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Original article

Evaluation of heavy metal contamination and accumulation in lettuce (*Lactuca sativa* L.) plants

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ABSTRACT

Lettuce is a plant species considered to be a bioindicator of the presence of heavy metals in soils, consumed fresh by the population, with the highest concentrations found in its leaves. The research was carried out in areas surrounding the White Ceramic Enterprise "Adalberto Vidal" of the Popular Council of Jamaica, in San José de las Lajas, Mayabeque, Cuba, with the aim of evaluating the extraction of heavy metals by lettuce plants under production conditions. A randomized sampling of soil and crop was carried out. The content of heavy metals was determined by X-Ray Fluorescence-Energy Dispersive X-Ray and the transfer coefficient in the crop was estimated. Values obtained were compared with the maximum permissible limits and hazardous levels for soil and plants, proposed in the literature. Results showed that the soil is contaminated by Ni, Cu and Pb metals. The accumulation of seven heavy metals studied (Fe, Co, Zn, Mn, Cu, Ni and Pb) was found in the crop at high levels and the highest accumulation is in the aerial part, which represents a danger for local food safety.

Key words: degradation, pollution, toxicity

INTRODUCTION

One plant species considered to be a bioindicator of heavy metals is lettuce (*Lactuca sativa* L.), which is used fresh by the population and it is in the edible part (leaves) where the highest concentration of these elements accumulates [(1)]. Despite the effort, in terms of environmental education, that has been made by the FITOPLANT Scientific Group of the UNAH (Agrarian University of Havana), even in the areas surrounding the White Ceramic Enterprise "Adalberto Vidal" (current joint venture since 2018 SANVIG, S.A.) of the Popular Council of Jamaica, this crop continues to be harvested for food and marketing purposes, since it does not show symptoms of toxicity under conditions of metallic stress [(2)].

Some plants and other organisms develop a complex physiological mechanism to minimize the negative effects of heavy metals, controlling the absorption, accumulation and translocation of these elements in the plant tissue. These mechanisms protect cells, causing plant tolerance to these elements, so that treatment techniques have their genesis in the ability of different organisms (plants and microorganisms) to degrade, extract or immobilize organic or inorganic contaminants in soil or water [(3)]. Therefore, studies attributed to the presence of heavy metals and some of their compounds in agricultural areas located over old smelters, refineries, aluminum factories, metallurgical industry and ceramic companies are important. Some of them precipitate in the sewage sludge, so the crop usually contains high concentrations of these metals [(4)].

It was scientifically demonstrated that in addition to causing some of the most serious environmental problems, exposure to heavy metals in certain circumstances is the cause of direct damage to humans, although they are considered trace elements, they are food toxins and their negative effects on health are manifested in the long term.

The action of different heavy metals has been reported on the increase of cancer cases, nervous system lesions and intellectual and mental retardation as a result of the consumption of water and food contaminated with these metals [(5-8)].

The aim of this study is to evaluate the heavy metal extraction capacity of lettuce plants under production conditions in the areas surrounding the wastewater discharge of the "Adalberto Vidal" Ceramic Enterprise in San José de las Lajas municipality.

MATERIALS AND METHODS

The study was carried out in an agricultural area adjacent to the discharge of waste from the White Ceramic Enterprise "Adalberto Vidal" in San José de las Lajas municipality, Mayabeque province (Figure 1), located at a distance of 200 m from the source of contamination and a profile of the "La Asunción" Farm, located in the same municipality, in an area not affected by waste, was established as the standard soil, it is a land not cultivated for more than 60 years, with spontaneous vegetation and does not receive any treatment.

Figure 1. a) Expulsion and transit of the Enterprise's residuals b) Production area

A completely randomized design was used to set up the experiment. For the matrices under study (soil and crop) five sampling points were taken with three replicates. Sampling was carried out following diagonals in the plot: beginning, middle and end, obtaining at the end a single sample as an average of sampling points.

Soil samples were taken manually at 0-20 cm depth, disregarding the surface layer, with an approximate separation of 1 m from the plot boundary. Samples of the lettuce crop (*Lactuca sativa* L.) var. Black Simpson were taken in this same condition at the time of harvest, carrying out determinations of heavy metals in root, stem and leaves. In addition, the transfer coefficient was calculated from the aerial part/root part ratio, which indicates where the metal ions are translocated to.

Soil samples were cleaned (removal of stones, plants, foreign matter) and sieved at 125 µm and then mixed with cellulose in a 4:1 ratio, after homogenization, "infinitely coarse" tablets were made up, pressed at 15 t. The irradiation of the pellets was carried out for 6 h of live time and the dead time was in all cases below 1 %.

The analyses were performed at the Analytical Laboratory of the Nuclear Physics Department of InSTEC by Energy-Dispersive X-Ray Fluorescence [9]. The determination of heavy metal concentration was performed with a high-resolution Si(Li) detector for X-ray spectrometry and a ^{238}Pu radioisotope source of low-energy photons by Energy-Dispersive X-Ray Fluorescence. An aluminum excitation chamber with a collimator for the characteristic radiation of 1 cm diameter and adjustable sample holder height was used. The minimum source-detector distance is 18 mm, direct radioisotope excitation was provided by AMERSHAND, which has a half-life of 87 years, an emission energy (U-L) of 12-17 KeV, with an α decay scheme. All spectra were processed with WinAxil code version 4.5.2.

For the determinations in vegetable samples of lettuce crop, after drying at 70 ºC, the aerial and root parts were separately homogenized by grinding them. Subsequently, 0.1 g of each part was weighed on an analytical balance (Sartorius BP121S) and subjected to a digestion process with HNO3, HCl (3:1) for 30 min. Levels of metals were determined by Atomic Absorption Spectrophotometry with air-acetylene flame in BuckScientific 210 VGP equipment, according to the procedure established and validated in the Environmental Analysis Laboratory [(10)].

The STATGRAPHICS Plus statistical program for Windows 5.1 was used for the analysis of the experimental data. The simple ANOVA test was performed, for which a significance level of 0.05 was established for a 95 % confidence interval. The Duncan's comparison were applied to determine between which levels the significant difference was established and for those mean values that presented significant differences, different letters were assigned to them.

In the case of the study of the soil samples, the statistical processing of the data consisted of calculating the confidence intervals of the means by treatments of the variables evaluated, for a confidence level of 95 %.

RESULTS AND DISCUSSION

Concentration of heavy metals in soil

Soil analysis results of samples in the agricultural areas surrounding the discharge of waste from the former White Ceramic Enterprise "Adalberto Vidal" in San José de las Lajas municipality showed that concentrations of the heavy metals evaluated (Cr, Co, Ni, Cu, Zn, Pb and Fe) were higher than those determined in the standard soil (Table 1); with the exception of Co, where the difference is five units, and Fe, 1.41 percentage units, since for this element we refer to the percentage of its quantity in the sample, because it was selected as the reference metal for the study; little influenced by anthropogenic sources, due to the high natural levels of this element in the earth's crust [11-13].

Table 1. Concentration of heavy metals (mg kg^{-1}) in soil that receives the waste from the White Ceramic Enterprise "Adalberto Vidal" in San José de las Lajas municipality

Samples	Cr ± std	Co ± std	Ni ± std	Cu ± std (mg kg^{-1})	Zn ± std	Pb ± std	Fe(%)± std
Pattern	118±42	15±4	84±29	364±98	117±40	90±27	4,54±1
Polluted	154±38	20±38	317±25	421±32	415±24	173±31	5,95±1
RV[a]	100	9	35	36	140	85	
IV[a]	380	240	210	190	720	530	
UPL[b]	100	50	75	100	300	100	
EC[c]	100	25	74	55	70	13	5

a- Reference Values (RV) and Intervention Values of the Dutch Norms [11]
b- Upper Permissible Limit in soils [12]
c- Reported values for the Earth's Crust [12]

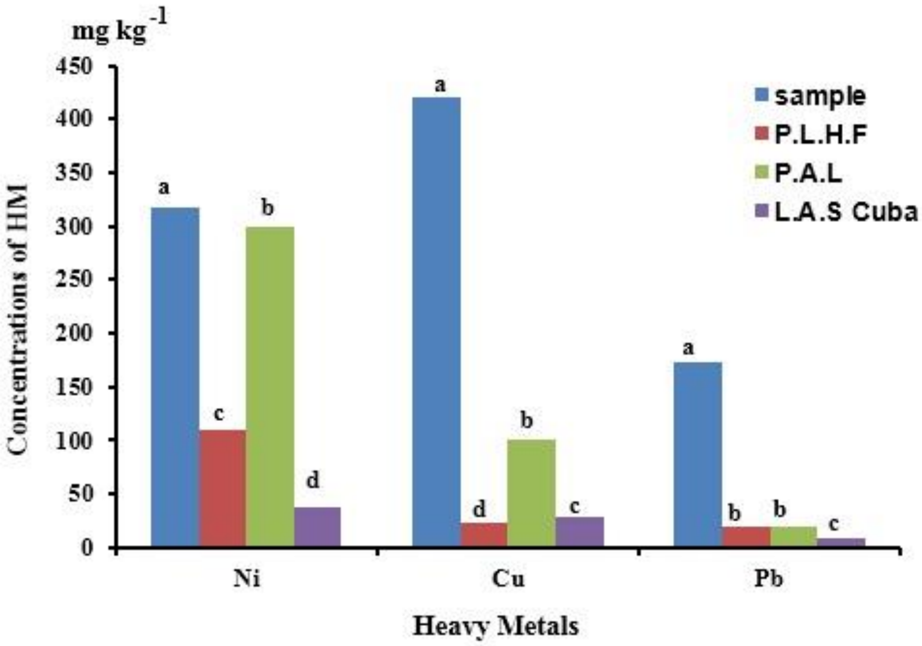
The concentration values of heavy metals in the contaminated soil are higher than the reference values, and in the case of nickel and copper, the value exceeds the intervention limit of these standards, which classifies the soil as moderately contaminated in Cr, Co, Zn and Pb and in need of urgent remediation, due to Ni and Cu concentrations. In turn, these values, with the exception of cobalt, are higher than those reported for the earth's crust and those proposed as Upper Permissible Limits [12].

For the rest of the elements analyzed, the decreasing order of contamination contributions is Zn, Ni, Pb, Cu and C, with values of 298, 233, 83, 57 and 36 units of differences respectively between the contaminated soil and the standard soil, which is equivalent to a percentage of 99.33 % for Zn and 310.66 % for Ni above the upper permissible limit, which are the ones that contribute the most [12].

These results are directly related to the wastes coming from the White Ceramic Enterprise "Adalberto Vidal", which has among its materials all kinds of clays and kaolins, coming from different provinces of the country: Pinar del Río (Club de Cazadores), Las Tunas (Dumañuelos), Isla de la juventud (Santa Elena), as well as feldspars coming from Sancti Spíritus (Pico tuerto), besides, many other oxides resistant to high temperatures, such as corundum (Al_2O_3), zirconium oxide (ZrO_2) or silicon carbide (SiC) and organic compounds such as methylene blue; clays containing heavy metals in the form of oxides such as: Iron (Fe), Manganese (Mn), Aluminum (Al), Silicon (Si), Barium (Ba), Titanium

(Ti), Zirconium (Zr), and Zinc (Zn), among other elements such as Calcium (Ca), Magnesium (Mg) and Sodium (Na) (1,7,10).

The metals with the highest concentrations in the soil according to the calculations are Ni, Cu and Pb (Figure 2); it can be seen that these elements are found in concentrations higher than those required by these established levels, obtaining statistically significant differences with respect to the three values with which they are compared.



Unequal letters differ significantly, according to Duncan for values with 95 % confidence.

Figure 2. Concentration of Ni, Cu and Pb compared to average levels acceptable for healthy food production and phytotoxic levels

Concentrations of these elements (Ni, Cu and Pb) are higher than those reported for the average of Cuban agricultural soils [(12-16)]. When comparing the concentration of these metals (Ni, Cu and Pb) with the permissible levels for healthy food (P.L.H.F), phytotoxic average levels (P.A.L) and levels in Cuban agricultural soils (L.A.S Cuba).

All these results have a chemical-biological influence on the crops produced in these areas, since, as it can be verified, the values with which they are compared are related to food safety, a property that takes interest in local food safety [(16)]. Besides, ratifying the importance of these studies, because the chemical elements that are studied in their majority are essential micronutrients for the growth and development of plants [(13,15)]. That is why the producer appreciates a growth and development of plants, which does not show symptoms of nutritional deficiency.

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Concentration of heavy metals in lettuce crops

When analyzing the concentration values obtained in the lettuce samples, in relation to some permissible levels, it can be seen that the crop is capable of extracting several toxic elements (Fe, Co, Zn, Mn, Cu, Ni, Pb), exceeding in all levels of sufficient or normal concentration in plants (S) [4,7,12] and also exceeding the excessive or toxic concentrations (E) for all the metals evaluated, except for Mn and Pb (Table 2).

Table 2. Heavy metal concentration (mg kg^{-1} of DM) in lettuce crop and its transfer coefficient value

Samples		Fe	Co	Zn	Mn	Cu	Ni	Pb
		mg kg^{-1} of DM						
Leaves		974	28	800	114	176	124	174
Roots		1 098	25	1 145	47	321	93	153
Stems		4 720	30	637	243	320	160	133
Permissible limits	S	--	--	30	30	5	0,1-5	--
in plants	T	--	--	50	300	5-20	10	10
	E	--	1	100	1 000	20-100	10-100	30-300
Transfer coefficient		5,19	2,32	1,26	7,6	1,55	2,94	2,01

(S) Sufficient or normal concentration levels in plants, (T) Tolerable levels in agricultural crops, (E) Excessive or toxic concentrations

There is a high accumulation of Fe, Zn and Cu in roots, stems and leaves, with a higher presence of Fe and Zn, which are elements considered toxic for agricultural crops [17]. For the lettuce crop it is not harmful, since it possesses physiological mechanisms that allow it to take them quickly and accumulate them in its aerial parts, making it possible for it to survive in the metallic stress conditions where the plant develops [18,19]. Therefore, it represents an economic reference crop model to study metal accumulation mechanisms, as well as the phytoextraction mechanisms used by phytoremediation, as a method to remediate areas vulnerable to heavy metal contamination [18].

In the case of the calculated transfer coefficient (Table 2), it was observed that for all the elements evaluated, the translocation is from the radical part to the aerial part, which corroborates the risk of cultivation in contaminated conditions [19], as it does not comply with food safety [18]. These pollution conditions by heavy metals in soils should not be allowed for the cultivation of agricultural species with food purposes [20,21], since in the case of lettuce there is a great accumulation of these elements very high in leaves, which is precisely its organ of consumption, besides fresh form and it is classified according to literature, as a bioaccumulator and bioindicator of heavy metals [22].

CONCLUSIONS

- The soils surrounding the old White Ceramic Enterprise's landfill in San José de las Lajas municipality are moderately contaminated with Cr, Co, Zn and Pb and in need of urgent remediation due to Ni and Cu concentrations, which shows that these are areas that are not suitable for agricultural use.
- The values of heavy metals in soil with the highest Ni, Cu and Pb concentration, exceed the permissible levels for healthy food, the average phytotoxic levels and the levels in Cuban agricultural soils.
- The lettuce crop, obtained under conditions of contamination by heavy metals, is shown as an accumulator of Fe, Co, Zn, Mn, Cu, Ni, Pb metals, exceeding in all levels of sufficient or normal concentration in plants.
- The transfer coefficient calculated for the lettuce crop indicates that all the metals studied are translocated from the root to the aerial part of the plant, being higher in order in the case of Mn, Fe, Ni, Co, Pb.

RECOMMENDATIONS

Continuing the physiological-biochemical studies of the species that are cultivated in other areas surrounding the dumping of waste from old White Ceramic Enterprise in San José de la Lajas municipality, to verify their level of toxicity, in order to warn about the danger to food safety in the locality.

BIBLIOGRAPHY

1. Santos OAA, Grana ALS, Carmenate RV, Goicochea CAB. Contaminación con metales pesados alrededor de la Empresa de Cerámica Blanca “Adalberto Vidal”, San José de las Lajas. Percepción del riesgo. Revista de Gestión del Conocimiento y el Desarrollo Local [Internet]. 2015;2(1):62–7. Available from: https://rcta.unah.edu.cu/index.php/RGCDL/article/view/763
2. Olivares Rieumont S, García Céspedes D, Lima Cazorla L, Saborit Sánchez I, Llizo Casals A, Pérez Alvares P. Niveles de Cadmio, Plomo, Cobre y Zinc en Hortalizas cultivadas en una zona altamente urbanizada de la Ciudad de La Habana, Cuba. Revista internacional de contaminación ambiental [Internet]. 2013;29(4):285–94. Available from: http://www.scielo.org.mx/pdf/rica/v29n4/v29n4a6.pdf
3. Alonso-Bravo JN, Montaño-Arias NM, Santoyo-Pizano G, Márquez-Benavides L, Saucedo-Martinez BC, Sánchez-Yáñez JM. Biorecuperación y fitorremediación de

suelo impactado por aceite residual automotriz. Journal of the Selva Andina Research Society [Internet]. 2018 [cited 31/08/2021];9(1):45–51. Available from: http://www.scielo.org.bo/scielo.php?script=sci_abstract&pid=S2072-92942018000100004&lng=es&nrm=iso&tlng=es

4. Delince W, Valdés Carmenate R, López Morgado O, Guridi Izquierdo F, Balbín Arias MI. Riesgo agroambiental por metales pesados en suelos con Cultivares de *Oryza sativa* L y *Solanum tuberosum* L. Revista Ciencias Técnicas Agropecuarias [Internet]. 2015;24(1):44–50. Available from: http://scielo.sld.cu/scielo.php?script=sci_arttext&pid=S2071-00542015000100006
5. Santana Báez S, Mendoza Martín M, Quevedo Villegas MC, Gutiérrez Disla EJ, Santana Báez S, Mendoza Martín M, et al. Revisión Sistemática sobre los efectos tóxicos de las nanopartículas metálicas en la salud de los trabajadores. Medicina y Seguridad del Trabajo [Internet]. 2018 [cited 31/08/2021];64(252):295–311. Available from: https://scielo.isciii.es/scielo.php?script=sci_abstract&pid=S0465-546X2018000300295&lng=es&nrm=iso&tlng=es
6. Puello Silva J, León Méndez G, Gómez Marrugo D, Muñoz Monroy H, Blanco Herrera L, Puello Silva J, et al. Determinación de metales pesados en humos metálicos presentes en ambientes informales de trabajo dedicados a la soldadura. Revista Colombiana de Ciencias Químico - Farmacéuticas [Internet]. 2018 [cited 31/08/2021];47(1):14–25. doi:10.15446/rcciquifa.v47n1.70653
7. Guzmán-Morales AR, Paz OC-L, Valdés-Carmenate R. Efectos de la contaminación por metales pesados en un suelo con uso agrícola. Revista Ciencias Técnicas Agropecuarias [Internet]. 2019;28(1). Available from: http://scielo.sld.cu/scielo.php?script=sci_arttext&pid=S2071-00542019000100004
8. Malpeli A. Contribución de la dieta a la exposición al plomo de niños de 1 a 7 años en La Plata. Encuentro de Centros Propios y Asociados de la CIC [Internet]. 2018;1. Available from: https://digital.cic.gba.gob.ar/handle/11746/8689
9. Yoko Y, Akiko H, Kenji M, Manabu M, Izumi N. High-Sensitive Determination of Inorganic Elements in Spinach Leaves by X-Ray Fluorescence Analysis and Its Application to Identification of Their Production Area. Bunseki Kagaku. 2007 [cited 31/08/2021];56(12):1053–61. Available from: https://jglobal.jst.go.jp/en/detail?JGLOBAL_ID=200902263363794095

10. Carmenate RV, la Paz OC, Arias MIB, Izquierdo FG, Morales ARG, Pérez MAM, et al. Fitogestión (FITOG-MP): tecnología para recuperar áreas contaminadas con metales pesados. Anuario Ciencia en la UNAH [Internet]. 2018;15(1). Available from: https://revistas.unah.edu.cu/index.php/ACUNAH/article/view/951/1339
11. Swartjes FA, Rutgers M, Lijzen JPA, Janssen PJCM, Otte PF, Wintersen A, et al. State of the art of contaminated site management in The Netherlands: Policy framework and risk assessment tools. Science of The Total Environment [Internet]. 2012 [cited 31/08/2021];427–428:1–10. doi:10.1016/j.scitotenv.2012.02.078
12. Kabata-Pendias A. Trace Elements in Soils and Plants [Internet]. Fourth Edition. 2010 [cited 31/08/2021]. 548 p. Available from: https://www.routledge.com/Trace-Elements-in-Soils-and-Plants/Kabata-Pendias/p/book/9781420093681
13. Muñiz O, Rodríguez M, Montero A, Miranda BC, De Aguiar AM, Araujo C. Criterios de calidad de los suelos cubanos en relación a metales pesados. 2014:6. Available from: https://xdoc.mx/preview/criterios-de-calidad-de-los-suelos-cubanos-en-relacion-a-metales-5ddc35ef3f621
14. Bernardis AC. Algunas propiedades químicas de dos especies vegetales forrajeras para establecer su origen geográfico. 2018 [cited 31/08/2021]; Available from: http://repositorio.unne.edu.ar/xmlui/handle/123456789/1562
15. Youssef MA, Abd El-Gawad AM. Accumulation and translocation of heavy metals in eggplant (*Solanum melongena* L.) grown in a contaminated soil. J. Energy Environ. Chem. Eng [Internet]. 2018;3:9–18. Available from: https://www.researchgate.net/profile/Mohamed-Youssef-15/publication/330318934_Accumulation_and_Translocation_of_Heavy_Metals_in_Eggplant_Solanum_melongena_L_Grown_in_a_Contaminated_Soil/links/5daf96e14585155e27f7dd2e/Accumulation-and-Translocation-of-Heavy-Metals-in-Eggplant-Solanum-melongena-L-Grown-in-a-Contaminated-Soil.pdf
16. MINISTERIO DE JUSTICIA. Decreto Ley 9/2020 Inocuidad de los Alimentaria (GOC-2020-675-076) [Internet]. 2020. [cited 31/08/2021]. Available from: https://www.gacetaoficial.gob.cu/sites/default/files/goc-2020-o76.pdf
17. Del Rio MN, Fu J, Euqué O. Riesgos y peligros relacionados con la inocuidad de los alimentos. Minal Cuba en Calidad y Técnología [Internet]. 2016 [cited 31/08/2021]; Available from: https://minalcuba.cubava.cu/2016/11/23/riesgos-y-peligros-relacionados-con-la-inocuidad-de-los-alimentos/
18. Lama Segura ER. Fitoextracción de plomo en suelos de tres parques por el girasol (*Helianthus annuus*) inoculado con el hongo micorrítico *Glomus intraradices*. 2018;

Available from: http://repositorio.lamolina.edu.pe/bitstream/handle/UNALM/3499/Lama-Segura-Eduardo-Rodolfo.pdf?sequence=1&isAllowed=y

19. Lopes NDR, Cheng Y, Shi W. Management of Soil Contaminants in Guinea-Bissau. International Journal of Environmental Monitoring and Analysis. [Internet]. Available from: https://www.researchgate.net/profile/Namir_Lopes/publication/330287942_Management_of_Soil_Contaminants_in_Guinea_-_Bissau/links/60d7fa1f299bf1ea9ec3b0a2/Management-of-Soil-Contaminants-in-Guinea-Bissau.pdf
20. Siddiqa A, Faisal M. Heavy metals: source, toxicity mechanisms, health effects, nanotoxicology and their bioremediation. In: Contaminants in Agriculture. Springer [Internet]. 2020;117–41. Available from: https://books.google.es/books?hl=es&lr=&id=cS3fDwAAQBAJ&oi=fnd&pg=PA116&dq=Heavy+Metals:+Source,+Toxicity+Mechanisms,+Health+Effects,+Nanotoxicology+and+Their+Bioremediation&ots=fhLsKJSimP&sig=WC5EBGnZpxHlaJV_Bw6YmVvjyAg#v=onepage&q=Heavy%20Metals%3A%20Source%2C%20Toxicity%20Mechanisms%2C%20Health%20Effects%2C%20Nanotoxicology%20and%20Their%20Bioremediation&f=false
21. Oficina Nacional de Normalización (NC). Contaminantes metálicos en alimentos-Regulaciones sanitarias. Tercera Edición. [Internet]. NC 493: 2015. p. 9. Available from: https://sistemas.mre.gov.br/kitweb/datafiles/Havana/pt-br/file/NC%20493%202015%20Contaminantes%20metalicos(1).pdf
22. González Oliva L, Ferro Díaz J, Rodríguez Cala D, Berazaín R. Métodos de inventario de plantas. In: Diversidad biológica de Cuba: métodos de inventario, monitoreo y colecciones [Internet]. 2017 [cited 31/08/2021]. p. 68–93. Available from: https://www.researchgate.net/profile/Carlos-Mancina/publication/321156956_Diversidad_biologica_de_Cuba_metodos_de_inventario_monitoreo_y_colecciones_biologicas/links/5a178b604585155c26a789e4/Diversidad-biologica-de-Cuba-metodos-de-inventario-monitoreo-y-colecciones-biologicas.pdf