HEAVY METALS ACCUMULATION OF *Stevia rebaudiana* FROM LEAVES AND FLOWERS EXTRACTION

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Abstract

AbstractHeavy metals accumulation in the Stevia rebaudiana from leaves and flowers extraction is reported. Heavy metals are the chemical elements with a specific gravity contain at least five (5) times the specific gravity of water. The heavy metals were found accumulated in animal and plant cells, leading to severe negative effect to the environments. The objective of the research is to investigate the heavy metals contain in Stevia plant (Stevia rebaudiana). The samples were purchased from Stevia Valley Taman Paya Dalam, Malacca Malaysia. The fresh leaves and flowers of Stevia plant were dried using oven equipment and were grinded until fine to make powder. The analysis of sample was carried out by using an Inductively Coupled Plasma Mass-Spectrophotometer (ICP-MS) to analyze the Cadmium (Cd), Chromium (Cr), Copper (Cu), Iron (Fe), Magnesium (Mg), plumbum (Pb), Selenium (Se) and Zinc (Zn). The result shows that heavy metals were contains Cd (0.6048 mg/kg), Cr (5.621 mg/kg), Cu (3.402 mg/kg), Fe (719 mg/kg), Mg (1.701 mg/k), Pb (1.682 mg/kg), Se (< 0.7644 μm/kg) and Zn(33.03 mg/kg) in leaves. Meanwhile, the results of heavy metals in flowers were Cd (0.2266 mg/kg), Cr (5.127 mg/kg), Cu (6.461 mg/kg), Fe (614 mg/kg), Mg (1.76 mg/kg), Pb (1.735 mg/kg), Se (< 0.7644 μm/kg) and Zn (31.46 mg/kg). Stevia rebaudiana plant contains eight heavy metals below permissible limit in plant and can be used as food products.

Keywords : *Heavy metals, Stevia rebaudiana, Leaves, Flowers, ICP-MS*

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INTRODUCTION

Stevia rebaudiana (Bertoni) is a family of Asteraceae is herbaceous perennial plant indigenous to Paraguay and Brazil where its leaves are used by the local Guarani Indians as natural sweetener for hundreds of years. About 150 stevia species are known, among them *Stevia rebaudiana* is the only one with significant sweet tasting properties (Soejarto *et al.,* 1983). A *Stevia rebaudiana* extracts having up to 300 times the sweetness of sugar, has garnered attention with the rise in demand for low-carbohydrate and low-sugar food alternatives. Because Stevia has a negligible effect on blood glucose, it is attractive as a natural sweetener to people on carbohydrate-controlled diets.

Fig. 1 Stevia rebaudiana Bertoni

Stevia rebaudiana plant (shown in Fig.1) is a worldwide importance today because its leaves are used as non-nutritive high potency sweetener primarily in Japan, Korea, China and South America. The consumption of stevia extract in Japan and Korea was about 200 and 115 tons/year, respectively (Kinghorn *et al.,* 2000). The extracts have been used for sweetening soft drinks such as diet coke, soju, soy sauce, dried seafood, candies, ice cream, chewing gum, yoghurt, and as well as in toothpaste and mouthwash in Japan, Korea, and Brazil (Carakostas *et al.,* 2008, Tedhani *et al.,* 2007).

Stevia rebaudiana plant is easily contaminated during growth, development and processing, and for this, an extensive research is needed to explore the characteristics of the heavy metal produced by the plant. The heavy metals produced from the herb and its toxicity of *Stevia rebaudiana* plant is not well documented, and scientific evidence is limited to establish *Stevia rebaudiana* plant as a medicinal plant. The toxicity of the heavy metals could affect the human health and may disturb the normal functions of control nervous system, liver, lungs, heart, kidney and brain, leading to hypertension, abdominal pain, skin eruptions, intestinal ulcer and different types of cancers. The heavy metals detected in the plant (leaves and flowers) are namely as cadmium (Cd), chromium (Cr), copper (Cu), iron (Fe), magnesium (Mg), plumbum (Pb), selenium (Se) and zinc (Zn). The extraction of all these heavy metals will be analysed using Inductively Coupled Plasma - Mass Spectrophotometer (ICP-MS) (AOAC, 1995).

Heavy metals are the chemical elements with a specific gravity that is at least five times the specific gravity of water. The specific gravity of water is 1.0 at 4 °C (39 °F). Specific gravity is a measurement of density of a given amount of a solid substance when it is being compared to an equal amount of water (Lide, 1992). Heavy metals are closely connected with environment deterioration and the quality of human life, and thus have aroused concern all over the world. There are 23 heavy metals that have been reported were the heavy elements which are antimony, arsenic, bismuth, cadmium, cerium, chromium, cobalt, copper, gallium, gold, iron, plumbum, manganese, mercury, nickel, platinum, silver, tellurium, thallium, tin, uranium, vanadium, and zinc (Glanze, 1996).

Many studies have investigated the presence of toxic contaminants in herbal plants. Trace elements such as zinc (Zn), manganese (Mn), chromium (Cr), copper (Cu), iron (Fe), plumbum (Pb), nickel (Ni) and vanadium (V) were being found in a traditional Chinese herb Jingi (Han *et al.,* 2008). The World Health Organisation (WHO) recommends limits for various medicinal plants of not more than 10 mg/kg Pb and 0.3 mg/kg Cd in the final dosage form of the plant material (WHO, 2007).

Heavy metals such as copper (Cu) and Zinc (Zn) are essential for normal plant growth and development since they are constituents of many enzymes and other proteins (Hall, 2001). Many species of plants have been successful in absorbing contaminants such as lead, cadmium, chromium, arsenic, and various radionuclides from soils. One of phytoremediation categories, phytoextraction, can be used to remove heavy metals from soil using its ability to uptake metals which are essential for plant growth (Fe,Mn, Zn, Cu, Mg,Mo, and Ni). Some metals with unknown biological function (Cd, Cr, Pb, Co, Ag, Se, and Hg) can also be accumulated (Cho-Ruk and Kurukote, 2006).

The high concentration of heavy metals in soils is reflected by higher concentrations of metals in plants, and consequently in animal and human bodies. The ability of some plants to absorb and accumulate xenobiotics makes them useful as indicators of environmental pollution (Buszewski *et al.,* 2000). Heavy metals from soil enter plants primarily through the root system. In general, plant roots are the most important site for uptake chemicals from soil (Miclean *et al.,* 2000).

In heavy metal polluted soils, plant growth can be inhibited by metal absorption. However, some plant species are able to accumulate fairly large amounts of heavy metals without showing stress, which represents a potential risk for animals and humans (Oliver, 1997). Heavy metal uptake by crops growing in contaminated soil is a potential hazard to human health because of transmission in the food chain (Brun *et al.,* 2001, Gincchio, *et al.,* 2002, Friesl, *et al.,* 2006).

Various instrument and methods have been used to determinate minerals and heavy metals in foods, *viz*., atomic absorption spectrophotometer (AAS), and Inductively Couple Plasma – Mass Spectrophotometer (ICP-MS). In this study, Inductively Couple Plasma – Mass Spectrophotometer was used to determinate minerals and heavy metals. Advantages of using an ICP-MS include its ability to identify and quantify all elements with the exception of Argon, multi-element technique,suitable for all concentrations from ultra trace levels to major components, detection limits are generally low for most elements with a typical range of 1 - 100 g/L, high sensitivity; good precision and accuracy, multi elemental analysis can be accomplished, and quite rapidly (Worley and Kvech 2000).

METHODS

Extraction for mineral analysis

The raw material consists of leaves and flowers of *Stevia rebaudiana* as a sample purchased from Stevia Valley Taman Paya Dalam, Malacca Malaysia. The fresh leaves and flowers of Stevia plant were dried using oven equipment and were grinded until fine to make powder. Leaves and flowers of *Stevia rebaudiana* plants of each weighed approximately 0.5 grams into a Teflon digestion vessel, to which was added exactly HNO_3 (65%, 7 mL) and H_2O_2 (30%, 1 mL). The vessels were then capped, and fitted into rotor segments and inserted into the microwave cavity. The samples were radiated for 20 minutes. Upon cooling, the vessels were uncapped and solutions transferred into 50 mL volumetric flasks.

Analysis of heavy metals using ICP-MS

The analysis of sample was carried out by using an Inductively Coupled Plasma Mass-Spectrophotometer (ICP-MS) instrument (Agilent 7500a) to analyze the cadmium (Cd), chromium (Cr), copper (Cu), iron (Fe), magnesium (Mg), plumbum (Pb), selenium (Se) and zinc (Zn). Three point calibration curves were constructed using five concentrations (0-500µg/L) of standards prepared in-house.

HNO_3 was used as a blank and all the analysis was performed in triplicates.

RESULTS AND DISCUSSION

Minerals are defined as naturally occurring inorganic elements having a characteristic crystalline structure and chemical composition, and that has been formed pursuant to the geological process (Higdon, 2002). Plants obtain minerals from the soil, and most of the minerals in human diets come directly from plants or indirectly via animal sources. Minerals from plant sources may also vary from place to place, because soil minerals content varies geographically. Minerals content in the microwave-digested samples were determined using an Inductively Coupled Plasma Mass-Spectrophotometer (ICP-MS).

Heavy metals in leaves and flowers of *Stevia rebaudiana* were presented eight parameters such as cadmium (Cd), chromium (Cr), copper (Cu), iron (Fe), magnesium (Mg), plumbum (Pb), selenium (Se) and zinc (Zn). Table I shows the total concentration of selected heavy metals of *Stevia rebaudiana* from leaves and flowers extraction.

roots and short growth while yield production is affected in by nutrient uptake and homeostasis. It is frequently being accumulated by agriculturally important crops and impairs animal and human health. Cd is being described as mobile in the soil, to easily absorb by plant than Pb (Alloway, 1995). Cadmium may cause decreased uptake of nutrient elements inhibition of various enzyme activities, induction of oxidative stress, including alterations in enzymes of the antioxidant defense system (Sandalio *et al.* 2001).

Heavy metals like Zn, Fe, Cu, Mn are essential for plant growth and important constituents of many enzymes of metabolic importance. Other metals like Pb, Cd, As, Se, Cr and Al are biologically non-essential and toxic above certain threshold levels. Chromium is toxic to plants and does not play any role in plant metabolism (Dixit *et al.,* 2002). Chromium (Cr) is toxic to higher plants at 100 $\mu M \cdot kg^{-1}$ dry weight (*Davies et al.,* 2002). The result shows that chromium (Cr) in leaves of *Stevia rebaudiana* was 5.621 mg/kg. Meanwhile, the result of chromium (Cr) in flowers was 5.127 mg/kg.

Table I. Heavy metals concentration of the leaves and flowers

Heavy metals	Unit	Leaves	Flowers
Cadmium (Cd)	mg/kg	0.6048	0.266
Chromium (Cr)	mg/kg	5.621	5.127
Copper (Cu)	mg/kg	3.402	6.461
Iron (Fe)	mg/kg	719	614
Magnesium (Mg)	mg/kg	1.701	1.76
Plumbum (Pb)	mg/kg	1.682	1.735
Selenium (Se)	µm/kg	<0.7644	<0.7644
Zinc (Zn)	mg/kg	33.03	31.46

The concentrations of cadmium (Cd) in plant tissues were exceeded the permissible limit of 2 mg/kg (Codex Alimentarius 2001a). The result shows that cadmium (Cd) in leaves of *Stevia rebaudiana* was 0.6048 mg/kg. Meanwhile, the result of cadmium (Cd) in flowers was 0.2266 mg/kg. Cadmium (Cd) being a highly toxic metal pollutant of soil inhibits

The uptake of the copper from soil by plants depends on the ability of the plants to transfer the metal across the soil–root interface and the total amount of Cu present in the soil (Agata and Ernest 1998). Copper (Cu) is necessary for plant growth in low concentrations, a structural part of enzymes, and is up taken as divalent cation (Cu^{2+}) or Cu

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chelate. Cu, however, is often present in high concentrations that are toxic enough to biota. The result shows that copper (Cu) in leaves of *Stevia rebaudiana* was 3.402 mg/kg. Meanwhile, the result of copper (Cu) in flowers was 6.461 mg/kg.

Iron (Fe) deficiency (iron chlorosis) is an important nutritional disorder in fruit trees that results from impaired acquisition and use of the metal by plants, rather than from a low level of Fe in soils. The most evident effect of Fe deficiency is a decreased content of photosynthetic pigments, which results in the relative enrichment of carotenoids over chlorophylls (Chl) and leads to the yellow colour that is characteristic of chlorotic leaves (Abadía *et al*. 2011). The result shows that iron (Fe) in leaves of *Stevia rebaudiana* was 719 mg/kg. Meanwhile, the result of iron (Fe) in flowers was 614 mg/kg.

Magnesium, a mineral present in most foods, is essential for human metabolism and for maintaining the electrical potential in nerve and muscle cells. Magnesium is a macro mineral, essential to the proper utilization of other minerals in the body, including calcium and phosphorus. Magnesium is also critical to energy production and to over 300 enzymatic reactions in the body. It helps metabolize carbohydrates, proteins and fats, plus other minerals and nutrients (Nature's Sunshine Products Follows Numerous Manufacturing Guidelines 2001). The result shows that magnesium (Mg) in leaves of *Stevia rebaudiana* was 1.701 mg/kg. Meanwhile, the result of magnesium (Mg) in flowers was 1.76 mg/kg. According to Marschner (1995), that 15 to 30% of the total magnesium in plants is associated with chlorophyll molecule. Magnesium is an essential element in biological systems. Magnesium occurs typically as the Mg^{2+} ion. It is an essential mineral nutrient for life..

Plumbum (Pb) is a highly toxic metal that is present in soils in concentrations generally lower than 100 µg g^{-1} (Swaine 1990), but can be as high as 300 µg g^{-1} in agricultural soils (Bradl 2005). Plumbum concentrations in coal are generally lower than 80 µg g^{-1} (Swaine 1990). Plumbum (Pb) is the one of the heavy metal which is a poison. Plumbum is the most common heavy metal contaminant in the environment. Plumbum (Pb) does not have any metabolic use, it is ubiquitous in the soil, and is readily accumulated in plants when available. The result shows that Plumbum (Pb) in leaves of *Stevia rebaudiana* was 1.682 mg/kg. Meanwhile, the result of plumbum (Pb) in flowers was 1.735 mg/kg. The concentration of plumbum in vegetables exceeded their permissible limit of 3 mg/kg^{-1} (Codex Alimentarius, 2001b).

Selenium (Se) is a trace mineral that is essential to good health but required only in small amounts (Thomson 2004, Goldhaber 2003). Some plants are tolerant to selenium, showing a high capability to accumulate this element without symptoms of toxicity. They are also capable of translocating and/or transforming selenium into bioactive compounds, which play an important role in both, human nutrition and phytoremediation (Ellis and Salt 2003). The interest of selenium speciation in plants is due to the antioxidant and anticarcinogenic properties of certain species. The result shows that selenium (Se) in leaves of *Stevia rebaudiana* was < 0.7644 mg/kg. Meanwhile, the result of selenium (Se) in flowers was < 0.7644 mg/kg.

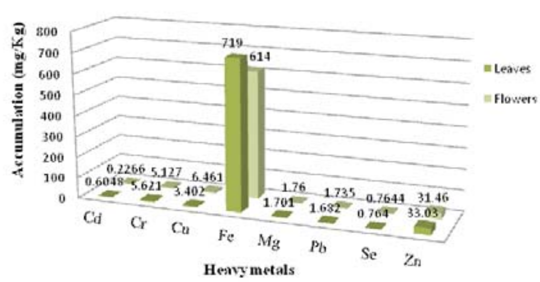

Fig. 2 Heavy metals accumulation of *Stevia rebaudiana*.

Zinc is an essential element for plant nutrition. It plays structural and/or catalytic roles in many enzymes such as Cu–Zn superoxide dismutase, alcohol dehydrogenase, RNA polymerase and DNA-binding proteins and is associated with the carbohydrate metabolism (Kim *et al.,* 2002, Broadley 2007). Moreover, zinc plays critical roles in the defence system of cells against reactive oxygen species (ROS), and thus represents an excellent protective agent against the oxidation of several vital cell components such as membrane lipid, chlorophyll and –SH groups of protein (Cakmak 2000, Zago and Oteiza 2001). However, when massively present in the environment, Zinc can reach optimal concentrations in all plant organs, thus inducing toxic effects and metabolic disorders. Zn inputs on soils are related with mining, industrial activities and agricultural practices (Bi *et al.,* 2006). Excessive Zn in plants can delay or diminish the growth (Andrade *et al.,* 2009) and root development (Lingua *et al.,* 2008) and causes leaf chlorosis (Wang *et al.,* 2009). The result shows that zinc (Zn) in leaves of *Stevia rebaudiana* was 33.03 mg/kg. Meanwhile, the result of zinc (Zn) in flowers was 31.46 mg/kg.

The heavy metals accumulation of *Stevia rebaudiana* from leaves and flower*s* extraction such as cadmium (Cd), chromium (Cr), copper (Cu), iron (Fe), magnesium (Mg), plumbum (Pb), selenium (Se) and zinc (Zn) are shown in Fig. 2.

CONCLUSIONS

The accumulation of heavy metals in leaves and flowers of *Stevia rebaudiana* contain eight heavy metals such as cadmium (Cd), chromium (Cr), copper (Cu), iron (Fe), magnesium (Mg), plumbum (Pb), selenium (Se) and zinc (Zn) below permissible limit in plant and can be used as food products.

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