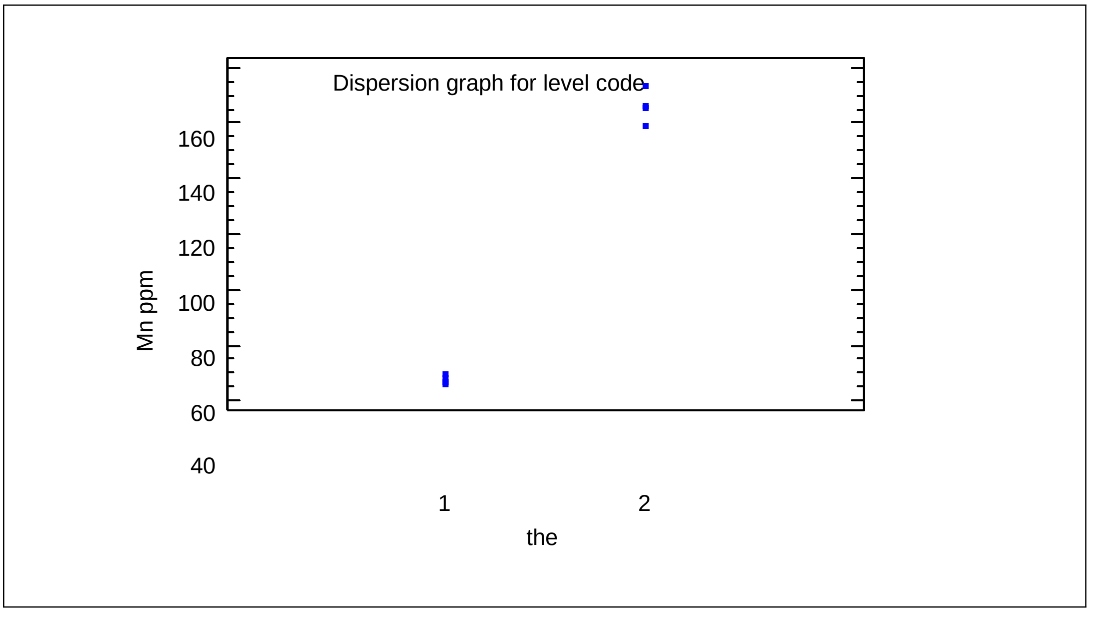
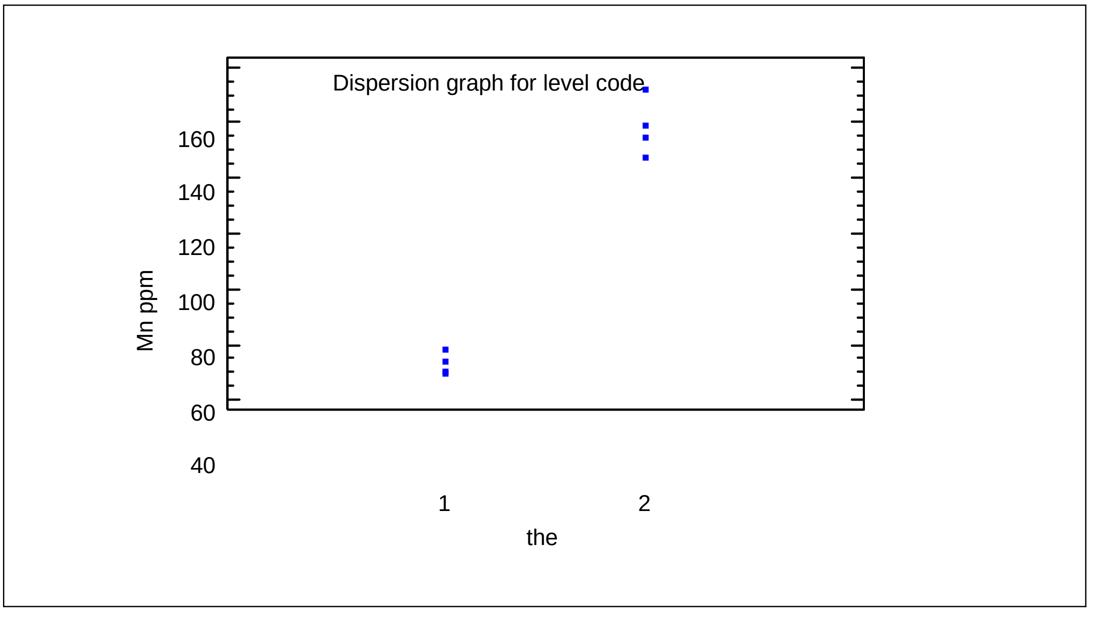
Graph 13: dispersion graph by level code (mg/kg Mn tot on mineral substrate)



Graph 14: dispersion graph by level code (mg/kg Mn tot on organic substrate)



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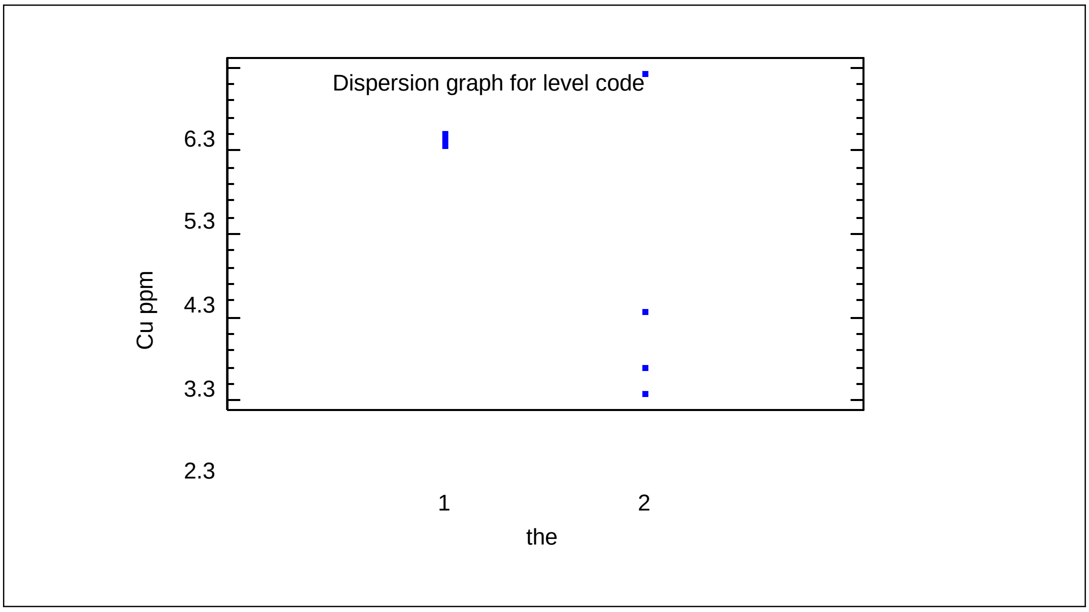
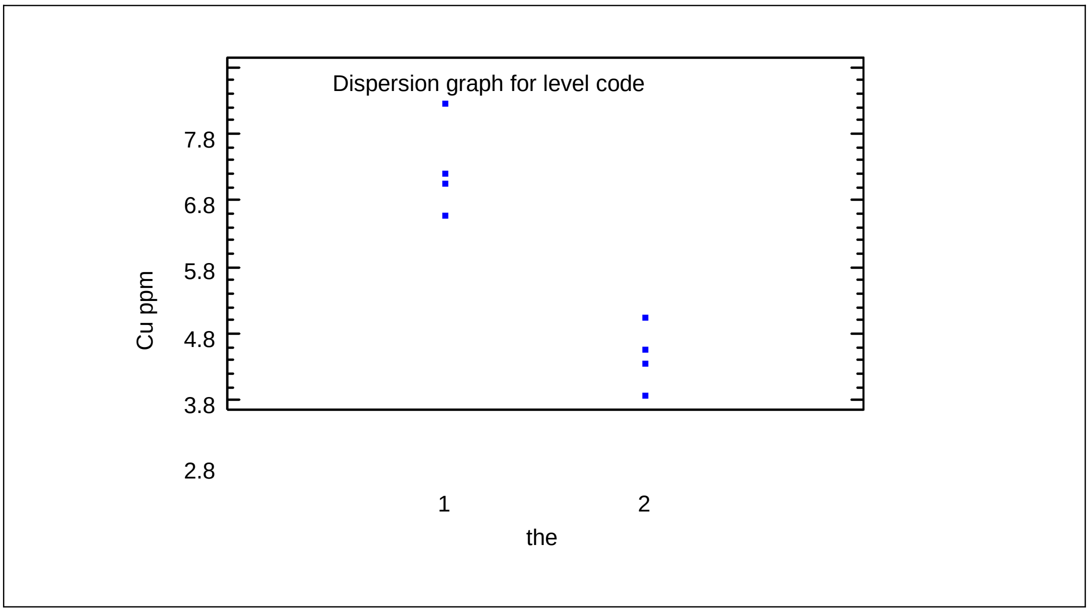
Graph 15: dispersion graph by level code (mg/kg Cu tot on mineral substrate)



Absence of significance (P = 0.05)

Graph 16: dispersion graph by level code (mg/kg Cu tot on organic substrate)



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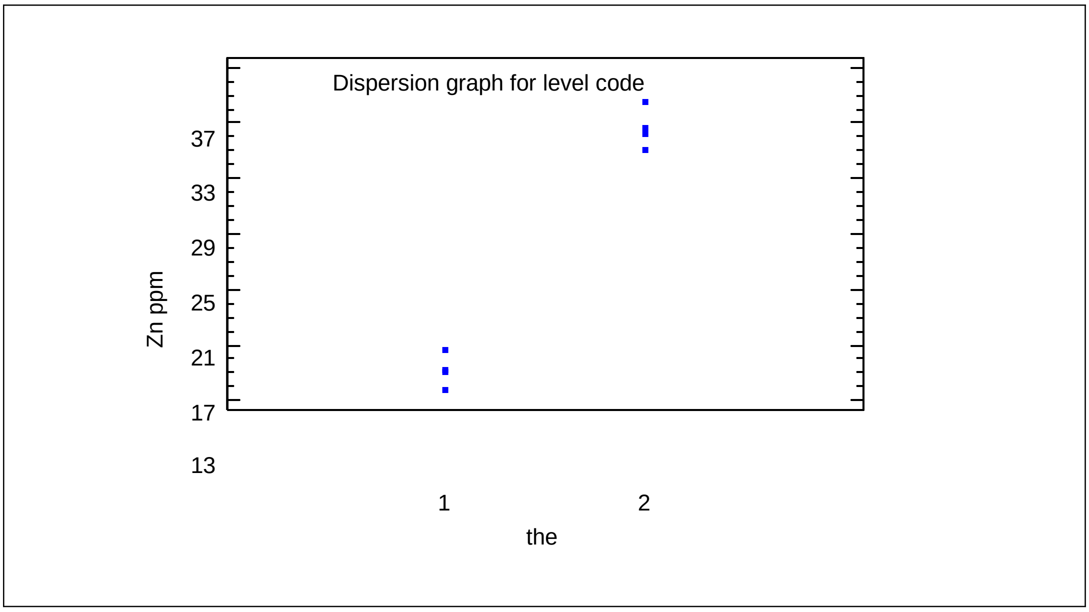
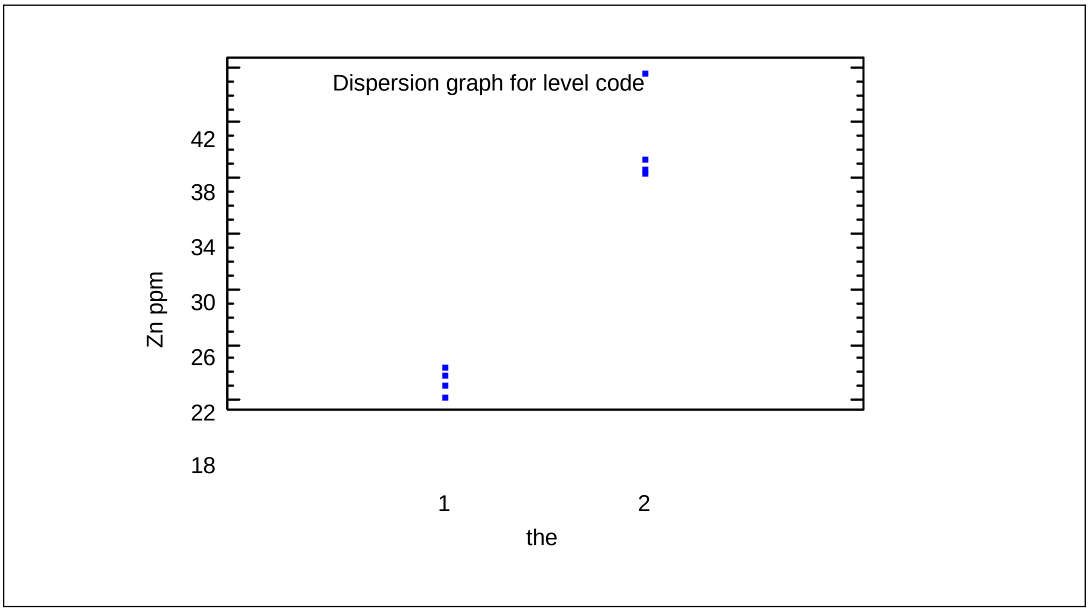
Graph 17: dispersion graph by level code (mg/kg Zn tot on mineral substrate)



Graph 18: dispersion graph by level code (mg/kg Zn tot on organic substrate)



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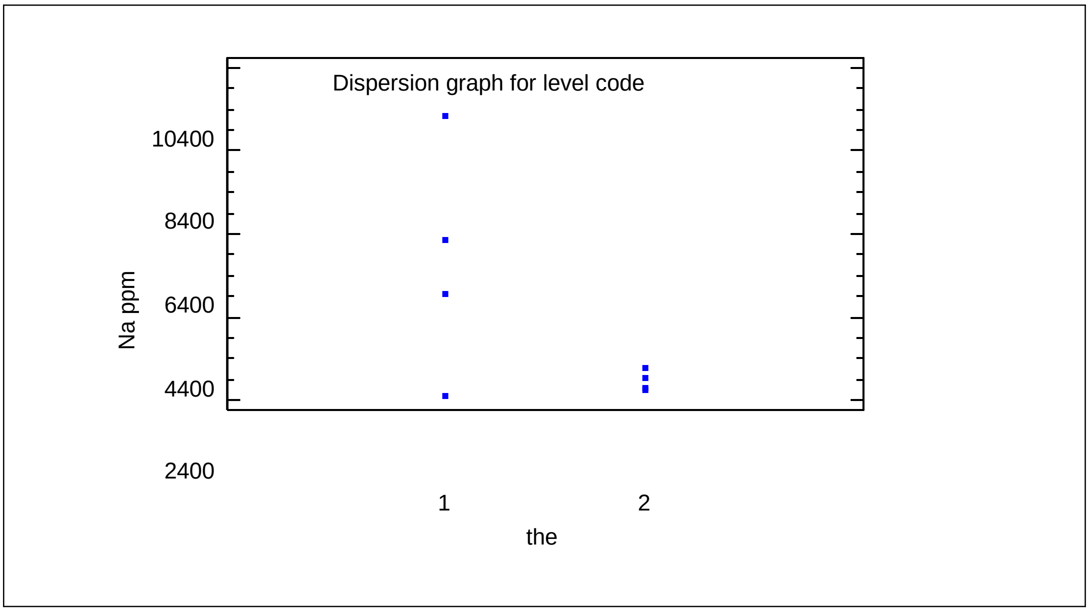
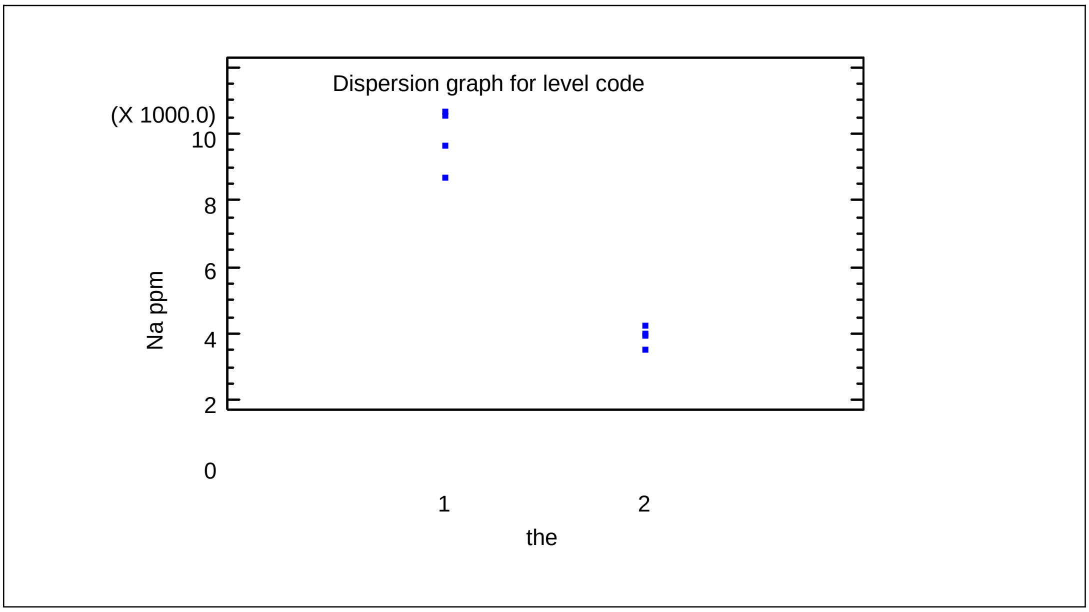
Graph 19: dispersion graph by level code (mg/kg Na tot on mineral substrate)



Absence of significance (P = 0.05)

Graph 20: dispersion graph by level code (mg/kg Na tot on organic substrate)



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Visual differences were found in the analytical procedure of plant tissues. The ashes produced for the determination of the cations and of the phosphorus had a different consistency and color, as well as the aqueous extract in an acid environment, as the following photos clearly show.

Aqueous ashes and extracts in an acid environment obtained from plant tissue samples of plants irrigated with treated or untreated water

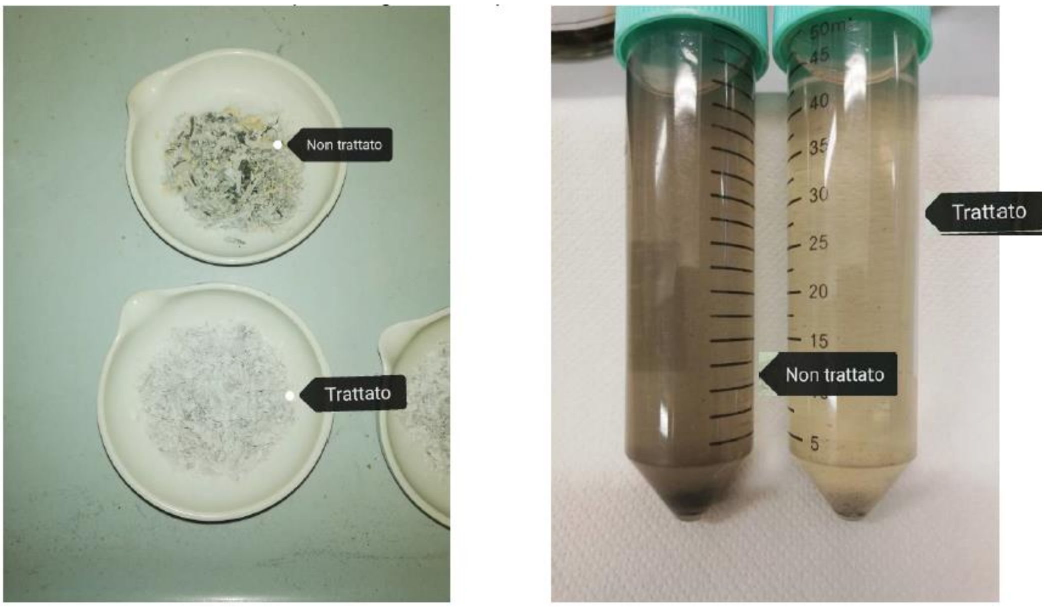

Figure 1:Treated (Trattato) and untreated (Non trattato) samples

Lastly, for the plants of the replicas not collected and left without irrigation (4 plants per thesis and by type of substrate, for a total of 16 pots), no differences in drying times were observed; the plants on a mineral substrate are, as was expected, prematurely dried (after 6 days from the suspension of irrigation) and earlier than those on organic substrates, dried after 12 days of non-irrigation.

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The results of the water analysis carried out on 10 May showed the absence of significant differences for the main parameters for the characterization of water for irrigation use (pH, electrical conductivity, carbonates and bicarbonates, calcium, magnesium, sodium, SAR). The results are reported in the attached Test Reports No. 19211/564 and 19211/565 issued on 13 May 2019.

COMMENT ON RESULTS AND FINAL CONSIDERATIONS

The results of the preliminary tests implemented with lettuce did not find statistically significant differences between the different treatments applied in terms of aerial biomass production; on the contrary, significant differences were found in relation to the endowment of mineral elements present in the plant tissues of lettuce plants.

Therefore, if the use of activated water, compared to untreated water, did not allow to obtain results in terms of increased production, different effects were found for the adsorption of the nutritive elements; it should be noted, however, that the amount of macroelements (N-P-K-Ca-Mg) found in the plants was low and below the sufficiency levels for the species in question.

Positive effects (increased adsorption for plants irrigated with treated water) were found for nitrogen, calcium, magnesium, manganese and zinc; on the contrary, for the elements potassium, iron and (only on organic substrate) for phosphorus, copper and sodium the adsorption was superior in the control plants (untreated water).

The different consistency of the ashes and the aqueous extracts in an acid environment obtained from plant tissues is believed to be the fruit of the different content of potassium, calcium and magnesium in the different samples.

In light of the results obtained and without well-defined information regarding the technology inherent in water treatment, it is not possible to provide a detailed and certain explanation of these outcomes. Below are some basic notions of the behavior of mineral elements in relation to root systems, concepts that could be useful for a better understanding of experimental results.

The adsorption by the calcium and magnesium roots is passive, while that of potassium, iron, manganese and zinc is active. The iron, to be adsorbed, needs a reduction of the trivalent form to the bivalent one, or to a chelation of the trivalent form; difficult to explain the opposite behavior obtained for iron and manganese, even if bibliographical data show an antagonism between the two elements (interferences). Nitric nitrogen is adsorbed both actively and passively.

Table 15 shows data concerning the methods of absorption of the mineral elements by the roots (average data obtained from studies dating back to the seventies); obviously, this data could vary from species to species.

Table 15: Different methods of absorption of the mineral elements by the roots

mineral element	% absorption mode		
	interception	mass flow	diffusion
nitrogen	2	98	-
phosphorus	3	6	91
potassium	2	20	78
calcium	28	72	-
magnesium	13	87	-
copper	70	20	10
iron	50	10	40
manganese	15	5	80
zinc	30	30	40

Segment from "The leaf diagnosis in horticulture", Massimo Valagussa, 2009

On the basis of these data, nitrogen, calcium and magnesium are absorbed mainly by mass flow, while phosphorus, potassium, but also manganese, by diffusion; iron, as well as by diffusion, together with copper, is also absorbed by interception (the roots transfer hydrogen ion to the colloids to activate exchange).

A greater knowledge of how the treatment acts on water could help more to explain the interesting results obtained in terms of endowment of mineral elements in plant tissues.

In consideration of the results obtained, it is possible to reflect the following, also in view of the possible implementation of further medium-long term field tests.

The preliminary tests were carried out in a container, with two different types of substrate (a very poor and organic mineral), where the supply of water (treated and untreated) was exclusive and, in terms of quantity, also significant.
The implementation of a new test should be carried out in our opinion in the open field and on a medium-long term crop cycle.
However, there are two issues that need to be addressed and clarified.

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The first arises from a declaration by the client about the inactivation that the treated water would exert on any added fertilizers. The subject would require further study. Is inactivation (and it is necessary to understand what is meant by this term) only on any fertilizers added to water (fertigation) or even on fertilizers/mineral elements present in the cultivation layer? If inactivation is also exercised on fertilizers distributed on the ground, the problem takes on a significant dimension; in the biological cycle of a soil (even not fertilized) the micro-organisms continuously "release" nutrients that are used by plants.
In this regard, it is believed that the implementation of a field test would require at least 4 experimental specimens, namely:

- Control with untreated irrigation water and without organo-mineral fertilization;
- Control with untreated irrigation water and organo-mineral fertilizer;
- Specimen with treated irrigation water and without organo-mineral fertilization;
- Specimen with treated irrigation water and organo-mineral fertilizer.

The aforementioned specimens would allow the verification of possible effectiveness of the system even in comparison with the current and conventional agricultural practices.
Further experimental specimens could be inserted in order to evaluate other aspects (if the client believes that they can be influenced by the use of activated water); as an example: Can treated water affect the water retention properties of the soil?
Note from Harvest Harmonics: This commentary reflects a misunderstanding on the part of the experimenters. It was mentioned that fertigation and other chemicals that were introduced via the irrigation system may affect the effectiveness of the Crop Harmonics signaling system, whereas fertilizers or chemicals applied outside of the irrigation system would not alter the effectiveness of the Crop Harmonics system.

The second issue is a limitation of the water treatment system: the need for minimum flow rates (liters/minute) to allow the instrument to effectively activate the liquid medium.
This limitation leads to the exclusion of the application of this technology in localized

irrigation systems (for example drip irrigation systems), systems that are increasingly favored in modern agriculture in order to obtain significant water savings.
This could be avoided if the system were able to treat the water present in collection tanks, before it is sent to the distribution lines.

Available for clarifications and further in-depth analysis.

For Minoprio Analisi e Certificazioni

Agronomist Doctor Massimo Valagussa

(with the collaboration of Dr. Piero Frangi of the Minoprio Foundation)

Vertemate con Minoprio, 10 June 2019