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2021-09
IJGHC, Vol.10, No.4, 301-312
https://repository.ub.edu.bi/handle/123456789/2368

E-ISSN: 2278-3229

IJGHC, September 2021 –November Sec. A; Vol.10, No.4, 301-312.
DOI: 10.24214/IJGHC/GC/10/4 /30112.

International Journal of Green and Herbal Chemistry

An International Peer Review E-3 Journal of Sciences

Available online at www.ijghc.com

Section A: Green Chemistry

Research Article

CODEN (USA): IJGHAY

Effect of wastewater phytoremediation process on the chemical composition of *Eichhornia crassipes* and *Pistia stratiotes.*

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Received: 04 July 2021; **Revised:** 26 August 2021; **Accepted:** 05 September 2021

Abstract: *Eichhornia crassipes* and *Pistia stratiotes* are known for their ability to remove pollutants from wastewater. However, the latter often represent an external aggression towards these two plants. The objective of this study is to investigate the variation in the elemental and secondary metabolite composition that are secreted by these two plants to deal with external aggressions. The different secondary metabolites were identified and quantified, according to the standard procedure, in Pistia stratiotes and *Eichhornia crassipes* species from two countries (Burundi and Côte d'Ivoire). The results of the phytochemical screening revealed the presence of flavonoids, tannins and polyphenols and the absence of sterols, alkaloids and terpenoids. The quantitative analysis revealed a high concentration of total flavonoids compared to tannins and polyphenols, regardless of the plant used (Eichhornia crassipes or Pistia stratiotes) or the country in which it was harvested. Moreover, the species harvested in Burundi are richer in these metabolites than those harvested in Côte d'Ivoire. Elemental analysis revealed the presence of macroelements (S, Na, Mg, Cl, P and K), microelements (Fe, Zn and Br) and trace elements (Mn, Si and Al).

Keywords: Secondary metabolites, Eichhornia crassipes, Pistia stratiotes, Elemental composition, phytochemical screening, External aggression.

INTRODUCTION

In recent years, floating macrophytes including water hyacinth (*Eichhornia crassipes*) and water lettuce (*Pistia stratiotes*), have been extensively studied as wastewater treatment agents to reduce parameters such as total dissolved solids (TDS), suspended solids (SS), pesticides, chlorides, ammonia nitrogen (NH_4-N), nitrate nitrogen (NO_3-N), ortho-phosphate (ortho-phosphate) and phosphorus (ortho-phosphate), suspended solids (SS), pesticides, chlorides, ammonia nitrogen (NH_4-N), nitrogen as nitrate (NO_3-N), ortho-phosphate (PO_4-P), total nitrogen Kjeldahl (TNK); Chemical Oxygen Demand (COD), Biochemical Oxygen Demand (BOD), turbidity, metals, etc.[1]. These aquatic plants offer a simple and inexpensive method of treating wastewater [2–4].

However, these pollutants often represent an external aggression towards these plants. In addition to these external aggressions, during the phytoremediation these macrophytes are in competition with the microphytes, (algae) as for their growth [2,5]. To do to these aggressions and inhibit the growth of microphytes; these macrophytes secrete chemical compounds called secondary metabolites [2,5,6]. These secondary metabolites are constituted of extremely diverse chemical compounds with various functions, each with well-defined biological activities [7]. They are extensively used in human, veterinary and agricultural therapy[8]. By phytochemical screening, it can be shown that these secondary metabolites are divided into several categories of terpenoids compounds, essential oils, alkaloids, lectinic and polypeptidic, phenolic and polyphenolic, quinonic, flavonic, flavonolic, tannic, and coumarinic [9]. Indeed, aquatic plants such as *Eichhornia crassipes* and *Pistia stratiotes* have various uses. *Eichhornia crassipes* has been used as animal feed and in fish farming [10].

We recognize many medicinal applications of *Pistia stratiotes*. Indeed, it is diuretic and is used against certain venereal diseases, hemorrhoids, and dysentery. It also helps to heal lung wounds caused by tuberculosis [11]. Moreover, these two plants are used in the production of biogas and bioethanol [12,13], compost, in the manufacture of paper [14]and as a biosorbent in the removal of toxic metals, organic matter and nutrients [5,15]. The chemical composition of a plant depends on the species, the season of its harvest and its environment, a knowledge of their chemical composition is essential if we want to consider prospects for use [16]. Several studies on the research of secondary metabolites of vegetables have been conducted for therapeutic purposes[7,9,17,18]. However, the secondary metabolite composition that are secreted by aquatic macrophytes to deal with external aggressions has been the subject of little work in the literature [6]. It is in this context that this study, which aims to evaluate the chemical and mineral composition of *Pistia stratiotes* and *Eichhornia crassipes*, collected in Burundi and Côte d'Ivoire, was conducted.

2. MATERIAL AND METHODS

2.1. Macrophysics sampling and Preparation of vegetal material: The vegetal material used was composed essentially of whole plants of *Eichhornia crassipes* and *Pistia stratiotes* collected respectively in the watershed of the Kinyankonge river, outlet of the discharges of the Buterere (Burundi) wastewater treatment plant (WWTP) and lakes n° 9 and 10 in Yamoussoukro (Côte d'Ivoire), between the N'Zuessy and Habitat neighborhoods. After simultaneous collection in both countries, part of the samples were dried at laboratory temperature (23-28°C) [4,5] for about a month until their mass stabilized in order to avoid any risk of oxidation of the polyphenols. Another part of these macrophytes was used to treat domestic wastewater of the countries and then dried as before after use cycle. The samples were then powdered to facilitate the penetration of the solvent during the extraction process and the manufacture of pellets that were used for elemental analysis by the X-Ray Diffractometer.

2.2. Secondary metabolites identification: Preliminary chemical analyses are necessary to have a general idea about the different families of secondary metabolites present in plants. The different classes of secondary metabolites have been highlighted through tests consisting in the appearance of colorations and/or specificities characteristic of them using appropriate reagents.

Phytochemical screening does not provide information on the structure of a specific molecule. It only highlights the presence of such or such chemical family which can contain different molecules, but of related structures in general, presenting one or more common characters [19]. This study was therefore the object of a photochemical screening to reveal the secondary metabolites contained in *Eichhornia crassipes* and *Pistia stratiotes* through chemical reactions. The **Table 1** presents the different classical methods that were used to identify the secondary metabolites [20,21].

Table 1 : Methods of identification of secondary metabolites

Secondary metabolites	Methodology	References
Sterols and Terpenes	Lieberman and Bürchard	Wagner, 1983
Polyphenols	Iron III trichloride testing	Wagner, 1983
Flavonoids	Reaction to cyanidine	Wagner, 1983
Catechic Tannins	Reaction to the Stiany	Ethel, 1922
Gallic Tannins	Reaction to the Stiany	Ethel, 1922
Alcaloids	Mayer's and Dragendorff's test	Harbon, 1998
Leucoanthocyanins	Reaction to cyanidine without addition of magnesium chips	Wagner, 1983
Anthocyanins	Acid-base reaction according to the coloring	Ethel, 1922

2.3. Dosage of Secondary Metabolites

2. 3.1. Dosage of total polyphenols (TPP): The dosage of PPT in the different extracts is performed by the colorimetric method of Folin-Ciocalteu [22]. It uses mainly the reagent of "Folin-Ciocalteu" which is a mixture of complexes of phosphotungstic ($H_3PW_{12}O_{40}$) and phosphomolybdic ($H_3PMo_{12}O_{40}$) acids of yellow color [22].

Principle: It is based on the oxidation in basic medium of the oxidizable functions of polyphenolics by the Folin-Ciocalteu reagent with the formation of a new molybdenum-tungsten complex (Mo_8O_{23}-W_8O_{23}). These molybdenum-tungsten complexes (metal oxides W_8O_{23}/Mo_8O_{23}) of blue color formed, present an absorption maximum at 765 nm with the formation of a phenolate ion whose intensity is proportional to the quantity of phenolic compounds present in the sample. The determination of the TPP is performed by comparing the difference in optical density (OD) observed with that obtained for a gallic acid standard of known concentration [23].

Figure 1 shows the calibration curve previously drawn with gallic acid under the same conditions as the samples to be analyzed. The results obtained are expressed as milligram equivalent of gallic acid per gram of dry matter (mg EGa/g Ms).

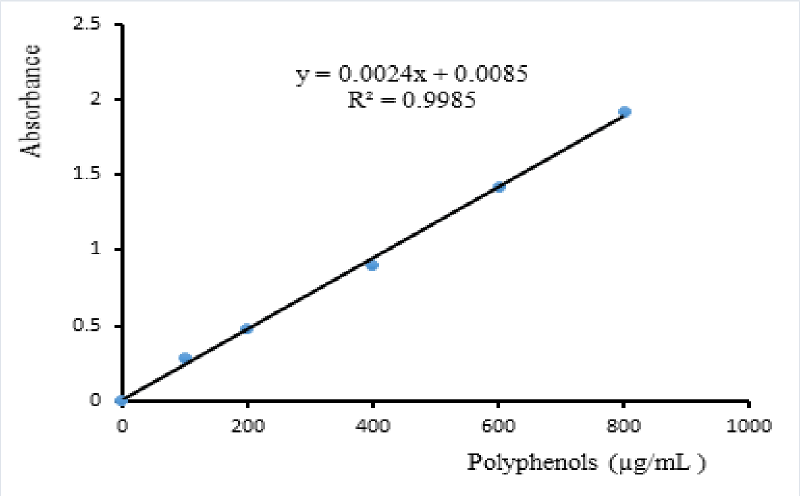

Figure 1: Calibration curve for the determination of total polyphenols

2.3.2. Dosage of Flavonoids: Total flavonoids were determined by the method of Marinova et al.[24]. A volume of 0.75 mL of 5% (w/v) sodium nitrite ($NaNO_2$) was added to 2.5 mL of extract in a 25 mL flask. 0.75 mL of 10% (w/v) aluminum chloride ($AlCl_3$) was added and incubated for minutes in the dark. After incubation, 5 mL of sodium hydroxide (1N NaOH) was added, and the volume was made up to 25 mL with distilled water. The mixture was shaken vigorously before being assayed with UV-visible Spectrophotometer. The reading was taken at 510 nm. The blank was prepared in the same way while replacing the extract with the extraction solvent. A calibration curve (**Figure2**) was performed with quercetin (0-1.5 g.L^{-1}) at different concentrations (0, 0.15, 0.3, 0.9, 1.2, 1.5 g. L^{-1}). The tests were carried out in triplicate and the flavonoid content is expressed as mg quercetin equivalent (QE) / kg fresh mass[23,25].

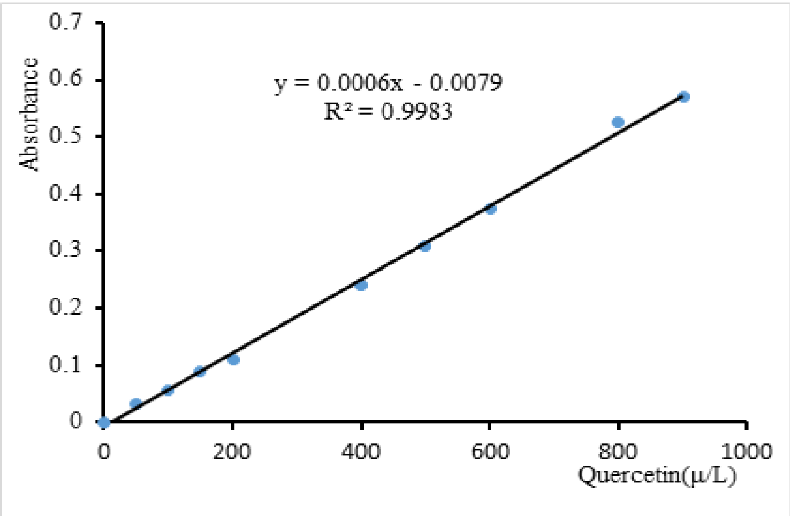

Figure 2: Calibration curve for the dosage of total flavonoids

DOI: 10.24214/IJGHC/GC/10/4/30112.

2.3.3 Dosage of condensed tannins: The determination is done by the vanillin method using vanillin and concentrated hydrochloric acid.

Principle: The principle of this assay is based on the fixation in acid medium of the aldehydic group of vanillin on carbon 6 of the ring A of catechin to form a red chromophore complex that absorbs at 500 nm [26].

Procedure: In a 50µL test tube of the extract, 3 mL of Vanillin/MeOH (4%) and 1.5 mL of fuming hydrochloric acid are introduced. After 15 min, the absorbance of the resulting mixture is measured with a UV-visible spectrophotometer at 500 nm against a blank. The calibration curve (**Figure 3**) is developed with the standard solution of catechin prepared at different concentrations. The results are expressed in milligram catechin equivalent per gram of dry matter (mg EC/g Ms) [27].

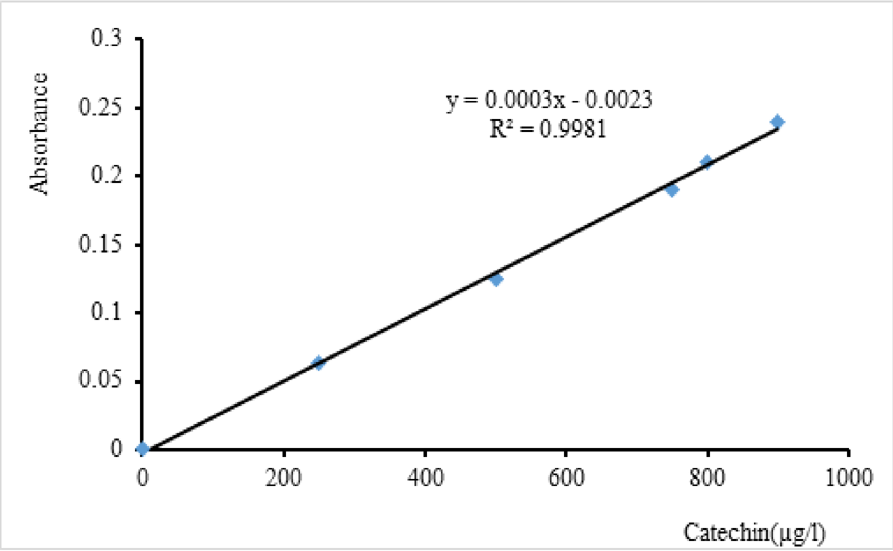

Figure 3: Calibration curve for the dosage of Tannins

3. RESULTS AND DISCUSSION

The chemical composition of a plant depends on the type of species, the season in which it was harvested and its environment. It is essential to know their chemical composition if one wants to consider prospects for their use. This section presents the analysis and interpretation of the results obtained during the elemental analysis, identification and characterization of secondary metabolites involved in the defense against external aggressions on *P. stratiotes and E. crassipes.*

3.1 Elemental composition of *P. stratiotes* and *E. crassipes*

- **Microelements: Figures 4 and 5** present the mineral content (macro and micro elements) of *P. stratiotes* and *E. crassipes* that were collected in Burundi and Côte d'Ivoire before and after using them in phytoremediation.

  Sulfur (S), Potassium (K), Calcium (Ca), Sodium (Na), Phosphorus (P), Magnesium (Mg) and Chlorine (Cl) constitute the macroelements evaluated. The analysis in **Figure 4** shows that *E. crassipes* is rich in Sulfur while *P. stratiotes* is rich in Potassium. It should be noted that the macrophytes collected in Burundi are rich in sulfur (459µg /L) than those collected in Côte d'Ivoire (280µg /L). On the other hand, the macrophytes collected in Ivory Coast are rich in

IJGHC, September 2021 –November Sec. A; Vol.10, No.4, 301-312.
DOI: 10.24214/IJGHC/GC/10/4/30112.

potassium (311µg /L) than those collected in Burundi (281µg /L). Our results are in agreement with those obtained by [28] who found in their work on monocotyledonous plants a macronutrient content greater than or equal to 1000 mg / kg dry matter. These differences in macronutrient concentrations between the two countries could be explained by the edaphic characteristics (temperature, precipitation etc.) of their environment. After phytoremediation, a decrease in macronutrient concentration is observed since part of the macronutrients is used by the macrophytes during their growth.

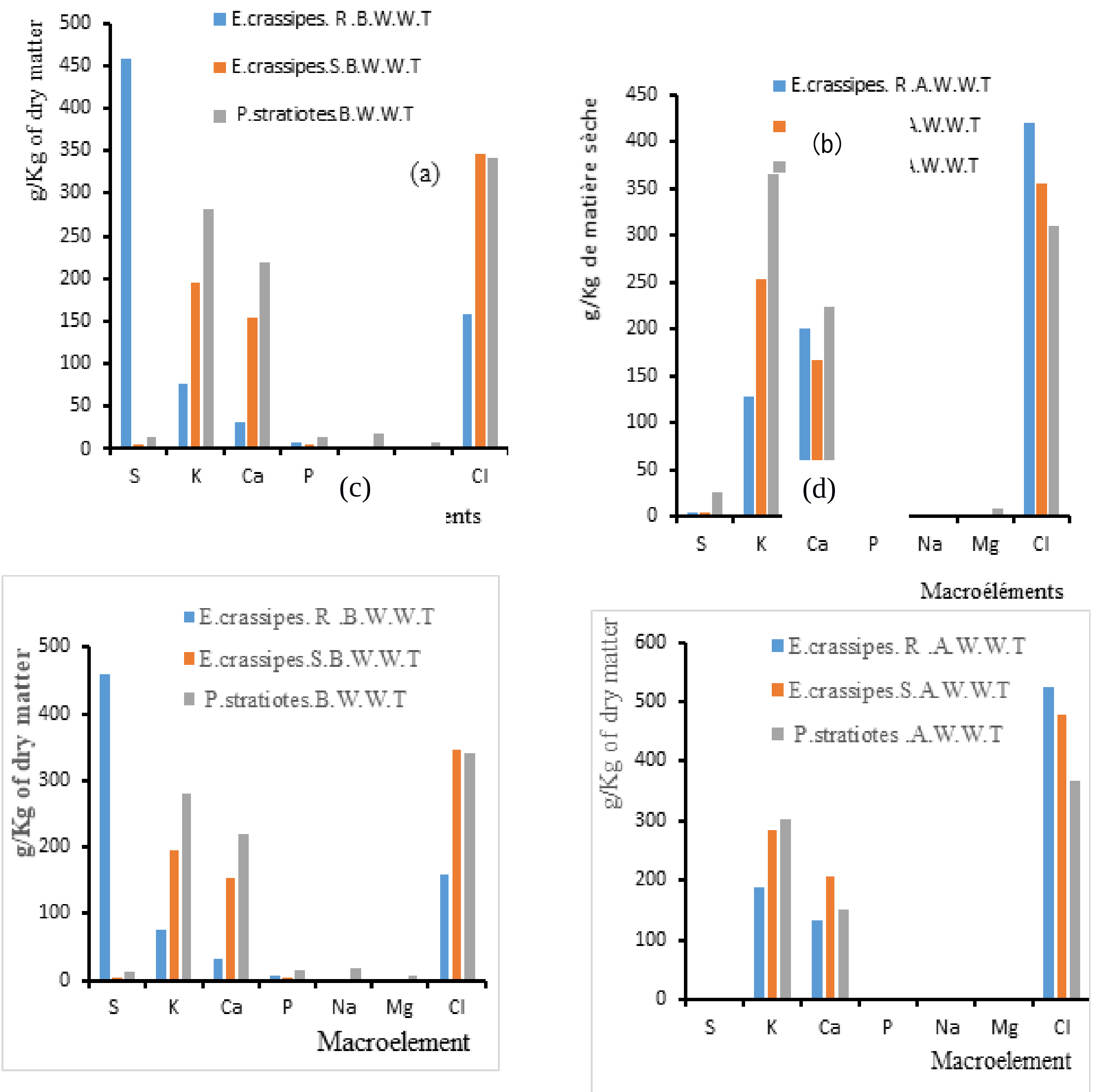

Figure 4: Macroelement composition of *E. crassipes* and *P. stratiotes* from Burundi before and after ((a) and (b)) and Côte d'Ivoire before and after ((c) and (d)) wastewater phytoremediation

***E. crassipes. R.B.W.W.T**: Root Eichhornia crassipes before Wastewater Treatment; **E. crassipes. R.A.W.W.T**: Root Eichhornia crassipes After Wastewater Treatment**; E. crassipes. S. B W.W.T**: Stem Eichhornia crassipes before wastewater treatment; **E. crassipes. S. A W.W.T:** Stem Eichhornia crassipes after wastewater treatment; **P. stratiotes. B.W.W.T:** Pistia stratiotes before wastewater treatment**; P. stratiotes A.W.W.T**: Pistia stratiotes after wastewater treatment.*

DOI: 10.24214/IJGHC/GC/10/4/30112.

- **Microelements:** Iron (Fe), Bromine (Br) and Zinc (Zn) are the microelements evaluated. Analysis of **Figure 5** shows that E. crassipes from Côte d'Ivoire is rich in iron (42.7g/kg dry matter) compared to Burundi (22.4g/kg dry matter).

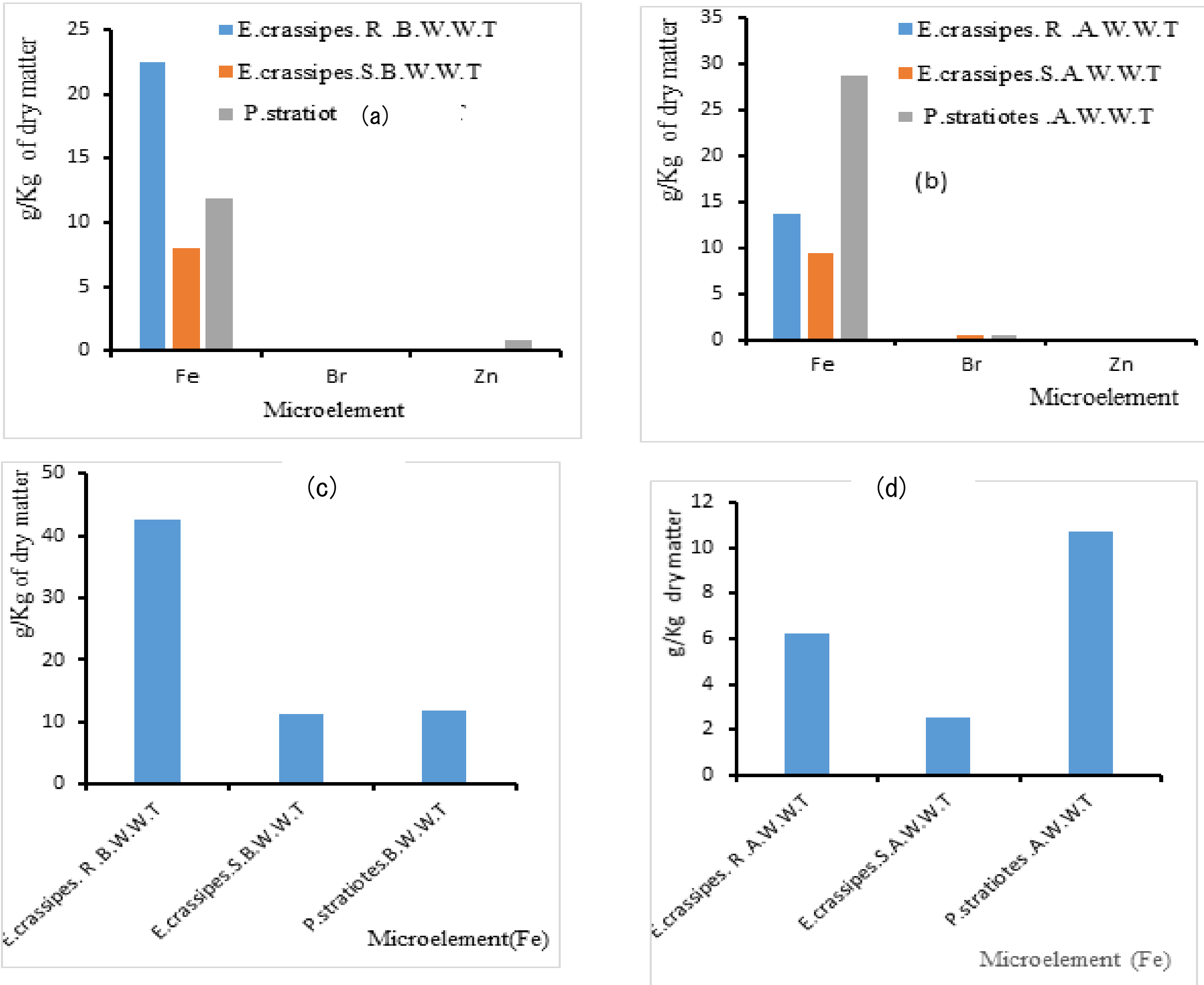

Figure 5: Microelement composition of *E. crassipes* and *P. stratiotes* from Burundi before and after ((a) and (b)) and Côte d'Ivoire before and after ((c) and (d)) wastewater phytoremediation

- **Trace elements:** Manganese (Mn), silicon (Si) and aluminum (Al) are the trace elements evaluated. **Figure 6** presents the trace element contents of *P stratiotes* and *E. crassipes* that were sampled in Burundi and Côte d'Ivoire before and after using them in phytoremediation. An analysis of **Figure 6** shows the absence of magnesium (Mg) and silicon (Si) in the roots of water hyacinth before wastewater phytoremediation, regardless of the country in which the plant was harvested. However, these elements were present in the stems of this plant at the beginning of the phytoremediation trials. After phytoremediation, a significant concentration of these elements in the roots of this plant was observed. This could be explained by the fact that this plant is often used for the elimination of metals [29]. For water lettuce where the whole plant was used; the presence of trace elements is noted at the beginning and at the end of the trials. The same is true for the stems of water hyacinth. This could be explained by saying that these trace elements concentrate in the aerial parts of the plant before their use cycle in phytoremediation.

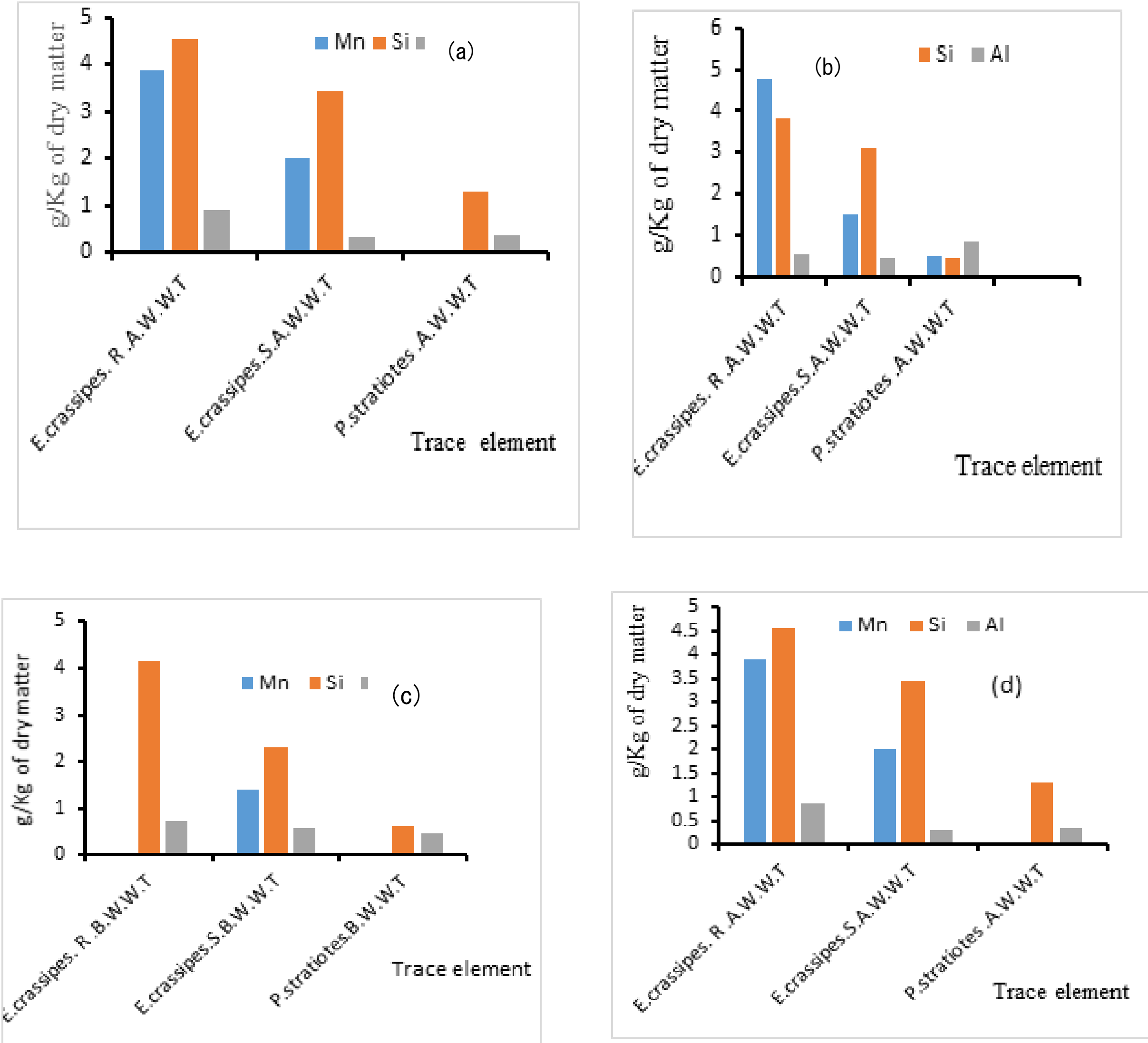

Figure 6: Trace element composition of *E. crassipes* and *P. stratiotes* from Burundi before and after ((a) and (b)) and Côte d'Ivoire before and after ((c) and (d)) wastewater phytoremediation

3.2 Secondary metabolites

3.2.1 Identification of secondary metabolites: Table 2 presents the results of the identification of secondary metabolites of *Eichhornia crassipes* and *Pistia stratiotes* from Burundi and Côte d'Ivoire by phytochemical screening.

The phytochemical screening of *Eichhornia crassipes* and *Pistia stratiotes* collected in Burundi and Côte d'Ivoire indicates the presence of chemical compounds such as polyphenols, flavonoids (flavonoid fibers, leucoanthocyanins) and catechic and gall tannins. We notice the absence in both countries of sterols, terpenes and alkaloids. These results corroborate with those found by [6].

DOI: 10.24214/IJGHC/GC/10/4/30112.

Table 2: Identification of secondary metabolites present in macrophytes.

		Côte d'Ivoire		Burundi	
		Eichhornia crassipes	***Pistia stratiotes***	***Eichhornia crassipes***	***Pistia stratiotes***
Sterols and terpenes		-	-	-	-
Polyphenols		+	+	+	+
Flavonoids	**Anthocyanins**	+	+	±	+
	Free flavonoids	±	+	±	+
	Leucoanthocyanins	+	±	±	+
Tannins	**Catechic**	+	±	±	±
	Gallic	+	±	±	+
Alcaloids		-	-	-	-

- : absent ; + : present ; ± : present as traces

3.2.2. Quantification of secondary metabolites: The results obtained during the quantitative analysis (**Figure 7**) come to confirm the observations made during the phytochemical screening process (**Table 2**). For the three families of secondary metabolites studied, we note that the macrophytes collected in Burundi are richer than those collected in Côte d'Ivoire. This could be explained by the environmental conditions (temperature, precipitation) as well as the state of the aquatic environment in which the macrophytes were collected. It should be noted that for both plants, phytoremediation contributes to a general decrease in secondary metabolite content. This could be explained by the fact that the latter were used to control aggressors and / or to eliminate microphytes (algae) that compete with macrophytes during phytoremediation[1]. Quantitative analysis (**Figure 7**) revealed a high concentration of total flavonoids compared to tannins and polyphenols, regardless of the plant used (*Eichhornia crassipes or Pistia stratiotes*) or the country in which it was harvested. These results are in agreement with those reported by Muhanon et al. [6].

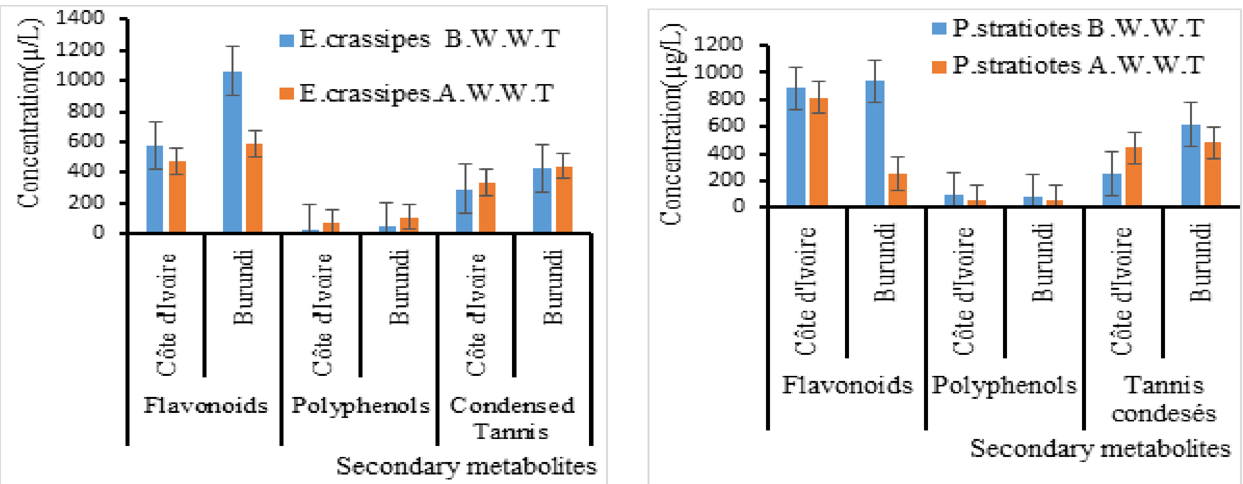

Figure 7: Comparison of the composition of macrophytes in secondary metabolites in Côte d'Ivoire and Burundi

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CONCLUSION

The elemental analysis allowed the identification of macroelements, microelements and trace elements contained in macrophytes before and after their use in wastewater treatment. The elemental composition of macrophytes varies from country to country. The phytochemical screening revealed the secondary metabolites contained in *E. crassipes* and *P. stratiotes*. The absence of sterols, terpenoids and alkaloids in the plants harvested in Côte d'Ivoire and Burundi was noted. The absence of metabolites suggests that they are not used by the plants to fight against the aggressors during the phytoremediation of the studied pollutants. The metabolites found in these plants are in large quantities in the form of flavonoids and tannins. Total polyphenols are present but in small quantities. This could lead us to think that these phenolic compounds are used by plants to fight against aggressors and/or inhibit the growth of microphytes.

ACKNOWLEDGEMENTS

The authors thank the Embassy of France in Burundi via Campus France for granting a scholarship to Pierre Ntakiyiruta to carry out his doctoral thesis work.

DISCLOSURE STATEMENT

No potential conflict of interest was reported by the author(s).

REFERENCES

1. Z.Ismail, S.Z.Othman, K.H.Law, A.H.Sulaiman, R. Hashim, Comparative Performance of Water Hyacinth (Eichhornia crassipes) and Water Lettuce (Pista stratiotes) in Preventing Nutrients Build-up in Municipal Wastewater. CLEAN – Soil, Air, Water [Internet] 2015 [cited 2020 Jan 23]; 43(4):521–31.
2. Z.Ismail, S.Z.Othman, K.H.Law, A.H.Sulaiman, R. Hashim, Comparative Performance of Water Hyacinth (Eichhornia crassipes) and Water Lettuce (Pista stratiotes) in Preventing Nutrients Build-up in Municipal Wastewater. CLEAN – Soil, Air, Water [Internet] 2015 [cited 2020 Jun 26]; 43(4):521–31.
3. P. Gupta, S. Roy, A. Mahindrakar, Treatment of Water Using Water Hyacinth, Water Lettuce and Vetiver Grass - A Review. Resources and Environment 2012; 2:202–15.
4. P. Ntakiyiruta B.G.H. Briton, G. Nsavyimana K.Adouby, D. Nahimana G. Ntakimazi, et al. Optimization of the phytoremediation conditions of wastewater in post-treatment by Eichhornia crassipes and Pistia stratiotes: kinetic model for pollutants removal. Environmental Technology [Internet] 2020 [cited 2020 Nov 17]; 41(28):1–26.
5. P. Ntakiyiruta, G. Nsavyimana , B.G.H.Briton, A. Kopoin, D. Nahimana, G. Ntakimazi , Actions combinées de Eichhornia crassipes et Pistia stratiotes pour traitement tertiaire de l'effluent des bassins facultatifs de la station d'épuration de Buterere, Burundi. Interanational jouranal of Biological and Chemical Sciences 2020; 14(7):2463–75.
6. Mahunon Ser, M.P.Aina , E.K.Kouassi, C.P.Agbangnan-Dossa, B.K.Yao, K. Adouby, Study of the variation in the chemical composition of Eichhornia crassipes exposed to external aggressions - Recherche Google. International Journal of Green and Herbal Chemistry

[Internet] 2017 [cited 2021 Mar 18]; 6(3):81–91.

7. S. Krief , Métabolites secondaires des plantes et comportement animal: surveillance sanitaire et observations de l'alimentation des chimpanzés (Pan troglodytes schweinfurthii) en Ouganda. Activités biologiques et étude chimique de plantes consommées. Sciences du Vivant [q-bio], Museum national d'histoire naturelle -MNHN PARIS, 2003; 348.
8. B. Abderrahmane, B. Mustapha, Etude théorique des métabolites secondaires des végétaux et des composés de synthèse sur le plan de l'activité biologique [Internet]. 2007 [cited 2020 Apr 22];Available from:
9. L. Lagunez Rivera, Etude de l'extraction de métabolites secondaires de différentes matières végétales en réacteur chauffé par induction thermomagnétique directe. 2006;
10. Mahunon Ser. Mécanismes d'élimination des polluants organiques et métalliques dans les processus de traitement des eaux usées par le lagunage à macrophytes flottants. 2017;
11. N. Mireille , F. Lecompte, B. Collange , M. Tchamitchian , Systèmes de culture et qualité de la laitue. Comment repenser les systèmes de culture pour réduire l'usage de pesticides et les risques de résidus ? Innovations Agronomiques 2010;9:67–84.
12. L.R.Kumar, S.K.Yellapu, X. Zhang, R.D.Tyagi, Energy balance for biodiesel production processes using microbial oil and scum. Bioresource Technology [Internet] 2019 [cited 2020 May 8]; 272:379–88.
13. R.Pantawong, A.Chuanchai, P. Thipbunrat, Y. Unpaprom, R.Ramaraj, Experimental Investigation of Biogas Production from Water Lettuce, Pistia stratiotes L. Emergent Life Sciences Research 2015;1:41–6.
14. O.Almoustapha, J. Millogo-Rasolodimby, Production de biogaz et de compost à partir de eichhornia crassipes, (mart) solms-laub (pontederiaceae) pour un développement durable en Afrique sahélienne. VertigO - la revue électronique en sciences de l'environnement [Internet] 2006 [cited 2020 May 5];(Volume 7 Numéro 2).
15. S.E.R.Mahunon, M.P. Aina, A.V.O.Akowanou, E.K.Kouassi, B.K.Yao, K. Adouby, et al. Optimization process of organic matter removal from wastewater by using Eichhornia crassipes. Environ Sci Pollut Res [Internet] 2018 [cited 2021 Mar 2];25(29):29219–26.
16. J.B.Saint-Lager, Étude de l'influence chimique exercée par le sol sur les plantes. Publications de la Société Linnéenne de Lyon [Internet] 1876 [cited 2021 Mar 20];4(1):50–84.
17. D. Guillaume, Z. Charrouf, Saponines et métabolites secondaires de l'arganier (Argania spinosa). Cahiers Agricultures 2005;14(6):509–16.
18. Jean B. Pharmacognosie, phytochimie, plantes médicinales (4e ed.). Lavoisier; 2009.
19. D. Marinova, F. Ribarova, M. Atanassova, Total phenolics and total flavonoids in Bulgarian fruits and vegetables. 2005;6.
20. Y. Nyamien , F. Adjé , F. Niamké, E. Koffi , O. Chatigre , A. Adima, et al. Effect of Solvents and Solid-Liquid Ratio on Caffeine Extraction from Côte d'Ivoire Kola Nuts (Cola nitida). 2013;4(1):5.
21. R.Algerieene, D. Et , U. Ziane, A.D.E.Djelfa , F. Des, S. Et, et al. Screening phytochimique de quelques plantes steppiques Artemisia Campestris et Teucrium Polium de la région de El Hamel wilaya de M ' Sila. 2016;

22. V.L.Singleton, J.A. Rossi, Colorimetry of Total Phenolics with Phosphomolybdic-Phosphotungstic Acid Reagents. Am J Enol Vitic 1965;16(3):144–58.
23. Y.Nyamien, F. Adje, F. Niamké, O. Chatigre, A. Adima, G.H.Biego, Caffeine and Phenolic Compounds in Cola nitida (Vent .) Schott and Endl and Garcinia kola Heckel Grown in Côte d ' Ivoire. 2014;4(35):4846–59.
24. D. Marinova, F. Ribarova, M. Atanassova, Total phenolics and total flavonoids in bulgarian fruits and vegetables. Journal of the University of Chemical Technology and Metallurgy 2005;40(3):255–60.
25. A.H.Facult, Screening phytochimique et identification spectroscopique des flavonoïdes d ' Asteriscusgraveolenssubsp . odorus. 2014;(January).
26. K. Mythili, C.U. Reddy, D. Chamundeeswari, P.K.Manna, Determination of Total Phenol, Alkaloid, Flavonoid and Tannin in Different Extracts of Calanthe Triplicata. Research & Reviews: Journal of Pharmacognosy and Phytochemistry 2014;2(2):40–4.
27. H. Gonzalo, F. F.Pilar, G. Javier, R.M.Angel, P. Del, Effect of different doses of quebracho tannins extract on rumen fermentation in ewes. Animal Feed Science and Technology [Internet] 2003 [cited 2021 Jan 29];109(1–4):65–78.
28. P. Raven, R. Evert, S. Eichhorn, and Bouharmont J. Biologie végétale. 2e édition. Bruxelles: De Boeck; 2015.
29. S. Rezania, M. Ponraj, A. Talaiekhozani, S.E.Mohamad , M.F.Md Din, S.M.Taib, et al. Perspectives of phytoremediation using water hyacinth for removal of heavy metals, organic and inorganic pollutants in wastewater. J Environ Manage 2015; 163:125–33.
30. S.Rezania, M. Ponraj, A.Talaiekhozani, S.E.Mohamad M.F.Md Din, S.M.Taib, et al. Perspectives of phytoremediation using water hyacinth for removal of heavy metals, organic and inorganic pollutants in wastewater. Journal of Environmental Management [Internet] 2015 [cited 2020 Feb 1]; 163:125–33.

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Online publication Date: 10.09.2021