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Determinant of the Alkaloidal Levels in *Cryptolepis Sanguinolenta* (Lindl.) Schtr

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This study is focused on the importance of the enrichment index of the soils on the alkaloidal levels of the plant organs of *Cryptolepis sanguinolenta*, a medicinal plant in two districts in the Eastern Region of Ghana. The extraction method was used to quantify the alkaloid in the organs while the irradiation method was also used to quantify the soil elements needed for the computation of the enrichment index of the soils. Results gathered show the enrichment of the soils to be less than 1. Therefore the soils of the locations are considered to be non-toxic. Pepease had an enrichment index of 0.22; Mamfe, 0.13 and Abonse, 0.14. The nature of the enrichment index of the soils fluctuated concurrently as the alkaloidal contents of the plant organs.

Key Words: *Cryptolepis sanguinolenta*, Enrichment index.

INTRODUCTION

Mineral elements influence alkaloid production, for example, cannabinoid production. Krejci[1] found increases related to unspecified "poor soil conditions". The influence of soil element concentrations on *Cannabis* has been reported by Haney and Kutcheid[2]. For example, soil elements have been shown to affect the production of cannabidiol (CBD), *delta*-8-tetrahydrocannabinol (THC) and cannabinol (CBN). Kaneshima *et al.*[3] have demonstrated the importance of optimal Fe levels for plant synthesis of THC. Latta and Eaton[4] reported on some mineral elements to be important for *delta*-9-tetrahydrocannabinol production, suggesting that some minerals may serve as enzyme co-factors. Coffman and Gentner *et al.*[5] also corroborated the importance of soil type and mineral content and observed a significant negative correlation between plant height at harvest and etrahydrocannabinol levels.

Kloke[6] and Kabata-Pendias and Pendias[7] proposed that the permissible or tolerable level of an element is the approximate concentration (or the threshold

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of the element's concentration) in soils above which crops produced are considered as unsafe for human or animal health. Lee *et al.*[8] proposed a pollution index [called Enrichment Index; (EI)] for soils underlain by black shales and slates in Korea. An enrichment index greater than 1 indicates that, on average, element concentrations are above the permissible level and any enrichment may be from anthropogenic inputs or natural geological sources[9, 10].

The research aims at the significance of the enrichment index (EI) of the soils and their relationship to the alkaloid content of the plant organs of the plant species, *C. sanguinolenta*.

EXPERIMENTAL

Soil and plant materials used in the study were collected from the environs of Pepease, Mamfe and Abonse in the Eastern Region of Ghana. The three settlements are located in the Kwahu South and Akuapem North Districts of the Region.

Determination of Enrichment Index: The soil samples were taken in March, June, September and December 2003. At each location, *viz.*, Pepease, Mamfe and Abonse, soil samples were taken within 5 cm radius around plants, which have been tagged for study. The concentrations of the toxic/trace elements of the soil samples were determined using the reactor (GHARR-1) of the Ghana Atomic Energy Commission (GAEC). The elements considered were arsenic copper, antimony, zinc, cobalt, chromium, thorium, uranium and vanadium (Appendix-2). The concentrations of the first four elements (As, Cu, Sb and Zn) were used to compute the enrichment index (EI) using the formula proposed by Lee *et al.*[8], as:

$$\text{Enrichment Index (EI)} = \frac{\Sigma\,(\text{Metal concentrations in soil/Permissible levels for metal})}{\text{Number of metals}}$$

Quantitative determination of alkaloid

Quantitative determination of alkaloid of the root, stem and leaf materials of the plant obtained from the locations were carried out on a monthly basis for one year (March 2002 to April 2003). Five replicates were prepared for each plant organ per location and the mean value computed. The plant materials were air-dried and ground to fine powder using the Manesty disintegrator. 50 g of the powders obtained in each case were Soxhlet extracted with hexane for 12 h to defat the powdered plant materials. The defatted powder in each case was taken and the alkaloid extracted with 500 mL of ethanol. The extracts were filtered and concentrated under reduced pressure using a rotary evaporator. The residue material was mixed with 200 mL of 10% aqueous acetic acid and allowed to stand overnight. The mixture was filtered with Whatmann No. 1 filter paper. The filtrate was basified to pH 10. The basic mixture was extracted with two equal volumes of 200 mL of chloroform. The chloroform extract was dried with anhydrous sodium sulphate and the solvent removed under reduced pressure. The weight of the evaporating dish was deducted from the weight of the evaporating dish plus the alkaloid residue to give the weight of the alkaloid contents and the percentage of alkaloid calculated using the following formula:

$$\text{Total alkaloid (\%)} = \frac{W}{Y} \times 100$$

where w = weight of alkaloid content extracted; Y = weight of powdered plant material.

RESULTS AND DISCUSSION

The results presented in Table-1 and Figs. 1a–c express the level of toxicity of the soils and the effect of the enrichment index of the soils on the alkaloid content of the plant species *C. sanguinolenta*, respectively.

Toxic/Trace element concentrations encountered in the soils of the locations

The results presented indicate the toxic/trace element concentrations (As, Cu, Sb and Zn) were used to compute the enrichment index of the soils of the locations. Table-1 shows the enrichment index of the soils gathered during the four quarters of the year of collection.

TABLE-1
ENRICHMENT INDEX OF THE SOILS OBTAINED FROM THE LOCATIONS

Locations	Months			
	March (1st quarter)	June (2nd quarter)	September (3rd quarter)	December (4th quarter)
Pepease	0.17 a (b) ±0.001	0.23 a (a) ±0.001	0.24 a (a) ±0.001	0.23 a (a) ±0.001
Mamfe	0.11 b (a) ±0.001	0.13 b (a) ±0.001	0.14 b (a) ±0.001	0.15 b (a) ±0.001
Abonse	0.12 b (a) ±0.001	0.17 b (a) ±0.001	0.16 b (a) ±0.001	0.11 b (a) ±0.001

Figures followed by the same letter in each column or row do not differ significantly ($P \leq 0.05$). Letters in brackets represent Duncan's range in a row.

The enrichment index values of the soils obtained at Pepease in March, June, September and December were statistically different from those of Mamfe and Abonse, but there was no statistical difference between the last two locations in all the four quarters (Table-1). Again, the enrichment index value of the soil obtained in the first quarter (March) at Pepease was also statistically different from the remaining three quarters (June, September and December) as shown in Table-1. The enrichment index values of the soils obtained at Mamfe and Abonse were not statistically different in the four quarters (March, June, September and December). The mean enrichment index values of the soils of the locations vary in the order of Pepease (0.22), Abonse (0.14) and Mamfe (0.13).

Effects of the enrichment index of the soils on the alkaloid content of the plant species, *C. sanguinolenta*

The results presented in Figs. 1a–c show the effect of the soil enrichment index values (EIs) on the alkaloid content of the plant species, *C. sanguinolenta*. Although there were some slight differences in the levels of the enrichment index, no significant differences were recorded in the enrichment index levels of the soils obtained from the locations at the following probability levels; $p < 0.05$, 0.001 and 0.0001. However, the differences did influence the synthesis of alkaloid in the plant species.

There was an overall increase in the enrichment value between March and December. The difference in the enrichment index between March and June led to a drop in the root alkaloid content with an increase in the leaf alkaloid and stabilization of the stem alkaloid content. The stabilization of the enrichment index values between June and December resulted in the stabilization of the root alkaloid content with further drop in the leaf alkaloid content and an increase in the stem alkaloid (Fig. 1a).

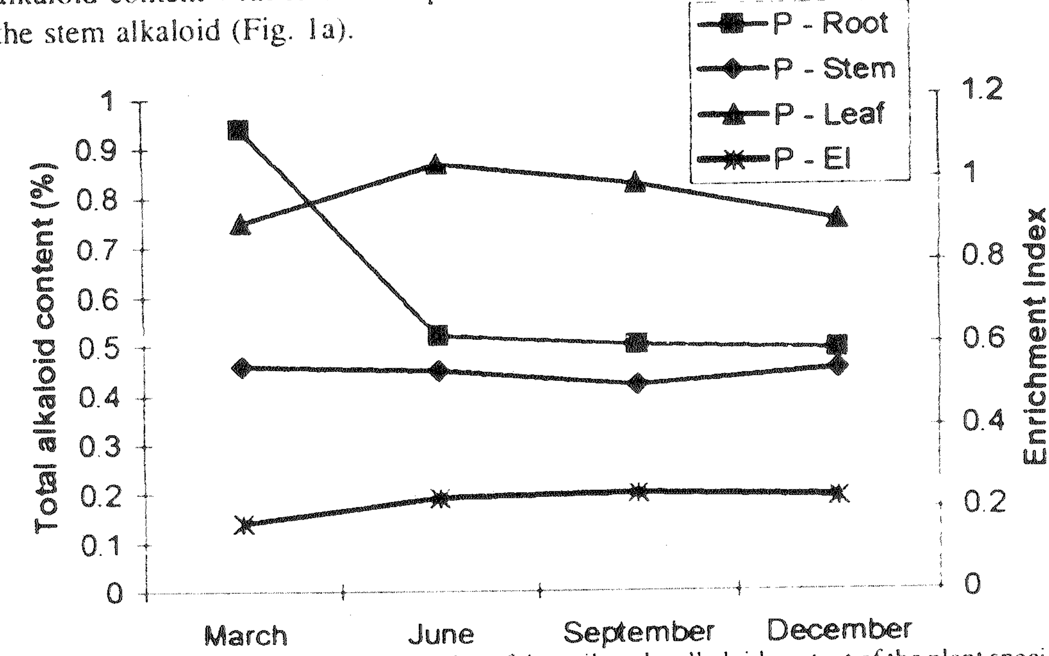

Fig. 1a. The effect of the enrichment index of the soil on the alkaloid content of the plant species, *C. sanguinolenta*, obtained from Pepease

The stable levels in the enrichment index throughout the year could still influence the alkaloidal levels of the plant at Mamfe. There was a drop in the root alkaloid content with an increase in the leaf alkaloid content and stabilization of the stem alkaloid content between March and June (Fig. 1b).

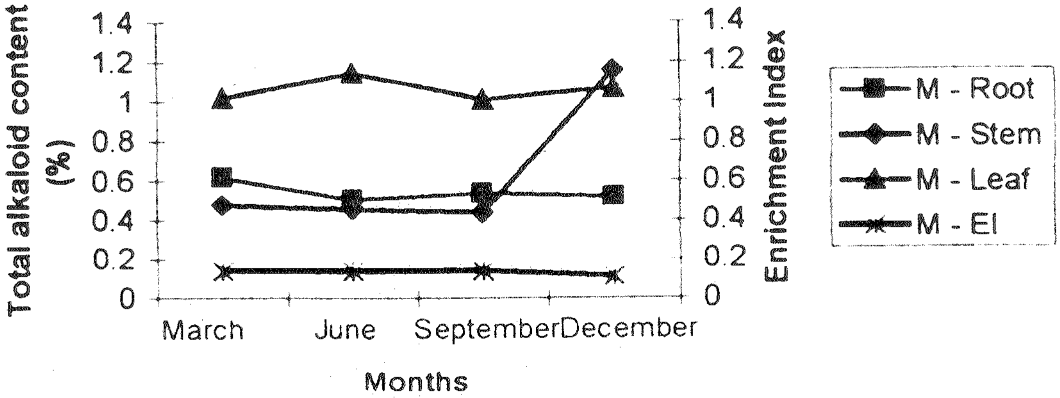

Fig. 1b. The effect of the enrichment index of the soil on the alkaloid content of the plant species, *C. sanguinolenta*, obtained from Mamfe

The stabilization of the enrichment index values between June and September led to an increase in the root alkaloid content, a drop in the leaf alkaloid content and stabilization of the stem alkaloid content (Fig. 1b). In Fig. 1b, a drop in the enrichment index values during the September and December periods led to a rise in the stem and leaf alkaloid contents with the stabilization of the root alkaloid content.

There were slight changes in the enrichment index of the Abonse soils. As presented in Fig. 1c, increase in the enrichment index during March and June resulted in the increase of the stem and leaf alkaloid contents with a drop in the root alkaloid (Fig. 1c). Slight decline in the EI values occurring between June and September resulted in increase in the root alkaloid content with a resultant fall in the stem and leaf alkaloid contents (Fig. 1c).

However, a change in the enrichment index values between September and December also resulted in an increase in the alkaloid contents of the stem and the leaf with a slight fall in the root alkaloid (Fig. 1c).

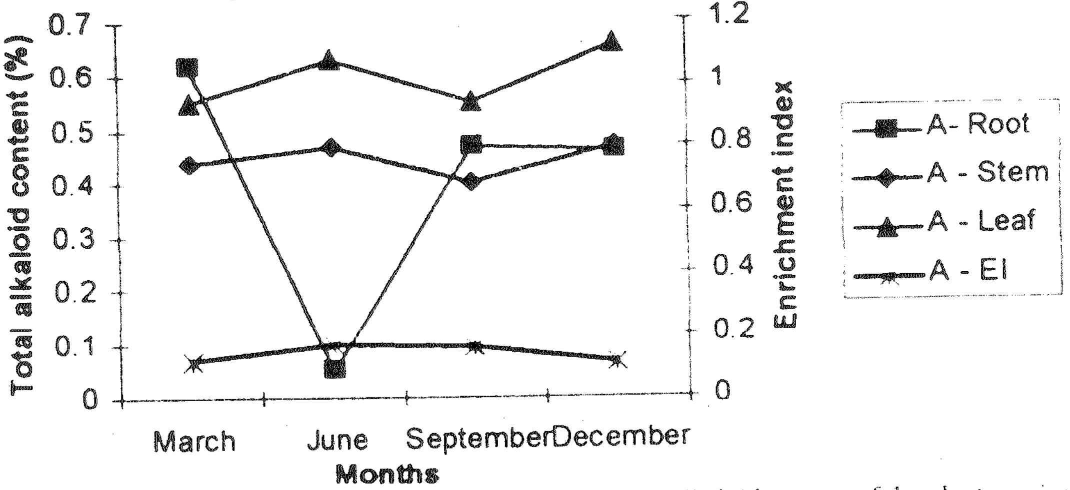

Fig. 1c. The effect of the enrichment index of the soil on the alkaloid content of the plant species, *C. sanguinolenta*, obtained from Abonse

Arsenic, copper, antimony and zinc are among the toxic/trace elements in the soils of the locations. Their concentrations were found to be below the normal or permissible levels of the world's average soils. Chon *et al.*[10] and Lee *et al.*[8] have reported on the high levels of potentially toxic elements, such as, cadmium and molybdenum as emanating from anthropogenic metal inputs in some geological soils. Pendias and Pendias[7] have also given specifications on the world average soils including As, Cu, Sb, Zn, etc indicating their toxic levels.

The enrichment index values of the soils of the locations are less than one indicating that they are non-toxic. Pepease had an EI value of 0.22, Abonse, 0.14 and Mamfe, 0.13. Lee *et al.*[8] have confirmed that soils with EI values less than 1 are not to be considered as enriched with toxic/trace elements.

The effect of the enrichment index of the soils on the total alkaloid content of the plant species *C. sanguinolenta*

Mineral elements whether in plant species and animals have been noted to play specific roles. The enrichment index values of the soils (As, Cu, Sb and Zn) of the locations affected the alkaloid content in the plant organs of the plant species *C. sanguinolenta* (Figs. 1a–c). Although the enrichment index difference of the locations was not significant (Table-1) their presence influences the synthesis of alkaloids in the plant species. Reports by Latta *et al.*[4] and Kaneshima *et al.*[3] proved the importance of some mineral elements, for example, optimal Mg and Fe levels in the synthesis of *delta*-9-tetrahydrocannabinol (THC) in *Cannabis* and suggested that these minerals may serve as enzyme co-factors. Coffman *et al.*[5] and Haney *et al.*[2] have also reported on the negative relationships between mineral nutrients and their involvement in the synthesis of plant species constituents.

Conclusion

The soils of the locations were endowed with toxic/trace elements in varying concentrations, comprising As, Cu, Sb and Zn (used for the computation of the enrichment index values) of the soils, which affected the levels of alkaloid content in the plant species *C. sanguinolenta* as presented in Figs. 1a–c. The enrichment index values (EIs) of the soils studied were below one. Therefore the soils of the locations could be classified as non-toxic and hence, herbal products prepared from the plant species obtained from the collection sites may be deemed to be safe from those toxic/trace elements.

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