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Potential of Heavy Metal and Mould Contamination in Rose Petal Tea

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Rose tea is an aromatic herbal drink made from rose flowers' fragrant petals and buds (after being dried). There are many beneficial components in rose tea, such as vitamin C, polyphenols, vitamin A, myrcene, quercetin, and other antioxidants. Although dried rose petal tea provides good benefits, there could be some potential for heavy metals, and mould contamination can occur during growth and storage. Heavy metal contamination may occur due to the absorption of these components through fertilizers during the growth process. Exposure of dried rose petals to humid conditions at room temperature from 5 d to 30 d caused mould contamination. In this research, the rose petals are characterized to investigate the existence of heavy metals from different farming methods: organic and inorganic fertilizer. Besides, the effect of shelf life on fungi or microorganism formation is also being studied. In this research work, the samples of rose plants were collected from two different locations using different types of fertilizer (organic and inorganic fertilizer). The samples undergo a pre-treatment process which comprised cleaning and drying roses. The analysis of heavy metal content was conducted using Inductively Coupled Plasma-Mass Spectrometer (ICP-MS) equipment. This study used qualitative approaches to investigate any physical changes of colour and appearance on the dried rose petals samples for every 10 d in 6 weeks duration of time. The current studies suggest that there could be a possibility that the content of copper (Cu) at 203.71 ppb and potassium (K) (162725.23ppb) components have reduced the chance of the dried rose petals being exposed to mould infection and have better resistance against any contamination during the storage period. In food application, this finding is valuable as to provide a longer shelf-life span for the rose tea during storage time. However, it is important to ensure the content was still within the permissible limit for food according to the required standard.

1. Introduction

Consumption of tea from the infusion of dried rose petals and buds has been increasing as the awareness of a healthy lifestyle becomes a trend in this modern era. Rose tea is made from rose flowers' fragrant petals and buds as an aromatic herbal beverage. There are many benefits of rose tea due to the high concentrations of vitamin C, polyphenols, vitamin A, minerals, myrcene, quercetin and other antioxidants (Kumari et al., 2021). Despite the goodness offered by dried rose petal tea, there could be some potential for heavy metals originating from the fertilizer during the growing process and mould contamination during the storage process. Since the consumption of rose tea consists of heavy metals and mould might contribute another bad effect on the human body, there are recognised need to investigate any connection effect between the type of fertilizer used to grow rose plant associated with the content of heavy metals and mould contamination occur on dried rose petals used for making rose tea.

Fertilizers are materials that carry single nutrients or multiple nutrients required by plants for growth. Organic fertilizer is natural materials from ores, animal or plant by-products, and compost (Hoang et al., 2021).

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Meanwhile, inorganic fertilizer is referred to as chemical or conventional fertilizers manufactured through chemical processes from ores or by direct synthesis. A complete fertilizer contains macronutrients such as nitrogen (N), phosphorus (P), and potassium (K) which are required to be reported for sale purposes. There are also micronutrients such as boron (B), chlorine (Cl), copper (Cu), iron (Fe), manganese (Mn), molybdenum (Mo), nickel (Ni), and zinc (Zn) present in the fertilizer in a minimal amount (Singh et al., 2022). Among those micronutrients, Mn, Fe, Cu, Zn, and Ni in the fertilizer are classified as heavy metals.

Gray mould, which is caused by *Botrytis cinerea* fungus, is a common disease found on a rose flower. It can be spotted in the form of water-soaked spots or flecks on the flower petals, and the infected petals turn into brown and white (Jafari et al., 2017). Therefore, in the dried rose petals production for rose tea application, it is crucial to ensure that the dried rose petals and bud are free from fungi contamination.

Previous research by dos Santos et al. (2018) has revealed a correlation between toxic elements found in rose petals and the change in seasons (winter and summer). Based on their analytical studies using Inductively Coupled Plasma-Atomic Mass Spectrometry (ICPMS) and Atomic Absorption Spectrometer (AAS) methods, it has proved that the toxicity level for components found inside the rose's petals is lower than the allowable concentration limits set by National Health Surveillance Agency (ANVISA) for inorganic contaminants in foods. Another important finding in their works has revealed that the content of minerals between rose petals and several other vegetables is broadly similar.

Food allergens from the consumption of flowers can cause disease and allergies. Even edible flowers are nutritious but may be exposed to pesticides, and contain natural harmful toxins, which may lead to asthma, allergies, and death (Purohit et al., 2021). The hazardous compound may originally be from the plant or the levels of the food chain. Two most common impurities in edible flowers are bacteria and chemical compounds. Hazardous bacteria may come from agricultural production, the food chain, and chemical impurities are primarily from agricultural production and the environment.

The works done by Salem et al. (2020) explained the correlation between the physicochemical properties of cultivated and uncultivated soil with the heavy metal content at four different seasons. It was reported that there could be soil contamination with heavy metal components (Cr, Cu, Cd, Mn, Zn, Ni, and Fe) due to chemical fertilizer for plant cultivation. The concentration of heavy metals is lower in the wet season compared to the dry season due to the presence of water. During heavy rainy seasons, most of the suspended materials which contain heavy metal chemical compounds may be flashed out through canals and adjoining the vast flood zone. The heavy metal uptake from the soil to the plant may vary if it is grown in an open field or greenhouse as reported by Li et al. (2017) and it is found that Cd, Mn, and Zn elements in cultivated vegetables are higher in greenhouse conditions.

The uptake of these heavy metal content from the soil into the plant was significantly influenced by the physicochemical properties of the soil and the environment around the plants. Perhaps the element interaction between Fe, Cd, and Mn was significant in metal element transportation in vegetables Salem et al. (2020). However, in the case of rose cultivation, there is no data comparison available yet to explain whether heavy metal content in roses may be affected by the farming method and other operating conditions, such as using a different type of fertilizer.

Apart from the risk of heavy metal contamination, rose tea production can also experience mould contamination. Mould or toxigenic fungi contamination on herbal tea, is primarily microbial contamination and is sourced from the water, air, and soils. Under the appropriate conditions, temperature, humidity, and nutrients, fungi can propagate well and colonize the available substrates (Krstić et al., 2021). Based on studies done by Krstić et al. (2021), it was found that the level of fungi contamination found in 14 types of herbal tea was still within the allowable limit (< 105 CFU/g). The objective of the present studies was to investigate the presence of heavy metals content in rose petals from different methods of farming (organic and inorganic fertilizer). In addition, any change in physical appearance of the dried rose petals were also observed during the storage time.

2. Materials and method

2.1 Preparation of samples

The rose flowers grown using organic fertilizer were collected from Pekan, Pahang. For inorganic samples, rose flowers grown using inorganic fertilizer were purchased from the nursery located in Kuantan, Pahang. Both rose flowers were stored at room temperature before the experiment began. The rose samples are appropriately washed using distilled water to clean from contaminants. The samples were washed gently. Then, samples were placed on the tray for the drying process in the oven (model FCH-9036A, Germany) at 70 ºC temperature. At this stage, water content inside the rose petals was removed, and the moisture content was measured.

2.2 Characterization of heavy metal content

The heavy metal concentration was determined using Inductively Coupled Plasma Mass Spectrometer (ICP-MS) equipment. There are eight (8) elements of heavy metals were determined, including Arsenic (As), Copper (Cu), Iron (Fe), Zinc (Zn), Lead (Pb), Cadmium (Cd), Nickel (Ni), and Potassium (K) in the rose petals from both samples (organic and inorganic). For this purpose, 0.5 g of dried samples were placed in the digestion vessel, and 6 mL of 2 % HNO_3 and 2 mL of H_2O_2 were added together to the digestion vessel. Then the vessel was placed in the microwave for the decomposition process before it was proceeded to the analysis process for heavy metal detection by using ICP-MS equipment. Then, liquid samples produced from the acid digestion were used for the heavy metal detection process using the ion extraction method concept inside the ICP-MS machine.

2.3 Physical Appearance Evaluation

Physical changes of colour and appearance were observed on the dried rose petals on organic and inorganic sample. The observation was made for every 10 d in 6 weeks. Changes of physical appearance and colour were recorded and compared accordingly.

3. Results and Discussions

3.1 Level of heavy metal and chemical content in dried rose petals grown by using organic and inorganic fertilizer

Table 1 and Table 2 show the 8 elements of heavy metal Table 1 and Table 2 show the 8 elements of heavy metal (As, Cu, Fe, Zn, Pb, Cd, Ni, and K) present in organic and inorganic samples. Based on the Malaysian Food Act (1983) and Regulations (1985), these 8 elements are the most dangerous element if it was consumed excessively by a human.

Table 1: Heavy metal content in dried organic rose petals sample

No	Parameter	Organic sample	Unit	Limitation in food**(ppm)
1	Arsenic (As)	*ND (<0.5)	ppb	0.100
2	Copper (Cu)	10.10	ppb	10.00
3	Iron (Fe)	55.82	ppm	1912.00
4	Zinc (Zn)	1.58	ppm	100.00
5	Lead (Pb)	*ND (<0.5)	ppb	6.00
6	Cadmium (Cd)	2.25	ppb	1.00
7	Nickel (Ni)	193.17	ppb	7.80
8	Potassium (K)	9864.87	ppb	6,000,000

*ND(<0.5) is referred to as not detected because concentration is less than 0.5 ppb
**Limitation in food value is based on the Malaysian Food Act (1983) and Regulation (1985)

Table 2: Heavy metal content in dried inorganic rose petals sample

No	Parameter	Inorganic sample	Unit	Limitation in food**(ppm)
1	Arsenic (As)	2.21	ppb	0.100
2	Copper (Cu)	203.71	ppb	10.00
3	Iron (Fe)	42.75	ppm	1912.00
4	Zinc (Zn)	1.38	ppm	100.00
5	Lead (Pb)	*ND (<0.5)	ppb	6.00
6	Cadmium (Cd)	*ND (<0.5)	ppb	1.00
7	Nickel (Ni)	35.35	ppb	7.80
8	Potassium (K)	162725.23	ppb	6,000,000

*ND(<0.5) is referred to as not detected because concentration is less than 0.5 ppb
**Limitation in food value is based on the Malaysian Food Act (1983) and Regulation (1985)

Based on data in Table 1 and Table 2, a similar trend was observed in As, Cu, and K for the inorganic sample. It has shown higher content of these components compared to the organic sample. As content in organic sample is very low, less than 0.5 ppb, and not detected by the ICPMS devices. K and Cu elements in inorganic sample have recorded 70 % to 90 % higher compared to the organic sample. The levels of As element observed in this study are far below to the allowable limit for As level for food by the Malaysian Food Act (1983) and Regulations (1985). The trend discussed above shows that inorganic fertilizer plays essential role in determining the content of As, Cu, and K elements in the rose petals. This is supported by the previous research from Lin et al. (2019), where it was found that the tea orchard treatment significantly decreased the Cd, Pb and As content in tea leaves and increased the amino acid of the tea and pH of the soil.

Surprisingly, these 4 elements, Ni, Cd, Zn, and Fe, have shown an opposite trend observed in Table 1 and Table 2, in which rose petals grown organically has shown higher level compared to the inorganically grown sample. As can be seen, Ni and Fe elements in organic sample, are 82 % and 23 % higher compared to inorganic sample. No significant difference between the both categories of samples was detected for Zn elements. A possible explanation to this might be that there are not true representation of the organic condition as the rose plant were grown on the soil directly which could be underestimate the existence of the elements which originally exist in the soil or leach into the soil from nearby area.

The Cd elements found in the inorganic sample are 2.25 ppb, which was considered low. The Cd content in the organic sample was not detected because it was too low. Lin et al. (2019) has reported a contradict trend related to Cd content uptake compared to this work. It is reported that Cd uptake in hemp cultivation found in seed and straw parts has increased proportionally with the amount of phosphate fertilizer added to soil and soil pH. Therefore, rose petal grown using chemical fertilizer should have higher Cd uptake than rose petal grown using organic fertilizer, but contrasting results were obtained in this work. However, it has previously been studied by Lu et al. (2017) that many factors may contribute to the existence of Cd naturally in soil and groundwater, including the atmosphere (from wildfires, ash, weathering of rock, airborne particles), cadmium in rocks, sediments, and soil, and anthropogenic Cd inputs into soil and combustion emissions, sewage sludge, landfills, traffic, metal industry, and mining. These findings suggest that there could any other resources of Cd naturally cause to the existence of these elements at low concentrations in rose petals samples grown organically. Pb elements appeared to be undetected in both types of the sample. Since the Pb elements has not been detected it is probably due to very low concentrations and cannot be detected, or they may not exist in the soil-grown organically and inorganically ways. In this study it has shown that the restricted level for Pb elements in the rose tea petal is safe according to the Malaysian Food Act (1983) and Regulations (1985), which is 6 ppm.

3.2 Physical appearance evaluation of dried rose petals grown by using different types of fertilizer

The physical appearance of the dried rose's petals grown in different fertilizers for 40 d is shown in Figure 1, Figure 2, Figure 3, Figure 4, and Figure 5. No significant difference in the physical appearance of the organic and inorganic dried rose petals samples in the first 20 d. The results revealed that in the first 20 d, samples of organic and inorganic dried rose petals have higher resistance to contamination. The higher resistance might be due to the drying process that has removed most of the water content in the samples, thus preventing the microorganism contamination.

As the storage time proceeded (day 25 to day 40), the organic dried rose petals' sample colour started to turn greyish and later blackish. Besides, a few spots of fungal growth appeared on the sample as the colour changed. The grey spots with fungal growth appear like *Botyris cinerea. Botyris cinerea* is an airborne plant pathogen that usually appears on rotten flowers or fruits under storage conditions (Kumari et al., 2021). On the other hand, the colour and appearance of the inorganic dried rose petals sample remained unchanged since day 1 of the storage period. Relative humidity from the atmosphere is one of the factors which may stimulate the growth of the fungal on the dried rose petals, especially on the organic sample.

(a) (b)

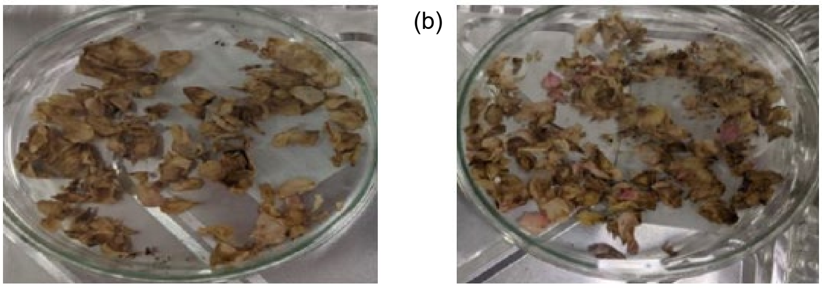

Figure 1: No colour changes and no mould formation on (a) organic rose petals, and (b) inorganic rose petal samples at Day 1

(a) (b)

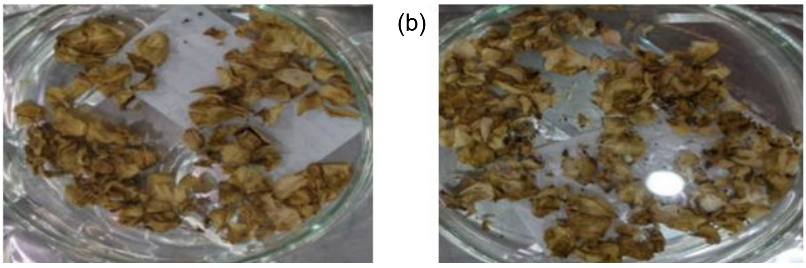

Figure 2: No colour changes and no mould formation on (a) organic rose petals, and (b) inorganic rose petal samples at Day 10

(a) (b)

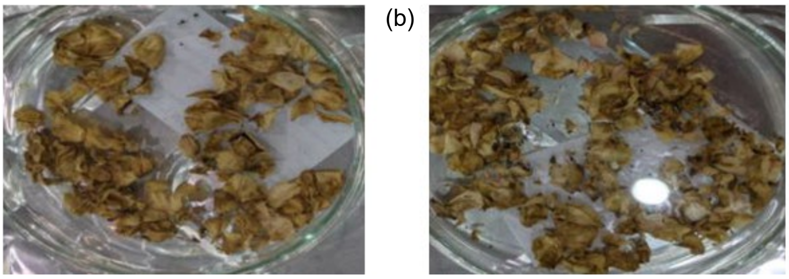

Figure 3: No colour changes and no mould formation on (a) organic rose petals, and (b) inorganic rose petal samples at Day 20

(a) (b)

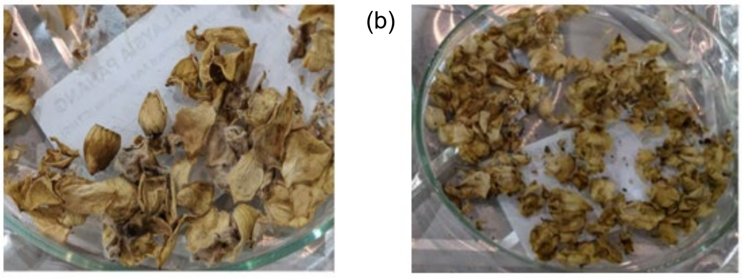

Figure 4: Grayish colour mould appeared on (a) organic rose petals, and no colour changes and no mould formation on (b) inorganic rose petal samples at Day 30

(a) (b)

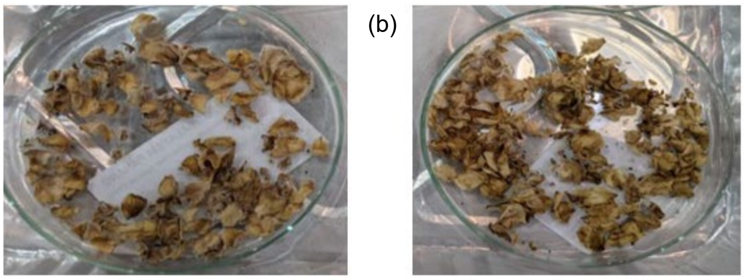

Figure 5: Grayish colour mould appeared on (a) organic rose petals, and no colour changes and no mould formation on (b) inorganic rose petal samples at Day 40

It is possible to hypothesis that the resistance of the inorganic sample towards contamination could be related to the chemical components absorbed by the flower during the growing process, as shown in Figure 1, Figure 2, Figure 3, Figure 4, and Figure 5. The high amount of copper (Cu) and potassium (K) in the chemical fertilizer of inorganic rose could protect the dried petals from microorganism contamination. These results are in agreement with those obtained by Nawaz et al. (2017) which revealed that by varying concentrations of Cu in chemical fertilizer provides antifungal properties to the basil plant oil extract (Nawaz et al., 2017). On the other hand, potassium (K) is an essential nutrient for plant production as it may improve insect and disease resistance in plants (Meena et al., 2016). The deficiencies of K in the soil during plant growth may increase susceptibility to diseases such as fungal infection.

4. Conclusion

Results of this work showed that the heavy metal contamination in rose petals might occur due to nutrient uptake from chemical fertilizer and naturally exist in the soil depending on the location of the plantation. Even though higher concentrations of heavy metal elements are detected in the sample of rose petal grown inorganically compared to organically grown rose, the concentration determined was still within the permissible range. The better resistance against any possible contamination and colour change during storage period of the inorganic dried rose sample might be due to the content of copper (Cu) at 203.71 ppb and potassium (K) at 162725.23 ppb has increased the resistance of the inorganic dried rose petals against any contamination during storage period since that the component from chemical fertilizer is beneficial in providing resistance to microbial activities and preventing disease infection during postharvest and beneficial for food preservation.

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