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Elemental interactions in soil and their impact on soil fertility under the influence of treated wastewater and biosolid

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Abstract
Treated wastewater and biosolids are increasingly used in agriculture as alternatives to well irrigation water and as organic fertilizers. While these practices offer benefits, they also present challenges. Both treated wastewater and biosolids contain essential plant nutrients and heavy metals, which vary based on their source. When applied to soil, these elements interact with each other and with soil properties, potentially affecting soil fertility. To investigate these interactions, a greenhouse pot experiment was conducted using a simulated soil, based on a randomized block design with 12 treatment combinations, four replications, and 48 pots. The goal was to examine how heavy metals, nutrients and soil properties such as pH, clay content, organic matter, and electrical conductivity influence soil fertility when treated with wastewater and biosolids. The interactions were classified into six groups, and their nutrient contributions were analyzed using regression analysis. The study found several significant interactions, with the highest contributions observed in macronutrients, particularly from the interactions between "soil properties x macronutrients" and "micronutrients x macronutrients." Significant contributions included nitrate (1016.05 mg/kg), potassium (783.07 mg/kg), calcium (4014.52 mg/kg), magnesium (475.46 mg/kg), and micronutrients like boron (2.02 mg/kg), zinc (11.98 mg/kg), copper (16.78 mg/kg), and iron (29.76 mg/kg). The present study offers new insights into how elemental interactions affect soil fertility, under the influence of treated wastewater and biosolids. It highlights the importance of understanding these interactions for the effective management of the interactively contributed plant nutrients in the presence of the applied wastewater and biosolids.

Keywords: *Heavy metals, Interactive behavior, Macronutrients interactions, Micronutrients interactions, Soil fertility*

Introduction

The growing global population, urbanization, tourism, climate change, excessive water consumption, technological advancements, intensive agriculture and livestock farming, and industrialization, coupled with the uneven distribution of water resources, have all contributed to an escalating demand for this vital resource (Abegunrin et al., 2016; Angelakis and Gikas, 2014). To address these challenges, the reuse of treated municipal wastewater (TMWW) and biosolids has become increasingly common. This practice is a prime example of the circular economy, and when specific criteria are met (Alcalde-Sanz and Gawlik, 2017), treated wastewater can enhance soil fertility. It is rich in nutrients beneficial for plants and can even assist in the restoration of infertile soils (Roig et al., 2012). TMWW contains significant quantities of nutrients necessary for plant growth, which are crucial for maintaining healthy ecosystems and agricultural productivity. These nutrients are divided into two categories: macronutrients and micronutrients, based

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on the quantity required by plants. Macronutrients include nitrogen, phosphorus, potassium, calcium, magnesium, and sulfur, while micronutrients encompass iron, manganese, zinc, copper, molybdenum, boron, chlorine, and nickel. Macronutrients are absorbed in large amounts and are vital for essential plant processes such as photosynthesis, cell division, energy transfer, and the synthesis of structural molecules (Tripathi et al., 2014). Nitrogen is integral to amino acids, proteins, and chlorophyll, influencing leaf and shoot growth; a deficiency in nitrogen leads to stunted growth and chlorosis (Abreu et al., 2025). Magnesium is critical in plant photosynthesis, and a deficiency leads to energy imbalances, as observed in tomato leaves (Zhu et al., 2025). Micronutrients, though required in smaller amounts, are equally important for plant health and productivity. These nutrients primarily function as cofactors for enzymes involved in various biochemical processes. Iron, for example, is necessary for chlorophyll synthesis and plays a role in electron transport during photosynthesis and respiration. Iron deficiency leads to interveinal chlorosis, particularly in younger leaves (Mengel et al., 2001). Nickel is the active component of urease, an enzyme that catalyzes the breakdown of urea into ammonia and carbamate (Shaba et al., 2022), and a lack of nickel can reduce growth and nitrogen uptake in plants (Arkoun et al., 2013). In the soil, these essential nutrients interact with heavy metals, organic matter, microorganisms, and other soil factors, creating a dynamic and ever-changing system. These interactions occur through various mechanisms, such as adsorption, desorption, fixation, and both synergistic and antagonistic competition for absorption (Demitrelos et al., 2022; Strawn, 2021). For instance, potassium (K) and magnesium (Mg) often compete for absorption, as seen in the K-induced magnesium deficiency in agricultural crops (Xie et al., 2021). Similarly, high phosphorus (P) concentrations can antagonize zinc (Zn) availability (Noulas et al., 2018). Mehnaz et al. (2019) observed that the addition of P to soil, in the presence of plants, led to a dual effect on nitrogen (N) in the soil—both enhancing nitrogen retention while also promoting N loss as N_2O, potentially through soil leaching. The antagonism between calcium (Ca), potassium (K), and magnesium (Mg) can also cause magnesium deficiency, as noted by Yan et al., (2020). Specifically, increasing soil potassium content was identified as a primary cause of magnesium deficiency in tomatoes grown in calcareous soils. A common occurrence in soil are the interactions among heavy metals and nutrients. Heavy metals in soils, such as Pb, Cd, As, Hg, Zn, Cu, and Ni can significantly affect plant growth, soil fertility, and environmental health. The presence of heavy metals can impact nutrient uptake (Umar et al., 2025), while nutrient availability and soil amendments along with soil properties such as pH,organic matter (OM), clay content (C) and electrical conductivity (EC) can, in turn, alter the mobility and toxicity of heavy metals (Kalavrouziotis et al., 2008; Koukoulakis et al., 2023). Both heavy metals and essential nutrients use similar transport mechanisms and absorption sites in plant roots. For instance, Cd competes with Zn and Ca for uptake due to their similar ionic radii and chemical behavior (Kubier et al., 2019). Zn also competes with Cd in the plant roots which could cause Zn deficiency (Tkalec et al., 2014). This competition, due to antagonism, can result in nutrient deficiencies when heavy metals are present in high concentrations. Zhang et al. (2023) found a negative correlation between soil total Nitrogen and Cr, Cu, Ni, Pb, meaning that these heavy metals are antagonistically related with N and could act as a limiting factor of nitrogen bioavailability. Also, Zn and P interact synergistically with P, K and Ca and these interactions contributed to the bioavailability of nutrients to groundnut (Aboyeji et al., 2020). In other cases, P due to its antagonistic effect may act as an inhibitory factor, for example it immobilizes Cd in soil, rendering it less available to plants (Seshadri et al., 2016). Similarly, Shen et al. (2020) suggest that the synergistic interactions of Cu, Zn, Mg, and Fe can be attributed to the similarity in their ionic radii and octahedral coordination geometry. Soil pH, OM, C and EC are factors that affect both nutrient availability and heavy metal mobility. Many heavy metals, such as Pb Cd and Zn, become more soluble and mobile at lower pH levels, increasing their potential for plant uptake (Kicińska et al., 2022). The bioavailability of nickel depends on pH, organic matter, Fe and Mn oxides, oxidation state of nickel, and the competitive interacttions with Zn and Cu (Ameen et al., 2019; Chauhan et al., 2008; Iyaka, 2011). OM in the soil can form complexes with both nutrients and heavy metals. These complexes can either reduce or enhance the mobility of both. For example, organic matter can form stable complexes with Cu, reducing its toxicity while improving the availability of nutrients like Fe and Zn (Rengel, 2004). Conversely, heavy metal accumulation can immobilize essential nutrients by

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forming insoluble compounds. In waterlogged or anaerobic soils, redox reactions can significantly alter the speciation and mobility of both heavy metals and nutrients. For example, the reduction of iron (Fe) and manganese (Mn) oxides can release heavy metals like cadmium (Cd) and arsenic (As) into the soil solution, making them more available for plant uptake (Suda and Makino, 2016). At the same time, this can affect nutrient availability, particularly for redox-sensitive elements such as Fe and Mn. The interactions between essential nutrients, heavy metals and soil properties in the soil play a crucial role in soil fertility and agricultural productivity and have important implications for the use of treated municipal wastewater (TMWW) in agriculture. On the one hand, they provide vital nutrients for plant growth; on the other, they can release harmful heavy metals into the soil, affecting plant health and soil quality. Antago-nistic interactions can lead to the fixation, adsorption, or precipitation of both nutrients and metals, reducing their availability to plants. The outcome of these interacttions depends on the balance between synergistic and antagonistic effects, as well as the specific types of metals involved. This research aims to explore how these complex interactions affect soil fertility, soil health, and productivity. By understanding these relationships, better management practices can be developed for the use of TMWW and biosolids in agriculture, ensuring that they enhance soil fertility while minimizing any potential risks associated with heavy metal contamination. The study also aims to investigate the beneficial effects of these interactions on soil health and productivity, contributing to more sustainable agricultural practices and environmental management.

Materials and Methods

Twelve treatments with equidistant levels of the studied variables were created in order to accurately study the interactions of the heavy metals Zn, Mn, Cu, Cd, Co, Cr, Ni and Pb with the macronutrients and micronutrients and their role in soil fertility.

Preparation of the simulation treatments

The experimental soil, a Clay Loam virgin soil, was collected from the top layer (0-30 cm) of uncultivated land on Zakynthos Island, Greece. Its physicochemical characteristics were determined using appropriate analytical methods (Table 1). Twelve treatments were applied to simulate different soil compositions, labeled T0 through T11. Each treatment consisted of a set amount of soil, biosolids, fine sand, CaO, and a heavy metal mixture, resulting in 48 pots with four replications per treatment. The aim was to create an equidistant particle composition for each treatment.

Table 2 presents the quantities added to each treatment. The preparation process began on a clean plastic surface, where soil was mixed with the specified amount of fine sand (FCL, particle size 1mm). The mixture was then placed into numbered rectangular plastic pots (45x21x18 cm). To prevent heavy metal leaching, the pot bottoms were sealed. The biosolid (Table 3), sourced from the local WTP of Zakynthos Island, was air- and sun-dried before being added in a 0.5 kg amount per pot. CaO was applied later due to COVID-19 restrictions. The pots were then incubated in a greenhouse, following the experimental design (Table 5). Aqueous inorganic salt solutions of Zn, Mn, Cu, Cr, Co, Cd, Ni, and Pb were added to treatments T1 to T11, while T0 served as the control. The solutions, prepared as per Table 4, were

Table 1. *Mean physical and chemical properties of the experimental soil*

S	C	Si	pH	EC	OM	$CaCO_3$	V.W.
%	%	%		mS/cm	%	%	g/cm^3
12	42	46	6.56	0.650	6.46	0.00	1.13
N-NO_3	P	K	Mg	Ca	Fe	Zn	Mn
mg/kg soil							
32	17	858	319	>2000	34.19	3.61	55.78
Cu	B	Cd	Co	Cr	Ni	Pb	
mg/kg soil							
17.21	1.55	0.61	0.82	0.038	1.09	9.66	

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Table 2. *Treatments applied to the experimental soil, in order to achieve the desired equidistant simulated physical and chemical soil properties*

Treatment code	Simulated treatment levels pH [a]	OM [b]	C [c]	Biosolids	Metal mixture	CaO[d]	Soil	Sand	Soil+sand mixture
		%	%	kg/pot	g/pot	g/pot	kg/pot	kg/pot	kg/pot
T0	6.79	6.82	18.0	0.5	0	1.29	1.83	8.17	10
T1	6.68	6.71	23.5	0.5	0.312	2.58	2.50	7.50	10
T2	6.72	6.72	32.7	0.5	0.625	3.87	3.15	6.85	10
T3	6.17	6.77	34.5	0.5	0.939	5.16	3.85	6.15	10
T4	6.77	6.79	35.0	0.5	1.249	6.45	4.52	5.48	10
T5	6.79	6.58	39.5	0.5	1.362	7.74	5.2	4.80	10
T6	6.58	6.19	37.0	0.5	1.878	9.03	5.7	4.30	10
T7	6.52	6.80	40.0	0.5	2.186	10.32	7.54	2.46	10
T8	6.80	6.83	43.5	0.5	2.498	11.61	7.22	2.78	10
T9	6.83	6.82	42.0	0.5	2.811	12.9	7.89	2.11	10
T10	6.62	6.83	45.5	0.5	3.123	14.19	8.57	1.43	10
T11	6.73	6.73	46.0	0.5	3.435	15.48	9.24	0.76	10

(a,b,c) Levels of pH, organic matter and C of soi, simulated by the CaO, OM and Sand and (d) CaO added for the enhancement of the pH simulated levels, formed by the addition of sand to soil.

Table 3. *Mean physical and chemical properties of the added biosolid*

pH	EC)	$CaCO_3$	$N\text{-}NO_3$	P	K	Mg	Ca	Fe	Na
	mS/cm	%	mg/kg soil						
7.06	4.425	12.5	754.650	209.04	2274	2805	9390	76.98	451.05
Zn	Mn	Cu	B	Cd	Co	Zn	Cr	Ni	Pb
mg/kg soil									
39.95	59.07	11.03	71.46	0.313	0.43	39.95	0.47	5.26	17.1

Table 4. *Metal mixtures per treatment applied to simulated experimental soil*

	$ZnSO_4$ $7H_2O$	$MnSO_4$ H_2O	$CuSO_4$ $5H_2O$	$Na_2Cr_2O_7$ $2H_2O$	$Co(NO_{3)})_2$ $6H_2O$	$Ni(NO_{3)})_2$ $6H_2O$	$Cd(NO_3)_2$ $4H_2O$	$Pb(NO_2)$
T0	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
T1	0.044	0.031	0.039	0.057	0.049	0.049	0.016	0.027
T2	0.088	0.062	0.078	0.115	0.098	0.098	0.032	0.054
T3	0.132	0.093	0.119	0.172	0.147	0.147	0.048	0.081
T4	0.176	0.124	0.156	0.229	0.196	0.196	0.064	0.108
T5	0.220	0.155	0.195	0.287	0.245	0.245	0.080	0.135
T6	0264	0.186	0.234	0.344	0.294	0.294	0.096	0.162
T7	0.308	0.217	0.273	0.401	0.343	0.343	0.112	0.189
T8	0.352	0.248	0.312	0.458	0.392	0.392	0.128	0.216
T9	0.396	0.279	0.351	0.516	0.441	0.441	0.144	0.243
T10	0.484	0.310	0.390	0.573	0.490	0.490	0.160	0.270
T11	0.440	0.341	0.429	0.627	0.539	0.539	0.176	0.297

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1	2	3	4	5	6	7	8	9	10	11	12
T11	T4	T10	T2	T0	T8	T7	T5	T1	T3	T9	T6
13	14	15	16	17	18	19	20	21	22	23	24
T11	T9	T8	T11	T4	T6	T5	T2	T7	T0	T10	T3
25	26	27	28	29	30	31	32	33	34	35	36
T0	T10	T7	T2	T11	T8	T4	T5	T1	T9	T3	T6
37	38	39	40	41	42	43	44	45	46	47	48
T7	T6	T3	T10	T8	T5	T1	T0	T2	T9	T11	T4

Table 5
The experimental randomized block design used in the present experiment (T0 to T12) treatments.

applied in 100 ml of deionized water to ensure even distribution of each metal. The 48 pots were incubated in the greenhouse under fluctuating conditions, with temperatures ranging from 15.3°C to 42.3°C and humidity levels between 35.2% and 87.2%.

Irrigation of the pots

During the incubation period, the pots were irrigated with treated wastewater or freshwater depending on the experimental treatment. The intervals between irrigations depended on the temperature and relative humidity of the greenhouse varying between 10 to 12 days.

Soil analysis

Five soil samplings were conducted throughout the experiment at the following time points: 0, 36, 187, 271, and 358 days. A total of 48 soil samples were collected per sampling and analyzed for their physicochemical properties. Soil mechanical analysis was performed using the hydrometer method (Gee and Bauder, 2018). pH was measured with a pH meter (electrochemical process), and electrical conductivity (EC) was assessed using the method of Miller and Curtin (2007). Calcium carbonate ($CaCO_3$) levels were determined through back titration (Bashour and Sayegh, 2007), and nitrate (NO_3^-) was analyzed according to Magdoff et al. (1990). Available phosphorus (P) was measured following the method of Olsen et el. (1954). The exchangeable cations, including potassium (K^+), calcium (Ca^{2+}), magnesium (Mg^{2+}), and sodium (Na^+), were determined using the protocol proposed by Sumner and Miller (2018).

Micronutrients such as zinc (Zn), manganese (Mn), iron (Fe), and copper (Cu) were extracted using DTPA and analyzed via atomic absorption (Lindsay and Norvell, 1978). Finally, the determination of heavy metals such as cadmium (Cd), cobalt (Co), chromium (Cr), nickel (Ni), and lead (Pb) was carried out using the AB-DTPA method.

Wastewater analysis

The TMWW used for the irrigation of the soil was analyzed according to (APHA, 2017), method 4500-H. The following methods were applied:
- pH: APHA 4500-HC B;
- EC (mmhos/cm): APHA 2510;
- COD (mg/L O2): APHA 5220D and by oxydation of dichromate using the colorimetric method;
- BOD5 (mg/L O2): APHA 5210D, i.e. the manometric method;
- Settling solids (mL/L): APHA 2540D, i.e. the weighing (static) method;
- Suspended solids (mg/L): APHA 2540C, i.e. the weighing (static) method;
- Kjeldahl nitrogen (mg/L N): APHA 4500-Norg B through the semi-micro Kjeldahl;
- Total P (mg/L P): APHA 4500-P E by means of the ascorbic acid method:
- Cadmium (mg/L Cd), chromium (mg/L Cr), manganese (mg/L Mn), nickel (mg/L Ni), lead (mg/L Pb), cobalt (mg/L Co), copper (mg/L Cu), zinc (mg/L Zn): APHA 3113-B;
-Zn was measured by atomic absorption spectroscopy, while the other metals were determined by graphi-

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te oven atomic absorption spectrophotometer (Shimadzu, AA-6300 model, equipped with GFA-EX7i graphite furnace, Kyoto, Japan).

Statistical analysis

The statistical analysis of the experimental data was performed by regression analysis, ANOVA of paired sample, I test, and descriptive statistics, using the package SPSS ver. 29. Linear, logistic and quadratic models were considered and the best fit was chosen.

Results and Discussion

Soil fertility is a fundamental characteristic that provides essential chemical elements for plant growth, including macronutrients (N, P, K, Ca, Mg, S) and micronutrients (Zn, Fe, Cu, Mn, B, Ni), in balanced proportions. The primary objective of this research is to explore the interactions between heavy metals, plant nutrients, and the physical and chemical properties of soil. This study follows a stepwise procedure to analyze these interactions and their effects on soil fertility.First, the research focuses on six specific types of interactions that influence nutrient availability for plants:
- Heavy metals x macronutrients
- Heavy metals x micronutrients
- Macronutrients x micronutrients
- Micronutrients x macronutrients
- Soil properties (pH, C, OM, EC) x macronutrients
- Soil properties (pH, C, OM, EC) x micronutrients

Then, these interactions were studied using regression analysis, with each nutrient as the dependent variable in the equations derived from experimental soil data. The regression equations were selected based on their statistical significance to assess nutrient contribution. The results, expressed in mg/kg of nutrient in soil, highlight the interactive contributions of the studied factors to soil fertility. The study aims to evaluate these contributions and, in the future, explore how these interactions can be utilized to enhance soil fertility. Ultimately, the research seeks to apply these findings to improve agricultural practices, with a focus on the practical use of elemental contributions for optimizing soil health.

Interactions between "heavy metals x macronutrients"

The interactions between heavy metals and macronutrients observed in this research were primarily synergistic, with very few showing antagonistic behavior. All interactions were statistically significant, as demonstrated by the representative interaction between PbxK (Fig. 1). In several cases, these interactions resulted in notably high contributions of macronutrients. The interactive contributions of heavy metals to macronutrients are detailed in Table 6. When comparing macronutrient contributions under the influence of heavy metals across the five soil samples, the maximum contributions observed were as follows:

a-NO_3 in 1016.04 mg/kg at the 5th sampling, (Table 6) (Zn$x$$NO_3$)

b- P in 82.08 mg/kg at the 2nd sampling (Table 6) (CuxP)

c- K in 783.08 mg/kg at the 2nd sampling, (Table 6) (ZnxK)

d- Ca in 4718.85 mg/kg at the 3rd sampling, (Table 6) (CrxCa)

d- Mg 475.46 mg/kg at the 5th sampling, (Table 6) (ZnxMg)

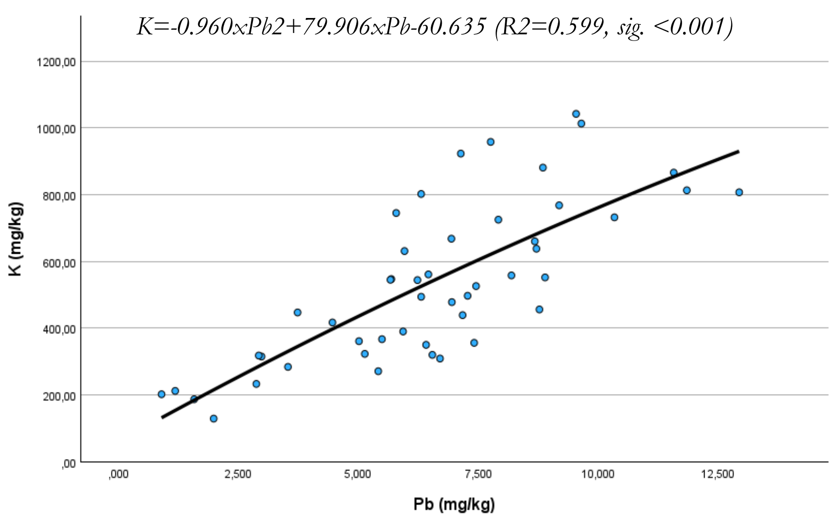

Figure 1
Interaction between heavy metal of Pb with macronutrient of K under the effect of treated waste water and biosolids (3rd soil sampling)

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Table 6. *Interactively contributed NO_3, P, K, Ca, Mg due to the interactions "heavy metals x NO_3, x P, x K, x Ca, x Mg respectively), under the effect of TMWW and biosolids per sampling*

Interaction	Contributed macronutrient	Mean interactive contribution (mg/kg soil)				
		1st sampling	2nd sampling	3rd sampling	4th sampling	5th sampling
ZnxNO_3	NO_3	124.69	200.57	-	-	1016.05
MnxNO_3		125.59	-	-	542.38	-
CuxNO_3		124.79	200.53	427.63	-	1016.04
CdxNO_3		124.73	200.57	427.62	-	-
CoxNO_3		124.73	-	-	-	-
CrxNO_3		124.73	-	-	542.36	1016.05
NixNO_3		124.73	200.57	-	-	1016.05
PbxNO_3		115.70	200.57	-	542.36	1016.04
ZnxP	P	74.12	82.07	50.73	-	38.89
MnxP		-	-	49.04	41.06	-
CuxP		74.13	82.08	50.70	-	38.88
CdxP		-	82.08	-	41.05	38.89
CoxP		-	-	50.73	41.05	-
CrxP		-	-	50.73	41.05	-
NixP		74.13	82.08	50.73	41.05	38.89
PbxP		-	82.07	50.74	41.04	-
ZnxK	K	761.78	783.08	534.59	-	701.36
MnxK		764.35	-	-	472.76	706.24
CuxK		761.71	783.05	534.59	473.17	701.35
CdxK		752.78	783.08	534.58	473.19	701.35
CoxK		761.78	783.08	534.58	473.19	701.35
CrxK		-	783.08	-	473.19	701.35
NixK		761.78	783.08	534.58	473.19	701.35
PbxK		761.74	783.08	534.57	473.20	701.36
ZnxCa	Ca	4014.04	3884.85	3046.73	-	-
MnxCa		4016.93	3885.98	3049.48	2847.98	-
CuxCa		4013.99	3884.68	3046.77	2847.98	-
CdxCa		4014.04	3884.83	3046.73	2858.04	-
CoxCa		-	3884.83	-	2847.98	-
CrxCa		-	3884.83	4718.85	-	-
NixCa		4014.04	3884.76	3046.73	2847.98	-
PbxCa		4014.04	3885.19	3046.73	2847.98	-
ZnxMg	Mg	292.02	318.45	294.16	-	475.46
MnxMg		292.23	318.36	-	336.49	-
CuxMg		292.05	318.45	294.16	336.50	475.46
CdxMg		292.00	318.45	294.17	336.50	475.46
CoxMg		292.00	318.46	294.17	336.50	475.46
CrxMg		-	318.46	-	336.50	475.46
NixMg		292.00	318.46	317.09	336.50	475.46
PbxMg		291.97	318.46	167.01	336.50	475.46

(*) Above data are statistically significant at $0.001<p<0.050$

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Among these macronutrients, Ca showed the highest interactive contribution at 4718.85 mg/kg, followed by NO_3 at 1016.04 mg/kg, and K at 783.08 mg/kg. Changes in the concentration of these nutrients across different sampling periods were likely influenced by soil processes, such as the release of NO_3 through biosolid decomposition (Table 6). Conversely, P showed a decrease in contribution, possibly due to fixation during the CuxP interaction (Table 6), while K decreased over time under the influence of ZnxK (Table 6). Ca decreased significantly between the 3rd and 5th samples due to the CrxCa interaction (Table 6), while magnesium (Mg) increased positively, influenced by the ZnxMg interaction (Table 6). These nutrient changes directly relate to soil fertility and crop growth, underscoring the importance of heavy metal and macronutrient interactions as a valuable nutrient source.

Interactions between "heavy metals x micronutrients"

The second interactive group involved the interaction of "heavy metals x micronutrients," which contributed significant amounts of micronutrients to the soil. This group of interactions proved to be an excellent source of micronutrients, with all interacttions being synergistic and statistically significant. A representative example of this is shown in Figure 2, which illustrates the interaction between CdxCu. The following maximum contributions of micronutrients were observed under the respective interactions:
a- Fe 24.51 mg/kg at the 1st sampling (Table 7) (MnxFe);
b- B 2.44 mg/kg at the 4th sampling (Table 7) (PbxB);
c- Zn 56.47 mg/kg at the 2nd sampling (Table 7) (CoxZn);
d- Mn 81.54 mg/kg at the 3rd sampling (Table 7) (CoxMn);
e- Cu 16.76 mg/kg at th 2nd sampling (Table 7) (PbxCu);
f- Ni 10.47 mg/kg at the 2nd sampling (Table 7) (CuxNi).

The highest contributions were observed for Mn, contributed by the interaction between CoxMn, followed by Fe (MnxFe). Regarding the quantitative changes in the concentration of these interactively contributed nutrients, Fe showed a decrease over time, likely due to fixation and changes in oxidation state (Table 7). A similar decrease was noted for B (Table 7). Zn exhibited variable changes, which remain poorly understood (Table 7). Mn also showed variable behavior (Table 7), while Cu declined with each sampling, though its initial concentration was lower (Table 7). Ni followed a similar trend, initially increasing in the second sample and then decreasing by the fifth sampling (Table 7).

Interactions between "macronutrients x micronutrients"

The above interactive groups of macronutrients and micronutrients seem to be very productive in terms of interactively produced micronutrients and an excellent source of the soil micronutrients. These interactions were mainly synergistic, for example the interaction

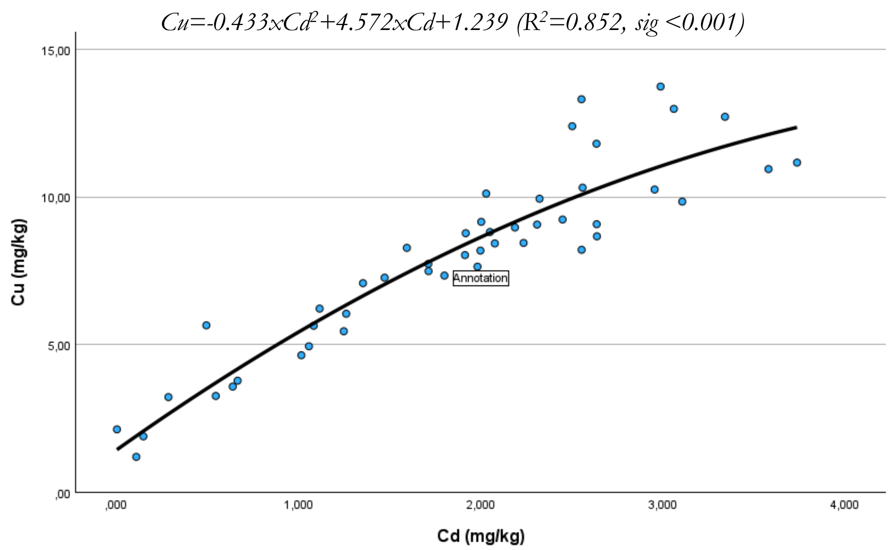

Figure 2
Interaction between heavy metal Cd with the micronutrient of Cu under the effect of treated waste water and biosolids (3rd soil sampling)

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Table 7. *Interactively contributed Fe, B, Zn, Mn, Cu, Ni due to the interactions "heavy metals x Fe, x B, x Zn, x Mn, xCu, x Ni respectively", under the effect of TMWW and biosolids per sampling*

Interaction	**Contributed micronutrient**	Mean interactive contribution (mg/kg soil)				
		1st sampling	2nd sampling	3rd sampling	4th sampling	5th sampling
ZnxFe	Fe	22.16	16.31	14.07	8.25	6.33
MnxFe		24.51	16.87	14.08	-	-
CuxFe		22.10	16.30	14.08	8.22	6.32
CdxFe		22.16	16.30	14.08	8.25	6.32
CoxFe		22.16	16.30	14.08	8.25	-
CrxFe		22.16	-	14.08	8.25	-
NixFe		22.16	16.38	14.08	8.25	6.32
PbxFe		22.13	16.30	14.08	8.24	6.31
ZnxB	B	2.02	2.04	1.16	-	-
MnxB		-	-	1.15	1.04	-
CuxB		-	2.02	1.93	-	-
CdxB		-	2.04	1.16	-	1.02
CoxB		-	2.04	1.16	-	1.02
CrxB		-	2.04	1.16	-	-
NixB		-	2.04	1.16	-	-
PbxB		-	2.44	1.13	-	-
MnxZn	Zn	8.66	-	5.26	52.61	3.98
CuxZn		8.66	11.49	11.21	-	3.98
CdxZn		8.66	11.51	5.26	-	3.98
CoxZn		-	11.49	5.26	56.47	3.98
CrxZn		8.66	11.49	5.26	56.47	3.98
NixZn		8.66	11.41	5.26	56.47	3.98
PbxZn		8.71	11.48	5.26	-	3.98
ZnxMn	Mn	75.73	-	81.53	69.61	49.56
CuxMn		75.70	51.41	-	-	49.57
CdxMn		75.70	51.41	-	-	49.56
CoxMn		75.70	51.41	81.54	-	49.56
CrxMn		75.70	-	81.54	-	49.56
NixMn		75.70	51.44	81.54	-	49.56
PbxMn		75.67	51.40	81.52	-	49.56
ZnxCu	Cu	14.90	16.75	7.92	7.27	5.77
MnxCu		14.90	16.75	-	-	5.76
CdxCu		12.50	16.74	7.92	7.27	5.76
CoxCu		12.50	16.74	7.92	7.27	5.76
CrxCu		12.50	16.75	-	7.27	5.76
NixCu		12.50	16.75	7.92	7.27	5.76
PbxCu		12.51	16.76	7.90	7.27	5.75
ZnxNi	Ni	1.14	10.43	1.76	1.11	0.95
MnxNi		1.14	9.01	1.87	-	0.95
CuxNi		1.16	10.47	1.74	1.11	0.95
CdxNi		1.09	10.30	1.75	1.11	0.95
CoxNi		1.09	10.34	1.75	1.11	0.95
CrxNi		1.09	10.34	1.75	3.44	0.95
PbxNi		1.08	9.99	1.77	1.11	0.95

(*) Above data are statistically significant at 0.001<p<0.050

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PxZn (Figure 3).
On the average, the greatest interactive contributions in this case were:
a- Zn 60.06 mg/kg at 4th sampling (Table 8) (KxZn);
b- Mn 103.65 mg/kg at 3rd sampling (Table 8) (MgxMn);
c- Cu 16.78mg/kg at 2nd sampling (Table 8) (KxCu);
d- Ni 12.54 mg/kg at 2nd sampling (Table 20) (NO_3xNi);
e- Fe 29.76 mg/kg at 1st sampling (Table 8) (CaxFe);
f- B 2.16 mg/kg at 2nd sampling (Table 8) (MgxB);
The highest interactive contributions due to these interactions were found in Mn followed by Zn, while the lowest were consistently seen in B.

Interactions between "micronutrients x macronutrients"

This interactive group significantly contributed to macronutrients, which are essential for plant growth and soil fertility. The interactions between microntrients and macronutrients were predominantly synergistic and statistically significant. A notable example of this synergistic interaction is the FexK interaction (Figure 4). The maximum contributions observed, based on the data presented in Table 9, are as follows:
a- NO_3 1016.05 mg/kg at the 5th sampling (FexNO_3):
b- P 82.19 mg/kg at 2nd sampling (FexP);

Table 8. *Interactively contributed Zn, Mn, Cu, Ni Fe, B due to the interactions "macronutrients x Zn, x Mn, x Cu, x Ni, x Fe, x B respectively", under the effect of TMWW and biosolids per sampling*

Interaction	Contributed micronutrient	Mean interactive contribution (mg/kg soil)				
		1st sampling	2nd sampling	3rd sampling	4th sampling	5th sampling
NO_3xZn	Zn	8.66	10.69	-	-	3.59
PxZn		8.66	11.75	5.26	60.06	4.78
KxZn		8.66	11.88	5.00	-	3.98
MgxZn		8.64	11.56	5.69	-	3.89
CaxZn		7.96	11.49	6.91	-	-
NO_3xMn	Mn	68.56	-	-	70.41	49.29
PxMn		75.85	-	80.28	71.23	-
KxMn		75.76	-	-	66.41	49.31
MgxMn		75.87	51.41	103.65	66.70	-
CaxMn		78.43	51.40	81.34	70.33	-
NO_3xCu	Cu	9.89	16.59	-	-	5.89
PxCu		11.17	16.99	8.86	6.40	6.21
KxCu		12.57	16.75	7.91	7.29	5.65
MgxCu		12.57	16.78	7.44	7.71	5.81
CaxCu		13.20	16.75	6.84	7.16	-
NO_3xNi	Ni	1.04	12.54	-	1.29	1.00
PxNi		1.12	10.85	1.75	1.11	0.53
KxNi		1.50	11.15	1.75	1.03	0.78
MgxNi		1.23	12.14	1.75	1.14	1.14
CaxNi		1.09	10.84	1.75	1.05	-
NO_3xFe	Fe	22.95	-	-	8.06	6.01
PxFe		21.64	-	13.04	7.63	5.74
KxFe		21.65	-	14.08	8.46	-
MgxFe		25.45	16.23	14.51	8.26	-
CaxFe		29.76	16.19	15.33	9.57	-
NO_3xB	B	-	-	-	1.28	1.08
PxB		-	2.06	1.17	-	1.03
KxB		2.02	2.04	-	0.85	-
MgxB		-	2.16	-	1.04	-
CaxB		2.02	1.49	-	-	-

(*) Above data are statistically significant at $0.001<p<0.050$

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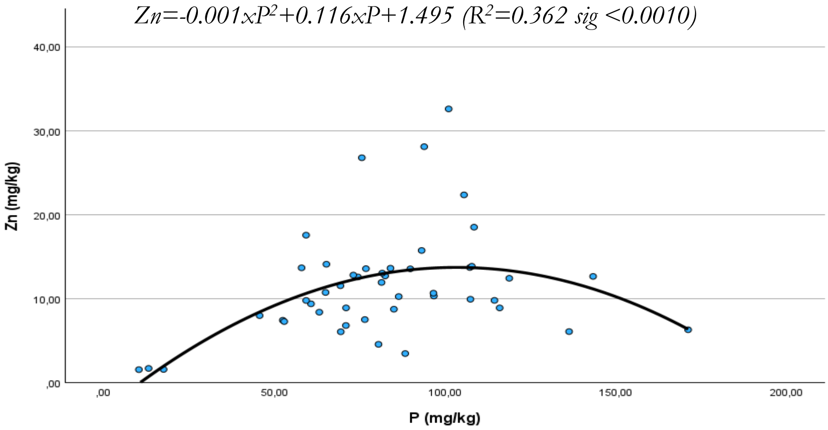

Figure 3
Interaction between macronutrient P and micronutrient Zn, under the effect of wastewater and biosolids, 2rd soil sampling

c- K 783.07 mg/kg 2nd sampling (BxK);
d- Ca 4014.06 mg/kg 1st sampling (FeXCa);
e- Mg 336.50 mg/kg 4th sampling (FexMg).
Among these, the most influential interactions were FexCa, followed by FexNO_3 and BxK, contributing significantly to Ca, NO_3 and K, respectively. Significant changes in interactive contributions occurred during the incubation period, as seen in Table 9. NO_3 concentration increased from 124.81 mg/kg to 1016.05 mg/kg under the FexNO_3 interaction, while P decreased from 82.19 mg/kg to 38.89 mg/kg under FexP. Similarly, K and Ca decreased under the interactions of BxK and FexCa, whereas Mg increased to 336.5 mg/kg. The Ca contribution showed a decreasing trend throughout the experiment.

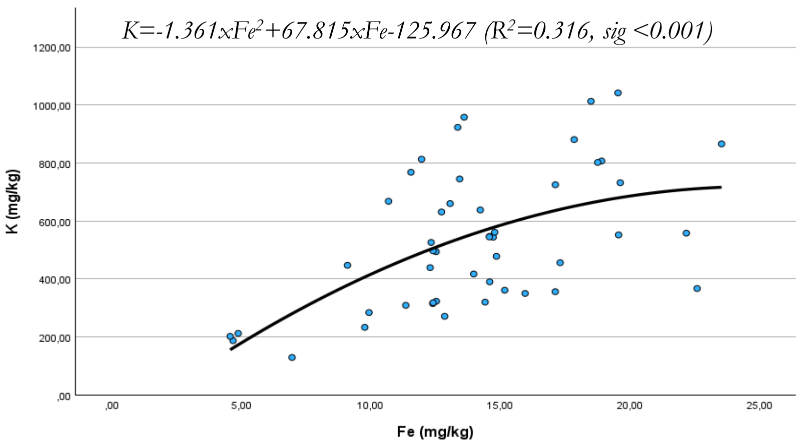

Figure 4
Interaction between micronutrient Fe and macronutrient K under the effect of wastewater and biosolids

Table 9. *Interactively contributed macronutrients due to the interactions "micronutrients x macronutrients", under the effect of TMWW and biosolids per sampling*

Interaction	Contributed Macronutrient	Mean interactive contribution (mg/kg soil)				
		1st sampling	2nd sampling	3rd sampling	4th sampling	5th sampling
FexNO_3	NO_3	124.81	-	-	-	1016.05
BxNO_3		124.73	-	-	542.36	1016.05
FexP	P	74.13	82.19	50.68	41.07	38.89
BxP		-	82.07	50.73	38.97	38.89
FexK	K	743.59	-	534.57	473.19	701.37
BxK		761.78	783.07	-	473.19	-
FexCa	Ca	4014.06	3884.81	3046.63	2847.94	-
BxCa		4014.04	3884.78	-	-	-
FexMg	Mg	292.11	318.49	294.18	336.50	-
BxMg		292.00	318.46	-	336.50	-

(*) Above data are statistically significant at $0.001<p<0.050$

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Interactions between "soil physical and chemical properties with macronutrients"

It has been found that the interactions between the soil" physical and chemical properties x macronutrients" can constitute an extremely effective source of macronutrients. The interactions between the examined nutrients and pH, OM, C, EC varied as they could be synergistic, antagonistic or a combination of the two. Organic matter had a mostly synergistic relationship with the nutrients for example the interaction OMxK (Figure 5). The results revealed that the interactions of this group contributed both Ca and NO_3 at the highest level, as seen in the following list of maximum interactive contributions (Table 10):

- NO_3 1061.02.mg/kg at 5th sampling (pHxNO3)
- P 82.06 mg/kg at 2nd sampling (OMxP);
- K 783.23 mg/kg at 2nd sampling (CxK);
- Mg 475.46 mg/kg at 5th sampling (OMxMg);
- Ca 4014.04 mg/kg at 1st sampling (ECxCa).

NO_3 and Mg contributions increased steadily. K, while it decreased from the 1st to the 4th sampling (761.89 mg/kg soil to 468.26 mg/kg soil), in the 5th sampling it increased due to the effect of the interaction CxK. P and Ca decreased throughout the experiment due to pHxP and pHxCa (Table 10).

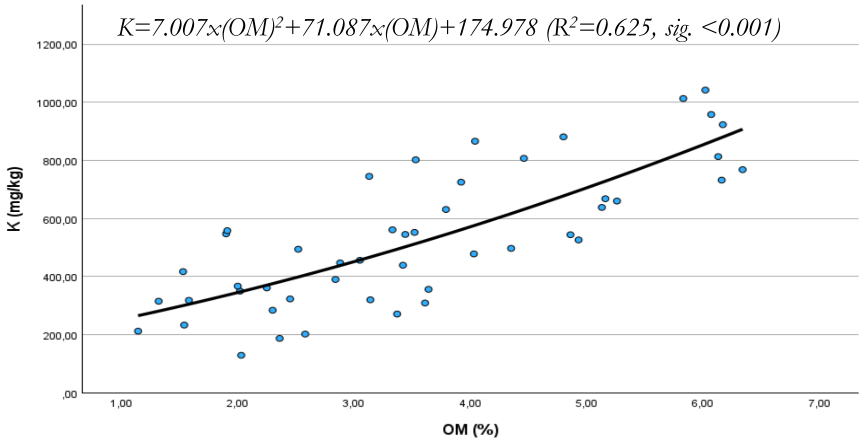

Figure 5
Interaction between soil OM and K under the effect of wastewater and biosolids at the 3rd soil sampling

Table 10. *Interactively contributed macronutrients due to the interactions of pH, OM, C, EC x macronutrients, under the effect of TMWW and biosolids per sampling*

Interactions	Contributed macronutrient	Mean interactive contribution (mg/kg soil)				
		1st sampling	2nd sampling	3rd sampling	4th sampling	5th sampling
$pHxNO_3$	NO_3	124.74	200.57	427.61	542.37	1016.02
$OMxNO_3$		124.72	200.56	-	-	-
$CxNO_3$		124.83	200.57	-	-	-
$ECxNO_3$		124.73	200.57	-	-	-
pHxP	P	-	82.06	50.83	41.06	38.89
OMxP		-	82.08	-	-	-
CxP		-	-	-	40.65	-
ECxP		74.13	82.07	50.73	-	-
pHxK	K	761.78	-	-	453.37	-
OMxK		761.80	783.07	534.58	473.20	701.35
CxK		762.18	783.23	534.37	473.24	701.52
ECxK		761.78	783.08	-	473.22	-
pHxMg	Mg	292.00	318.45	-	336.51	-
OMxMg		291.99	318.45	294.17	336.50	475.46
CxMg		291.53	318.54	293.64	336.41	475.46
ECxMg		292.00	318.47	-	336.45	
pHxCa	Ca	4014.02	3884.82	3046.71	2847.99	-
OMxCa		4014.03	3884.84	3046.73	2847.98	-
CxCa		4014.01	3884.62	3059.46	2847.79	-
ECxCa		4014.04	3884.82	3046.73	2848.00	-

(*) Above data are statistically significant at 0.001<p<0.050

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Interactions between "soil properties (pH, OM, C, EC) x micronutrients

The following data highlight the maximum nutrient contributions from interactions between soil properties and micronutrients (Table 11):

- Fe: 22.16 mg/kg at 1st sampling (OMxFe);
- B: 2.05 mg/kg at 2nd sampling (OMxB);
- Zn: 11.95 mg/kg at 2nd sampling (OMxZn);
- Mn: 81.54 mg/kg at 3rd sampling (pHxMn);
- Cu: 17.31 mg/kg at 2nd sampling (OMxCu);
- Ni: 10.87 mg/kg at 2nd sampling (OMxNi This interactive group demonstrated statistically significant synergistic interactions, resulting in high nutrient contributions (Figure 6). The highest contribution was observed with the pHxMn interaction, followed by OMxFe. Notably, OM emerged as a key factor,,

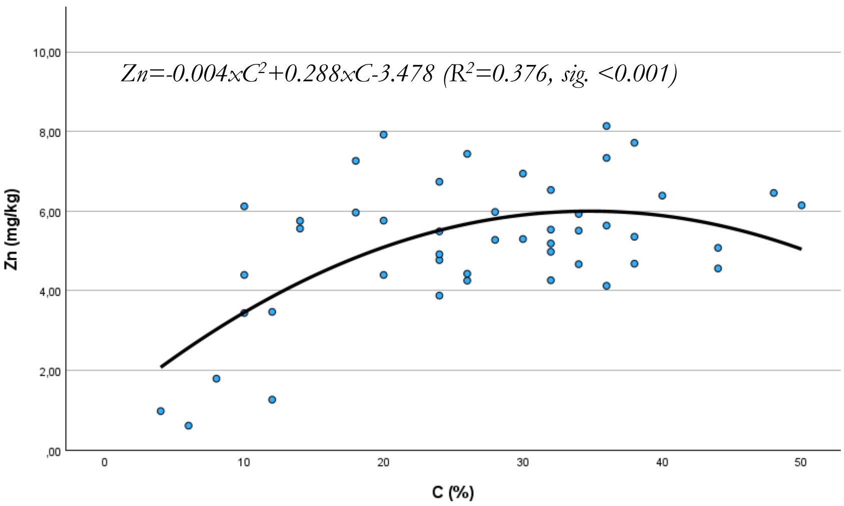

Figure 6
Interaction between soil clay (C) and Zn under the wastewater and biosolids at the 3rd soil sampling

Table 11. *Interactively contributed micronutrients due to the interactions of pH, OM, C, EC x micronutrients, under the effect of TMWW and biosolids per sampling*

Interactions	Contributed micronutrient	Mean interactive contribution (mg/kg soil)				
		1st sampling	2nd sampling	3rd sampling	4th sampling	5th sampling
pHxFe	Fe	-	16.29	14.08	8.24	6.30
OMxFe		22.16	-	-	8.25	-
CxFe		22.16	-	14.12	8.47	-
ECxFe		22.08	16.46	-	8.28	6.40
pHxB	B	2.01	-	0.81	1.03	1.04
OMxB		-	2.05	-	-	-
CxB		-	2.04	-	-	-
ECxB		2.01	2.04	1.12		1.03
pHxZn	Zn	8.67	11.49	5.27	56.47	9.98
OMxZn		8.65	11.95	5.26	-	3.98
CxZn		9.23	11.72	5.32	-	3.98
ECxZn		8.66	11.48		-	
pHxMn	Mn	-	-	81.54	-	-
OMxMn		75.70	-	-	-	49.56
CxMn		75.08	51.31	79.71	71.76	-
ECxMn		75.70	51.41	81.54	-	-
pHxCu	Cu	12.49	16.75	-	7.26	5.76
OMxCu		12.51	17.31	7.91	7.27	5.76
CxCu		12.28	16.23	7.71	7.37	5.76
ECxCu		12.50	16.75	-	7.30	5.77
pHxNi	Ni	1.09	10.35	-	1.11	-
OMxNi		1.08	10.87	1.75	1.10	0.95
CxNi		0.60	10.34	1.94	1.10	0.95
ECxNi		1.09	10.35	-	1.14	0.95

(*) Above data are statistically significant at $0.001<p<0.050$

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interacting significantly with most micronutrients (Fe, B, Zn, Cu, and Ni). This finding warrants further investigation to better understand OM's role in supplying plants with interactive micronutrients. Special attention should be given to the effectiveness of these interactions, as they appear highly impactful based on the results. Summarizing, the results of the interactive contributions in macro and micronutrients indicate potential additions to soil through the following interactive groups:

(a) Heavy metals x Macronutrients
(b) Macronutrients x Micronutrients
(c) Micronutrients x Macronutrients
(d) Soil properties x Macronutrients
(e) Soil properties x Micronutrients

Calcium (Ca)

Contributed by (a) group: 4718.85 mg/kg soil, under the interaction of CrxCa;
Contributed by (c) group: 4014.06 mg/kg soil, under the interaction of FexCa;
Contributed by (d) group: 4014.04 mg/kg soil, under the interaction of ECxCa.

Nitrate (NO_3)

Contributed by (a) group: 1016.04 mg/kg soil, under the interaction of ZnxNO_3.

Phosphorus (P)

Contributed by (a) group: 82.08 mg/kg soil, under the interaction of CuxP.
Contributed by (c) group: 82.19 mg/kg soil, under the interaction of FexP.
Contributed by (d) group: 82.06 mg/kg soil, under the interaction of OMxP

Potassium (K)

Contributed by (a) group: 783.08 mg/kg soil, under the interaction of ZnxK.
Contributed by (d) group: 783.23 mg/kg soil, under the interaction of OMxK.

Magnesium (Mg)

Contributed by (a) group: 475.46 mg/kg soil, under the interaction of ZnxMg.
Contributed by (c) group: 336.5 mg/kg soil, under the interaction of FexMg.
Contributed by (d) group: 475.46 mg/kg soil, under the interaction of OMxMg.

Manganese (Mn)

Contributed by (b) group: 103.65 mg/kg soil, under the interaction of MgxMn.
Contributed by (e) group: 81.54 mg/kg soil, under the interaction of pHxMn.

Zinc (Zn)

Contributed by (b) group: 60.06 mg/kg soil, under the interaction of KxZn.
Contributed by (e) group: 11.95 mg/kg soil, under the interaction of OMxZn.

Copper (Cu)

Contributed by (b) group: 16.78 mg/kg soil, under the interaction of KxCu.
Contributed by (e) group: 17.31 mg/kg soil, under the interaction of OMxCu.

Iron (Fe)

Contributed by (b) group: 29.76 mg/kg soil, under the interaction of CaxFe.
Contributed by (e) group: 22.16 mg/kg soil, under the interaction of OMxFe.

Conclusions

Elemental interactions have been occurring in nature since the emergence of biological life on Earth. These interactions supply the soil with essential plant nutrients, positively impacting both biotic and abiotic systems. One of the key characteristics of these elements is their ability to interact in either synergistic or antagonistic ways, playing a crucial role in maintaining and sustaining life. Since these interact-tions can produce plant nutrients, this study focuses on the interactive role of heavy metals in soil fertility. The elemental interactions in the soil were examined in relation to the generation of essential macron-trients and micronutrients. The data from this study lead to several important conclusions: Elemental in-teracttions can generate significant quantities of plant nutrients, improving soil fertility. This process war-rants further investigation, particularly in the context of treated wastewater and biosolid reuse. Notable interactive nutrients included Ca at 4014.52 mg/kg, Mn at 103.65 mg/kg, K at 783.46 mg/kg, and Zn at 60.06 mg/kg. The interactive groups of "micron-trients x macronutrients" and "soil properties x ma-cronutrients" produced the highest total interactive quantities of macronutrients, at 6279.52 mg/kg and 6371.19 mg/kg, respectively. In contrast, the interact-tion between "soil properties x micronutrients" yiel-ded the smallest total nutrient quantity of 145.88 mg/kg. Although some interactive factors, such as pH

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and EC, had antagonistic effects on nutrient availability, they could also increase nutrient concentrations under specific synergistic conditions. High concentrations of certain elements, like calcium, in the experimental soil contributed significantly to soil fertility across all subcategories examined. While B interacted with heavy metals, macronutrients, and soil properties, its overall interactive contribution remainned low. The interactions that generate plant nutrients should be further studied, particularly in the context of treated wastewater and biosolid reuse, as they may offer alternative sources of nutrients for improving soil fertility. Given the complexity of nutrient interactions in soil, further research is needed to understand their implications for plant growth, as well as interactions at the soil-plant interface. Additionally, the long-term impact of irrigating with treated wastewater on these interactions should be explored, especially as its use in agriculture increases. It is critical to continue studying the elemental interactive contributions to soil fertility. This research could lead to practical applications, such as complementing inorganic fertilizers with nutrient contributions from elemental interactions, thereby reducing crop fertilization costs and promoting environmental sustainability.

Statement of conflict of interest.
We hereby declare that we do not have any conflict of interest.

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