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The Uptake of Nutrients by Maize from Soil Treated by Carbamazepine

Diploma Thesis

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Declaration

I hereby declare that this master's thesis, titled " The Uptake of Nutrients by Maize from Soil Treated by Carbamazepine" is my own dissertation, completed under the supervision of my thesis supervisor and consultant, and that all sources have been referenced and accepted using full references. As the author of the thesis, I announce that I did not infringe on any third-party copyrights while writing it.

Dawit Abate Yimer

Prague, 20th April 2021

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The Uptake of Nutrients by Maize from Soil Treated by Carbamazepine

Summary

A pot experiment with maize plant and soil from Suchdol experimental field has been set up at the outdoor precipitation-controlled vegetation lobby, Department of Agro-environmental Chemistry and Plant Nutrition, Czech University of Life Sciences to investigate the effect of Carbamazepine (CBZ) on the yield and uptake of nutrient by the maize crops. The maize (*Zea mays L.*) was grown in 45 pots filled with a mixture of soil and CBZ. Eight maize plant was sown at the beginning and thinned to four at the 14 days after sowing, regularly irrigated to 60% of the respective soil maximum water holding capacity. Five treatments were set up in 9 replicates with a control, carbamazepine concentration (CBZ) of 0.1 ppb, 1 ppb, 10 ppb CBZ, and 1 ppm CBZ/5kg of dry soil. Among the nine replicates of each treatment, 3 of them were harvested at the 30 days after sowing (DAS), the other 3 at the 60 DAS, and the remaining at the maturity (90 DAS). With each harvest, a soil sample was collected to analyse plant available nutrients. Plant biomass was harvested separately, both fresh and dry weight of plant taken and the content of nutrient in the maize measured.

The results showed no significant effect of CBZ on the maize biomass yield at all the three maize growing stages. However, the application of CBZ in the soil had a significant effect on the concentration of N in maize biomass, while no significant effect on the concentrations of P, K, and Mg. At the first maize harvesting time, the addition of 1ppm CBZ significantly decreased the concentration of N in maize biomass by 18.1 % as compared to the control. The findings also showed that the presence of CBZ in the soil had a significant impact on the uptake of N and K, while no significant effect on the uptake of P and Mg. The application of 1ppm and 1ppb CBZ reduced N uptake by 31.2 % and 44.1 %, respectively, in the first harvest. Additionally, the uptake of K decreased by 81.7% at the CBZ application rate of 1ppb CBZ. Furthermore, CBZ treatments induced a significant effect on the concentration of soil P and K. The addition of 1ppb CBZ significantly decreased the concentration of soil P by 12.4% at the first harvest. As a result, the addition of 1ppm CBZ treatment significantly reduced soil K concentration at third-round harvest, showing a decrease of 20.8% in comparison to the control. Therefore, based on our findings, Overall, we can conclude from our research that the addition of carbamazepine to soil can affect maize nutrient uptake and soil nutrient supply during the early growing stages at higher CBZ concentrations

Keywords: Pharmaceuticals, Carbamazepine, Nutrients, Interactions, Maize

Table of contents

LIST OF TABLES

LIST OF FIGURES

APPENDICES

1. Introduction

Our society uses more and more pharmaceutical chemicals designed to affect our bodies. There are currently more than 4,000 pharmaceuticals in use (Boxall et al., 2012). Pharmaceuticals are designed to be excreted from the body after their impact has been felt. This Pharmaceuticals for veterinary and human beings and their metabolites enter the environment through several routes (Henschel et al., 1997). The main source of pharmaceuticals, used by humans, in the environment is household sewage and most of these treatment plants do not eliminate pharmaceuticals, therefore, they find their way into our surface water (Fent et al.,2006). Water is needed for the movement of nutrients and sugars from the soil to the plants. Reclaimed Wastewater (RW) is increasingly used to irrigate farmland and to reduce agricultural water shortages worldwide. This use has contributed to questions about soil pollution by pharmaceuticals and personal care products (PPCPs) and human health hazards linked with dietary crop intake (Liu et al., 2020). These compounds can be absorbed by plants and even retained within the environment for years. A large majority of the studies performed in this area have focused on pharmaceutical uptake into plants and what compounds can be up taken and their concentrations (Liu et al., 2020).

Carbamazepine (CBZ) is a pharmaceutical product that is widely found in biosolids, reclaimed wastewater, and agricultural soils (Kinney, et al 2006). Because of its extensive use, it is widely available in wastewater treatment plants with sewage systems. And such pharmaceutical products are not efficiently eliminated by the wastewater treatment plant and can be taken to water systems (Zhang et al., 2008). CBZ in reclaimed irrigation water, biosolids, and manure are added to soils where they can impact soil microbiota and can be consumed, stored, and metabolized by plants. Therefore, in this study, the effect of CBZ application to the soil on the yield and uptake of nutrients by the maize was investigated.

2. Scientific hypothesis and objectives

2.1. Hypothesis

The addition of Carbamazepine in soil could influence the yield and uptake of nutrients by maize (Zea mays) and the availability of nutrients in the soil.

2.2. Objectives

The main objective of this thesis is to determine how the presence of carbamazepine in the soil can influence the growth and composition of plants, specifically in maize, as well as the availability of nutrients in the soil.

3. Literature Review

3.1. Plant nutrients

Agriculture continues to satisfy the basic needs of humans and their civilization by serving as a source of food, clothes, housing, medicine, and leisure (Chandrasekaran et al., 2010). Accordingly, to ensure the efficient growth and development of both vegetative and reproductive tissues, all plants must all needed nutrients from their environment. Based on the relative quantities required for plant growth, these nutrients are divided into two major groups of macronutrients and micronutrients. These minerals accomplish several roles: as osmotic solutes to retain sufficient water potential, as cofactors in enzymatic reactions, as structural components in macromolecules, and as ionized species. To provide a load balance in cellular compartments (Table 1). Macronutrients are commonly present in plants at concentrations greater than 0.1 % but micronutrients are generally found at concentrations less than 0.01 % of dry tissue weight. The macronutrients are nitrogen (N), potassium (K), calcium (Ca), magnesium (Mg), phosphorus (P), and sulfur (S). Manganese (Mn), iron (Fe), boron (B), copper (Cu), molybdenum (Mo), chlorine (Cl), zinc (Zn), and nickel (Ni) are examples of micronutrients. These 14 minerals, along with the elements carbon (C), hydrogen (H), and oxygen (O), are generally accepted as important for the growth and development of all plants. Cobalt (Co), sodium (Na), silicon (Si), selenium (Se), iodine (I), and vanadium (V), are also necessary for plant growth (Grusak, 2001). The content of these all elements varies depending on the chemical behaviour of each nutrient.

Essential elements needed for crop growth have different characteristics based on their chemical nature and mobility. Their chemical nature classification is observed from the point of metals and non-metals. K, Ca, Mg, Fe, Zn, Mn, Cu, Co, and V, etc are metals, and C, H, O, N, P, S, B, Si, etc, are non-metals. Depending on the behaviour of their movement, for example, N, P, K are highly mobile, whereas Zn is moderately mobile. S, Fe, Cu, Mn is less mobile, and Ca and B are immobile (Chandrasekaran et al., 2010). Plants require a widespread root chain for soil nutrient uptake. Nutrients move towards the surface of the roots by mass flow, diffusion, and/or root interception could occur. The nutrients reaching the surface of the root could be absorbed by plants either actively by the expenditure of energy or passively without the need for energy. As a result, an improved understanding of plant nutrition would help to boost crop productivity and nutritional value for the continuously increasing human population (Karthika et al., 2018).

Table 1**.** Plant nutrients are based on biochemical behavior (Karthika et al., 2018; Mengel and Kirkby 2001).

Nutrient	**Biochemical functions**
N	A component of amino acids, amides, proteins, nucleic acids, nucleotides, coenzymes, chlorophyll, and other organic compounds.
S	Cysteine, cystine, methionine, and S proteins are also cysteine-containing proteins. Both components are lipoic acid, adenosine-5′-phosphate, thiamine pyrophosphate, glutathione, biotin and 3-phosphoadenosin.
P	Sugar phosphates, nucleic acids, nucleotides, coenzymes, phospholipids, phytic acid, and other compounds. Has an important function in ATP-dependent reactions.
B	Complexes of mannitol, mannan, polymannuronic acid, and other cell wall constituents. Cell elongation and nucleic acid metabolism are also facilitated by this protein.
K	More than 40 enzymes require it as a cofactor. The primary cation in forming cell turgor and preserving cell electroneutrality.
Ca	A part of the middle lamella of the cell wall. It is needed as a cofactor by certain enzymes involved in the hydrolysis of ATP and phospholipids. It functions as a second messenger in metabolic regulation.
Mg	Many enzymes involved in phosphate transfer need it. a component of the chlorophyll molecule
Cl	Required for the photosynthetic reactions that result in the evolution of O_2.
Mn	Some dehydrogenases, decarboxylases, kinases, oxidases, and peroxidases use it to work. Involved in the evolution of photosynthetic O_2
Fe	A cytochrome and non-haem iron protein constituent implicated in photosynthesis, N_2 fixation, and respiration.
Ze	Many enzymes contain it, including alcohol dehydrogenase, glutamic dehydrogenase, and carbonic anhydrase.
Cu	In enzymes such as tyrosinase, monoamine oxidase, uricase, cytochrome oxidase, phenolase, lacases and plastocyanine, ascorbic acid oxidase is used.
Ni	A component of urease. A component of hydrogenases in N_2-fixing bacteria
Mo	Nitrogenase, nitrate reductase, and xanthine dehydrogenase also contain this element.

3.1.1. Nitrogen (N)

An average 78-79 % of N is available in the atmosphere as inert (N_2), which is not beneficial to plants and therefore is not directly absorbed. There are various forms of N (N_2, NO_2, NH_3, NH_4^+, NO_3^-, C-NH_2), from which plants uptake N mainly in the form of NH_4^+, NO_3^- depending on plant species and soil conditions such as pH (Leghari et al., 2016). Nitrogen, upon elimination, gains the −3 valence for assimilation and absorption. Nitrate reductase and nitrite reductase are the two main enzymes that ensure the conversion of nitrate (NO_3^-) to ammonium (NH_4^+). The movement of nitrogen to higher plants takes place mainly as nitrate and amino acids, mostly by xylene from the roots to the upper parts of the plant. High-affinity H+ coupled symporters of the NRT (Nitrate Transporter) family mediate the NO_3^- uptake. Nitrate reduction and assimilation happen mainly in the shoot (Karthika et al., 2018). N is among the nutrient, which is required in larger quantities by plants as compared to other nutrients. Nitrogen, which is a very mobile element, flows well between the atmosphere, the soil, and the living organisms. (Nitrogen can be present in soils and plants, as well as in the water we drink and the air we breathe. It is also essential for life: it is a critical component of DNA, which defines our genetics). Nitrogen-adequate plants hold from 1 to 5% of N (10,000–50,000 ppm or mg kg^{-1} of dry matter). Under reduced conditions, such as in the case of rice, N is taken in ammonia form. Furthermore, N-use productivity in crops is higher at standard soil pH range (6.5-7.0), but nitrogen being a macronutrient is maximally usable at higher pH, although at the same time availability does not mean improved utilization because increased pH disturbs plant root growth and all other plant parts work (Leghari et al., 2016). On the other hand, soil composition (percentage of sand, silt, and clay) is theoretically included in the nitrogen control scheme. N is not retained by sandy or coarse soils whereas, sandy loam, and loam soils have the highest ability to hold N for plants. Therefore, the use of N is higher in certain crops grown underclay and the loamy soil. The soil texture can be improved with the use of organic manure. This improves the productivity of nitrogen usage in several ways. In general, the greatest crop removal is found to be the maximum nutrient removal; but, as nitrogen supply declines, so does its quality. Optimum availability of N is essential for proper plant intake (Leghari et al., 2016).

Considering the signs of a leaf, it is worth remembering the presence of nitrogen atoms in chlorophyll molecules. The nitrogen shortage caused noticeable signs, which could also be readily observed by visual inspection: the plant slowed down its development and the leaves appeared uniformly yellow basal leaves were especially affected by the symptoms (Rustioni et al., 2018). In addition, the effects of nitrogen deficiency on photosynthetic CO_2 assimilation, PSII (Photosystem) photochemistry, and photoinhibition have been studied in maize (*Zea mays*) plants grown under natural illumination. Nitrogen-deficient plants had slightly lower CO_2 assimilation ability but showed no difference in the maximum efficiency of PSII photochemistry (Lu and Zhang, 2000).

3.1.2. Phosphorus (P)

Apart from (N), (P) is a critical resource for plant growth and productivity. Phosphorus in soils is almost entirely in the form of orthophosphate, with total P concentrations usually ranging from 500 to 800 mg/kg dry soil (Mengel and Kirkby, 2001). Its concentration in plants varies from 0.05 to 0.5% of the overall plant dry weight, its fixation in the form of aluminum/iron or calcium/magnesium phosphates makes it unavailable for plant uptake. Due to soil fixation of P, its availability in soil is infrequently adequate for optimum plant growth and production (Malhotra et al., 2018). Phosphorus is required in lower quantities than other macronutrients, ranging from 0.1 to 0.5 % in plant leaf dry weight (Osman, 2013). Plant roots consume P either HPO_4^{2-} or H_2PO_{4-}. Meanwhile, since soil concentrations of these ions are on the micromolar scale (M), high-affinity active transport systems are needed for P uptake across the plasma membrane of root epidermal and cortical cells (Shen et al., 2011). Adsorption of phosphate decreases with rising pH until a pH value of 6 - 6.5 is obtained (Mengel and Kirkby, 2001). P is important for energy conservation and conversion in photosynthesis and respiration. P, including ADP and ATP (adenosine diphosphate and triphosphate) and DPN and TPN (di- and triphospiridine nucleotide), is a component of RNA and DNA complexes, which are the major components of genetic material, seeds have the highest concentration of P in a mature plant, and P is required in large quantities in young cells of shoots and root tips, where metabolism is high and cell division is rapid, P aids in root growth, flower initiation, and seed and fruit growth, and P has been shown to reduce the occurrence of disease in some plants and has been shunned (Uchida, 2000). P deficient leaves are more curly than younger leaves, often seen on older leaves that are covered with new leaves, and lately the maturation of small-headed plants. Plants with a phosphorus deficiency can stay darker green than average plants and show purple discoloration on the underside first, then across the plant. When P deficiency is serious, leaf tips will die. Plants grow slowly, stems are small and shortened, and maturation is postponed. P deficient plants also show poor tilting and curling (Pandey et al., 2020).

3.1.3. Potassium (K)

Potassium (K) is one of the essential plant nutrients needed for plant growth and physiology. Potassium is not only a part of plant structure, but it also acts as a regulator in a variety of biochemical processes related to protein synthesis. Various physiological processes, including stomatal control and photosynthesis rely on K (Hasanuzzaman et al., 2018). Rattan (2015) declare that Potassium is taken up by the roots, in its cationic form, i.e., K^+. Kirkby (2012) the weathering of K-bearing minerals is the primary natural cause of K^+. K occurs in a complex balance of the soil and in plants it is exceptionally mobile. In healthy plant tissues, the K concentration ranges from 1 to 5%, however K does not form a structural part of the plant, but it does play a regulatory function in plant metabolism and growth (e.g., organic acid anions and inorganic anions) and insoluble anions and therefore facilitates in stabilizing the pH between 7 and 8, which is the best for most enzyme reactions. Dotaniya et al. (2016) said that soil pH influences the activity of plant nutrients and affects the concentration of K in crops. K carries positive charges and is negatively affected by positive cations in acidic soil, i.e., H^+ and Al^{3+}; and in higher pH soil by Ca^{2+} and Mg^{2+}. The presence of hydrogen ions decreases the susceptibility to fix K in low-pH conditions. However, utilizing pH-increasing modifications, H^+ is neutralized and K ions are more likely to travel closer to the soil colloidal surface where they may be vulnerable to fixation. Besides K is extremely mobile in plants, and the translocation of K takes place through both the xylem and the phloem. The transport of K^+ takes place in the xylem from root to shoot. Whereas the transport of K^+ through phloem is to sink organs (e.g., roots and rhizomes). There may be seeds or fruits in the shoot or tubers and the roots of the storage roots (beets) (Karthika et al., 2018). Chlorosis with the scorching of plant leaves and yellowing of the edges of the leaf are typical symptoms of K deficiency. This is one of the first symptoms of K deficiency in the middle and lower stages. K deficiency in plants allows plants to die faster than they should. This effect can be also quicker if plants are subjected to drought or high temperatures, as poor K absorption decreases plant tolerance to temperature rises and drought, resulting in less water flow in plants, and other consequences such as uneven fruit ripening, poor pest resistance, and weak and unhealthful roots (Dotaniya et al., 2016).

3.1.4. Magnesium (Mg)

Magnesium (Mg) has numerous biochemical roles in biological processes. Among the essential mineral nutrients needed by plants, Mg plays an important role in phloem loading and transporting photoassimilates to sinking organs, such as roots, shoot tips and seeds. In vegetative parts the Mg requirement is 1,5–3,5 g/kg for optimum growth of the plantation, and in soil solutions the Mg concentration is between 0.125 mmol L^{-1} and 8,5 mmol L^{-1}, which are sufficient to sustain plant growth (Guo et al., 2016). Mg is taken up by plants as Mg^{2+} (Karthika, Rashmi, & Parvathi, 2018). Magnesium has a variety of primary roles in plants. Photophosphorylation (such as ATP formation in chloroplasts), photosynthetic carbon dioxide (CO2) fixation, protein synthesis, chlorophyll formation, phloem loading, photoassimilate partitioning and utilization, reactive oxygen species processing, and photooxidation in leaf tissues are all influenced by Mg (Cakmak and Yazici, 2010).

Magnesium (Mg) plant deficiency is a common problem concerning the production and efficiency of agriculture and forestry systems. While various studies have looked at the impact of Mg deficiency on biomass and photosynthetic CO_2 assimilation (Jákli and Tränkner, 2019). Magnesium (Mg) can have both a direct and indirect impact on the disease as an indispensable mineral ingredient for plants and microbes. Since nutrition is part of a delicately balanced interdependent system formed by plant genetics and the environment, balanced nutrition is important for disease tolerance expression (Huber and Jones, 2013). Slight effect of Mg deficiency during the vegetative growth period, however, do not usually result in low yield until permanent shifts, such as a decrease in the amount of grain per year in cereals, occur (Kirkby, 2012).

3.2. Maize

Maize (Zea mays. L) was one of the first plants cultivated by farmers 7,000 to 10,000 years ago, and corn cobs were discovered in caves in several archaeological sites in Mexico, dated to be over 5000 years old (Piperno and Flannery, 2001). Corn is a yearly plant generally referred to as maize and is part of the grass family, i.e., poaceae having short life cycle and requiring warmer weather conditions. It’s well known that maize is a very essential human food and raw material for various industrial activities, and it is also an important livestock feed (Kumar and Jhariya, 2013). Cultivated throughout the world and it is a good source of food. Maize can also be converted into several products such as starch, pulp, oils, drinks, glue, industrial alcohol, and fuel ethanol (Ranum et al., 2014).

3.2.1. Maize climatic adaptation

Some scholars have empirically examined climatic adaptation of maize, Maize is a warm-weather plant and does not thrive in areas where the average daily temperature is below 19 °C or the average summer months are under 23 °C. For every millimetre of water used, approximately 10 to 16 kg of grain are produced. Jones (1985) observed that corn can be grown on a wide variety of soils but the most suitable is the one well-drained with the soil type (deep loam and silt loam) containing sufficient organic matter and available nutrients. Corn can be grown in soils with a pH from of 5.0 to 8.0, but corn is moderately salinity-sensitive and 90 % relative yield was achieved with an electrical conductivity of approximately 1.8 dS m^{-1}. Soil salinity can have a major effect on the absorption of a variety of nutrients but reduced dry matter production is most likely the result of reduced soil water and increased toxicity of sodium chloride and sulfate in soil solution. (Larson and Hanway, 1977). Although the minimum temperature for plant growth is 10 °C, germination at soil temperatures between 16 and 18 ° C would be quicker and less variable. At 20 °C, maize is expected to emerge within five to six days. The critical temperature that adversely affects yield is approximately 32° C. A frost-free period range between 120 to 140 days is required to prevent damage and frost can damage maize at all stages of growth. Although the growth point is below the soil surface, new leaves will form, and frost damage will not be too serious. Leaves of mature plants are easily weakened by frost, and grain filling can be adversely affected (Plessis, 2003). Rainfall throughout the corn-growing period should be in the range of 460–600mm in the temperate regions, and in the tropics, corn does the greatest with 600–900mm of rain during the growing season (Fageria et al., 2011). Soil pH is thus defined as the "master soil component," influencing a wide range of soil biological, chemical, and physical properties, as well as processes influencing plant growth and biomass yield (Neina, 2019).

3.2.2. Uptake of nutrients by maize

It is well known that the nutrient concentration of a plant is influenced by not just the availability of that nutrient but also the supply of other nutrients, and that light, temperature, water, and humidity are factors that influence the environment. The amount of accumulation calculated in the sample may provide general guidance for the absorption of nutrients by high-yield corn. The deposition of nutrients in the corn grown on Brazilian Oxisol during the crop growth cycle (Tables 2 and 3). Macro and micronutrient accumulation dramatically improved with rising plant age. The Total approximate concentration of macronutrients for N, P, K, Ca, and Mg is 199.44, 21.19, 186.89, 41.64 and 29.10 kg, respectively. In comparison, the cumulative concentration of macronutrients during the growth period for micronutrients Fe, Mn, Cu , Zn, and B is 376.33, 67.07, 534.36, 2254.12 and 145.75 g respectively (Fageria et al., 2011).

Table 2 Macronutrients accumulation of corn plants during the crop growth cycle (Fageria al., 2011).

Plant Age in Days	**N (kg ha^{-1})**	**P (kg ha^{-1})**	**K (kg ha^{-1})**	**Ca (kg ha^{-1})**	**Mg (kg ha^{-1})**
18	1.91	0.13	1.78	0.28	0.13
35	31.39	2.41	33.56	4.54	2.74
53	143.14	10.37	189.84	27.59	15.77
69	147.27	13.50	197.56	37.47	23.18
84	186.40	20.75	237.8	51.78	34.42
119 (straw)	72.03	4.47	152.56	33.41	20.46
119 (grain)	127.42	16.71	34.33	8.22	8.64
Total	199.44	21.19	186.89	41.64	29.10
N > K > Ca > Mg > P					

Table 3. Micronutrient’s accumulation of corn plants during the crop growth cycle (Fageria et al., 2011).

Plant Age in Days	**Zn (g ha^{-1})**	**Cu (g ha^{-1})**	**Mn (g ha^{-1})**	**Fe (g ha^{-1})**	**B (g ha^{-1})**
18	1.75	0.60	3.63	75.36	0.61
35	28.59	11.74	46.32	811.36	10.80
53	145.52	59.22	295.73	897.99	81.08
69	223.47	69.54	418.05	1513.46	90.22
84	319.81	79.32	700.27	1890.49	133.22
119 (straw)	184.37	53.32	452.16	2048.24	103.12
119 (grain)	192.00	13.75	82.21	205.88	42.62
Total	376.33	67.07	534.36	2254.12	145.75
Fe>Mn > Z > B > Cu					

3.2.3. Requirement of nutrients in maize

Adequate supply and balance of essential nutrients are needed for the better yield of maize (Barbieri et al., 2008). Uptake of nutrients and their accumulation differ between agroecosystems, but these values can serve as guidance for assessing the nutritional requirements and status of the corn crop (Fageria et al., 2011). Grain yields of newer corn hybrids are higher than those of older hybrids at various stages of N fertility (Ding et al., 2005). Below Table describes nutrient accumulation in grain and straw of sorn plants to produce 1t of Grain

Table 4. Nutrient accumulation in grain and straw of corn plants to produce 1t of grain

Fageria et al. (2011)

Nutrient	Amount of nutrient required to produce 1t of grain
Nitrogen	24 kg
Phosphorus	3 kg
Potassium	23 kg
Calcium	5 kg
Magnesium	4 kg
Zinc	46 g
Copper	8 g
Manganese	65 g
Iron	27 g
Boron	18 g

Yields of newer corn hybrids are higher than those of older hybrids at various stages of N fertility (Ding et al., 2005). In other research conducted by Setiyono et al. (2010) using the QUEFTS (quantitative evaluation of the fertility of tropical soils) approach, they were able to approximate two boundary lines that described the minimum and maximum internal nutrient efficiencies, which were 40 and 83 kg of grain kg $^{-1}$ N, 225 and 726 kg of grain kg $^{-1}$ P, and 29 and 125 kg of grain kg $^{-1}$ K, respectively. As it is mentioned earlier seventeen chemical elements have been recognized as essential in plants. In the absence of these nutrients, plants cannot complete their life cycles and perform natural physiological functions (Osman, 2013). Healthy plants would have a deep green color to show that they consist of a sufficient nutrient (Sabri et al., 2020). In turn, the deficiency of nutrients causes both physical and physiological damage to the plants.

3.3. The use of wastewater in crop production

The use of wastewater in irrigation is much more common than many people believe. Worldwide an approximate of 20-million-hectare arable land is currently reported to be irrigated with wastewater. It is also expected that the unreported use of wastewater in agriculture will be significantly higher. It is mostly common in urban and peri-urban areas of the developing world, where insufficient economic resources and institutional capacity restrict the establishment and operation of adequate wastewater collection and treatment facilities (Liebe and Ardakanian, 2013).

3.3.1. The use of wastewater for irrigation

Prehistoric civilizations (for example, ancient Egyptians, Mesopotamians, Minoans, and Indus valley societies) used wastewater for irrigation. Important historical evidence suggests that the ancient Minoans used wastewater irrigation for agriculture around 3500 BC. (Tzanakakis et al., 2007). Wastewater use in agriculture is much more widespread (Liebe and Ardakanian, 2013). The shortage of quality freshwater for agriculture has made the use of wastewater (WW) a common choice (Khurana and Singh, 2012). The use of treated wastewater for agricultural irrigation is becoming more widespread, particularly in arid and semi-arid areas. For instance, more than 85%, 71%, and 46% of treated wastewater are used for agricultural irrigation in Israel, Spain, and California, respectively (Sato et al., 2013). Besides Sato et al. (2013), report indicates that out of 181 countries only 55 countries have available data on the generation, treatment, and usage of wastewater. Treated wastewater is often used for agricultural purposes, but in humid regions, this end-use is not important. The condition is different in developing countries' arid and semi-arid regions, such as North America, Australia, and southern Europe, where treated wastewater is mostly used for irrigation, considering the growing competition between agriculture and other sectors for water. Hence, agriculture is the industry that uses the most water supplies in the world, accounting for nearly 70 % of global water consumption (Frenken and Gillet, 2012). Over the last 50 years, world overall planted area has increased by 12 % and irrigation supplies have doubled in that period. Around the same time, climate change, global warming, shifting trends of weather, the rise of the world's population, and modern eating patterns have ensured that populations around the world now face water shortage issues. Reused wastewater is an alternative form of drainage that is both inexpensive and healthy in terms of human health and the environment as it increases water supply and decreases reliance on groundwater and surface water supplies (Gallego et al., 2019). With population growth and social and economic advancement, water scarcity is becoming a major concern worldwide. As a main alternative water supply, wastewater can be used in irrigation to compensate for water scarcity. Wastewater irrigation has a long tradition of growth and has experienced diverse processes in emerging and developed countries. Untreated drainage of wastewater can have various environmental issues (Zhang and Shen, 2019). Thus, farms with restricted access to irrigation water may benefit from treated wastewater effluent recovery, reducing stress on traditional supplies, particularly during dry seasons (Vergine et al., 2017). The freshwater shortage has resulted in increased use of treated water as an alternative and efficient source of crop irrigation (Paltiel et al., 2016).

As per the classification of Zhang and Shen .(2019) wastewater is divided into 3 major categories, urban water, which is domestic sewage, from commercial establishments, industrial sewage, and stormwater, and another urban run-off. The subsequent one is treated water**,** which is the treatment plant shall meet the requirements to ease its emissions or health hazard also reclaimed (recycled) water which is regulated wastewater that can be used officially in managed conditions for possible use as irrigation. Most of the produced wastewater is therefore not treated, and most of the untreated wastewater is used by small-scale farmers with no capacity to maximize the amount of efficiency of the wastewater they collect for irrigation. Many farmers in water-scarce developed countries irrigate wastewater because:

- ✓ it is the only supply of water available for irrigation throughout the year
- ✓ wastewater irrigation implies lower energy costs if deep groundwater is the preferred clean water source
- ✓ wastewater as irrigation decrease cost related to purchase of fertilizer as the wastewater could be source of nutrients by itself (Sato et al., 2013).

Wastewater reuse in agriculture must follow the rigorous requirements for wastewater management in watercourses. Another aspect that appears to support the re-use of wastewater is that it is both a fertilizer and a reservoir of water (Vazquez-Montie et al.,1996). Increasing industrial reuse of processed effluent supports aims such as the promotion of organic production, the protection of limited water supplies, and the preservation of environmental sustainability. Wastewater irrigation will also lower purification and fertilization costs, as soils and crops act as biofilters, and wastewater provides nutrients (Haruvy, 1997).

3.3.2. The negative impact of wastewater in crop production.

Agricultural use of rather treated or untreated wastewater and the re-use of agricultural water is a complicated question, involving a wide range of different elements, such as food processing, water quality and water treatment, hydrology, health concerns as well as socio-economic issues for those concerned, involving users, and overall environmental risks (Huibers and Van Lier, 2005). Wastewater is a source of contamination that can influence the environment if good practices are not implemented. Although populations and urban areas are rising at exponential rates and water shortage is increasing, it is expected that the use of wastewater in irrigation will continue to expand in areas where fresh water is limited in the immediate future (Liebe and Ardakanian, 2013). Even if, recycling and using wastewater for crop production provides nutrients (phosphorus, nitrogen, potassium, and calcium) they have also adverse effect like the assimilation of pharmaceuticals themselves by the plants and excessive development due to over fertilizations (Asano and Pettygrove, 1987).

The chemical composition of WW (wastewater) differed surprisingly in terms of its heavy metal content, pH, electrical conductivity (EC), biological oxidation demand (BOD), chemical content. Demand for oxidation (COD), alkalinity, and hardness. Land application of all types of wastewater soil EC, cation exchange capacity (CEC), overall and DTPA-extractable heavy-metal/micro-nutrient material, usable macro-nutrient content (N, P, and K) with substantial decreases in surface soil calcium carbonate content (Khurana and Singh, 2012). Water contain micropollutants such as endocrine disruptors and pharmaceuticals due to their persistence after the most traditional wastewater treatments (Barbera et al., 2020).

Toxicity happens when a particular ion is taken up by the plant and accumulates in concentrations resulting in damage or decreased yield. The most prevalent contamination from the use of reclaimed residential wastewater is caused by domestic detergent discharges or from processing plants. In addition to its direct effect on the plant, sodium in irrigation water can have an impact on the soil structure, decreasing the rate at which the water may travel to the soil as well as the soil aeration ability. If the rate of penetration is significantly reduced, it may be difficult to provide the plant with enough water for good growth (Asano and Pettygrove, 1987).

3.3.3. Treatment of wastewater before application to the soil

The presence of biological and chemical pollutants may affect the agricultural environment, as well as the health of farmers and consumers (Petousi et al., 2019). Wastewater reuse is an essential component of efficient water supply management; water reuse from multiple wastewater sources for the elimination of toxins, nutrients, and pathogens offers an incentive for water protection (Cynthia , 2011).

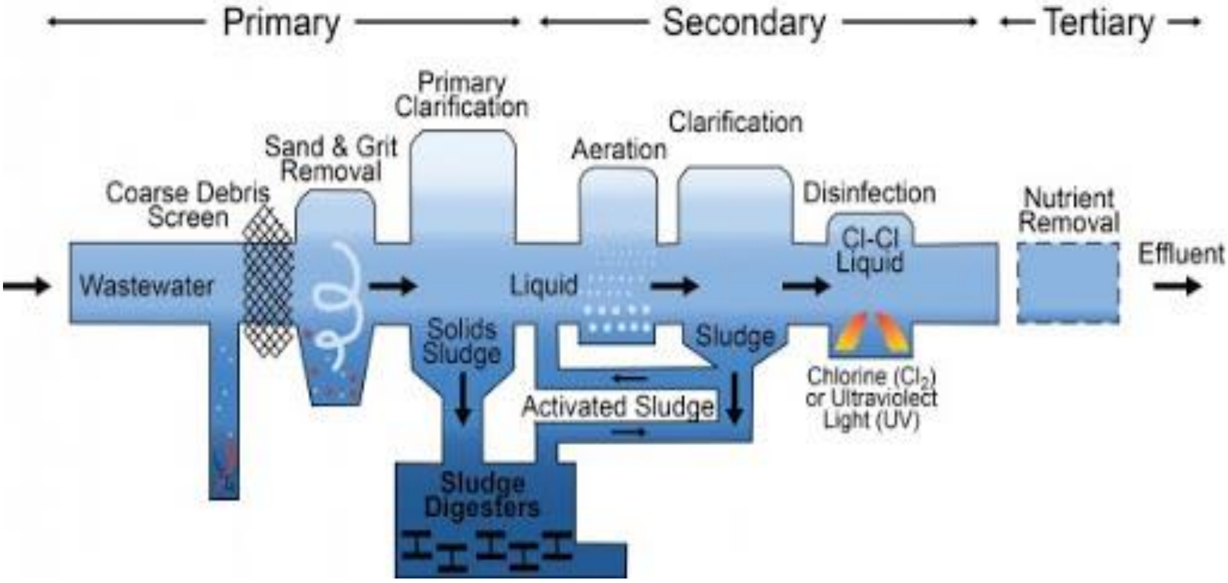

Figure 1. Flow diagram of the wastewater treatment process (u.s. wastewater treatment factsheet) (University of Michigan, 2017).

As domestic drainage joins the wastewater treatment plant (WWTP), the first step of treatment is the primary treatment (Figure 1) where gross solids are separated from the water. This is achieved in small stages first by using screens to eliminate large floating matter, such as plant matter then water is channeled into the grit trap, followed by slowing the water, causing huge sediments, such as rocks and sand, to settle down to the bottom of the chamber. Then the water flows through a finer mesh, the domestic water reaches the sediment tank, where the flow of water is further decreased. This causes sediments that are floating in the water to fall to the bottom of the tank; these main sediments are then collected and are the starting point for the phase of sludge or biosolids. After the first sedimentation tank, the water is already full of organic matter and transfers through secondary treatment. In secondary treatment, 90% of the organic matter still in the water is eliminated through biological processes. To do something with the WWTP enlist the help of microorganisms which under the right conditions, consume organic matter, providing a cost-effective and efficient method for the removal of organic substances. The water then reaches the second sedimentation chamber, where the water is slowed down again, and the suspended particles are then able to settle out of the water. Finally, the water undergoes a phase of disinfection before discharge, and can differ depending on the plant, such as the use of ozone and ultraviolet radiation; but the most prevalent form of disinfection due to cost-effectiveness is chlorine. In most situations, the disinfection stage is the last step before the wastewater can be discharged back into the environment or used in other uses (University of Michigan, 2017).

Sewage treatment plants are a vital aspect of pollution prevention, but sewage treatment plants are unable to significantly eliminate many water-borne pharmaceuticals. Moreover, multiple factors play a role in wastewater disposal systems which should be precisely modified to increase the effectiveness of opioid abatement, thus mitigating future environmental risks (Zuccato et al., 2006). On the other hand, the main conventional methods used for treating polluted industrial wastewater are chemical precipitation, flotation, chemical oxidation, biological methods, Adsorption/filtration, and electrochemistry (Qin et al., 2015). Emerging pollutants – i.e., contaminants with recent significance – include a broad variety of chemical substances, such as pharmaceuticals, personal care goods, surfactants, plasticizers, and synthetic additives, which are not used in environmental surveillance programmes (García-Medina et al., 2020).

3.4. Pharmaceuticals

Pharmaceuticals are intended to cure and treat illness and helping people to stay healthy. However, the active medicinal ingredients (APIs) in these drugs, either as the original drug or its metabolites, can be released into the environment and may be present at very low yet detectable concentrations (Cunningham et al., 2006). They help us follow the new way of life and contribute to our wellbeing and high living standards (Kummerer, 2010). Pharmaceuticals are widely present in both marine and industrial ecosystems because of human activity and subsequent discharges of wastewater effluent to the environment (Carvalho et al., 2014). PPCPs incorporated into the soil by irrigation are primarily accumulated in surface soil, and certain forms of PPCPs can be stored in groundwater under extensive or long-term irrigation (Qin et al., 2015). Additionally, pharmaceuticals are crucial for the wellbeing of both humans and animals, after their use, the metabolites are excreted and enter the environment through different pathways. Pharmaceuticals and metabolites have been present in the environment (surface water, groundwater, potable water, etc. water, sediment, sludge, and manure from sewage) for decades (Klatte et al., 2017). However, there are currently more than 4,000 pharmaceuticals in use, in personal care goods (Boxall et al., 2012). Pharmaceuticals and their metabolites from the use of veterinary and human beings enter the atmosphere through several routes (Henschel et al., 1997). The ongoing release of human and veterinary drugs into the environment is primarily due to the production practices, disposal of unused or expired items, and excretions. Many of these compounds or their bioactive metabolites end up in soil and sediments due to poor disposal mechanisms and their properties (Dı´az-Cruz et al., 2003). As a result of increased wastewater production, their efficient use in agriculture has been also increased dramatically (Qadir et al., 2010). In the effect of all WWTPs, nine pharmaceuticals were found in at least one sampling campaign. Acetaminophen, diclofenac, and amoxicillin were the most abundant drugs, followed by atenolol, ketoprofen, clarithromycin, carbamazepine, and doxycycline; mean concentrations (considering all plant measurements) were 3914 ± 2620, 2065 ± 739, 2002 ± 2170,1223 ± 1042, 961 ± 1003, 356 ± 370, 233 ± 100, 196 ± 189, respectively (Palli et al., 2019). Also, a study has been performed at the German urban waste treatment plant (STP) by According to Ternes (1998), more than 80% of the selected drugs were detected in at least one urban STP effluent with concentrations of up to 6.3 g L^{-1} (carbamazepine) due to insufficient removal of drug residues during the transit through the STP, resulting in contamination of the receiving waters.

3.4.1. The assimilation of pharmaceuticals by crops

More attention is given to plant absorption of new chemical substances including pharmaceuticals (Wu et al., 2012). PPCPs are taken up by roots and aerial tissue roots (parts which are completely exposed in air) take PPCPs through mass flow or diffusion through the roots of dissolved compounds (Bartrons and Peñuelas. 2017; Miller et al., 2016). Human actions are the primary source of PPCPs in the ecosystem and are concentrated in urban, agricultural, and industrial wastewater treatment plants. PPCs in reclaimed irrigation water, biosolids, and manure are added to soils where they can impact soil microbiota and can be consumed, stored, and metabolized by plants. Airborne absorption of PPCPs can also occur through the deposition of volatilized compounds and aerosols, as well as through direct interaction with irrigation water or alteration materials. PPCs affect plants, their microbiota, and food web that feed on them, including humans (Bartrons and Peñuelas, 2017). It has been well known that when pharmaceuticals introduced to the growing medias of plants such as corn, they are likely to take these substances out of their enviromnet and retain them in their tissues (Shenker et al., 2011; Carter et al., 2014; Dodgen et al., 2015).

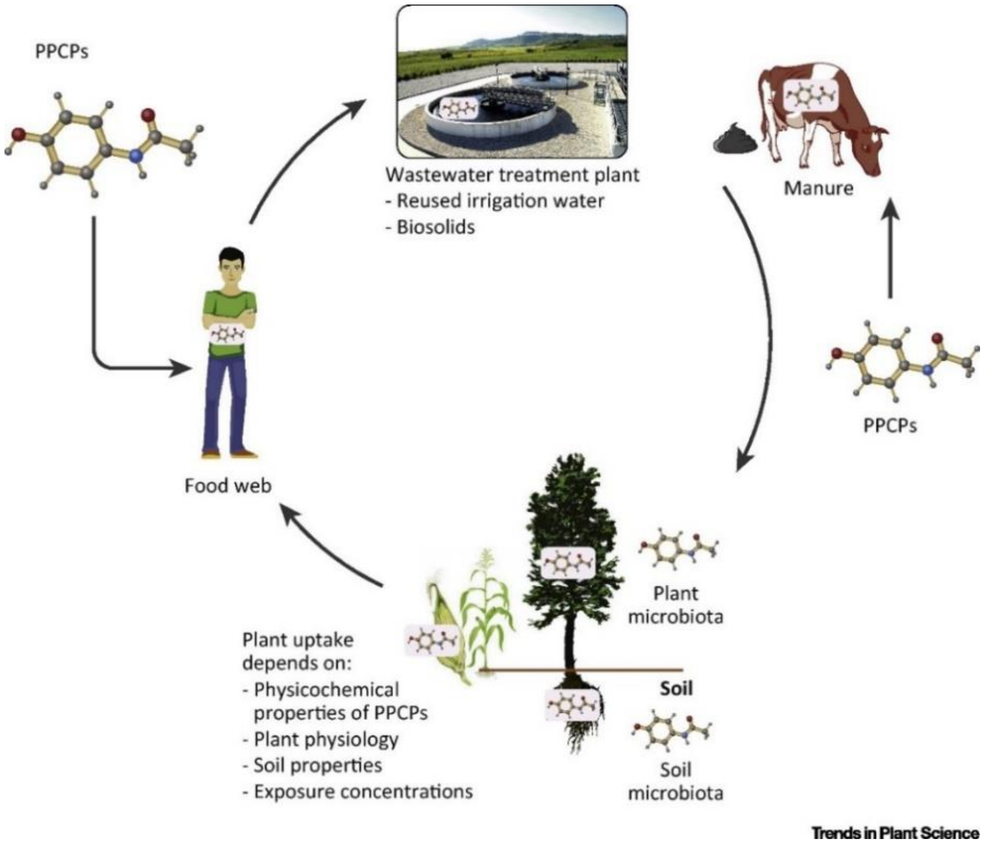

Figure 2. The sources and fates of pharmaceutical and personal care products in plants and the environment (Bartrons and Peñuelas, 2017).

The plant uptake depends on the physicochemical properties of PPCPs, plant physiology, soil properties, and exposed concentration however, wastewater is the key way to bring this pharmaceutical into the environment (Figure 2). The involvement and potential adverse effects of active pharmaceuticals in the ecosystem are presently an emerging subject of concern in environmental sciences, since these compounds, manufactured to interfere with receptors and processes in target species, are constantly discharged into the environment by wastewater treatment plants, such as sewage sludge and wastewater. As a result, non-target organisms can be exposed during their life if their environments become polluted with these chemicals. Detrimental consequences have been reported on both marine and terrestrial invertebrates when exposed to environmentally significant amounts of carbamazepine (Oliveira et al., 2015). Suggesting the CBZ absorption may be called passive and is not limited to root membranes. The concentration of CBZ in the leaves corresponded to the age of the leaf – the highest amount was seen in the cotyledon leaves (2354 µg kg $^{-1}$). The concentration of CBZ in the real leaves was lower in the youngest (top) leaf (462 µg kg $^{-1}$) in plants exposed to higher levels of CBZ (>10 000 µg L^{-1}). The amounts of carbamazepine in the stem and roots were slightly lower than those found in the leaves. This may mean that CBZ is primarily translocated by water mass flow and is thus localized and stored to the maximum degree in mature/older leaves. The concentrations of carbamazepine in the stem and roots were slightly smaller than those found in the leaves, meaning that carbamazepine is primarily translocated by the movement of water mass; it is therefore localized and accumulated in the mature / older leaves to the maximum degree (Shenker et al., 2011). Hydrophobicity of a compound is typically used to interpret the absorption of organic compounds into plant roots. A positive linear association between root uptake and hydrophobicity was found for neutral PPCPs, indicating that hydrophobicity was a key factor influencing the uptake of neutral root PPCPs. This model cannot, however, be generalized to ionic PPCPs additional processes, such as electrical attraction or repulsion and ion traps, may induce root aggregation in ionizable PPCPs. Ions usually cross biomembranes (e.g., plasma membrane, tonoplast) at a lower rate than neutral molecules, and thus molecular dissociation can contribute to reduced root aggregation, as shown in a recent review, ionic pharmaceuticals showed lower uptake relative to neutral ones. The acidic PPCPs can be partially detached, resulting in at least two species: the undissociated acid and its associated anion. Anions are usually poorly absorbed by plants since plant cells have a negative electrical potential in the cell membrane, and this contributes to repulsion of the negatively charged anion. A process that may contribute to the aggregation of acidic compounds in plant cells is called an ion trap. When the pH of the external solution is below the pH of the cells, the non-dissociated acid outside the cell can spread rapidly to the cell. However, due to higher pH, detachment of the weak acid can occur within the cell. As the ion is much less able to permeate membranes than its neutral molecule, the acid is trapped inside the cell. For example, generally low root accumulation of acidic PPCPs, including diclofenac, naproxen, gemfibrozil, atorvastatin, and ibuprofen, was observed in a hydroponic sample (Wu et al., 2015 ; Wu et al., 2013).

3.4.2. The effect of pharmaceuticals on crops

Reclaimed water provides a significant amount of nutrients required for plant growth, such as nitrogen and phosphorus. However, residual soil PPCPs can harm soil species (microorganisms and fauna), crops, and even humans. However, short-term adverse effects need comparatively high concentrations, bioavailability, and/or precise toxicity (e.g., receptor-mediated effects). To date, most experiments have focused on possible acute effects, but trace soil PPCPs are more likely to cause permanent effects (Qin et al., 2015). A few studies have shown that trace PPCPs are toxic to soil microorganisms or fauna (e.g., destroy their population structure and diversity), which could then cause possible effect on nutrient organic matter decomposition and then nutrient uptake. The findings are mainly limited to antibiotics. For example, the addition of oxytetracycline hydrochloride and penicillin could reduce soil bacterial biomass (Colinas et al., 1994). The organization of soil microbial populations may be broken down by antibiotics (Westergaard et al., 2001). Soil PPCPs can also theoretically impact soil microbial processes (e.g., biodegradation, nitrification, enzyme activities, and soil respiration) and functional biodiversity (Waller and Kookana, 2009).

Additionally, PPCPs residues in reclaimed water can theoretically be consumed by crops. Reclaimed water with trace amounts of PPCPs may encourage crop growth, but their accumulation in crops may also cause morphological and physiological effects. Young seedlings of plants are more open to PPCPs than mature seedlings. Triclosan at 10 mg kg^{-1} in the silt loam soil greatly inhibited the root elongation of rice plants grown in the environment chamber. Increasing the concentration of triclosan to 50 mg kg^{-1} substantially slowed root elongation and the development of rice and cucumber plants. When soya seedlings grown in ½ strong Hoagland solution in a greenhouse were exposed to moderately high amounts of bisphenol A (e.g., >7.0 mg L^{-1}), the chlorophyll content decreased dramatically, contributing to a decline in photosynthesis (Qin et al., 2015).

3.4.3. Carbamazepine

Carbamazepine (CBZ) is a white or nearly white crystalline powder that is used in the therapy of epilepsy and trigeminal neuralgia. The analytical formula of CBZ is $C_{15} H_{12} N_2O$ and the elemental analysis is about C, 76.25% H, 5.12% N, 11.86% O,6.77%. IUPAC ID os 5H-dibenzo[b,f]azepine-5-carboxamide. CBZ having melting point of 189–193°C (Alrashood, 2016). CBZ is an anti-epileptic treatment listed as class II in the biopharmaceutics classification scheme (BSC) (low solubility and high permeability) (Zakeri-Milani et al., 2009).

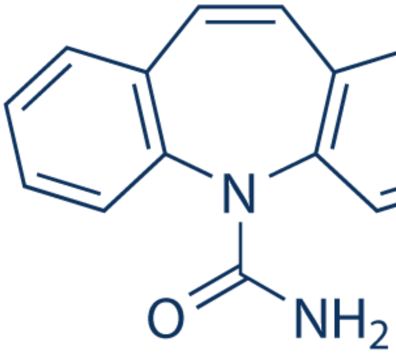

Figure 3. Chemical structure carbamazepine CBZ (Silski-Devlin et al., 2020).

CBZ is a pharmaceutical product that is widely found with biosolids in recovered wastewater and agricultural soils (Kinney et al., 2006). Because of its wide use, it is widely available in wastewater treatment plants with sewage systems. However, such medications are not efficiently eliminated by the wastewater treatment plant and can be taken to water systems. CBZ is an example of a wastewater treatment plant incorrectly withdrawn drugs (Zhang et al., 2008). CBZ has been found to occur in WWTP wastewater, biosolids, and soil at concentrations ranging from 0.24 to 2.10 mg L^{-1}, 7.8 to 258 mg kg $^{-1}$, and 0.0065 to 7.5 mg kg^{-1}, respectively. CBZ has been noted to have a low elimination efficiency in WWTPs of ≤ 10 % and has a half-life of more than 365 days in both WWTPs and soil (Knight et al., 2018). Prescription medication CBZ was measured at concentrations of 93.6 ng L^{-1} in recycled wastewater. Concentrations of these compounds can be seasonally dependent, and sorption, water solubility, and charged functional groups on the molecules may depend on preservation and bioavailability in soil samples (Chefetz et al., 2008). This same pharmaceutical was found with average concentrations of 271.2 µg kg $^{-1}$ for salbutamol, 66.4 µg kg L^{-1} for carbamazepine, 92.95 µg kg L^{-1} for sulfamethoxazole, and 11.43 µg kg L^{-1} for trimethoprim in biosolids planned for land use (Lapen et al., 2008).

3.4.3.1. Reaction of carbamazepine with soil

The adsorption nature of CZB on agricultural soils was found to be dependent on soil fertilization (directly related to its organic matter content): higher organic matter content resulted in higher sorption ability. The findings show that CBZ is not extensively adsorbed to soils in the case of those exposed to mineral fertilization. Contaminated soils (due to the widespread use of effluent for field irrigation and application of sludge) can also be a possible source of carbamazepine input into neighboring surface and ground waters through leaching (Calisto and Esteves, 2012). The existence of CBZ in the soil ecosystem has been reported repeatedly, and effective removal strategies are critical. There are many creative approaches, including physical, chemical, physicochemical, and biological methods for the reduction of organic compounds in soil. The focus was on the implementation of bioremediation technologies for the oxidation of CBZ in soil. However, the use of biotechnology for CBZ treatment has been hampered by its low degradation efficiency and lengthy degradation period. Biodegradation removed less than half of the CBZ after 80 days of incubation. After incubation for 120 days, the biodegradation rate of CBZ was just 5.82–21.43%. With the rigor of industrial standards and the enhancement of land economic value, effective and fast soil remediation methods are important (Zou et al., 2020). According to the estimation of Chen et al. (2013), the fate and transport of PPCPs incorporated into the soil by recycled water irrigation are highly reliant on their sorption and degradation in soil. The sorption and degradation of PPCPs are nearly responsible for from 27 to 98% and from 0 to 70% for the fate of the total PPCPs mass introduced in sandy loam soil, respectively.

Chemical oxidation, as an effective and promising strategy for the remediation of organic soil contamination, has advantages of comparatively short remediation duration, low cost, and performance against multiple pollutants. $Na_2S_2O_8$, H_2O_2, $KMnO_4$ and ozone oxidants are commonly used and evaluated in the chemical oxidation of organic pollutants. Hypochlorite, as a comparatively weak oxidant, has also been used in water treatment, but has seldom been recorded for soil remediation (Zou et al., 2020). The effect of NaOCl dose the control test showed that CBZ could not be degraded in the absence of NaOCl; however, when the NaOCl dosage was increased from 0 to 100 mmol kg^{-1}, the degradation efficiency of CBZ improved drastically. After 4 h of the reaction, the removal rate of CBZ rose from 41.3 to 85.1 %, with the NaOCl dose rising from 25 to 75 mmol kg^{-1} at the Fe^{2+} /NaOCl molar ratio of 2:1. The result suggested that an improvement in the NaOCl dose could greatly facilitate the removal rate of CBZ. This could lead to an enhanced generation of reactive radicals (HO percent) at relatively high concentrations of NaOCl, which was necessary for CBZ to withstand competition from other soil components. However, a further increase in NaOCl concentration (100 mmol kg^{-1}) resulted in less change in CBZ degradation performance (89.6 %). The explanation for this could be that excessive NaOCl may respond with HO percent to form ClO%. The pH conditions (3, 5, 7, 8, 14, 9, 11) were related to the Fe^{2+}/H_2O_2 and $Fe^{2+}/Na_2S_2O_8$ processes. The highest

CBZ degradation efficiency (82.6%) could be achieved at pH 3 in the Fe^{2+}/H_2O_2 phase. Persulfate is a competitive oxidant with good reactivity, but the result showed that the degradation of CBZ by the Fe^{2+}/NaOCl process was higher than that of the $Fe^{2+}/Na_2S_2O_8$ process in the 3–11 pH range. The maximal degradation efficiency of CBZ for $Fe^{2+}/Na_2S_2O_8$ at pH 5 (85.05%) was still lower than that of Fe^{2+}/NaOCl at pH 7 (90.35%). The result showed that the effect of the acidic or alkaline atmosphere on the removal of CBZ in soil by the Fe^{2+}/NaOCl process was negligible. CBZ elimination pattern under varying temperature conditions (30, 40, 50, 60 °C). During the first 15 minutes of the reaction, the CBZ removal rate rose dramatically with an increase in temperature. After 5 minutes of reaction, the degradation rates of CBZ at 30, 40, 50 and 60 °C were 37.7, 40.0, 45.3 and 70.1 % respectively. The result revealed that the temperature rise will dramatically reduce time for the reaction to achieve equilibrium, from 4h at ambient temperature to 1h after heating. Increased temperatures could increase the effective likelihood of collisions between NaOCl and CBZ molecules, which could lead to the degradation of CBZ by the Fe^{2+}/NaOCl mechanism (Zou et al., 2020).

3.4.3.2. Effect of carbamazepine on plant growth and nutrient composition

The concentrations of CBZ in nutrient solution and of cucumber xylem sap nutrient solution throughout harvest were 65.9 and 76.1 μg L^{-1}, respectively. These two values were statistically alike, suggesting that carbamazepine absorption can be considered passive (Shenker et al., 2011). The mean soil water partition coefficient (K_d) measured using soil and pore water concentrations for both treatments was 1.5 ± 0.09 and 1426.3 ± 553.0 L kg $^{-1}$ for CBZ and verapamil ($C_{27}H_{38}N_2O_4$), respectively. The lower sorption coefficients are most likely due to the lower organic carbon and soil clay content used in this study. CBZ and verapamil were taken in a dose-dependent way at all exposure doses, including an environmentally realistic 5 μg/kg. A connection plot between pore water and leaf concentration shows that the improved bioavailability of APIs (Active Pharmaceutical Ingredient) in pore water is primarily responsible for this increased absorption of zucchini. For CBZ, leaf concentrations rose from 0.2 ± 0.02 mg kg $^{-1}$ to 821.9 ± 120.2 mg kg $^{-1}$ (DW) at the maximum degree of therapy (10 mg kg $^{-1}$). Visible symptoms of necrosis in older leaves have been observed in plants treated with CBZ at concentrations greater than 4 mg kg^{-1} (burnt leaf edges and white spots). The necrosis rate improved with an increase in the concentration of CBZ and was first detected 1 week after germination. These visible results corresponded to a large difference between the above-ground biomass ($p<0,05$) and the controls for the 8−10 mg kg^{-1} treatments where biomass had reached a limit of 60% of the control. There was also a marked decline in root development in all CBZ procedures, hitting just 30 per cent of the control at the maximum stage of therapy and being slightly different for >1 mg kg $^{-1}$ compared to control ($p<0,05$) (Carter et al., 2015).

Accumulation of CBZ on C. Pepo (Cucurbita pepo) fruiting, like female C. Pepo flowers were not able to set fruit when the leaf concentrations were ≥ 14 mg kg $^{-1}$. In addition to the noted variations in the chlorophyll content of old and young plants, there was a clear variation in the presentation of old and young leaves. This may mean that vital nutrients were transferred to the young leaves, leaving old leaves lacking in nutrients such as N. Absolute N concentrations in young and old leaves relative to N concentrations in young leaves increased dramatically ($p<0,05$) as CBZ concentrations in soil increased from 2 mg kg $^{-1}$ to 10 mg kg $^{-1}$. However, no major variations were found in the overall N concentrations of the old leaves (p = 0.23 to 1.0). The concentration of N in the young leaves was smaller than in the old leaves. This was surprising due to the usual versatility of N within plants where N is prioritized for areas of new growth. N is also an essential part of proteins, carbohydrates, hormones, and chlorophyll. While chlorophyll content decreased in old leaves with growing concentrations of CBZ in the soil, the use of N in the plant can be inhibited in the presence of CBZ (Knight et al., 2017).

4. Material and Methods

4.1. Soil collection and description

The soil was collected from the top 20 cm of Suchdol experimental field Czech university of life science, (50°7′N, 14°22′E) soil type is chernozem, with a soil pH 6.90 and the textural class is silt clay loam soil. The collected soil was air-dried, sieved to 10 mm and 5 kg of soil (dry weight) was filled to 45 pots with 6 L holding capacity.

4.2. Experimental design

Carbamazepine was purchased from Sigma Aldrich in the form of powder and came in a resealable glass bottle and was kept at 4°C, the pot experiment was performed in the open-air rainfall-controlled vegetation hall of the Department of Agro-environmental Chemistry and Plant Nutrition. Plants of maize (Zea mays L.) were grown in 6L plastic pots filled with a mixture of soil and CBZ mixtures 5 kg (d.w.) of soil. To achieve the aims of the study 5 treatments were set up in 9 replicates (3 replicate for the first harvest, 3 replicate second harvest, and the rest 3 for harvest at maturity) including control, carbamazepine concentrations (CBZ) of 0,1 ppb, 1 ppb of CBZ, 10 ppb CBZ and 1 ppm of CBZ/5kg dry soil. Among the nine replicates of each treatment, 3 of them were harvested at the 30 days after sowing (DAS), the other 3 at the 60 DAS, and the remaining at the maturity of 90 DAS (Table 5).

All pot received basal fertilization 3 days before the sowing in a form of water solution. The amount of applied nutrients was as follows: 100 mg N/kg (dw) of soil (NH_4NO_3), 80 mg K/kg (dw) of soil and 32 mg P/kg (dw) of soil (K_2HPO_4) all treatments were fertilized.

Term of harvest	**Treatments CBZ**				
	Control	0,1 ppb	1 ppb	10 ppb	1 ppm
First round harvest pot No.	1,2,3	4,5,6	7,8,9	10,11,12	13,14,15
Second round harvest pot No.	16,17,18	19,20,21	22,23,24	25,26,27	28,29,30
Third round harvest pot No.	31,32,33	34,35,36	37,38,39	40,41,42	43,44,45

Table 5. The experimental design corresponds to the CBZ concentration and the number of pots.

4.3. Crop cultivation

The soil is thoroughly mixed with carbamazepine and fertilizer before enters the pot after the establishment of the pots and the addition of the CZB, 8 maize seeds were sown and thinned to 4 plants at a depth of 2 cm on 5th June 2020. Irrigated prior to sowing to facilitate optimal germination of the seeds. Irrigation was also extended daily to 60 % of soil maximum water holding capacity. Thinning of seedlings were applied after at the stage of 3rd leaf development (2weeks).

4.4. Crop harvest and soil sampling

Separate plant parts (leaves, roots, and grain) of maize were harvested on 21st July 2020, 24th August 2020, and 24th September 2020 respectively and stored in separate bags. Both plant and soil samples were held in a freezer (-40 C) before extraction. From the 1st, 2nd, and 3rd harvest roots of plants from each pot is washed vigorously on a wire mush by running demineralized water to extract soil particles and then dried in open air. All plant parts were oven-dried at 70°C, dry weight of biomass and root was taken and milled. After each harvest of plants, a 50 g of soil samples were collected.

4.5. Soil and plant analysis

The concentration of nutrients in maize were determined after digestion of milled 0.5 g of maize biomass and 0.25 g of root with the mixture of 8 ml concentrated nitric acid HNO_3 (Analytika) and 2 ml (30% v/v) H_2O_2 (Analytika) in Ethos 1 microwave oven (Milestone). Similarly, the extraction of soil was done using 0.01 M $CaCl_2$ (1:10 w/v).

Then concentrations of nutrient in both soil and plant were determined by optical emission spectrometer with inductively coupled plasma ICP-OES (Varian Vista Pro, Varian Australia) and that of higher potassium concentrations were determined using flame atomic absorption spectrometer F-AAS (Agilent AA285S, Agilent Australia). In this experiment both, the plant and soil samples are analyzed, nutrient content have been done determined, and the effect of carbamazepine was analyzed.

4.6. Statistical methods

The data were compared using one-way ANOVA, followed by a post-hoc TUKEY test. The statistical studies were carried out using the IBM SPSS Statistics 26 program (2020). In addition, MS Excel 2020 was used to compute means, standard deviations and draw figures.

5. Results

5.1. Maize biomass yield

5.1.1. Fresh weight

The fresh maize biomass yield was not significantly affected by the application of CBZ. The highest fresh biomass was recorded from the control pot (38.4 g pot $^{-1}$) while the lowest fresh biomass was recorded from the pot receiving 1 ppm CBZ (32,3 g pot $^{-1}$) on the first-round harvest (30 days after sowing). Again, at the second-round harvest (60 days after sowing), the highest and the lowest fresh biomass yield was from the control pot (93.7 g pot $^{-1}$) and from the pot receiving 1 ppm CBZ (86.9 g pot $^{-1}$) respectively. And when we come to the third-round harvest (90 days after sowing), the highest fresh biomass yield was recorded from the pot receiving 1ppm CBZ (127.9 g pot $^{-1}$), while the lowest fresh biomass was recorded from the control pot (112.3 g pot $^{-1}$). Moreover, the yield of fresh biomass decreased as the concentration of CBZ increased for the round 1 and 2 however, the opposite was true in the case of round 3.

Term of harvest	**Treatments CBZ**				
	Control	0,1 ppb	1 ppb	10 ppb	1 ppm
Round 1	38,4^{a} *(2,5)*	36,2^{a} *(1,9)*	33,7^{a} *(0,5)*	34,7^{a} *(3,8)*	32,3^{a} *(2,0)*
Round 2	93,7^{a} *(2,2)*	92,4^{a} *(8,5)*	89,5^{a} *(1,1)*	88,5^{a} *(3,1)*	86,9^{a} *(3,0)*
Round 3	112,3^{a} *(8,1)*	114,4^{a} *(21,0)*	111,6^{a} *(8,8)*	122,2^{a} *(11,1)*	127,9^{a} *(6,1)*

Table 6. The fresh biomass yield of maize (g pot^{-1}). All the values are means (n=3). The standard deviations are indicated in italic brackets, different letters indicate significance difference between treatments of the same round.

5.1.2. Dry weight

The dry biomass yield was not significantly affected by the application of CBZ. At the first-round harvest (30 days after sowing), the highest dry biomass was recorded from the control pot (5.33 g pot^{-1}), while the lowest dry biomass yield was recorded from the pot receiving 1 ppb CBZ (4.05 g pot^{-1}). At the second-round harvest (60 days after sowing), the highest and the lowest dry biomass yield was from the pot receiving 0.1 ppb CBZ (16.4 g pot $^{-}$

^{1}) and from the pot receiving 1 ppb CBZ (14.2 g pot $^{-1}$) respectively. And when we come to the third-round harvest (90 days after sowing), the highest dry biomass was recorded from the pot receiving 1ppm CBZ (32.12 g pot $^{-1}$), while the lowest recorded from the control treatments (26.62 g pot $^{-1}$) (Table 7). The dry maize biomass yield decreased with the increment in the concentration of CBZ at the rounds 1 and 2 however, the opposite was true in the case of round 3. The dry biomass yield is consistent to the fresh biomass yield.

Term of harvest	**Treatments CBZ**				
	Control	0,1 ppb	1 ppb	10 ppb	1 ppm
Round 1	5,33^{a}	4,50^{a}	4,05^{a}	5,04^{a}	4,82^{a}
	(0,49)	*(0,21)*	*(0,52)*	*(0,99)*	*(0,40)*
Round 2	14,48^{a}	16,37^{a}	14,19^{a}	14,65^{a}	14,68^{a}
	(0,64)	*(1,77)*	*(0,53)*	*(0,99)*	*(1,53)*
Round 3	26,62^{a}	27,21^{a}	30,27^{a}	30,24^{a}	32,12^{a}
	(0,30)	*(4,35)*	*(4,87)*	*(3,23)*	*(0,66)*

Table 7. The dry biomass yield of maize (g pot^{-1}). All the values are means (n=3). The standard deviations are indicated in italic brackets, and different letters indicate significance difference between treatments of the same round.

5.2. Concentrations of major nutrients in the maize biomass

5.2.1. Nitrogen

The N concentration in the maize biomass was significantly affected by the application of CBZ only at the first round of harvest. The addition of 1ppm CBZ significantly decreased the concentration of N in the maize biomass as compared to control at round 1 but in the rest of the treatments the concentration of N was not significantly different than the control. However, the concentration of N in the maize biomass grown on 1ppm, 1ppb, 10ppb, and 0.1ppb CBZ treatments as compared to control decreased by 18.1%, 9.5%,7.0%, and 7.0%, respectively in the first-round harvest. In the second-round harvest, the concentration of N in the maize biomass grown on the 1ppb, 0.1ppb, 10ppb, and 1ppm CBZ decreased by 12.3%, 11.62 %, 8.5 %, and 7.26 %, respectively as compared to the control. At the third round of harvest, the concentration of N in maize biomass grown on 1ppb,10ppb, and 0.1ppb CBZ treatments as compared to control decreased by 4.7%, 7.4%, and 7.9%, however the pot receiving 1ppm CBZ increased by 3.4% as compared to control. The concentration of N was higher for the first harvest and then exhibited a decrease at the second and third harvest times (Figure 4).

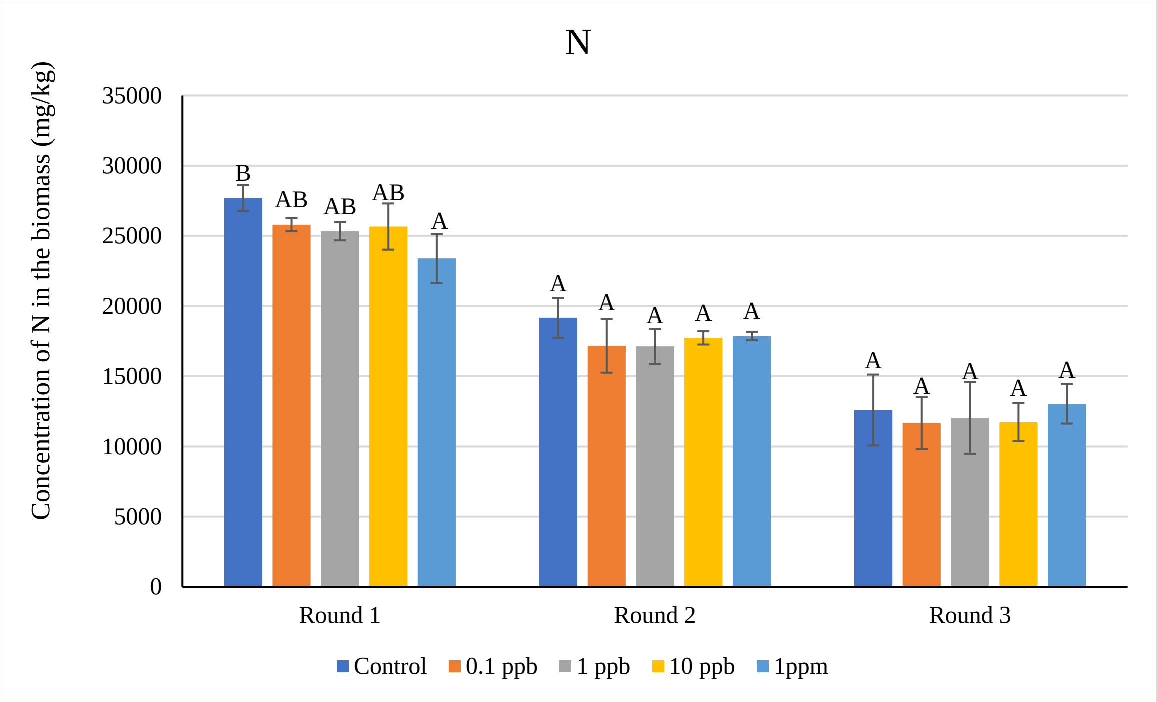

Figure 4. N concentration in the maize biomass (mg kg^{-1}). Different letters indicate significance difference between treatments of the same round.

5.2.2. Phosphorous

Apart from nitrogen, the concentration of P in maize biomass did not vary significantly (mg kg^{-1}) from the control in each of the three harvests. At the first harvest time, the concentration of P in maize biomass grown on 1ppm, 10ppb, 1ppb, and 0.1 ppb CBZ treatments as compared to control decreased by 27.8%, 18.77%, 18.49%, and 7.9%, respectively. In the second-round harvest, the concentration of P in the maize biomass of 1 ppb, 1 ppm, and 10 ppb CBZ treatments decreased by 20.0%, 17.4%, and 1.6%, respectively as compared to the control, however the pot receiving 0.1ppb CBZ increased by 2.16 % as compared. In addition, the concentration of P in maize biomass grown on 1 ppb, 0.1 ppb, 1 ppm, and 10 ppb CBZ treatments decreased by 50.02 %, 40.35 %, 30.94 %, and 9.7 %, respectively, as compared to the control at the third-round harvest. Furthermore, the concentration of P in the sound round harvest was 46.32% and 36.7% higher than in the first and third round harvests, respectively. The concentration of P in maize biomass was lower during the first harvest, increased during the second harvest, and then decreased during the third harvest (Figure 5).

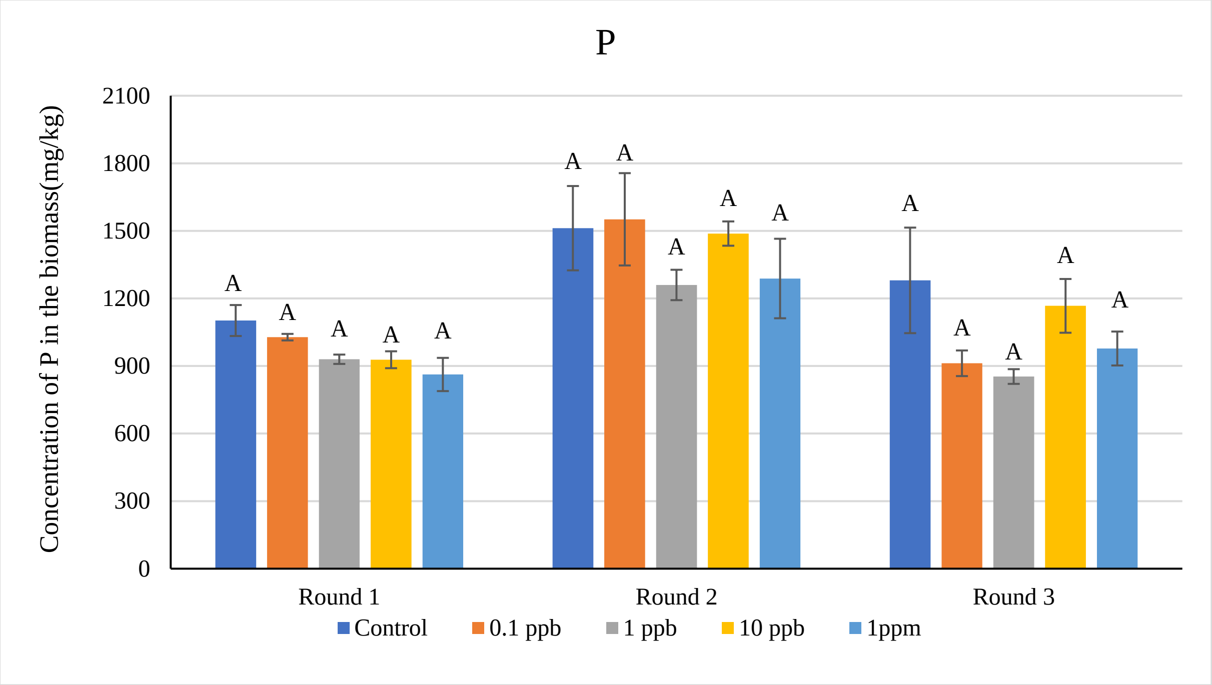

Figure 5. P concentration in the maize biomass (mg kg^{-1}). Different letters indicate significance difference between treatments of the same round.

5.2.3. Potassium

Following phosphorus, none of the treatments demonstrated any significant difference in K concentration of maize biomass (mg kg $^{-1}$) as compared to the control. The concentration of K in maize biomass grown on 1ppb, 0.1ppb, and 1ppm treatments as compared to control decreased by 18.8%, 7.98%, and 5.72% respectively, however, 10 ppb treatment increased by 9.65 % in the first round. In the second round, the concentration of K in maize biomass decreased by 54.82%, 34.12%,23.14%, and 16.21% as compared to the control in the pot receiving 0.1 ppb, 1ppb,1ppm, and 10 ppb treatments. And for the third round the concentration of K in maize biomass grown on 10 ppb, 0.1ppb,1ppb, 1 ppm treatments as compared to the control decreased by 17.86%, 17.86%, 10.07%, and 9.18% respectively. Concentration K was higher for the first and second collection time and then exhibited a radical decrease at the third collection time (Figure 6).

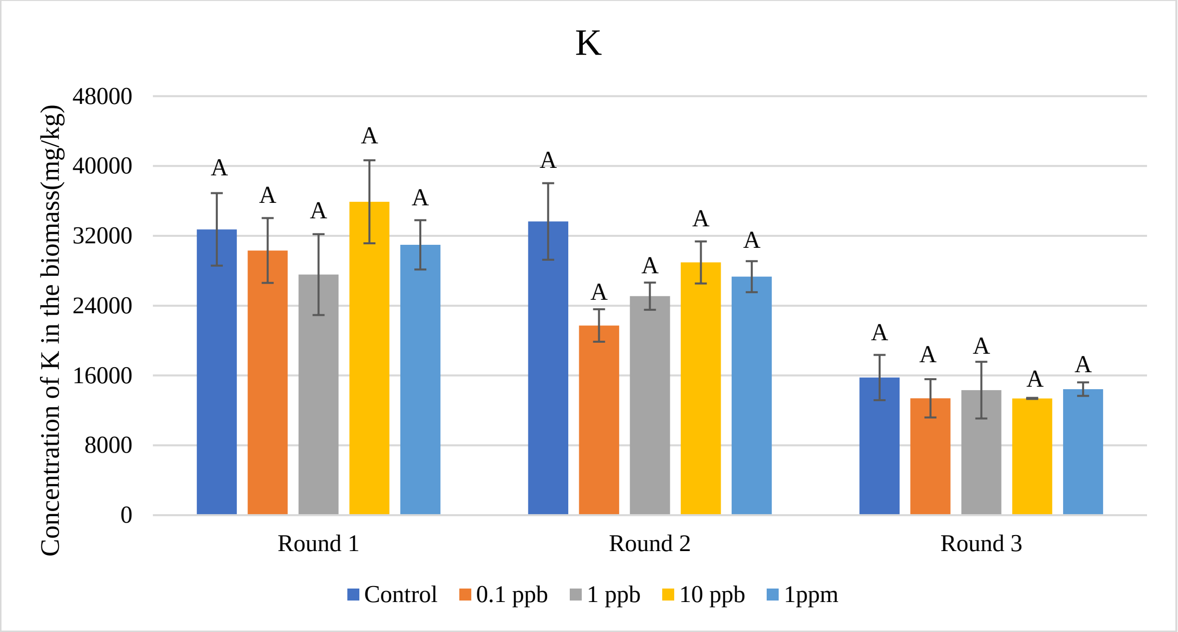

Figure 6. K concentration in the maize biomass (mg kg^{-1}). Different letters indicate significance difference between treatments of the same round.

5.2.4. Magnesium

Magnesium, similar to phosphorus all treatments had no significant difference in Mg concentration of maize biomass (mg kg^{-1}) compared to the control. At the first harvest time the concentration of Mg in maize biomass grown on 1ppb, 1ppm, 0.1ppb, and 10ppm, as compared to control decreased by 20.05%, 11.3%, 10.2%, and 5.72% respectively. In the second round, the concentration of Mg in maize biomass decreased by 23.12%, 10.7 %,10.52%, and 10.49 % as compared to the control in the pot receiving 1 ppb, 10 ppb,1ppm, and 0.1 ppb treatments. And for the third round the concentration of Mg in maize biomass grown on 1 ppm, 10 ppb, and 0.1ppb as compared to the control decreased by 26.8 %, 3.85 %, and 3.42 % however the pot receiving 1 ppm CBZ increased by 26.8% as compared to the control. During all three harvests, the Mg concentration in maize biomass remained relatively constant (Figure 7).

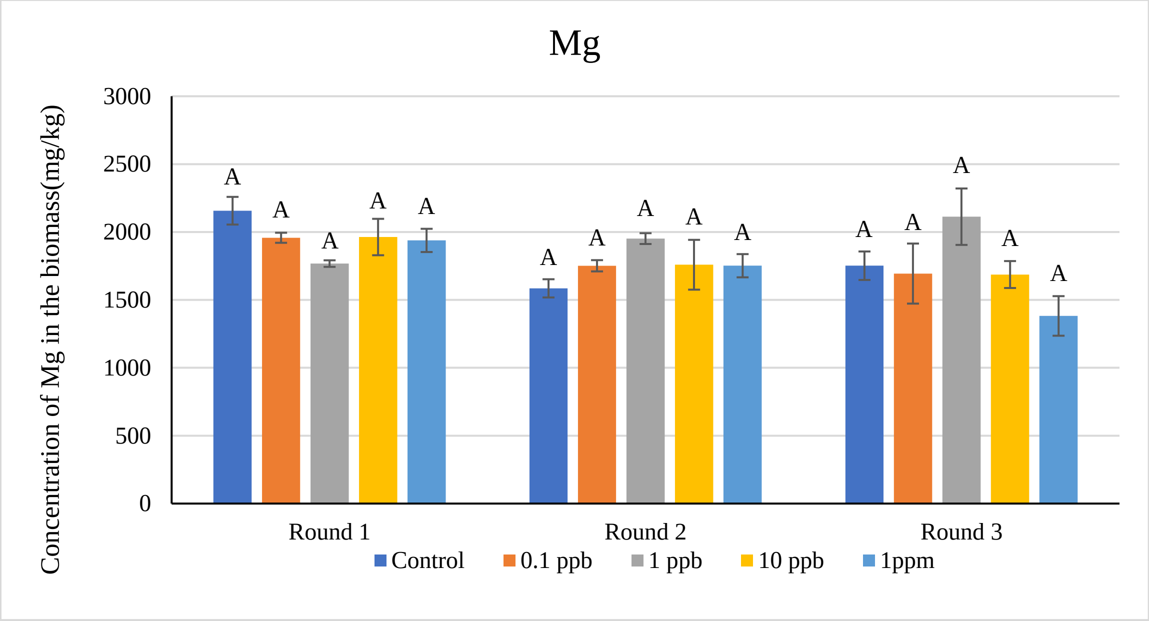

Figure 7. Mg concentration in the maize biomass (mg kg^{-1}). Different letters indicate significance difference between treatments of the same round.

5.3. Uptake of major nutrients by maize

5.3.1. Nitrogen

Nutrient uptake is the process by which plants acquire all the elements required for growth. The results showed that applications of different CZB levels had a significant effect) on the uptake of N. The maximum uptake of N (418 mg pot^{-1}) was found in the pot receiving 1 ppm CBZ from the third-round harvest, while the minimum uptake of N (102.5 mg pot^{-1}) was found in the pot receiving 1ppb CBZ from the first-round harvest.

The addition of 1ppm and 1 ppb CBZ in the soil significantly decreased the uptake N in round 1, but the rest of the treatments were not significantly different than the control N. The N uptake increased with the growing stage of maize. In the first-round harvest, N uptake by maize grown on 10ppb, 0.1ppb, 1ppm, and 1ppb treatments decreased by 15.12%, 27.10%, 31.17%, and 44.09%, respectively, as compared to the control. In the second round, the uptake of N by maize of 1ppm, 10ppb, and 1ppb treatments decreased by 5.7%, 6.54%, and 14.3% respectively as compared to the control however, in the 0.1ppb treatment the uptake of N increased by 0.83 % as compared to the control. In the third round, the uptake of N in 0.1 ppb CBZ treatment decrease by 7% as compared to the control however in the 10 ppb, 1 ppb, and 1 ppm treatments the uptake of N increased by 4.9%, 8.07%, and 24.5%, respectively as compared to the control (Table 8).

Term of harvest	**Treatments CBZ**				
	Control	0,1 ppb	1 ppb	10 ppb	1 ppm
Round 1	147,7^{b} *(17,5)*	116,2^{ab} *(6,7)*	102,5^{a} *(11,1)*	128,3^{ab} *(16,2)*	112,6^{a} *(9,6)*
Round 2	277,0^{a} *(11,8)*	279,3^{a} *(21,5)*	242,7^{a} *(9,5)*	260,0^{a} *(22,2)*	262,1^{a} *(25,2)*
Round 3	335,7^{a} *(69,4)*	313,7^{a} *(35,8)*	362,8^{a} *(84,1)*	352,2^{a} *(18,5)*	418,0^{a} *(36,5)*

Table 8. Uptake of N by maize biomass (mg pot-1) all values are means (n=3). Different letters indicate a significant difference between treatments of the same round.

5.3.2. Phosphorous

Following nitrogen, there was no significant effect of CBZ application on P uptake. The uptake of P increased with the growing stage of maize. The maximum uptake of P (35.2 mg pot^{-1}) was found in the pot receiving 10 ppb CBZ from the third-round harvest, while the minimum uptake of P (3.1 mg pot^{-1}) was found in the pot receiving 1ppb CBZ from the first-round harvest (Table 9).

In the first round, P uptake by maize grown on 10ppb, 0.1ppb, 1ppm and 1 ppb treatments decreased by 12.5%, 17.4%, 28.6%, and 74.2% respectively, as compared to the control. In the second round, the uptake of P by maize 0.1 ppb, 10 ppb, 1ppm, and 1 ppb CBZ treatments decreased by 4.7%, 22%, 41.5%, and 48.6% respectively as compared to the control. In the third round, the uptake of P in 1ppm,1ppb, and 0.1 ppb CBZ treatments decreased by 2.8%, 14.5%, and 17.8%, respectively as compared to the control, however, in the 10ppb treatment the uptake of P increased by 8.9 % as compared to the control.

Term of harvest	**Treatments CBZ**				
	Control	0,1 ppb	1 ppb	10 ppb	1 ppm
Round 1	5,4^{a} *(1,4)*	4,6^{a} *(0,3)*	3,1^{a} *(1,6)*	4,8^{a} *(1,0)*	4,2^{a} *(0,4)*
Round 2	26,6^{a} *(6,8)*	25,4^{a} *(4,2)*	17,9^{a} *(1,2)*	21,8^{a} *(1,1)*	18,8^{a} *(2,1)*
Round 3	32,3^{a} *(8,2)*	27,4^{a} *(5,8)*	28,2^{a} *(8,0)*	35,2^{a} *(3,9)*	31,4^{a} *(1,8)*

Table 9. Uptake of P by maize biomass (mg pot^{-1}) all values are means (n=3). Different letters indicate significance difference between treatments of the same round.

5.3.3. Potassium

Following phosphorus, potassium was the third nutrient analyzed, and the findings revealed that various CZB levels had a significant effect on maize K uptake. The addition of 1ppb in the soil significantly decreased the uptake of K in the first-round harvest, but the rest of the treatments was not significantly different than the uptake of K by the maize grown on the control treatment. The maximum uptake of K by maize (533.4 mg pot^{-1}) was found in the control pot from the second-round harvest, while the minimum uptake of K (109.3 mg pot^{-1}) was at the 1ppb CBZ treatment from the first-round harvest.

In the first round, uptake of K by maize grown on 10 ppb, 1 ppm, 0.1 ppb, and 1 ppb treatments decreased by 11.3%, 33.6%, 45.6%, and 81.7 %, respectively, as compared to the control. In the second round, the uptake of K by maize decreased by 11.3%, 33.6%, 45.6%, and 81.7 % as compared to the control in the pots receiving 10 ppb, 1ppm, 1 ppb, and 0.1 ppb CBZ. However, in the third round, the uptake of K in 0.1 ppb, 10 ppb, 1ppb, and 1 ppm CBZ treatments increased by 29.3%, 22.85%, 12.8%, and 1.3%, respectively as compared to the control (Table 10).

Term of harvest	**Treatments**				
	CBZ				
	Control	0,1 ppb	1 ppb	10 ppb	1 ppm
Round 1	198,6^{b} *(45,8)*	136,4a^{b} *(15,9)*	109,3^{a} *(31,0)*	178,5a^{b} *(20,2)*	148,7^{ab} *(5,6)*
Round 2	533,4^{a} *(274,0)*	354,8^{a} *(36,3)*	355,4^{a} *(10,4)*	422,9^{a} *(17,5)*	399,3^{a} *(18,9)*
Round 3	358,4^{a} *(113,7)*	363,2^{a} *(71,4)*	440,3^{a} *(158,3)*	404,3^{a} *(41,3)*	463,5^{a} *(15,5)*

Table 10. Uptake of K by maize biomass (mg pot^{-1}) all values are means (n=3). Different letters indicate significance difference between treatments of the same round.

5.3.4. Magnesium

The last nutrient analyzed was Mg. According to the results, CBZ implementation had no significant effects on Mg uptake. The uptake of Mg increased with the growing stage of maize. The maximum uptake of Mg (55.0 mg pot^{-1}) was found in the pot receiving 1 ppb CBZ from the third-round harvest, while minimum uptake Mg (6.6 mg pot^{-1}) was also found in the pot receiving 1 ppb CBZ from the first-round harvest (Table 11).

In the first round, the uptake of Mg by maize grown on 10ppb, 0.1ppb, 1ppm, and 1 ppb treatments decreased by 11.2%, 14.7%, 25.3%, and 65.2% respectively, as compared to the control. In the second round, the uptake of Mg by maize of 1ppb, 0.1ppb and 10 ppb CBZ treatments decreased by 3.5%, 12.5% and 22.3%, respectively as compared to the control however, in the 1ppm treatment the uptake of Mg increased by 72.6% as compared to control. In the third round, the uptake of Mg in 10ppb,0.1ppb and 1ppm CBZ treatments decreased by 0.19%, 12.3% and 15.1% respectively as compared to the control, however, in the 1ppb treatment the uptake of Mg increased by 7.6 % as compared to the control.

Term of harvest	**Treatments CBZ**				
	Control	0,1 ppb	1 ppb	10 ppb	1 ppm
Round 1	$10,9^{a}$ *(2,5)*	$9,5^{a}$ *(1,6)*	$6,6^{a}$ *(3,6)*	$9,8^{a}$ *(1,4)*	$8,7^{a}$ *(1,3)*
Round 2	$29,6^{a}$ *(8,1)*	$26,3^{a}$ *(8,2)*	$28,6^{a}$ *(2,9)*	$24,2^{a}$ *(2,9)*	$51,1^{a}$ *(2,1)*
Round 3	$51,1^{a}$ *(8,4)*	$45,5^{a}$ *(2,1)*	$55,0^{a}$ *(23,7)*	$51,0^{a}$ *(5,7)*	$44,4^{a}$ *(5,1)*

Table 11. Uptake of Mg by maize biomass (mg pot^{-1}) all values are means (n=3). Different letters indicate significance difference between treatments of the same round.

5.4. Availability of major nutrient in the soil

5.4.1. Phosphorus

This is measured by the number of elements in the soil that can be taken up by plant roots and serve as essential nutrients for growing plants. In the first round, the addition of 1 ppb CBZ significantly decreased the concentration of soil P by 12.4% as compared to the control. However, the rest of the treatments were not significantly different than the control P in second and third round harvests (Figure 8). The concentration of P in the soil was slightly higher at the first round by 6.3% and 14% as compared to the second and third round harvest.

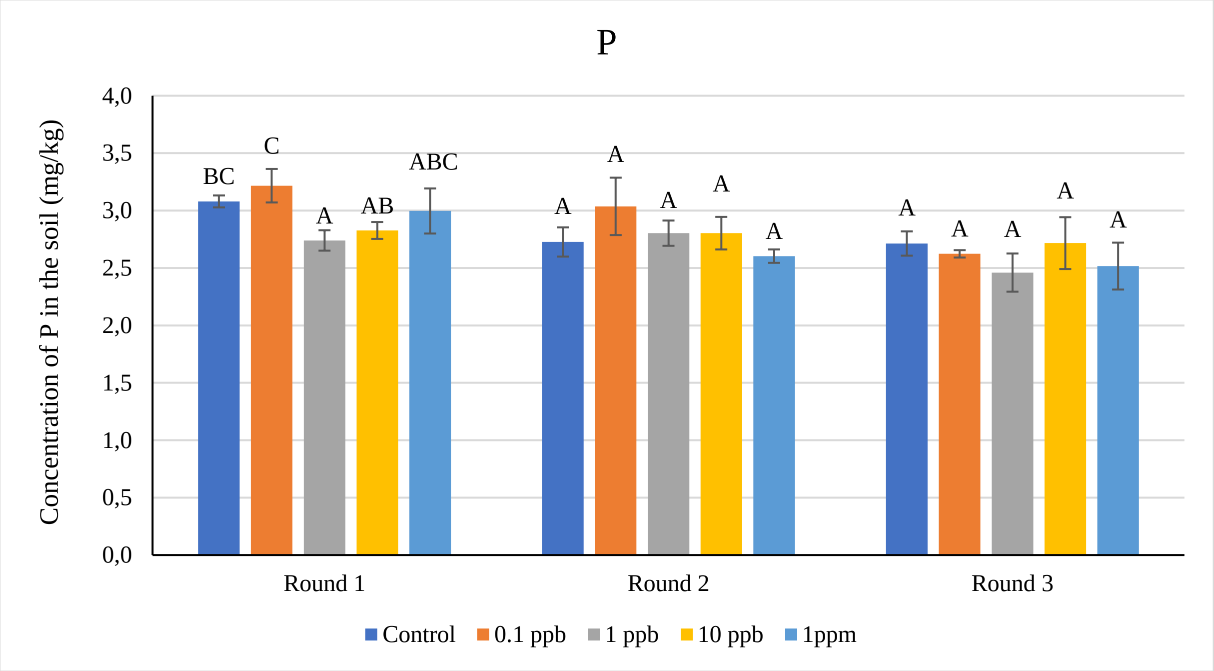

Figure 8. The available P concentration in the soil sample after each harvest (mg kg^{-1}). Different letters indicate significance difference between treatments of the same round.

5.4.2. Potassium

Only at the third round of harvest did the addition of 1 ppm CBZ significantly reduce the available K concentration in the soil sample by 20.8 % to the control. But in the rest of treatments there were no significant. The concentration of K was significantly higher in the first round as compared to the second and third round in the soil. The concentration of soil K was higher in the first-round harvest by 31.3% and 7.8% as compared to the second and third round harvests (Figure 9).

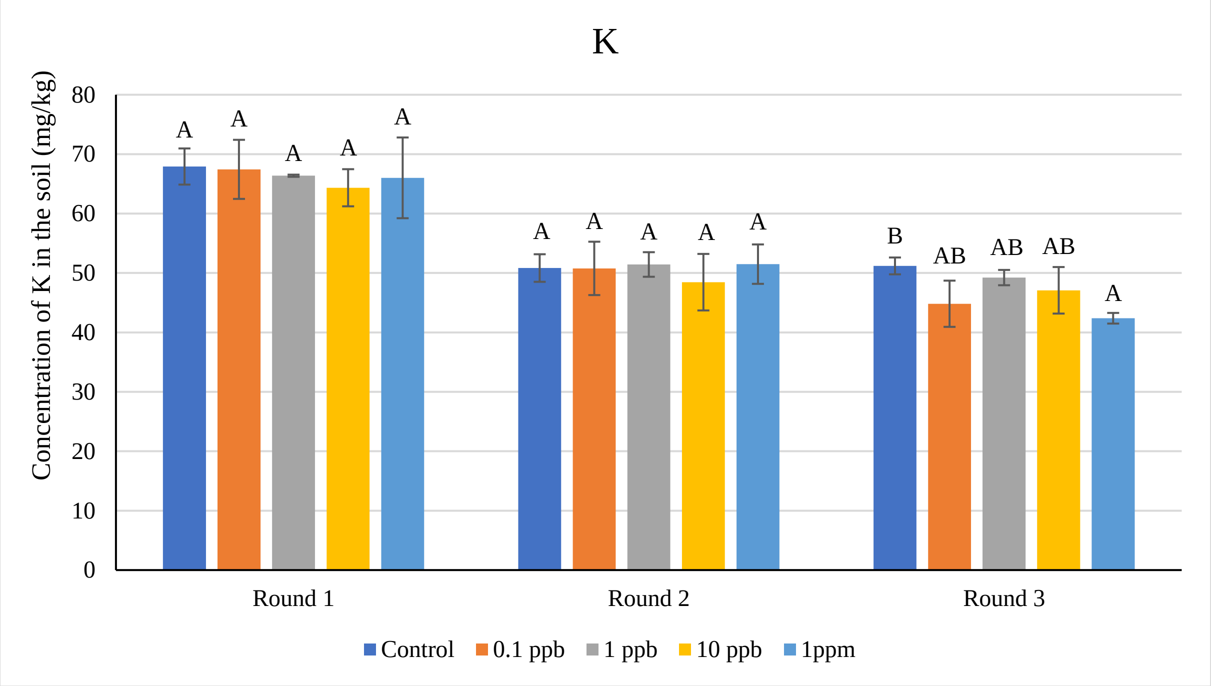

Figure 9. The available K concentration in the soil sample after each harvest (mg kg^{-1}). Different letters indicate significance difference between treatments of the same round.

5.4.3. Magnesium

Mg was the last nutrient analyzed, followed by P; at all three round harvests, there was no significant difference of Mg concentration in the soil between treatments. The concentration of Mg in the soil was at the same level in the first and second round however it was slightly lower in round. The concentrations of soil Mg were higher by 1.6% and 9.9% as compared to the second and third round harvests (Figure 10).

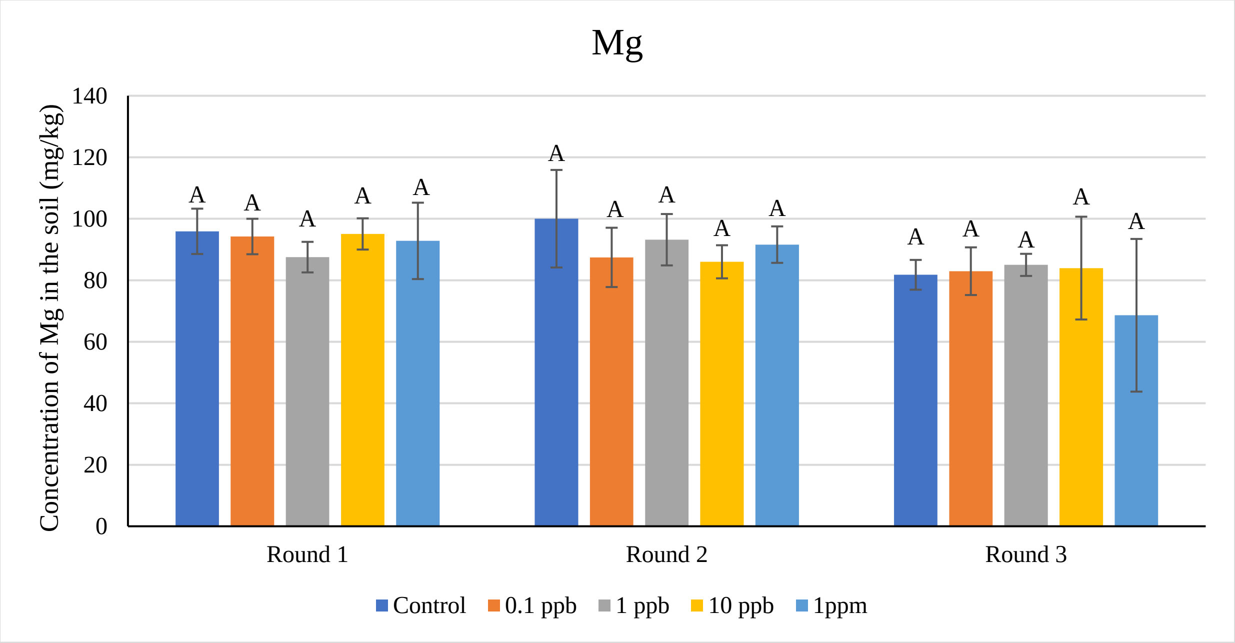

Figure 10. The available Mg concentration in the soil sample after each harvest (mg kg^{-1}). Different letters indicate significance difference between treatments of the same round.

6. Discussion

6.1. Biomass yield

According to the findings, there was no significant difference in maize fresh and dried biomass yield between all CBZ treatments among all three consecutive harvests. This result is consistent with the findings of other studies. For example, based on the study of Knight et al. (2017), the biomass yield of the *(C. pepo)* plant was not affected by the application of CBZ in the soil at very high rate of 20 mg kg^{-1}. This was again in agreement with the finding of Shenker et al. (2011), as they reported no significant difference in the biomass yield of the Cucumber plant by the application of CBZ at 1000 μg L^{-1}. Overall, the biomass yield from the various CBZ treatments coincided with earlier study findings. Even if several studies reported no significant impact on plant biomass yield, the weight of fresh and dry biomass was decreased as the concentration of CBZ increased in the first and second harvest however, the opposite was true for the third harvest. Our finding also supported by Knight et al. (2017) by saying there was no significant difference in overall biomass yield between all the treatments and the controls, but the general number of fruits decreased as CBZ concentration in the soil increased, and the number of fruits harvested from each round varied this shows that the CBZ treatments had negative effects on plant development. For example, the highest and the lowest fresh biomass was recorded from pots receiving 1 ppm at round 3 (127,9 g pot^{-1}) and 1 ppm at round 1(32,3 g pot^{-1}) respectively same is true for dry biomass the highest and the lowest dry biomass was recorded from pots receiving 1 ppm at round 3 (32.12 g pot^{-1}) and 1 ppm at round 1(4.05 g pot^{-1}), respectively (Table 6 and 7**).** Carbamazepine can be converted by plants into its active metabolite, 10,11-epoxide-carbamazepine. In a previous analysis, approximately 40% of the molar fraction of carbamazepine was detected in the form of the epoxy metabolite (Goldstein et al.,2014). The active epoxy carbamazepine was likely present in the maize leaf at concentrations comparable to carbamazepine in our sample, compared to epoxy carbamazepine, this could cause less of a danger to the plant.

Opposite to result of this study, Carter et al. (2015) reported a significant decrease in both above and below ground biomass after the application of CBZ to soil. While the significant was significantly only at higher concentrations (8 to 10 mg kg^{-1}). Additionally, they observed that plants treated with low CBZ concentration (0.005 mg kg^{-1}) induced a change in the plant hormone concentration, which increased the synthesis of auxin (IAA) and cytokinin (iP). So, since plant hormones play critical roles in the plant growth as well as biotic and abiotic stress responses, changes in their concentrations could possibly have a major impact on plant growth. Both of these plant hormones are responsible for a variety of processes, cell proliferation in the shoot and root apical meristems, as well as cell differentiation and organ outgrowth in the peripheral region (Eric et al., 2015). Excess iP and IAA production may therefore be responsible for stimulating shoot growth, possibly as a compensatory mechanism, therefore the effects CBZ on the iP and IAA could induce effect on the maize biomass yield.

6.2. The uptake and concentration of nutrients in the maize biomass

All CBZ treatments did not induced any significant effect on the concentration of P, K, and Mg by maize at all harvests. However, the addition of 1ppm significantly decreased the concentration of N in the biomass as compared to control in the first harvest. The addition of 1ppm CBZ in the soil significantly decreased the concentration of N by 18.1% in the biomass (Figure 4). The concentration of N was higher for the first harvest and then exhibited a decrease at the second and third harvest times. This is consistent with the findings of an earlier report. Knight et al. (2017), when CBZ concentrations in soil increased, N concentrations in young leaves increased as compared to the control. But at harvests, N concentrations in the controls were in the critical range for (*C. pepo*) leaves.

Concentration of P was lower for the first collection time and then revealed an increase in the second harvest and a decrease again in the third harvest (Figure 5). However, the addition of 1ppm in all harvests decreased the concentration of P in the biomass by 27.8%, 17.8%, and 30.94% in round 1,2 and 3 respectively. Subsequently the addition of 1ppb decreases the concentration of biomass P by 18.49%, 20.02%, and 50.0% at rounds 1, 2, and 3 respectively. N, P, and K concentrations are high in early-stage growth samples. Because of the dilution of carbohydrates and other structural solids, the abundance of those nutrients decreases as the plant matures and enters the bloom level (Jarrell and Beverly, 1981). This might be the reason for the concentration of N, P and K decrease at the second and third harvesting times. K concentration in the biomass almost similar in all treatments compared to that of the control (Figure 6). Concentration K was higher for the first and second collection time and then exhibited a radical decrease at the third collection time. For example, by the addition of 0.1 ppb in the soil decreased the K concentration by 7.98%, 54.82%, and 17.86% following the arrangement in all harvests. Mg concentration in the biomass is all most similar through all the harvesting times (Figure 7) this result was also supported by (Knight et al.,2017). The application of CBZ had no major effect on Mg uptake and concentration, which may be attributed to the high abundance of K in our soil sample compared to Mg. Thus, decreased Mg absorption by plants may be due to ion rivalry. This is consistent with some studies (Gransee and Führs, 2013), which show a decrease in Mg absorption by plants when there is a rise in Mg concentration in soil samples.

We observed that different CBZ applications had a major impact on the uptake of N and K (Table 8 and Table 10). In the first harvest, the addition of 1ppm and 1ppb significantly decreased the uptake of N by 31.7% and 44.1% respectively. And the addition of 1ppb significantly decreased the uptake of K in the first harvest by 81.7% as compared to the control. P and Mg had no significant effect on the uptake however the P and Mg uptake decrease as the concentration of CBZ increased such as the addition of 1 ppb in the soil decreased the up-take P by 74.2%, 48.6%, and 17.8% separately in all harvests. Accordingly, the addition of 0.1ppb in the soil decreased the uptake Mg by 14.7%, 12.5%, and 12.3% respectively. Knight et al. (2017) found that the concentration of K in young leaves from the 10 mg kg^{-1} CBZ treatment was significantly different from the control.

Generally, the uptake of N, P and K increased with the growing stage of maize. Optimal availability this nutrient is needed for proper plant uptake (Leghari et al, 2016; Mengel and Kirkby, 2001) and for these plants require a widespread root chain for into the soil to uptake the needed amount of nutrient. Then the nutrients moved towards the surface of the roots by mass flow, diffusion, and/or root interception could reach the surface of the root and then absorbed by plants either actively by the expenditure of energy or passively without the need for energy (Karthika et al., 2018). However, Sun et al. (2018) findings explicitly proved that pharmaceuticals induced morphological indicators improved at higher concentrations, with the effect being more noticeable in roots than shoots. Carter et al. (2015) also observed carbamazepine treatment at a concentration of (>1 mg kg^{-1}) in the zucchini plant showed a significant reduction in root development, the change in the root development could have occurred in our experiment and caused reduction on the uptake of N and K.

N is a critical component of proteins, hormones, and chlorophyll (Karthika et al., 2018). Sun et al. (2018) mentioned that with increasing CBZ treatment rates, the levels of chlorophyll a and chlorophyll b decrease. Since chlorophyll content decreased as CBZ concentrations in the soil increased, the use of N inside the plant could be influenced by the presence of CBZ. Besides the reduction in chlorophyll content indicates that the plant's photosynthetic potential is decreased. Our experiment demonstrated that the N and K uptake, and the availability were affected so this finding is directly related to N since root plants grew aboveground biomass first, they absorbed more N and K in the early stages. Carter et al. (2015) observed changes in plant hormone balances when carbamazepine > 4ppm in the soil is applied and as long as our maximum concentration was 1 ppm, we can’t see such an effect in maize. To date, there has been relatively little research into API-induced toxicity in plants. Accordingly, these all are our possible reasons for the decrement in the accumulation of plant nutrients and uptake, and further work is required to confirm or disprove these assumptions.

6.3. Availability of major nutrients in the soil

CBZ treatments induced a significant effect on the concentration of soil P and K. The addition of 1ppb CBZ significantly decreased the concentration of soil P (Figure 8) in the first harvest. P concentrations in the soil were slightly higher in the first collection time then it was decreasing negligibly then it remained almost constant for the remaining growing season. The concentration of soil K significantly affected by the addition of 1ppm CBZ treatment at the third-round harvest. But the rest of the treatments were not significantly different than the control (Figure 9). The concentration of K in the soil was relatively lower in second and third round harvest as compared to the firsts round.

In general, P and K concentrations in soil were smaller than controls in the first harvest for P and in the third harvest for K. This usually implies low nutrient availability from CBZ treatment. Knight et al. (2017) mentioned that K behavior was different based on the harvesting time in which its exhibited radical increase while the first collection and then remained constant which was a similar finding. This was again in agreement with the finding of Carter et al. (2015) as they reported the existence of APIs in the soil seems to be affecting P and K composition, though this was only seen in the carbamazepine treatments. K behavior was different based on the harvesting time in which its exhibited radical increase while the first collection and then remained constant. Mg concentration was constant throughout the harvest which was a similar finding (Knight et al.,2017).

Generally, the application rate of CBZ was affecting the availability of nutrients as their availability decrease depending on the application rate of CBZ for P and K. Uchida. (2000) reported that P is needed in the large quantity in young cells of shoots and root tips, where metabolism is high and cell division is rapid. But in our experiment, clearly shows the role of CBZ physicochemical properties in influencing plant absorption and toxicity and availability of major nutrients in the soil. In the other experiment contacted by Carter et al. (2015) observed that carbamazepine treatment showed a significant reduction in root development. This might be the reasons for the reduction of soil K and P.

Plant uptake and soil nutrients are also influenced by soil pH (Mengel and Kirkby, 2001). The pH of the soil will change after pharmaceuticals are added to the soil matrices. The degree of pH change seems to be influenced by both pharmaceutical physicochemical properties and soil composition, as shifts in relation to controls and across time were not uniform for all treatments by CBZ (Carter et al., 2016). However, in our experiment the soil pH was ranging between (6,61- 6,79) (Appendix A). The research soils selected for study were graded as both "silty sand" and "clayey loam." They investigate this with a broader variety of pharmaceuticals and soil types, but our experiment is limited to one kind of soil (silt clay loam soil), which may be the reason for the lack of a significant difference. These are our potential explanations for the decline in nutrient concentration in the soil caused by the application of CBZ. Further research is needed to validate or refute these hypotheses.

7. Conclusion

A pot experiment was set up in a greenhouse to investigate the effect of CBZ on the yield and uptake of nutrients by maize to investigate the effect of CBZ on the yield and uptake of nutrients by maize. Based on the result, the application of CBZ at all rates (0.1 ppb, 1 ppb, 10 ppb CBZ, and 1 ppm CBZ/5kg of dry soil) was not able to induce any significant effect on the fresh and dry maize biomass yield. However, there was insignificant decline of fresh and dry maize biomass as the concentration of CBZ increased for the first and second harvest time and insignificant increment in the case of third harvest time. On the other hand, the application of CBZ at the rate of 1ppm significantly decreased the concentration of N in maize biomass only at the first harvest time, while insignificant effect on the concentrations of P, K, and Mg. The significant decline of N concentration at the first harvest by the addition of 1ppm CBZ was by 18.1% compared to the control. Furthermore, the application of 1ppm CBZ significantly decreased the uptake of N at the first round of harvest, while the application of 1ppb significantly decreased the uptake of K at the second round of harvest. In the first harvest, the application of 1ppm and 1ppb CBZ decreased N uptake by 31.2% and 44.0%, respectively again 1ppb CBZ treatment reduced K uptake by 81.7 %. Furthermore, CBZ treatments had an impact on the concentration of P and K in soil. In the first harvest, the addition of 1ppb CBZ significantly reduced the concentration of soil P by 12.4%. As a result, the addition of a 1ppm CBZ treatment significantly reduced soil K concentration at third-round harvest, resulting a 20.75 % decrease in comparison to the control. There have been very few studies on the impact of CBZ on nutrient absorption and its association with plant nutrients in the soil, so we highly encourage further research on the topic. Overall, we can conclude from our research that the addition of carbamazepine to soil can affect maize nutrient uptake and soil nutrient supply during the early growing stages at higher CBZ concentrations.

8. References

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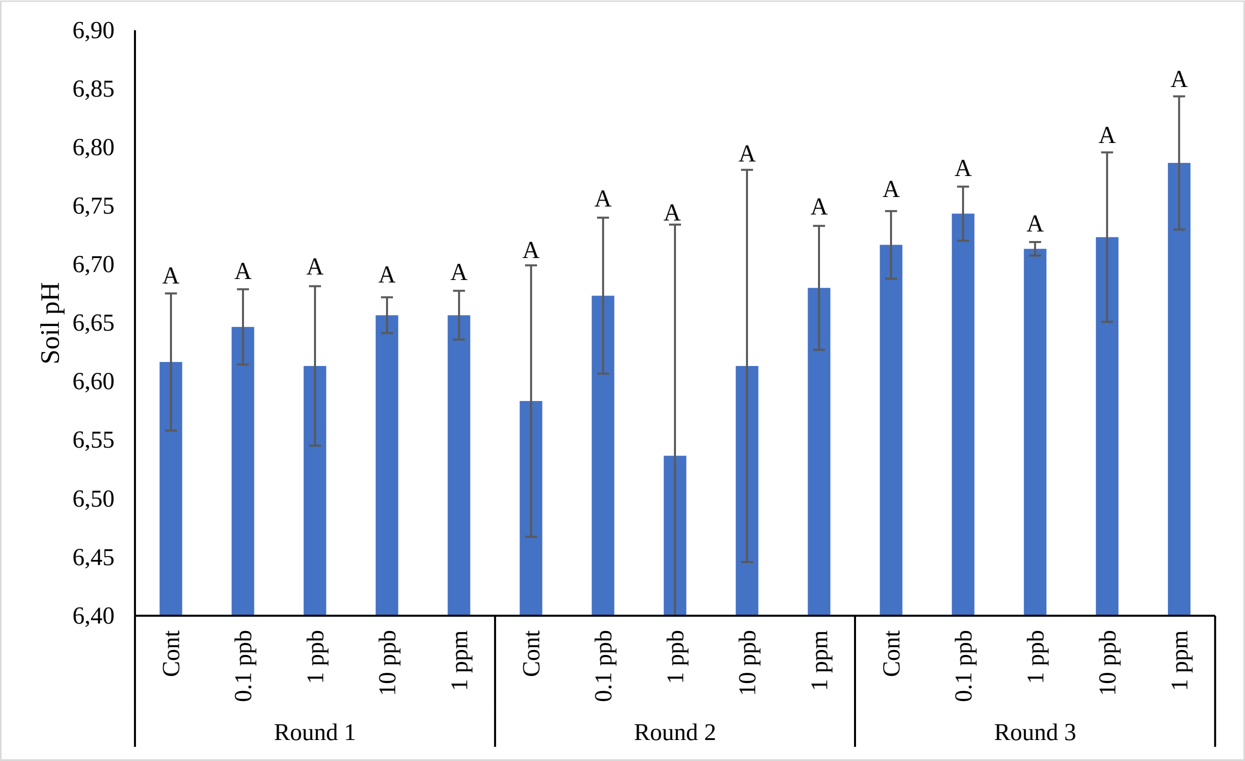
9. List of Appendices

Appendix A

Soil pH during the experiment

Term of harvest	Treatments CBZ				
	Control	0,1 ppb	1 ppb	10 ppb	1000 ppm
Round 1	6,62^{a} *(0,06)*	6,65^{a} *(0,03)*	6,61^{a} *(0,07)*	6,66^{a} *(0,02)*	6,66^{a} *(0,02)*
Round 2	6,58^{a} *(0,12)*	6,67^{a} *(0,07)*	6,54^{a} *(0,20)*	6,61^{a} *(0,17)*	6,68^{a} *(0,05)*
Round 3	6,72^{a} *(0,03)*	6,74^{a} *(0,02)*	6,71^{a} *(0,01)*	6,72^{a} *(0,07)*	6,79^{a} *(0,06)*

Annex 1. Soil pH All the values are means (n=3). The standard deviations are indicated in italic brackets, different letters indicate significance difference between treatments of the same round.



Annex 2. Soil pH graph. Different letters indicate significance difference between treatments of the same round.

Appendix B

Photographs of the experimental site's preparation and the first weeks after sowing

Appendix C

Photographs comparing maize plants 30 days after sowing and irrigation

Appendix D

Photographs comparing maize plants 60 days after sowing