ACCUMULATION OF COPPER IN VEGETABLES AND FRUITS

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Abstract. Soil pollution is a component that contributes to the increase of environmental pollution. The level of soil pollution is given by its degree of contamination with various pollutants, heavy metals, etc. Because agricultural products in general, respectively fruits and vegetables in this case, extract water and nutrients from the soil to develop, it is necessary to study how a certain degree of soil contamination leads to obtaining crops which contain a percentage of those toxic elements that can produce long-term sickness in people who currently consume such contaminated products. The paper presents the accumulation and transfer of copper from soil to vegetables (radishes and carrots, parsley and spinach leaves), berries (strawberries, black currants) and plums. In the experiments, fertile soil was used in which different amounts of copper sulphate were added (c1 = 33.2 $mg \cdot kg^{-1}$, c2 = 72.4 $mg \cdot kg^{-1}$, c3 = 265.1 $mg \cdot kg^{-1}$, c4 = 378.2 $mg \cdot kg^{-1}$) in four concentrations, taking as reference a sample of uncontaminated soil (c0 = 17.6 $mg \cdot kg^{-1}$). The resulting and well-homogenized soil was added to pots in which vegetable seeds, shrubs and trees in the second year of fruiting were planted. The paper aims to highlight the presence of copper in soils and its accumulation in the vegetative parts of vegetables and fruit consumed by humans. The correct diagnosis of nutritional deficiencies in plants is important in maintaining their growth and development. Excessive accumulation of heavy metals is a danger to the health of the human body.

Keywords: accumulation, copper, fruits, vegetables.

Introduction

Heavy metals are hazardous contaminants in food and the environment, and they are non-biodegradable. Source of contamination includes adding of manure, sewage sludge, fertilizers and pesticides to soils [1]. Other sources are: mining and processing metal ore, untreated wastewater for irrigation, the harvesting process, storage and/or at the point of sale [2].

Heavy metal elements, such as lead (Pb), cadmium (Cd), arsenic (As) etc., have toxic effects on human health. Toxic metals can accumulate persistently in the body throughout life. Other metallic elements such as copper (Cu) and zinc (Zn) are important nutrients for humans, but excessive ingestion can also adversely affect human health. For example, an excess of Cu can cause acute stomach and intestinal pain and liver damage [3].

Also, copper is bound to increase the availability of this nutrient if plants can take up the complex. However, some complexes are apparently less available, probably due to the fact that they are too large to be absorbed. Leaves turn yellowish, at the trees rough bark accompanied by gumming also occurs. Correction of the deficiency has been obtained with soil applications of copper sulphate, foliar chelate or bordeaux sprays in early spring or soil applications of copper has been observed with high applications of nitrogen, phosphorus, or zinc fertilizers [4].

Many studies identify the risks in relation to increased soil metal concentration and their plant uptake and thus affect food quality and safety [3; 5; 6].

For example, [7] reported the high level of Cu = 703 $mg \cdot kg^{-1}$ in the contaminated soil from Dabaoshan mine. The paper [8] presents potential health risk of heavy metal accumulation in vegetables irrigated with polluted river water. The mean concentration of copper found in the agricultural soil was Cu = 69.013 $mg \cdot kg^{-1}$. The mean concentrations of copper in edible parts of selected vegetables were Cu = 9.373 $mg \cdot kg^{-1}$.

In Romania, the paper [9] presents heavy metal content in vegetables and fruits cultivated in Baia Mare mining area and health risk assessment, the results showed that copper is more likely to accumulate in vegetables 1.4-196.6 $mg \cdot kg^{-1}$ than in fruits 1.9-24.7 $mg \cdot kg^{-1}$. In the paper [10] heavy metal concentrations varied among different fruit plums due to their different absorption capacity and the regional soil and atmospheric degree of pollution. Copper content in the plum leaf was higher than the threshold value at most locations. The determined value of copper in the fruit of plum was much lower than the limit value.

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In a literature review, [11] reported papers dealing with the influence of pollutants on the vegetable content and their impact on human health [12] and studies on the potential pathway of consuming vegetables grown on contaminated soil. Earlier studies confirmed that legumes and root vegetables are

tend to be low and medium accumulators and leafy vegetables are high accumulators. Carrot cultivars exhibited significant differences in accumulated concentrations of Cu.

Paper [13] presented summary of literature on the content of copper in different vegetables grown in diverse parts of the globe. The content of copper in: carrot roots = 0.007-11 $mg \cdot kg^{-1}$, radish roots = 0.02-16.8 $mg \cdot kg^{-1}$, spinach leaves = 0.02-14.5 $mg \cdot kg^{-1}$, parsley leaves = 6.28- 15.2 $mg \cdot kg^{-1}$.

Paper [14] presents uptake and translocation of Cu from a the nutrient solution with 0.5 $\mu mol \cdot l^{-1}$ Cu, applied continuously on the growth of strawberries in peat and perlite. The results showed that more than 65% of Cu was located in the roots, crowns and leaf blades each represented 10% while only 0.5% was located in the flowers.

Paper [15] presented the composition of mineral elements in black and red currant cultivars. The lowest amount was obtained in copper. The data obtained in this study do confirm that black and red currants represent a valuable source of different nutrients.

For the study were chosen root vegetables, leafy vegetables, berries often grown in greenhouses, strawberries, and fruits found in the forest, but they are also found in bushes and grasslands. Among the fruit trees, plum trees are the most common in our country's hill and mountain area. These vegetables and fruits were chosen to highlight the accumulation of copper in the vegetative parts of these plants, often consumed by humans.

Carrots (*Daucus carota* L.) and radish (*Raphanus sativus* L*)* are biennial or annual herbaceous plants with a pivotal soil root. They are grown in middle texture soils that are loose and rich in humus. As active principles they contain: carbohydrates, carotenes, potassium, calcium, phosphorus, vitamins A, B, C, K, proteins, fats, cellulose, mineral salts [16].

Spinach (*Spinacia oleracea* L.) and parsley (*Petroselium hortense*) are herbaceous plants, having a pivoting root in the soil, with basal leaves willing in rosette, long or short petiolate, having characteristic smell at parsley. They are grown in middle texture soils that are fertile permeable, loose, rich in humus and high moisture. As active principles they contain: carbohydrates, lipids, chlorophylls, Na, K, Ca, Mg, Cu, I, vitamins A, B1, B2, C, apiol, myristicin, terpineol, cymene [16].

Strawberries (*Fragaria moschata*) are herbaceous plants with a rhizome in the soil, covered with leaf residues, the stem is covered with hairs, they have trifoliate leaves and white flowers. The fruits are in a globose receptacle and are tasty and fragrant. They grow very well in humus-rich soils. They contain active principles: salts, oily substances, salicylic acid, iodine, vitamins A, B, C. Black currant (*Ribes nigrum* L.) is a shrub with lobed leaves and pubescent veins, with yellow-green flowers. The fruit is a black globular berry. It is spread in moist, swampy areas. As active principles it contains: sugars, organic acids, tannins, flavonoids, vitamins, potassium salts, anthocyanins, volatile oils [16].

Plums (*Prunus domestica*) is a tree grown in soil with a shallow branched root. The fruit is an elongated oval, blue-black drupe. It contains sugars, cellulose, mucilage, vitamins and mineral salts [16].

In this study, we investigated the contents of Cu in soil and vegetables, and fruit crops to evaluate the translocation factors between soils and plants with the purpose to assess potential health risks to the population.

Materials and methods

The plant species chosen for this study are root vegetables (carrots and radishes), leafy vegetables (spinach and parsley), berries (strawberries and black currants) and plums, because they are among the most cultivated and consumed vegetables and fruits for their high nutrients and vitamins.

The properties of the soil were: pH 5.05, total nitrogen 1.68%, total phosphorus 0.52%, total potassium 0.85%, electrical conductibility 1.12, moisture 63.7%.

The solutions with concentrations of c1, c2, c3 and c4 were prepared individually using copper sulphate as reagent, the solvent used in the preparation of the solutions being distilled water. To obtain mixtures of solutions of Cu for each of the concentrations of c1 = 1.5%, c2 = 3.0%, c3 = 4.5% and c4 = 6.0% individually prepared, equal parts were taken from each solution, and were mixed until homogenizing resulting in the mixture. In parallel as reference samples, vegetables and fruits were planted in pots with uncontaminated fertile soil c0 = 0%.

The pots in which the vegetables and fruits were planted were loaded with fertile soil that was mixed and homogenized in turn with each of the four solutions of different concentrations. The added soil was 1000 ml of Cu mixture solution per 20 kg of soil for each pot. In the experiments, heavy metal loading was performed by initially loading the soil with each of the four mixture concentrations of Cu, without supplementing until harvest.

The experiments took place between March and September 2019. For radishes, carrots, parsley and spinach, seeds were put in pots, for strawberries, stolons were planted in pots and kept in the greenhouse during the vegetation, they were constantly watered. The conditions in the greenhouse during the experiment were measured daily, the temperatures were between 18-30°C, the humidity between 32-88%. Harvesting was done at 30 days for radishes, at 33 days for parsley, at 45 days for carrots and spinach and at 35 days for strawberries.

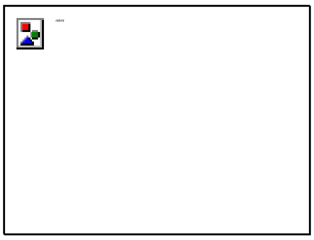
For black currants cuttings were planted in pots and for plums trees in the 3rd year of fruiting, transferred to pots, and kept in an open space, but covered to keep the shade and protect them from the rain. The atmospheric conditions during the experiment were between 14-33°C and the humidity between 28-62%. Harvesting was done at 112 days for black currants and 5 months after planting for plums.

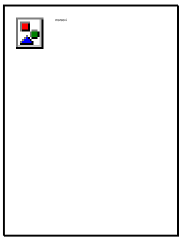
The metal content of the vegetables, fruits and soil samples was measured using flame atomic absorption spectrometry (FAAS, GBC 932AA or GFAAS, GBC Savant AAZ) [17]. The methods and techniques chosen are in accordance with the recommendations elaborated by the Institute of Pedological and Agrochemical Research of Bucharest and the Romanian Standards regarding the elaboration of the pedological and agrochemical studies [18; 19].

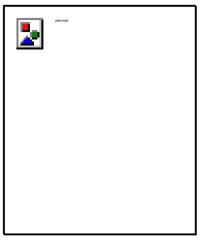
Since plants can accumulate metals from the soil, the translocation factor (TF) of heavy metals from soils to crops, called accumulation factor, the uptake factor or concentration factor, were calculated as follows [1; 5; 6; 20; 21]. This factor has been determined for the assessment of the level of phytoextraction in plants.

$$TF = \frac{heavy \quad metal \quad concentration \quad in \quad crop}{heavy \quad metal \quad concentration \quad in \quad sol}$$

Aspects from the harvest of the vegetable and fruit crops are shown in Figure 1.


Radishes


Carrots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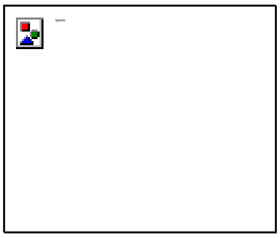
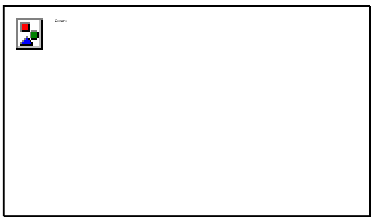
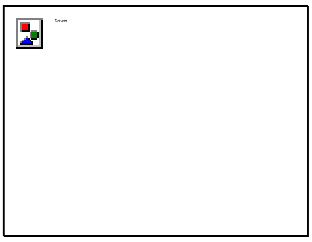

Spinach


Strawberries


Black currants

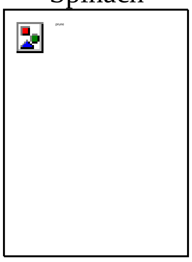

Plums

Fig. 1. **Vegetable and fruit species used in the experiments**

Results and discussion

In Romania, copper is a heavy metal predominant in soils. Copper limits are regulated so that the normal vegetable content is 20 mg·kg^{-1} and the alert threshold is 100 mg·kg^{-1} [23].

Figure 2 shows the copper content of the soil in the plants. It is observed that the trend of the copper content in vegetables, both roots and leafy, and in strawberries, which are herbaceous plants, is increasing when increasing the copper content in the soil, but only up to the value of 265.1 $mg \cdot kg^{-1}$ Cu in the soil. For the highest value of copper in the soil (378.2 $mg \cdot kg^{-1}$), the copper content decreases in all studied vegetables and strawberries, but in currants and plums copper continues to increase. The explanation is that the first is a shrub and the other is a fruit tree, and they have a longer vegetation period.

Uptake of heavy metals by plants tends to increase when increasing the concentration as long as it is within a certain range. When the concentration goes beyond the range, the uptake will decrease because plant roots are injured, thus causing a lower absorbing ability. Therefore, it is easy to make an error if an area's soil pollution status is determined simply from the contents of pollutants in the seeds. The enrichment factors quantify the relative differences in bioavailability of metals to plants and is a function of both soil and plant properties [1].

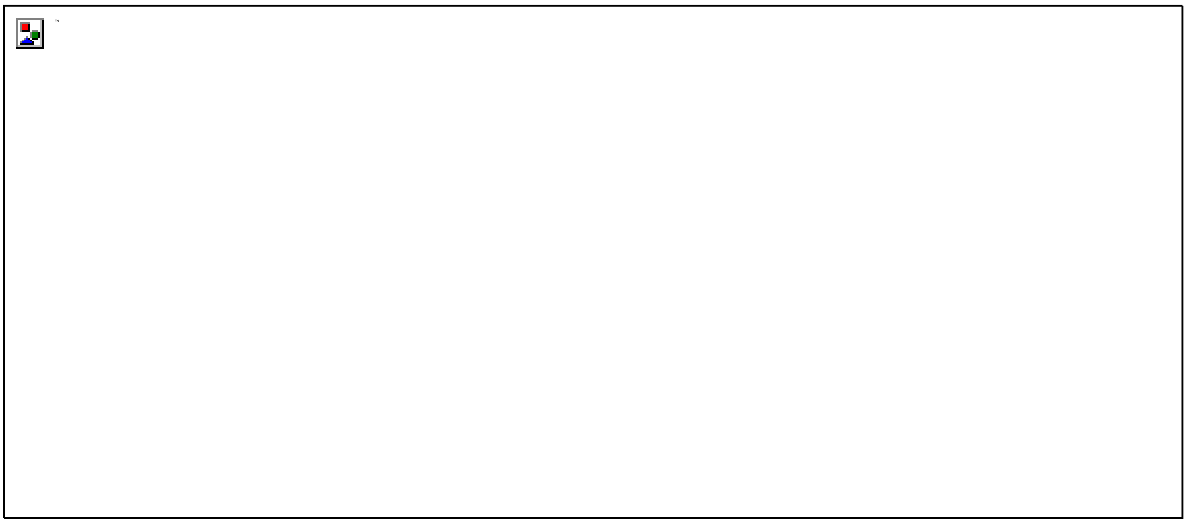


Fig. 2. **Content of copper from soil in plants**

Very small amounts of copper are needed by a tree. When adequately supplied, it moves easily from old to new leaves. When deficient, however, it becomes immobile so that young leaves first exhibit deficiency symptoms. More than half of the copper in trees is located in the chloroplasts and participates in photosynthetic reactions. It is also found in other enzymes involved with protein and carbohydrate metabolism [4].

Depending on the vegetation period and the type of the plant, the atmospheric conditions, the type of the soil in which it grows, the plant can absorb more or less copper from the soil. Vegetables have a short vegetation period (30-60 days) and intense biological activity in the soil, so they absorb more copper in a short time. Shrubs and fruit trees have a longer vegetation period (2-5 months), until the fruit is ripe, so the substances absorbed from the soil are stored in other vegetative parts (stems, branches, leaves).

As the paper [22] presents, the high copper content in vegetables could explain growth delay in fresh weight and plant height when their soil copper concentration exceeds the admissible level.

The concentrations of copper in the edible parts of the vegetable types and fruits decreased in the order:

- for uncontaminated soil: radishes > carrots > spinach > parsley > strawberries > plums > black currants;
- for moderately contaminated soil: spinach > strawberries > carrots > parsley > radishes > black currants > plums;
- for maximum contaminated soil: strawberries > spinach > black currants > carrots > radishes > parsley > plums.

The mobility of metals from soil to plants is a function of the physical and chemical properties of the soil and of vegetable species, and it is altered by innumerable environmental and human factors [1].

The concentrations of Cu, presented in the paper [3], in the edible parts of the six vegetable types decreased in the order of legume vegetables > leafy vegetables > solanaceous vegetables > root vegetables > melon vegetables > stalk vegetables.

Figure 3 presents the translocation factor depending on soil contamination with copper for different vegetable and fruit species.

Regarding the accumulation of copper from soil in plants, we see from Figure 3 that the translocation factor decreases as the amount of copper in the soil increases. Maximum values of the transfer factor are found in the case of root vegetables, respectively radishes = 0.514 and carrots = 0.432, and also for spinach leaves = 0.391, parsley leaves = 0.256 and minimum values for black currants = 0.102 and plums = 0.165. Strawberries are herbaceous plants with a short vegetation period, and the accumulation (0.235) was not very high.

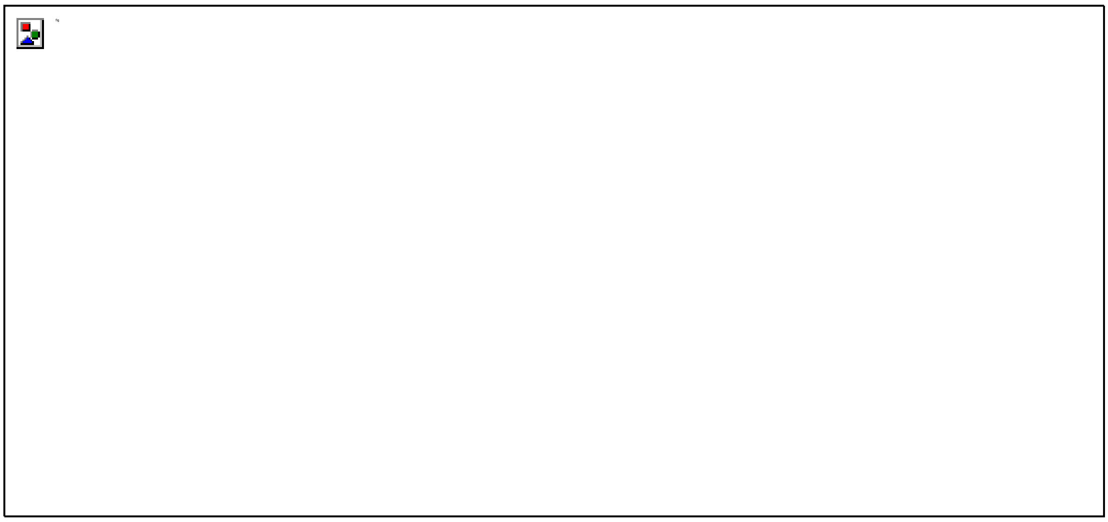


Fig. 3. **Accumulation of copper from soil in plants**

The values of the transfer coefficient for uncontaminated soil in the studied crops were radishes > carrots > spinach > parsley > strawberries > blackcurrants > plums, while for contaminated soils it decreases spinach > strawberries > carrots > parsley > radishes > blackcurrants > plums.

The transfer coefficient shows the bio-availability of copper from the soil in the edible parts for the studied crops, as well as the capacity of phytoextraction plants. It is known that values higher than 1 of the transfer coefficient in plants show that they can be good phytoremediating agents for soils.

The results obtained in the study show low bio-accumulation (< 0.18) of copper in the soil for parsley, currants and plums and medium bio-accumulation for spinach, strawberries, carrots and radishes (0.21-0.54). No value of the transfer coefficient exceeds 1, so the studied plants are not good agents for phytoremediation.

The paper [1] reports higher values for the transfer coefficient of copper for vegetables, radishes = 0.978, spinach leaves = 3.223 and parsley leaves = 1.107. It may be due to the presence of natural copper in the soil and its lower retention in the soil than other toxic cations.

Higher transfer factors reflect relatively poor retention in soils or greater efficiency of vegetables to absorb metals. Low transfer factor reflects the strong sorption of metals to the soil colloids [1].

The TF values for Cu, from paper [6], for various vegetables varied greatly between the plant species and locations. The transfer factor for Cu is very low compared to other elements in all varieties of vegetables (spinach = 0.106, radish = 0.084, carrots = 0.101). The difference in the TF values between locations may be related to soil nutrient management and soil properties.

Conclusions

From the experimental results obtained after contaminating the soil with heavy metals and their absorption by vegetables and fruits, the following conclusions can be drawn:

1. The analysed plants have proven to have different bio-accumulation capacity, depending on the plant species, the vegetative part of the plants and the concentration of solution of copper added in soil.
2. The accumulation of copper in vegetables and fruits is high in those grown on soils contaminated with little copper and decreases as copper is found in a large amount in the soil, which shows that both vegetables and fruits have defence mechanisms, so at certain concentrations of copper in the soil it is no longer extracted from the soil, instead the plants may show necrosis on the leaves, may wither or may stop evolving.
3. The translocation factor is a measure of the phytoextraction capacity of plants, TF of copper from the soil into the roots and leaves of vegetables was medium (0.21-0.54) and in the fruits it was low (< 0.18). The studied plants cannot be good agents for phytoremediation soil because $TF < 1$.
4. All over the world, more and more attention is being given to soil metal contamination, because through the food chain (soil - plant - human body) human health is endangered.

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Author contributions

Conceptualization, P.A.; methodology, V.I. and F.V.; investigation, P.A., V.I. and F.V.; writing – original draft preparation, P.A., V.I. and F.V.; writing – review and editing, P.A. and V.I.; project administration, P.A. All authors have read and agreed to the published version of the manuscript.

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