UNIVERSITE DE YAOUNDE I

CENTRE DE RECHERCHE ET DE FORMATION DOCTORALE EN SCIENCE DE LA VIE, SANTE ET ENVIRONNEMENT

UNITE DE RECHERCHE ET DE FORMATION DOCTORALE SCIENCES DE LA VIE

DEPARTEMENT DE BIOCHIMIE

THE UNIVERSITY OF YAOUNDE I

CENTRE FOR RESEARCH AND TRAINING IN GRADUATE STUDIES IN LIFE, HEALTH AND ENVIRONMENTAL SCIENCE

RESEARCH AND DOCTORATE TRAINING UNIT IN LIFE SCIENCE

DEPARTMENT OF BIOCHEMISTRY

LABORATORY FOR PHYTOBIOCHEMISTRY AND MEDICINAL PLANTS STUDIES

LABORATOIRE DE PHYTOBIOCHIMIE ET D'ETUDE DES PLANTES MEDICINALES

ANTIMICROBIAL AND BIOCONTROL AGENTS UNIT

UNITE DES AGENTS ANTIMICROBIENS ET DE BIOCONTROLE

EFFECTS OF THE INTERACTION BETWEEN GENOTYPE AND ARBUSCULAR MYCORRHIZAL FUNGI INOCULATION ON COCOA (*Theobroma cacao* L.) SEEDLINGS GROWTH AND CADMIUM ACCUMULATION

submitted as a partial fulfilment of the requirements for the award of the degree of Doctorat/PhD in Biochemistry

Option: Biotechnology and Development

By

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Registration Nº: 14U2726

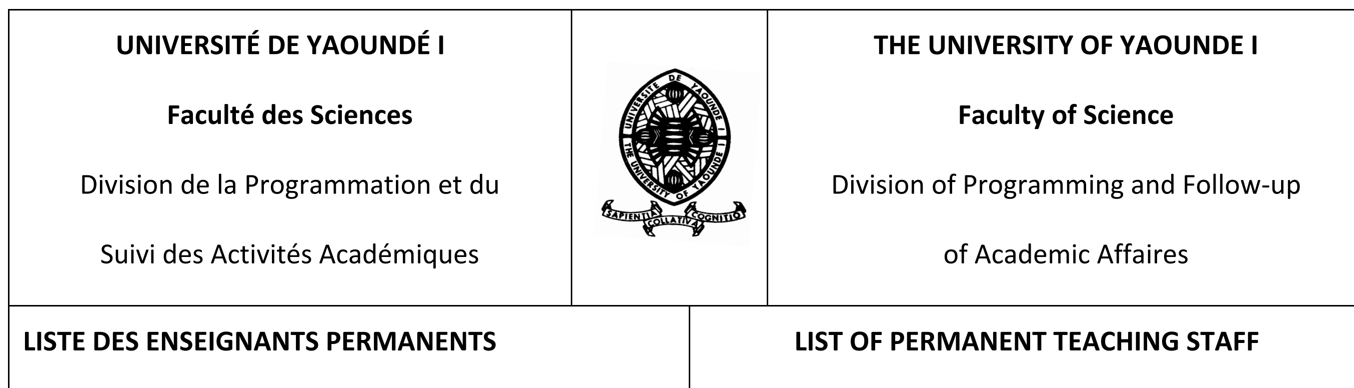
MSc. Biochemistry

Co-supervised by

NANA WAKAM Louise
Associate Professor, UY1

FEKAM BOYOM Fabrice
Professor, UY1

Academic Year: 2024/2025



UNIVERSITÉ DE YAOUNDÉ I Faculté des Sciences Division de la Programmation et du Suivi des Activités Académiques		THE UNIVERSITY OF YAOUNDE I Faculty of Science Division of Programming and Follow-up of Academic Affaires
LISTE DES ENSEIGNANTS PERMANENTS		LIST OF PERMANENT TEACHING STAFF

ACADEMIC YEAR 2024/2025

(By Department and Grade)

DATE OF UPDATE 16 January 2025

ADMINISTRATION

1. **DEAN:** OWONO OWONO Luc Calvin, *Professor*
2. **VICE-DEAN/ DPSAA:** NDJIGUI Paul-Désiré, *Professor*
3. **VICE-DEAN / DSSE:** NYEGUE Maximilienne Ascension*, Professor*
4. **VICE-DEAN / DRC:** NOUNDJEU Pierre, *Associate Professor*
5. **Head of Administrative and Financial Division:** NDOYE FOE Florentine Marie Chantal, *Associate Professor*
6. **Head of Academic Affairs, Education and Research Division/ DAARS:** AJEAGAH Gideon AGHAINDUM, *Professor*

1- DEPARTMENT OF BIOCHEMISTRY (BC) (44)			
N°	NOMS ET PRÉNOMS	GRADE	OBSERVATIONS
1.	BIGOGA DAIGA Jude	Professor	In duty
2.	FEKAM BOYOM Fabrice	Professor	In duty
3.	KANSCI Germain	Professor	In duty
4.	MBACHAM FON Wilfred	Professor	In duty
5.	MOUNDIPA FEWOU Paul	Professor	*Head of DEPARTMENT*
6.	NGUEFACK Julienne	Professor	In duty

7.	NJAYOU Frédéric Nico	Professor	In duty
8.	OBEN Julius ENYONG	Professor	In duty
9.	ACHU Merci BIH	Associate Professor	In duty
10.	BEBEE Fadimatou	Associate Professor	In duty
11.	BEBOY EDJENGUELE Sara N.	Associate Professor	In duty
12.	FONKOUA Martin	Associate Professor	In duty
13.	AKINDEH MBUH NJI	Associate Professor	In duty
14.	ATOGHO Barbara MMA	Associate Professor	In duty
15.	AZANTSA KINGUE GABIN BORIS	Associate Professor	In duty
16.	BELINGA née NDOYE FOE F. M. C.	Associate Professor	*Head DAF / FS*
17.	DAKOLE DABOY Charles	Associate Professor	In duty
18.	DONGMO LEKAGNE Joseph Blaise	Associate Professor	In duty
19.	DJUIDJE NGOUNOUE Marceline	Associate Professor	In duty
20.	DJUIKWO NKONGA Ruth Viviane	Associate Professor	In duty
21.	EFFA ONOMO Pierre	Associate Professor	*VD/FS/Univ Ebwa*
22.	EWANE Cécile Annie	Associate Professor	In duty
23.	KENGNE NOUEMSI Anne Pascale	Associate Professor	In duty
24.	KOTUE TAPTUE Charles	Associate Professor	In duty
25.	LUNGA Paul KEILAH	Associate Professor	In duty
26.	MANANGA Marlyse Joséphine	Associate Professor	In duty
27.	MBONG ANGIE M. Mary Anne	Associate Professor	In duty

28.	MOFOR née TEUGWA Clotilde	Associate Professor	*DEAN FS / UDs*
29.	NANA Louise épouse WAKAM	Associate Professor	In duty
30.	NGONDI Judith Laure	Associate Professor	In duty
31.	Palmer MASUMBE NETONGO	Associate Professor	In duty
32.	PECHANGOU NSANGOU Sylvain	Associate Professor	In duty
33.	TCHANA KOUATCHOUA Angèle	Associate Professor	In duty

34.	BAKWO BASSOGOG Christian Bernard	Lecturer	In duty
35.	ELLA Fils Armand	Lecturer	In duty
36.	EYENGA Eliane Flore	Lecturer	In duty
37.	FOUPOUAPOUOGNIGNI Yacouba	Lecturer	In duty
38.	KOUOH ELOMBO Ferdinand	Lecturer	In duty
39.	MADIESSE KEMGNE Eugenie Aimée	Lecturer	In duty
40.	MANJIA NJIKAM Jacqueline	Lecturer	In duty
41.	MBOUCHE FANMOE Marceline J.	Lecturer	In duty
42.	OWONA AYISSI Vincent Brice	Lecturer	In duty
43.	WILFRED ANGIE ABIA	Lecturer	In duty
44.	WOGUIA Alice Louise	Lecturer	In duty

2- DEPARTMENT OF BIOLOGY AND ANIMAL PHYSIOLOGY (BPA) (49)			
1.	AJEAGAH Gideon AGHAINDUM	Professor	*DAARS/FS*

2.	DJIETO LORDON Champlain	Professor	In duty
3.	DZEUFIET DJOMENI Paul Désiré	Professor	In duty
4.	ESSOMBA née NTSAMA MBALA	Professor	*CD et Vice DEAN/FMSB/UYI*
5.	KEKEUNOU Sévilor	Professor	*Head of DEPARTMENT*
6.	MEGNEKOU Rosette	Professor	In duty
7.	NJAMEN Dieudonné	Professor	In duty
8.	NOLA Moïse	Professor	In duty
9.	TAN Paul VERNYUY	Professor	In duty
10.	TCHUEM TCHUENTE Louis Albert	Professor	*Service Inspector / Coord.Progr./MINSANTE*
11.	ZEBAZE TOGOUET Serge Hubert	Professor	In duty

12.	ALENE Désirée Chantal	Associate Professor	*Vice DEAN/ UEb*
13.	ATSAMO Albert Donatien	Associate Professor	In duty
14.	BILANDA Danielle Claude	Associate Professor	In duty
15.	DJIOGUE Séfirin	Associate Professor	In duty
16.	GOUNOUE KAMKUMO Raceline épse FOTSING	Associate Professor	In duty
17.	JATSA BOUKENG Hermine épse MEGAPTCHE	Associate Professor	In duty
18.	KANDEDA KAVAYE Antoine	Associate Professor	In duty
19.	LEKEUFACK FOLEFACK Guy B.	Associate Professor	In duty
20.	MAHOB Raymond Joseph	Associate Professor	In duty
21.	MBENOUN MASSE Paul Serge	Associate Professor	In duty

22.	MOUNGANG Luciane Marlyse	Associate Professor	In duty
23.	NOAH EWOTI Olive Vivien	Associate Professor	In duty
24.	MONY Ruth épse NTONE	Associate Professor	In duty
25.	MVEYO NDANKEU Yves Patrick	Associate Professor	In duty
26.	NGUEGUIM TSOFACK Florence	Associate Professor	In duty
27.	NGUEMBOCK	Associate Professor	In duty
28.	TADU Zephyrin	Associate Professor	In duty
29.	TAMSA ARFAO Antoine	Associate Professor	In duty
30.	TOMBI Jeannette	Associate Professor	In duty
31.	YEDE	Associate Professor	In duty

32.	AMBADA NDZENGUE GEORGIA ELNA	Lecturer	In duty
33.	BASSOCK BAYIHA Etienne Didier	Lecturer	In duty
34.	ETEME ENAMA Serge	Lecturer	In duty
35.	FEUGANG YOUMSSI François	Lecturer	In duty
36.	FOKAM Alvine Christelle Epse KENGNE	Lecturer	In duty
37.	FOSSI TANKOUA Olivia Epse DJEUTCHOUANG SAYANG	Lecturer	In duty
38.	GONWOUO NONO Legrand	Lecturer	In duty
39.	KOGA MANG Dobara	Lecturer	In duty
40.	LEME BANOCK Lucie	Lecturer	In duty
41.	MAPON NSANGOU Indou	Lecturer	In duty

42.	METCHI DONFACK Mireille Flaure EPSE GHOUMO	Lecturer	In duty
43.	NDENGUE Jean De Matha	Lecturer	In duty
44.	NGOUATEU KENFACK Omer Bébé	Lecturer	In duty
45.	NJUA Clarisse YAFI	Lecturer	*Head of Div. U. Bamenda*
46.	NWANE Philippe Bienvenu	Lecturer	In duty
47.	YOUNOUSSA LAME	Lecturer	In duty
48.	ZEMO GAMO Franklin	Lecturer	In duty
49.	KODJOM WANCHE Jacguy Joyce	Assistante	In duty

3- DEPARTMENT OF BIOLOGY AND PLANT PHYSIOLOGY (BPV) (37)			
1.	AMBANG Zachée	Professor	*Head of DEPARTMENT*
2.	BIYE Elvire Hortense	Professor	In duty
3.	DJOCGOUE Pierre François	Professor	In duty
4.	MBOLO Marie	Professor	In duty
5.	NDONGO BEKOLO	Professor	In duty
6.	ZAPFACK Louis	Professor	In duty
7.	ANGONI Hyacinthe	Associate Professor	In duty
8.	DJEUANI Astride Carole	Associate Professor	In duty
9.	MAHBOU SOMO TOUKAM Gabriel	Associate Professor	In duty
10.	MALA Armand William	Associate Professor	In duty

12.	NGALLE Hermine BILLE	Associate Professor	In duty
13.	NGONKEU MAGAPTCHE Eddy L.	Associate Professor	*CT/MINRESI*
14.	TONFACK Libert Brice	Associate Professor	In duty
15.	TSOATA Esaïe	Associate Professor	In duty
16.	ONANA Jean Michel	Associate Professor	In duty

17.	DIDA LONTSI Sylvere Landry	Lecturer	In duty
18.	GONMADGE Christelle	Lecturer	In duty
19.	MAFFO MAFFO Nicole Liliane	Lecturer	In duty
20.	MANGA NDJAGA JUDE	Lecturer	In duty
21.	NNANGA MEBENGA Ruth Laure	Lecturer	In duty
22.	NOUKEU KOUAKAM Armelle	Lecturer	In duty
23.	NSOM ZAMBO EPSE PIAL Annie Claude	Lecturer	*On Secondment/UNESCO MALI*
24.	GODSWILL NTSOMBOH NTSEFONG	Lecturer	In duty
25.	KABELONG BANAHO Louis-Paul-Roger	Lecturer	In duty
26.	KONO Léon Dieudonné	Lecturer	In duty
27.	LIBALAH Moses BAKONCK	Lecturer	In duty
28.	LIKENG-LI-NGUE Benoit C	Lecturer	In duty
29.	TAEDOUNG Evariste Hermann	Lecturer	In duty
30.	TEMEGNE NONO Carine	Lecturer	In duty

31.	BOLIE Hubert	Assistant	In duty
33.	MACHE NKOUANDEU Pasma	Assistante	In duty
34.	MAFFO FOKOU Adèle	Assistante	In duty
35.	METSEBING Blondo-Pascal	Assistant	In duty
36.	NTONMEN YPNKEU Amandine Flore	Assistante	In duty
37.	ONANA EBODE Clotaire	Assistant	In duty

4- DEPARTMENT OF INORGANIC CHEMISTRY (CI) (28)			
1.	GHOGOMU Paul MINGO	Professor	*Minister in Charge of Mission PR*
2.	NANSEU NJIKI Charles Péguy	Professor	In duty
3.	NDIFON Peter TEKE	Professor	*CT MINRESI*
4.	NGOMO Horace MANGA	Professor	*Vice Chancelor/UB*
5.	NJIOMOU C. épse DJANGANG	Professor	In duty
6.	NJOYA Dayirou	Professor	In duty

7.	ACAYANKA Elie	Associate Professor	In duty
8.	EMADAK Alphonse	Associate Professor	In duty
9.	KAMGANG YOUBI Georges	Associate Professor	In duty
10.	KEMMEGNE MBOUGUEM Jean C.	Associate Professor	In duty
11.	KENNE DEDZO GUSTAVE	Associate Professor	In duty
12.	MBEY Jean Aimé	Associate Professor	In duty

13.	NDI Julius NSAMI	Associate Professor	*Head of DEPARTMENT*
14.	NEBAH Née NDOSIRI Bridget NDOYE	Associate Professor	*Senator/SENATE*
15.	NYAMEN Linda Dyorisse	Associate Professor	In duty
16.	PABOUDAM GBAMBIE AWAWOU	Associate Professor	In duty
17.	TCHAKOUTE KOUAMO Hervé	Associate Professor	In duty
18.	BELIBI BELIBI Placide Désiré	Associate Professor	*Head Service/ ENS Bertoua*
19.	CHEUMANI YONA Arnaud M.	Associate Professor	In duty
20.	KOUOTOU DAOUDA	Associate Professor	In duty
21.	MAKON Thomas Beauregard	Lecturer	In duty
22.	NCHIMI NONO KATIA	Lecturer	In duty
23.	NJANKWA NJABONG N. Eric	Lecturer	In duty
24.	PATOUOSSA ISSOFA	Lecturer	In duty
25.	SIEWE Jean Mermoz	Lecturer	In duty
26.	BOYOM TATCHEMO Franck W.	Assistant	In duty
27.	DANTIO NGUELA Christian Brice	Assistant	In duty
28.	LEKENE NGOUATEU Reine	Assistant	In duty

5- DEPARTMENT OF ORGANIC CHEMISTRY (CO) (36)			
1	Alex de Théodore ATCHADE	Professor	*DEPE/Univ. Bertoua*

2	DONGO Etienne	Professor	*Vice-DEAN/FSE/UYI*
3	NGOUELA Silvère Augustin	Professor	*Head of DEPARTMENT/UDS*
4	PEGNYEMB Dieudonné Emmanuel	Professor	*Rector UBertoua/ Head of DEPARTMENT*
5	MBAZOA née DJAMA Céline	Professor	In duty
6	MKOUNGA Pierre	Professor	In duty

7	AMBASSA Pantaléon	Associate Professor	In duty
8	EYONG Kenneth OBEN	Associate Professor	*Director/HTTTC/UBda*
8	FOTSO WABO Ghislain	Associate Professor	In duty
10	KAMTO Eutrophe Le Doux	Associate Professor	In duty
11	KENMOGNE Marguerite	Associate Professor	In duty
12	MVOT AKAK CARINE	Associate Professor	In duty
13	NGOMO Orléans	Associate Professor	In duty
14	NGO MBING Joséphine	Associate Professor	*Head of Unit MINRESI*
15	NGONO BIKOBO Dominique Serge	Associate Professor	*Head Div./MINESUP*
16	NOTE LOUGBOT Olivier Placide	Associate Professor	*Dir ENS/Uty Bertoua*
17	NOUNGOUE TCHAMO Diderot	Associate Professor	In duty
18	TABOPDA KUATE Turibio	Associate Professor	In duty
19	TAGATSING FOTSING Maurice	Associate Professor	In duty
20	OUAHOUO WACHE Blandine M.	Associate Professor	In duty
21	ZONDEGOUMBA Ernestine	Associate Professor	In duty

22	MELONG Radius	Lecturer	In duty
23	MESSI Angélique Nicolas	Lecturer	In duty
24	MUNVERA MFIFEN Aristide	Lecturer	In duty
25	NGNINTEDO Dominique	Lecturer	In duty
26	NONO NONO Éric Carly	Lecturer	In duty
27	OUETE NANTCHOUANG Judith Laure	Lecturer	In duty
28	SIELINOU TEDJON Valérie	Lecturer	In duty
29	TCHAMGOUE Joseph	Lecturer	In duty
30	TSAFFACK Maurice	Lecturer	In duty
31	TSAMO TONTSA Armelle	Lecturer	In duty
32	TSEMEUGNE Joseph	Lecturer	In duty
33	NDOGO ETEME Olivier	Assistant	In duty
34	NGUEMDJO CHIMEZE Valery Wilfried	Assistant	In duty

6- DEPARTMENT OF RENEWABLE ENERGIES (ER) (1)			
1.	BODO Bertrand	Professor	Head of Department

7- DEPARTMENT OF COMPUTER SCIENCE (IN) (25)			
1.	ATSA ETOUNDI Roger	Professor	*Head de Division des SI/MINESUP*
2.	FOUDA NDJODO Marcel Laurent	Professor	*Academic Inspector General / MINESUP*
3.	NDOUNDAM René	Professor	In duty
4.	ABESSOLO ALO'O Gislain	Associate Professor	*CT1/MINFOPRA*
5.	MELATAGIA YONTA Paulin	Associate Professor	In duty
6.	TSOPZE Norbert	Associate Professor	In duty

7.	AMINOU HALIDOU	Lecturer	*Head of DEPARTMENT*
8.	DJAM Xaviera YOUH - KIMBI	Lecturer	In duty
9.	DOMGA KOMGUEM Rodrigue	Lecturer	In duty
10.	EBELE Serge Alain	Lecturer	In duty
11.	EKODECK Stéphane Gaël Raymond	Lecturer	In duty
12.	HAMZA Adamou	Lecturer	In duty
13.	JIOMEKONG AZANZI Fidel	Lecturer	In duty
14.	KOUOKAM KOUOKAM E. A.	Lecturer	In duty
15.	MESSI NGUELE Thomas	Lecturer	*Head of DEPARTMENT/ Computer Eng./U Ebolowa*
16.	MONTHE DJIADEU Valery M.	Lecturer	In duty
17.	NZEKON NZEKO'O Armel Jacques	Lecturer	In duty

18.	OLLE OLLE Daniel Claude Georges Delort	Lecturer	*Deputy Director/ ENSET Ebolowa*
19.	TAPAMO Hyppolite	Lecturer	In duty
20.	BAYEM Jacques Narcisse	Assistant	In duty
21.	MAKEMBE. S. Oswald	Assistant	*Director CUTI*
22.	MAXWELL NDOGNKON MANGA	Assistant	In duty
23.	NDOM Francis Rollin	Assistant	In duty
24.	NGUIMEYA TSOFACK Baudoin	Assistant	In duty
25.	NKONDOCK. MI BAHANACK. N.	Assistant	In duty

8- DEPARTMENT OF MATHEMATICS (MA) (36)			
1.	AYISSI Raoult Domingo	Professor	*Head of DEPARTMENT/D. ENSPY*
2.	KIANPI Maurice	Associate Professor	In duty
3.	MBANG Joseph	Associate Professor	In duty
4.	MBEHOU Mohamed	Associate Professor	*Head de Division/ENSPY*
5.	MBELE BIDIMA Martin Ledoux	Associate Professor	In duty
6.	NOUNDJEU Pierre	Associate Professor	*VDRC/FS/UYI*
7.	TAKAM SOH Patrice	Associate Professor	In duty
8.	TCHAPNDA NJABO Sophonie B.	Associate Professor	*Director/AIMS Rwanda*
9.	TCHOUNDJA Edgar Landry	Associate Professor	In duty

10.	AGHOUKENG JIOFACK Jean Gérard	Lecturer	*Head Cellule MINEPAT*
11.	BOGSO ANTOINE Marie	Lecturer	In duty
12.	BITYE MVONDO Esther Claudine	Lecturer	In duty
13.	CHENDJOU Gilbert	Lecturer	In duty
14.	DJIADEU NGAHA Michel	Lecturer	In duty
15.	DOUANLA YONTA Herman	Lecturer	In duty
16.	KIKI Maxime Armand	Lecturer	In duty
17.	KOKOMO AYISSI Eric Brice	Lecturer	In duty(Transfer from the University of Douala)
18.	LOUMNGAM KAMGA Victor	Lecturer	In duty
19.	MBAKOP Guy Merlin	Lecturer	In duty
20.	MBATAKOU Salomon Joseph	Lecturer	In duty
21.	MENGUE MENGUE David Joël	Lecturer	*Head Dpt /ENS Uty Ebolowa*
22.	MBIAKOP Hilaire George	Lecturer	In duty
23.	NGUEFACK Bernard	Lecturer	In duty
24.	NIMPA PEFOUKEU Romain	Lecturer	In duty
25.	OGADOA AMASSAYOGA	Lecturer	In duty
26.	POLA DOUNDOU Emmanuel	Lecturer	*Internsihip*
27.	TENKEU JEUFACK Yannick Léa	Lecturer	In duty
28.	TCHEUTIA Daniel Duviol	Lecturer	In duty
29.	TETSADJIO TCHILEPECK M. Eric.	Lecturer	In duty

30.	EBODE ATANGANA Pie Désiré	Assistant	In duty

31.	FOKAM Jean Marcel	Assistant	In duty
32.	GUIDZAVAI KOUCHERE Albert	Assistant	In duty
33.	MAMA ASSANDJE Prosper	Assistant	In duty
34.	MANN MANYOMBE Martin Luther	Assistant	In duty
35.	MEFENZA NOUNTU Thiery	Assistant	In duty
36.	NYOUMBI DLEUNA Christelle	Assistant	In duty

9 - DEPARTMENT OF MICROBIOLOGY (MIB) (25)

1.	ESSIA NGANG Jean Justin	Professor	*Head of DEPARTMENT*
2.	KOUITCHEU MABEKU Epse KOUAM Laure Brigitte	Professor	In duty
3.	MUNE MUNE Martin Alain	Professor	In duty
4.	NYEGUE Maximilienne Ascension	Professor	*Vice-DEAN / DSSE*
5.	RIWOM Sara Honorine	Professor	In duty
6.	SADO KAMDEM Sylvain Leroy	Professor	In duty

7.	ASSAM ASSAM Jean Paul	Associate Professor	*DEAN/FASA/UDs*
8.	BOUGNOM Blaise Pascal	Associate Professor	In duty
9.	NJIKI BIKOÏ Jacky	Associate Professor	In duty
10	TCHIKOUA Roger	Associate Professor	*Head of the Academic Affairs*

11	EHETH Jean Samuel	Lecturer	In duty
12	ESSONO Damien Marie	Lecturer	In duty
13	EZO'O MENGO Fabrice Télésfor	Lecturer	In duty
14	LAMYE Glory MOH	Lecturer	In duty
15	MEYIN A EBONG Solange	Lecturer	In duty
16	MONI NDEDI Esther Del Florence	Lecturer	*Chief of service/DAAC/UYI*
17	NKOUDOU ZE Nardis	Lecturer	In duty
18	NKOUE TONG Abraham	Lecturer	In duty
19	NGOUENAM Romial Joël	Lecturer	In duty
20	NJAPNDOUNKE Bilkissou	Lecturer	In duty
21	TAMATCHO KWEYANG Blandine Pulchérie	Lecturer	In duty
22	SAKE NGANE Carole Stéphanie	Lecturer	In duty
23	TOBOLBAÏ Richard	Lecturer	In duty

24	ZO'O EZO'O Fabrice Télesfor	Assistant	In duty
25	MAYI Marie Paule Audrey	Assistante	In duty

10. DEPARTEMENT OF PHYSICS (PHY) (47)			
1.	BEN- BOLIE Germain Hubert	Professor	In duty
2.	BIYA MOTTO Frédéric	Professor	*DG/HYDRO Mekin*

3.	DJUIDJE KENMOE ép. ALOYEM	Professor	In duty
4.	EKOBENA FOUDA Henri Paul	Professor	*Vice-Rector. Uty Ngaoundéré*
5.	ESSIMBI ZOBO Bernard	Professor	In duty
6.	EYEBE FOUDA Jean sire	Professor	In duty
7.	FEWO Serge Ibraïd	Professor	In duty
8.	HONA Jacques	Professor	In duty
9.	NANA ENGO Serge Guy	Professor	In duty
10.	NANA NBENDJO Blaise	Professor	*Head of DEPARTMENT/Uty. Bertoua*
11.	NDJAKA Jean Marie Bienvenu	Professor	*Head of DEPARTMENT*
12.	NJANDJOCK NOUCK Philippe	Professor	In duty
13.	SAIDOU	Professor	*Head of centre/IRGM/MINRESI*
14.	SIEWE SIEWE Martin	Professor	In duty
15.	SIMO Elie	Professor	In duty
16.	TABOD Charles TABOD	Professor	*DEAN FS/Univ/Bda*
17.	TCHAWOUA Clément	Professor	In duty
18.	WOAFO Paul	Professor	In duty
19.	ZEKENG Serge Sylvain	Professor	In duty
20.	VONDOU Derbetini Appolinaire	Professor	In duty

21.	ENYEGUE A NYAM épse BELINGA	Associate Professor	*Head of Div./ENSPY*

22.	FOUEJIO David	Associate Professor	*Head of Unit/MINADER*
23.	MBINACK Clément	Associate Professor	In duty
24.	MBONO SAMBA Yves Christian U.	Associate Professor	In duty
25.	MELI'I Joelle Larissa	Associate Professor	In duty
26.	MVOGO ALAIN	Associate Professor	In duty
27.	NDOP Joseph	Associate Professor	In duty
28.	WAKATA née BEYA Annie Sylvie	Associate Professor	*Secretay General /UYII*
29.	WOULACHE Rosalie Laure	Associate Professor	*Absent*
30.	ABDOURAHIMI	Lecturer	In duty
31.	AYISSI EYEBE Guy François Valérie	Lecturer	In duty
32.	CHAMANI Roméo	Lecturer	In duty
33.	DJIOTANG TCHOTCHOU Lucie Angennes	Lecturer	In duty
34.	EDONGUE Hervais	Lecturer	In duty
35.	KAMENI NEMATCHOUA Modeste	Lecturer	In duty
36.	LAMARA Maurice	Lecturer	In duty
37.	NGA ONGODO Dieudonné	Lecturer	In duty
38.	OTTOU ABE Martin Thierry	Lecturer	Head Div./DIPD/UYI
39.	SOUFFO TAGUEU Merimé	Lecturer	In duty
40.	TCHAPET NJAFA Jean-Pierre	Lecturer	Adj Head of service/FLASH/UYI
41.	TEYOU NGOUPO Ariel	Lecturer	In duty
42.	TOGUEU MOTCHEYO Alain Bertrand	Lecturer	In duty

43.	WANDJI NYAMSI William	Lecturer	In duty

44.	ETEME Armand Sylvin	Assistant	In duty
45.	NGO MOUELLAS épouse LOTHIN	Assistante	In duty
46.	TCHODIMOU Carole	Assistant	In duty
47.	SOUFFO TAGUEU Merimé	Assistant	In duty

11- DEPARTMENT OF GEOLOGY (ST) (33)			
1.	BISSO Dieudonné	Professor	*Head of DEPARTMENT*
2.	EKOMANE Emile	Professor	*Head Div./Uté Ebolowa*
3.	GANNO Sylvestre	Professor	In duty
4.	NDJIGUI Paul-Désiré	Professor	*Vice-DEAN /DPSAA*
5.	NGUEUTCHOUA Gabriel	Professor	*CEA/MINRESI*
6.	NGOS III Simon	Professor	In duty
7.	NKOUMBOU Charles	Professor	In duty
8.	ONANA Vincent Laurent	Professor	*Head of DEPARTMENT/Uty. Eb.*
9.	YENE ATANGANA Joseph Q.	Professor	*Head Div. /MINTP*
10.	TCHOUANKOUE Jean Pierre	Professor	In duty

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Numerical Distribution of Faculty Members of the Faculty of Sciences at the University of Yaoundé I

NOMBRE D'ENSEIGNANTS					
DEPARTMENT	**Professors**	**Associate Professors**	**Lecturers**	**Assistants**	**Total**
BCH	08 (01)	25 (15)	11 (05)	00 (00)	**44 (21)**
BPA	12 (02)	18 (06)	16 (06)	01 (01)	**47 (15)**
BPV	08 (02)	10 (03)	14 (06)	06 (03)	**39 (14)**
CI	06 (01)	14 (02)	05 (01)	03 (01)	**28 (05)**
CO	06 (01)	14 (05)	12 (03)	02 (00)	**33 (09)**
ER	01 (00)	/	/	/	**01 (0)**
IN	03 (00)	03 (00)	13 (01)	06 (00)	**24 (01)**
MAT	01 (00)	08 (00)	20 (01)	07 (01)	**36 (02)**
MIB	05 (03)	05 (01)	13 (06)	01 (01)	**24 (11)**
PHY	17 (01)	11 (04)	15 (01)	04 (02)	**45 (08)**
ST	10 (00)	14 (05)	09 (02)	00 (00)	**33 (07)**

Total	67 (11)	122 (41)	128 (32)	30 (09)	342 (88)

For a total of **360 (94),** including:

- Professors: **67 (11)**
- Associate Professors: **122 (41)**
- Senior Lecturers: **128 (32)**
- Assistants: **30 (09)**
- () = Number of Women **94**

DEDICATION

TO

The **Great Tamghe family**, especially to **Ghomsi Sen Alicia Kathe**.

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LIST OF ABBREVIATIONS AND ACRONYMS

Symbol	
A	Absorbance
AF	Accumulation Factor
AMF	Arbuscular Mycorrhizal Fungi
AmBcAU	Antimicrobial & Biocontrol Agents Unit
ANOVA	Analysis Of Variance
APX	Ascorbate Peroxidase
BCA	Biocontrol Agent
BCF	Bioconcentration Factor
BSA	Bovin Serum Albumin
BW	Body Weight
CAT	Catalase
CEC	Cation Exchange Capacity
Cd	Cadmium
CFU	Colony-Forming Units
Chl	Chlorophyll
Chl AcEq	Chloragenic Acid Equivalent
CIRAD	International Cooperation Centre of Agricultural Research for Development
DNA	Desoxyribo Nucleic Acid
DPPH	2, 2-Diphenyl-1-picrylhydrazyl
DW	Dry Weight
EDTA	Ethylene Diamine Tetra-Acetic Acid
EFSA	European Food Safety Agency
FAO	Food and Agriculture Organization
Fe	Iron
FW	Fresh Weight
GAE	Gallic Acid Equivalent
GI	Growth Inhibition
Gim	*Gigaspora margarita*
Glh	*Glomus hoi*
Gli	*Glomus intraradices*
GPX	Guaicol Peroxidase
GSH	Gluthation
Hg	Mercure
HPLC	High Performance Liquid Chromatography
ICCO	International Cocoa Organisation
ICP-OES	Inductively Coupled Plasma Optical Emission Spectrometry
IRAD	Research of Institute for Agricultural Development
LMW	Low Molecular Weight
MCC	Metal Chelating Capacity
MDA	Malondialdehyde

MFP	AMF inoculum propagated with Leek (*Allium porum*)
MFSC	Mycorrhizal inoculum propagated with the combination of sorghum (*Sorghum bicolour)* and cowpea (*Vigna unguiculata*).
MFSG	AMF inoculum propagated with the combination of sorghum (*Sorghum bicolour*) and groundnut (*Arachis hypogaea* L)
MTs	Metallothioneins
NRAMP	Natural Resistance-Associated Macrophage protein
ONCC	National Cocoa and Coffee Office
PAH	Polycyclic aromatic hydrocarbon
PAL	Phenylalanine Amonia Lyase
Pb	Lead
PC	phytochelatine
PCA	Principal Component Analysis
pH	Pydrogene Potential
PPO	Polyphenol Oxydase
PC	Principal Component
QEq	Quercetin Equivalent
RCF	Root Colonisation Frequency
RDW	Root Dry Weight
RL	Root Lenght
ROS	Reactive Oxygenated Species
Sb	Antimoine
SCA	Scavenging Activity
Scd	*Scutellospora dipurpurescens*
SDW	Shoot Dry Weight
SL	Shoot Lenght
SOD	Superoxide Dismutase
SODECAO	Cocoa Development Society
T. cacao	*Theobroma cacao*
TAMF	Total Aerobic Mesophilic Flora
TBA	Thiobarbituric acid
TCA	Trichloracetic Acid
TDI	Tolerable Dose Intake
TF	Translocation Factor
TI	Tolerence Index
WCF	World Cocoa Foundation
WHO	World Health Organisation
WTO	World Exchange Organization
ZIP	Zinc Iron Regulatory Protein

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ABSTRACT

The presence of cadmium (Cd) in agricultural products, particularly cocoa, is mainly due to soil contamination. Genetic varietal selection and the use of arbuscular mycorrhizal fungi (AMF) appear to be sustainable strategies to overcome this issue. This study aimed at evaluating the impact of the interaction between cocoa varieties and AMF on the toxicity and accumulation of Cd in cocoa seedling under semi-controlled conditions. A preliminary evaluation of the accumulation profile and sensitivity to Cd was conducted on 16 hybrid cocoa genotypes from clonal farm. Subsequently, the effect of an AMF inoculum consortium derived from various propagation hosts on growth, Cd accumulation, and some biochemical and mycrhizospheric parameters in the hybrid genotype with the highest accumulation profile was assessed. The results showed that soil treated with Cd significantly ($P<0.0001$) reduced all growth parameters in most cocoa hybrids compared to the control treatments. Analysis of Cd translocation factors (TF) revealed a significant difference among hybrids, with *SNK16*UPA143* showing the highest value (97%) and *T79/501*SNK13* the lowest (19%). Investigation on the hybrid *SNK16*UPA143* demonstrated improved growth parameters due to root colonization by AMF. Furthermore, root absorption and translocation of Cd to the aerial parts of young cocoa plants significantly decreased, with varying patterns concerning propagation systems. AMF inoculum used induced significant ($P<0.0001$) high reduction of cadmium translocation and accumulation in cocoa plantlets by 31% to 89%. AMF activity resulted in a high concentration of glomalin, a regulation of mycorhizospheric pH, and an increase in soil microbiota (+926%). Biochemically, AMF inoculation increased the metal-chelating capacity (+107%) of young cocoa plant extracts. Principal component analysis revealed a negative correlation between Cd retention and the biosynthesis of soluble flavonoids (-36%), soluble sugars (-68%), free amino acids (-86%), prolin (-94%), and malondialdehyde (MDA) (-27%). There was also a decrease in the specific activities of antioxidant enzymes such as superoxide dismutase (SOD, -43%), phenylalanine ammonialyase (PAL, -22%), guaiacol peroxidase (GPX, -100%), ascorbate peroxidase (APX, -85%), catalase (-71%), polyphenol oxidase (-76%), and fumarase (-77%). These results support a promising strategy of using selected varieties and standardizing mycorrhizal efficiency to mitigate Cd accumulation in cocoa through propagation hosts.

Keywords: *Theobroma cacao* L., genotype selection, arbuscular mycorrhizal fungi, propagation host, cadmium, toxicity, accumulation

RESUME

La sélection génétique variétale et l'utilisation de champignons mycorhiziens à arbuscule (CMA) apparaissent comme des stratégies durables pour reduire laccumulation du cadmium (Cd) chez le cacaoyer. Ce travail visait à évaluer l'impact de l'interaction entre les variétés de cacao et les CMA sur la toxicité et l'accumulation de Cd par les jeunes plants de cacaoyers en conditions semi-contrôlées. Une évaluation préalable du profil d'accumulation et de la sensibilité au Cd a été menée sur 16 génotypes hybrides de cacao issus de parcelles clonales. Ensuite, l'effet d'un consortium d'inoculum de CMA provenant de divers hôtes de propagation sur la croissance, l'accumulation du Cd, et certains paramètres biochimiques et mycorhizosphériques chez le génotype hybride ayant montré le meilleur profil accumulateur a été évalué. Les résultats montrent que le traitement du sol avec du Cd a globalement entraîné une réduction drastique de tous les paramètres de croissance chez la plupart des hybrides de cacao par rapport aux témoins. L'analyse des facteurs de translocation (TF) du Cd a révélé une différence significative ($P<0,0001$) entre les hybrides, avec *SNK16*UPA143* présentant la valeur la plus élevée (97 %) et *T79/501*SNK13* la plus faible (19 %). Les études sur l'hybride *SNK16*UPA143*, ont démontré une amélioration des paramètres de croissance par colonisation racinaire par les CMA. L'absorption racinaire et la translocation du Cd dans les parties aériennes des jeunes plants de cacao ont considérablement diminué, avec des schémas variables selon les systèmes de propagation. L'inoculum résultant de la combinaison de sorgho (*S. bicolour*) et d'arachide (*A. hypogaea*) a induit une translocation significativement faible du Cd entre les racines et les parties aériennes (TF = 9 %). L'action des CMA s'est traduite par une concentration importante de glomaline, une regulatin du pH de la mycorhizosphère, une augmentation de la conductivité et du microbiote du sol (+926%). Sur le plan biochimique, l'inoculation par les CMA a augmenté la capacité chélatrice de métaux (+108 %) des extraits des jeunes plants de cacao. L'analyse en composantes principales a révélé une corrélation négative entre la rétention de Cd et la biosynthèse des flavonoïdes solubles (-36 %), des sucres solubles (-68 %), des acides aminés libres (-87 %), de la prolin (-94 %), et de la malondialdéhyde (MDA) (-27 %). Il y a eu également une baisse des activités spécifiques des enzymes antioxydantes telles que la superoxyde dismutase (SOD, -43 %), la phénylalanine ammoniac-lyase (PAL, -28 %), la guaïacol peroxydase (GPX, -100 %), l'ascorbate peroxydase (APX, -85 %), la catalase (-71 %), la polyphénol oxydase (-76 %), et la fumarase (-78 %). Ces résultats soutiennent une stratégie prometteuse de l'utilisation de hybrides sélectionnées et la standardisation de l'efficacité mycorhizienne pour atténuer l'accumulation de Cd dans le cacaoyer via l'hôte de propagation.

Mots-clés : *Theobroma cacao* L., sélection génotypique, champignons mycorhiziens à arbuscules, hôte de propagation, cadmium, toxicité, accumulation.

INTRODUCTION

INTRODUCTION

Cocoa beans, from *Theobroma cacao* L., are the primary ingredient for chocolate and cocoa powder. According to Follana (2022), the top five cocoa-producing countries are Ivory Coast, Ghana, Indonesia, Ecuador, and Cameroon. Approximately 74.8% of the world's cocoa comes from Africa, while Asia/Oceania and the Americas contribute 19.8% and 5.3% respectively (Assoua *et al.,* 2022; Fountain, 2020; Statista, 2023). Cocoa is a vital crop in social programs in Colombia and Cameroon, which aim to alleviate rural poverty, promote peace, employ youth, develop rural areas, and increase of growth domestic product (GDP). With an estimated production of 300,000 tons in the 2022/2023 season (Statista, 2023), this crop represents a significant economic opportunity for Cameroon in the post-COVID-19 era.

However, a current global concern is the presence of cadmium (Cd) in cocoa tissues and products and their potential health effects. Recent studies have found that cocoa beans, pod husks, and cocoa-based products accumulate significant amounts of Cd, a non-essential, highly toxic heavy metal that can cause severe health issues when ingested (Barraza *et al.,* 2017; Bertoldi *et al.,* 2016). This alarming discovery necessitates a reassessment of the risks posed to human health and food safety. Notably, Cd affects cocoa plants worldwide, including those in Africa, South America, Central America, and Asia (Odoh *et al.,* 2019; Pereira *et al.,* 2017; Gramlich *et al.,* 2018; Bertoldi *et al.,* 2016). This indicates a global link between Cd availability and cocoa plants. In some production areas, Cd concentrations in cocoa beans often exceed 0.6 mg/kg, the upper limit for compliance with the European Union (EU) regulations implemented in 2019 (EU, 2014; Meter *et al.,* 2019). Addressing this issue is urgent.

Short-term strategies to reduce Cd uptake in cocoa involve agronomic modifications, such as using low-accumulating cocoa varieties, though the classification of these varieties based on their sensitivity to Cd remains unclear (WCF, 2018). Thus, screening and breeding low Cd-accumulating cocoa varieties are urgently needed. However, there is still a lack of understanding of heavy metal tolerance and accumulation characteristics in *T. cacao* due to complex and unclear environmental conditions across different study areas. Identifying low Cd-accumulating cocoa varieties is crucial as it would help to screen the pivotal genes responsible for Cd accumulation resistance and facilitate cocoa breeding.

The use of naturally occurring microorganisms with detoxification properties has been proposed as an eco-friendly alternative for cleaning heavy metal-polluted environments (Lata

et al., 2019). Among these soil microbes, arbuscular mycorrhizal fungi (AMF) have shown great potential in helping plants cope with stress caused by chemical contamination (Gong *et al.,* 2013). They aid in phytoremediation by increasing the absorptive area, enhancing the plant's antioxidant response, and improving heavy metal sequestration (González-Guerrero *et al.,* 2010). Additionally, AMF exhibit various plant growth-promoting traits (Porras-Soriano *et al.,* 2009) and improve soil quality (Chern *et al.,* 2007). Therefore, using AMF could be a promising, sustainable, and environmentally friendly strategy to reduce or eliminate Cd contamination in cocoa.

Hypothesis:

Based on the existing knowledge, we hypothesize that plant genotype and AMF can interfere and play a crucial role in Cd accumulation and resistance in cocoa plants.

Objectives:

This study aims to investigate the effects of the interaction between genotype and AMF on cocoa seedlings growth and cadmium accumulation. Specifically, this study aimed to:

1. Evaluate the sensitivity of some cocoa seedling genotypes to Cd stress and their accumulation profiles,
2. Investigate the effects of AMF on Cd stress and accumulation in cocoa seedlings with high accumulation profiles,
3. Assess biochemical modifications in cocoa seedlings following Cd intoxication and AMF treatments.

CHAPTER I:

LITERATURE REVIEW

CHAPTER I: LITERATURE REVIEW

I.1 The host plant (*Theobroma cacao* L.)

I.1.1 History and description of the cocoa trees

The cocoa tree (Figure 1) is originally from the tropical rainforests of Central America, specifically in the Upper Amazon basin near the Andes, as suggested by Vos *et al*. (2003). The Mayan civilization in Mexico domesticated and cultivated this tree since ancient times and gave it the name "*Theobroma cacao*," with "Theobroma" meaning food of the gods and "cocoa" derived from the term "kakaw," which means cocoa beans. The Mayans both consumed the beans and used them as a form of currency, as mentioned by Crown & Hurst (2009). In the 18th century, the cocoa tree was introduced to Africa from Ghana, and it has been cultivated in Cameroon since 1892, according to Chauveau, (1991) and Assoumou, (1977) respectively.

The cocoa tree, scientifically known as *Theobroma cacao* L., belongs to the diploid species of the Malvaceae family. It is an evergreen tree that typically grows up to 8 meters in height (Motamayor *et al.,* 2002). However, in forest environments, it can reach a height of 10 meters. The tree has a straight main stem with light and whitish wood covered by a thin and smooth brown bark. Flowering usually begins 2-3 years after planting, and from this point onwards, the tree produces flowers throughout the year. The tree reaches its maximum yield between 7 and 10 years. The flowers are approximately one centimeter in size, and only a small percentage (2%) of them are pollinated within their 48-hour lifespan. The compatibility for fertilization in the ovary varies depending on the origin of the cocoa tree. Generally, Forastero Haut-Amazoniens and Trinitario trees are self-incompatible but inter-compatible. However, most Trinitarios can only be cross-pollinated with self-compatible trees, similar to the Lower Amazonian Forestero, which includes the African Amelonado variety. The fruit of the cocoa tree is an elongated berry, or pod, measuring around 15-25 centimeters and containing approximately 25-75 seeds. These seeds have a brown or reddish colour and are surrounded by a whitish mucilage. Cocoa trees are cultivated in tropical and humid regions located within 20 degrees north and south of the equator, as mentioned by Ndo *et al*. (2023).

Figure 1: *Theobroma cacao* L; plant bearing ripe (yellow) and unripe (green) pods (Ghomsi, 2024)

I.1.2 Systematics of the cocoa tree

Cacao is classified based on the physical characteristics of its pods and seeds (Lawi & Adhitya, 2018), There are three main groups: Criollo, Forastero, and Trinitario (Daguenet, 1982). The Forastero group, specifically the Amazon Forastero, dominates the global cacao market, accounting for 95% of the market share and 70% of global cultivation (Afoakwa, 2016). On the other hand, Criollo and Trinitario cacao trees make up only 4-5% of the market. These varieties, along with is known for their high quality and fine flavour, which can range from spicy and fruity to caramel, nuts, and vanilla (Badrie *et al.,* 2015). In contrast, bulk cacao lacks these distinct flavours (Afoakwa, 2016). The cacao tree we know today is the result of centuries of crossing and selection, with its origins dating back approximately 35,000 years (Verna, 2013). Different cultivars exhibit variations in pod appearance, bean yield, flavour characteristics, and disease resistance. These differences can be attributed to the genetic composition of the beans, the cacao plant's origin and growth conditions, as well as its geographical location (Van de Walle *et al.,* 2016). Forastero cacao, which originates from the Amazon region and is predominantly grown in West Africa, is a robust and high-yielding variety capable of withstanding adverse conditions (Beg *et al.,* 2017; Van de Walle *et al.,*

2016). It also possesses moderate resistance to diseases and pests, making it a popular choice for producing cheaper cacao beans (Rusconi & Conti, 2010). Forastero cacao beans are typically small, flat, and purple in colour, with a strong cacao flavour. The shape of the cacao pod varies, resembling both Criollo pods and Amelonado. The fruit itself is furrowed with a mostly smooth surface and a blunt or rounded tip. Immature Forastero fruits are usually green and turn yellow when mature, sometimes displaying a red pigmentation in unripe pods (Badrie *et al.,* 2015; Efombagn *et al.,* 2008). The fruit wall is thick and hard, with a woody mesocarp. Criollo cacao, which was cultivated by the Mayas over 1,500 years ago, can be found in Venezuela, Central America, Madagascar, Papua New Guinea, the West Indies, Sri Lanka, East Timor, and Java (Beg *et al.,* 2017; Lachenaud & Motamayor, 2017). This variety is known for its unique and homozygous genotype. Criollo beans are small and range in colour from white to light purple due to the limited presence of anthocyanin. Criollo pods are oblong and pointed, with intense surface rugosity and thin walls (Argout *et al.,* 2011). However, Criollo cacao is highly susceptible to diseases, resulting in lower yields (Albores-Flores et al., 2018). Over time, Criollo hybrids emerged, incorporating genotypes from the Forastero variety. These hybrids, known as Trinitario, are now cultivated worldwide (Argout *et al.,* 2011; Bidot Martinez *et al.,* 2017). Trinitario cacao is a hybrid of Amazon Forastero and Criollo varieties. This fine flavour variety is primarily found in the West Indies and has been spread to other regions such as Venezuela, Ecuador, Cameroon, Samoa, Sri Lanka, Java, and Papua New Guinea (Motamayor *et al.,* 2003). Trinitario cacao exhibits high phenotypic and genetic diversity (Badrie *et al.,* 2015). The beans of this variety come in various colours, and it has a moderate level of resistance to pests and diseases (Van de Walle *et al.,* 2016).

I.1.3 The cocoa genetic diversity

The genetic diversity of *Theobroma cacao* L. in Cameroon forms a critical foundation for understanding genotype-dependent differences in cadmium accumulation, a growing concern for cocoa safety and market access. Cameroon, as one of the world's leading cocoa producers, possesses a rich cocoa genetic pool shaped by historical introductions and breeding efforts (Zhang *et al.,* 2016). Cocoa was first introduced from Nigeria in the late 19th century, primarily as Forastero seeds, followed by Trinitario seeds from Trinidad. In the 1950s, Upper Amazon (UA) clones from Ecuador and Peru were imported for breeding purposes (Zhang *et al.,* 2016). These introductions, combined with local selections and breeding efforts, have shaped the current genetic landscape of Cameroonian cocoa (Efombagn *et al.,* 2008; Nwaga, 1984).

Research institutes such as IRAD, CIRAD, and SODECAO have played pivotal roles in developing and releasing hybrid varieties and clones adapted to local conditions, balancing productivity with quality and disease resistance (Efombagn *et al.,* 2007; Junior *et al.,* 2017).

Molecular studies using microsatellite (SSR) and single nucleotide polymorphism (SNP) markers have revealed extensive genetic diversity and structuring within Cameroonian cocoa germplasm. Efombagn *et al.* (2008) analysed 400 farm accessions, 95 GenBank accessions, and 31 reference clones, demonstrating high allelic richness and heterozygosity, with farm populations clustering into groups related to traditional Amelonado types and hybrids involving Upper Amazon and Trinitario genetics. This genetic structuring corresponds closely with geographical origin and breeding history, as confirmed by Laurent *et al.* (1994) and Livingstone *et al.* (2011). Such structuring is critical for conservation strategies and breeding programmes, ensuring the preservation of unique genetic resources and effective deployment of improved varieties. However, challenges remain, including mislabelling and redundancy within germplasm collections, which complicate breeding and conservation efforts (Eyango *et al.,* 2025).

The diversity of cocoa varieties cultivated in Cameroon reflects this genetic complexity. The local SNK clones (Sélection Nkoemvone) are widely grown in the East region and are appreciated for their excellent flavour and aroma, despite lower yields and disease resistance (Junior *et al.,* 2017). ICS and UPA clones, imported from Ecuador and Peru, are valued for their high yield and disease resistance but have coarser flavour profiles (Efombagn *et al.,* 2009; Junior *et al.,* 2017). The T clones, derived from Ghana's Tafo selections, are well adapted to West African conditions, offering early bearing, pest and disease resistance, and good bean quality (Asare, 2022; Junior *et al.,* 2017). The SCA clones from Brazil, introduced in the 1980s, also contribute to yield and resistance improvements (Efombagn *et al.,* 2009; Nwaga, 1984). Hybrid progenies resulting from crosses among these groups (e.g., *SNK 10*IMC 67, SNK13*ICS95, UPA143*SNK64, IMC67*SNK109, BBK1418*MO20, ICS1*SNK37, T79/501*SNK13, SNK16*T60/1174, T79/501*SNK109, UPA143*SNK64, T60/887*SNK64, T79/501*SNK16, SNK64*T40/1170, T60/1174*UPA134, SCA12 × ICS40, SNK16*UPA143*) are central to breeding programmes aiming to combine desirable traits (Nwaga, 1984; Ribeyre *et al.,* 2017).

Phenotypic and genotypic diversity is further maintained and enhanced by agroforestry systems and farmer selection practices. Studies in southern Cameroon reveal that cocoa agroforests harbour diverse tree species and maintain genetic connectivity between cultivated and wild cocoa populations, fostering resilience and ecosystem services (Sonwa *et al.,* 2007; Laird *et al.,* 2007). Participatory selection programmes have identified cocoa accessions with resistance to black pod disease caused by *Phytophthora megakarya*, a major threat in Cameroon, providing valuable material for breeding (Nyassé *et al.,* 2003). The integration of molecular tools with traditional knowledge and agroecological practices is thus key to sustainable cocoa production.

I.1.4 Global cocoa production

According to the International Cocoa Organization's (ICCO) Quarterly Bulletin of Cocoa Statistics (2025a), global cocoa production for the 2024/25 season is forecasted to reach approximately 4.84 million tonnes, marking a 7.8% increase compared to the previous season (ICCO, 2025a). This rebound follows a contraction in the 2023/24 season, where production declined by nearly 12.9% to 4.37 million tonnes due to adverse weather conditions and disease outbreaks in key producing regions (ICCO, 2025b). Despite these fluctuations, the long-term trend reflects growing global demand for cocoa products, driven by expanding markets in Asia and sustained consumption in Europe and North America. West Africa remains the dominant cocoa-producing region, with Côte d'Ivoire and Ghana as the two largest producers, accounting for approximately 38% and 12% of global production respectively (Statista, 2025). Cameroon, Nigeria, and other West African countries contribute an additional 14%, with Cameroon alone producing around 7% of the world's cocoa (Statista, 2025; Business in Cameroon, 2025). South America, led by Ecuador and Brazil, produces about 14% of global cocoa, while Southeast Asia contributes roughly 10%, with Indonesia as a major player (Statista, 2025; World Population Review, 2025). Cameroon's cocoa sector is experiencing a positive trajectory, with production expected to rise by 6.7% in the 2024/25 season to approximately 285,000 tonnes, nearing its recent record highs (Business in Cameroon, 2025). This growth is attributed to favourable farmgate prices, reaching up to CFA6,000 per kilogram, incentivizing farmers to increase production despite challenges such as aging plantations and pest pressures. The top cocoa-producing countries worldwide are illustrated in Figure 2.

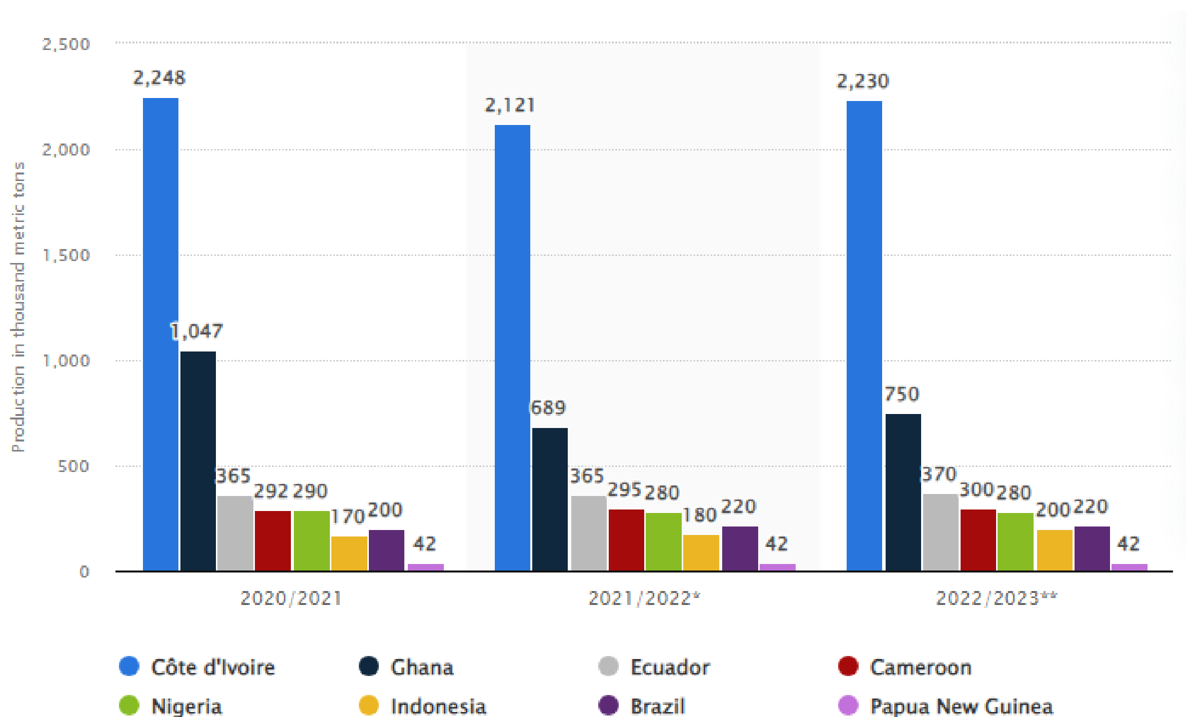


Figure 2: Top 8 cocoa-producing countries (Statista, 2023)

I.1.5 Importance of cocoa and cocoa derivatives

I.1.5.1 Nutritional and medicinal importance

The cocoa tree is grown for its beans used as raw materials in the manufacture of food, pharmaceutical or cosmetic products. Cocoa has long been equated with a food/medicine (Badrie *et al.,* 2015). Cocoa bean is composed of caffeine (0.2%), sugar (11%), protein (11%), tannin (6%), theobromine (1.2%), butter (54%), cellulose (9%), water (5%), and pentosan (7.5%) (Tuenter *et al.,* 2020). It is an excellent source of essential minerals, for instance, 100g of cocoa pouder conteint: magnesium (499 mg), iron (13.86 mg), phosphorus (734 mg), zinc (6.81 mg), copper (3.79 mg), manganese (3.84 mg), selenium (14.3 mg), calcium (around 125 mg), and potassium (1524 mg) (Cinquanta et al., 2016; NatureClaim, 2024). In addition to macronutrients and minerals, cocoa provides small amounts of B vitamins such as riboflavin (vitamin B2) and niacin (vitamin B3), which are important for energy metabolism and nervous system function (Sesso, 2022, NatureClaim, 2024)

Medicinally Cocoa is a nutrient-rich functional food with numerous health benefits. It contains bioactive compounds like flavonoids, theobromine, and magnesium (Khan et al., 2014; Martín

et al., 2013). Cocoa consumption has been associated with improved cardiovascular health, reduced blood pressure, and enhanced endothelial function (Ellam & Williamson, 2013). Its antioxidant and anti-inflammatory properties contribute to neuroprotection, cognitive enhancement, and potential prevention of neurodegenerative diseases (Latif, 2013). Cocoa may also aid in weight management by improving mitochondrial biogenesis and glucose uptake in muscles (Latif, 2013). Additionally, cocoa exhibits antimicrobial effects against oral pathogens, potentially benefiting dental health by reducing biofilm formation and caries development (Fideles *et al.,* 2023). The beneficial effects of cocoa on cancer, diabetes control, cardiovascular disease and hepatitis prevention have been reported (García-Cordero *et al.,* 2023; Garza-Juárez *et al.,* 2023; Martin *et al.,* 2013; Sun, 2016).

I.1.4.2 Socio-economic importance of cocoa

The amount of cocoa beans produced worldwide is always changing. According to ICCO (2023b), it grew from 31.63% of total tonnes in 2009/2010 to 50.01% of total tonnes in 2022 (ICCO, 2023b). In four of the top five cocoa-producing nations in the world Côte d'Ivoire (43%), Ghana (20%), Nigeria (6%), and Cameroon (6%), Africa provides more than 75% of the global cocoa production, mostly by small producers. Ecuador (7%), Brazil (4%), Indonesia (3%), Malaysia and some Caribbean countries supply the remaining portion of global production (Appiah, 2023; ICCO, 2023a). Africa is the main producer of cocoa beans, Europe is the major consumer and the major processing nation of cocoa beans are Netherland and USA (Statista, 2022). According to Mukete *et al*. (2018), cocoa beans are utilized in the chocolate industry to create a variety of derivatives, including cake, paste, butter, liquor, and cocoa powder.

One of the most significant agricultural products in Cameroon is cocoa. In fact, along with coffee, it accounts for 40% of primary sector exports. For the rural populations in the Center, South, South-West, Littoral, and East regions, it is their primary source of income. This industry contributes roughly 2% of the GDP of the entire country, 6% of the GDP of the primary sector, and 30% of the GDP of the agricultural products for export and processing subsector (Mukete *et al.,* 2018). On a social level, cocoa is estimated to provide more than 200 billion CFA francs annually to about 400,000 farming families, who rely mostly on it for their income (ONCC & Etoa, 2007). The National Cocoa and Coffee Office (ONCC) released results for the 2022–2023 cocoa season, and the regions that represent 36-50 percent, 31-54 percent,

6-98 percent, and 4-99 percent of purchase declarations, respectively, are the Central, South–West, Littoral, and South (ONCC, 2023).

I.1.3 Cocoa production constraints

The Cameroonian cocoa sector is experiencing two major problems: losses in the fields (Bose *et al.,* 2023) and the deterioration in the quality of commercial cocoa (Manga *et al.,* 2020).

I.1.3.1 Field yield losses

Cocoa production in Cameroon suffers significant field yield losses, primarily due to the intense parasitic pressure exerted by black pod disease, caused predominantly by *Phytophthora megakarya*. This pathogen is recognized as the most destructive agent of black pod rot in West and Central Africa, with losses reported to reach **up to 80%** under favorable environmental conditions (Bailey *et al.,* 2016; Ndoungue *et al.,* 2018). The disease's severity is particularly pronounced in humid cocoa-growing regions of Cameroon, where climatic conditions such as frequent rainfall and high humidity create an ideal environment for pathogen proliferation and spore dispersal (Deberdt *et al.,* 2008). In addition to black pod disease, other factors exacerbate yield losses. The aging of cocoa plantations and the demographic profile of farm managers—many of whom are older and less inclined to adopt improved agronomic practices—contribute to declining productivity (Hinzen *et al.,* 2010). The low adoption rate of selected, disease-resistant planting material further limits the sector's resilience against *Phytophthora* infections (Nwaga et al., 1984). Climate variability compounds these biotic stresses. Irregular rainfall patterns and droughts not only facilitate the spread of *Phytophthora* spores by creating wet conditions conducive to infection but also induce physiological stress in cocoa trees. Heat stress and water deficits reduce photosynthetic efficiency and increase evapotranspiration, weakening plants and lowering yields (Bomdzele & Molua, 2023). Moreover, insect pests such as defoliating caterpillars (*Anomis leona*), psyllids (*Tyora tesmanni*), and mirids (*Sahlbergella* spp., *Distantiella* spp., *Helopeltis* spp.) attack young shoots and pods, causing direct damage and creating entry points for secondary infections (Bisseleua, 2008; Djuideu *et al.,* 2021).

I.1.3.2 Deterioration in the quality of commercial Cameroonian cocoa beans

- **Poor bean fermentation**

Poor fermentation of cocoa beans in Cameroon significantly contributes to cocoa quality deterioration. Insufficient fermentation time and inadequate postharvest practices result in

beans with high acidity, slaty or violet colouration, and incomplete flavour development, all of which are undesirable for chocolate production (Niemenak *et al.,* 2014; Levai *et al.,* 2015). These poorly fermented beans retain excess polyphenols and organic acids, leading to bitterness and astringency, and are more likely to be downgraded or rejected in international markets due to their inferior sensory and physical qualities (Levai *et al.,* 2015; Kongor *et al.,* 2016). The use of inappropriate fermentation methods, such as plastic bags or insufficient turning, further exacerbates these problems by limiting microbial activity essential for optimal flavour and colour development (Guehi *et al.,* 2010). Consequently, the prevalence of poorly fermented beans undermines the commercial value and reputation of Cameroonian cocoa, highlighting the need for improved fermentation protocols and farmer training to enhance bean quality and marketability.

- **Polycyclic aromatic hydrocarbon (PAH) content**

Post-harvest processing of cocoa, particularly the drying process, is partly responsible for the decline in the quality of commercial cocoa. In several regions, planters dry the beans in kilns or bitumen using hydrocarbons as fuel, which leads to the impregnation of the seeds with polycyclic aromatic hydrocarbons (PAHs) that are carcinogenic to humans (Ngwang & Meliko, 2021). This led to the rejection in 2012 of nearly 2000 tonnes of cocoa from Cameroon (Assoua *et al.,* 2022). Nevertheless, the Cameroonian government has decided to tackle the problem by launching a vast awareness campaign on good drying practices and support for cocoa producers and buyers. Today the safety drying techniques of cocoa beans are in implementation in different cocoa production area of Cameroon.

- **Cadmium (Cd) content**

The South West & Centre Regions of Cameroon with 43.40% and 36.81% respectively are the heavy cocoa production zones in Cameroon. Whereas, the South Region with 9.59%, Littoral with 5.87%, and East with 3.22% are the average cocoa producing areas in Cameroon. On the other hand, North West and West of Cameroon are the mild cocoa-producing regions (ONCC, 2023). However, south west which account for 43.40% is a volcanic area with a lot of Cd in the soil. Furthermore, cocoa farmers in Cameroon frequently use fertilizers and which raises the soil's availability of Cd. In fact, in a study carrying by Tsufac *et al.* (2020) it was found that, 65% of farmers used chemical fertilizers, with NPK and urea being the most popular. The

fruits from the plants grown there, including cocoa, are prone to accumulate more of this heavy metal as a result (Manga *et al.,* 2020). This accumulation leads to a decline of marketability and then, rejection on the international market; according to Agritrade, 2013, the cocoa from Latin America is most affected by cocoa cadmium content and followed by those from west and central Africa. The National Cocoa and Coffee Board and the Cocoa and Coffee Inter-Professional Council assert that the difficulty in meeting these quality standards prevents Cameroon's cocoa from commanding premium prices and from competing in niche markets with cocoa from other competitors (Assoua *et al.,* 2022). Cocoa bean Cd concentration data in different cocoa producing countries reported in different studies is highlighted in the Table I below. It shows clearly that there is a geographical difference, with the highest bean Cd concentrations reported for cocoa grown in Central and South American countries and very low concentration from those in African countries.

Table I: Cadmium concentrations in cocoa beans from several studies around the world (Blommaert, 2023)

Sample origin	Bean Cd content average (µg kg-1)	Processing	Study source
Ecuador	0.94	P, UF	(Chavez *et al.,* 2015)
	0.78	UP, UF	(Barraza *et al.,* 2018)
	2.68	UP, UF	(Barraza *et al.,* 2019)
Brazil	0.10	P,F	(Knezevic, 1979)
	0.55	P, F	(Quintino *et al.,* 2017)
Cameroon	0.05	P, F	(Vītola and Ciproviča, 2016
Colombia	12.0	UP, F	(Albarrcín *et al.,* 2019)
Costa Rica	2.20	UP, UF	(Furcal-Beriguete and Torres-Morales, 2020)
Ghana	0.02	P, F	(Vītola and Ciproviča, 2016
	0.3	P, F	(Takrama *et al.,* 2015)
Indonesia	0.52	P, F	(Knezevic, 1979)
	0.76	P, F	
Ivory coast	0.05	P, F	(Yapo *et al.,* 2014)
	0.12	P, F	(Knezevic, 1979)
Malaysia	1.14	P, F	(Knezevic, 1979)
Nigeria	0.02	P, F	(Vītola and Ciproviča, 2016)
Peru	0.96	UP, UF	(Rosales-Huamani *et al.,* 2020)
	1.31	P, F	(Knezevic, 1979)
Trinidad & Tobago	0.98	P, F	(Ramtahal *et al.,* 2015)
	1.00	UP, UF	(Lewis *et al.,* 2018)
Asia	0.33	UP, F	(Bertoldi *et al.,* 2016)
Central and south America	0.62	UP, F	(Bertoldi *et al.,* 2016)
East Africa	0.51	UP, F	(Bertoldi *et al.,* 2016)

P stands for peeled; UP stands for unpeeled; F stands for fermented; UF stands for unfermented; and R stands for roasted.

I.2 The heavy metal

I.2.1 Description of heavy metals

Hazardous heavy metals in soils are defined as elements with relatively high atomic weights and densities that pose significant ecological and human health risks due to their toxicity, persistence, bioavailability, and potential to accumulate in organisms (Briffa *et al.,* 2020). These metals include cadmium (Cd), mercury (Hg), arsenic (As), lead (Pb), chromium (Cr), and others, whose hazard depends on their chemical speciation, soil properties, and exposure pathways (Vodyanitskii, 2016; Young, 2013). According to Vodyanitskii (2016) classification based on maximum permissible additions (MPA) in soils, highly hazardous metals with MPA below 1 mg/kg include beryllium, selenium, thallium, antimony, and cadmium, while moderately hazardous metals such as vanadium, mercury, nickel, copper, chromium, arsenic, and barium have MPAs between 1 and 10 mg/kg, and metals like zinc fall into the low hazard category. This classification highlights metals like cadmium (Cd) and mercury (Hg) as having considerable to high ecological risks, while chromium (Cr), copper (Cu), arsenic (As), and lead (Pb) often pose moderate to low risks depending on local concentrations (Ahmad et al.,2021).

I.2.2 Cadmium

Cadmium is a transition metal (Figure 3) that is silvery white with blue undertones. Cd is not required for the growth of animal or plant organisms (Borsari, 2011). On the other hand, its physical and chemical properties, which are similar to those of zinc and calcium, allow it to overcome biological barriers and accumulate in tissues. Furthermore, the order of accumulation of this element in plant tissue is as follows: root, stem, leaves, pods or fruits, and cocoa beans. This suggests that the cocoa bean contains the least amount of Cd when compared to other plant tissues (Wang *et al.,* 2023).

Figure 3: Cadmium metal

Cd is naturally scarce in the Earth's crust. Cd levels in unpolluted soils range between 0.1 and 2 ppm, with the majority of the time being less than 1 ppm (Kabata-Pendias, 2004). However, human behaviours (agricultural or industrial) lead to Cd enrichment of soils. Indeed, industrial activity and urban traffic pollution contribute to soil, surface, and groundwater pollution (Kubier *et al.,* 2019). Furthermore, agricultural chemicals, such as insecticides, may include various hazardous metals that contribute to agricultural soil contamination as present in Figure 4 (Dewi *et al.,* 2022). Phosphate fertilizer application contributes to Cd enrichment of soils (Niño-Savala *et al.,* 2019). Indeed, according to Mortvedt & Beaton (1995), more than 50 mg of Cd/kg can be observed with certain phosphate fertilizers.

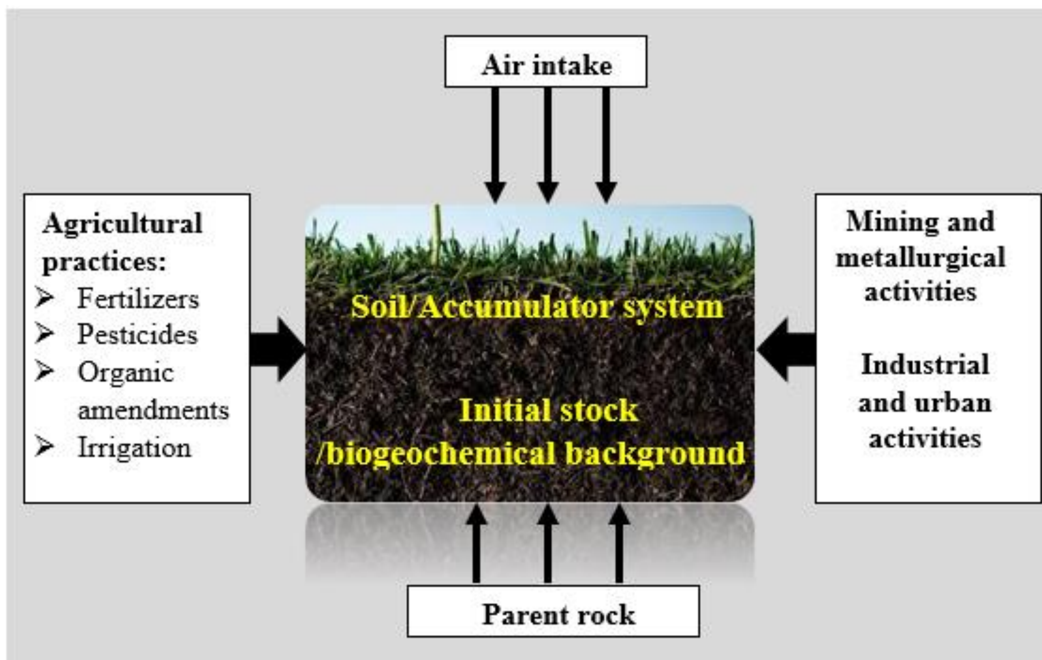


Figure 4: Origins of cadmium in soil (Ghomsi, 2024)

I.2.2.1 Chemical properties of cadmium

The chemical properties of Cd make this metal a unique chemical element. It is classified as a transition metal and is represented by the symbol Cd on the periodic table. With an atomic number of 48, the most abundant form of Cd in the environment is ionic form Cd^{2+}. Its coordination flexibility and lack of redox chemistry are related to its completely filled electron configuration (d^{10}), its ability to accept electrons let to classify it as a Lewis acid (Borsari, 2011). Thus, the electron cloud associated with Cd^{2+} is polarizable and its intermediate ionic radius is 0.95 Å (Shannon, 1976). As a result, Cd has the ability to form covalent bonds with other atoms and form complexes of high selectivity. Its ionic radius similar to that of the calcium ion (Ca^{2+}), 1.00 Å, gives it a higher affinity for ligands such as sulfur compared to Ca^{2+}, whose electron configuration is that of noble gases (Shannon, 1976). Then, the complexes formed between Cd^{2+} and the ligands oxygen and nitrogen are less stable than those formed with Ca^{2+}. In biochemistry, Cd induces a strong complexation of proteins and biomolecules such as glutathione (Remelli *et al.,* 2016). Zinc and Cd are quite similar electropositive metals, classically Cd is perceived as an analogue of Zn elements and belongs to the same group (electronic d10 configuration), yet Zn2+ has a smaller ionic radius (0.74 Å (six-fold coordination) than Cd (Hussein *et al.,* 2022). Reacting with strong acids, it is easily soluble in strong nitric acid and diluted, it remains poorly soluble in concentrated hydrochloric and sulphuric acids unless it is processed. The metal Cd is dissolved with the release of hydrogen in the above acids described, but also in acetic acid, a weak acid (Helmke, 1999).

1.2.2.2 The toxicity of cadmium and associated risks to human health

Cadmium toxicity is link to the absorbed dose, the route and the time of exposure; this can lead to various disorders and can also result in excessive damage due to oxidative stress induced by free radical formation (Balali-Mood *et al.,* 2021; Jaishankar *et al.,* 2014). According to the International Agency for Research on Cancer (IARC), the cancer risk associated with Cd has earned it a Group I list as a human carcinogen. Its primary targets are the lung, pancreas, endometrium, and breast (IARC, 2018). Renal dysfunction, nephropathy, osteoporosis, diabetes, periodontal disease, hypertension, age-related macular degeneration, and cardiovascular disease are other disorders associated with Cd (Ashizawa *et al.,* 2012). Generally, complications due to Cd exposure appear after a long incubation period, i.e. chronic Cd toxicity, and acute Cd poisoning occurs only rarely (Chaney, 2015; Clemens *et al.,* 2012); Chaney, 2015). In the human body, the biological half-life of Cd varies between 20 and 30 years, where it gradually accumulates in human organs (Perry *et al.,* 1976). The kidney in the

human body is the preferred target organ of this toxicant, which is reflected in the frequency of renal dysfunction observed (Sigel *et al.,* 2013). However, damage to the respiratory system, liver and bones can also be detected. The *Itai-itai* disease observed in Japan in the previous century is a painful illustration of this poison (Nguyen, 2020). Poisoning of humans by Cd occurs through nutrition. Foods with relatively higher concentrations of Cd include organ meats, shellfish, grains, and seeds (Charkiewicz *et al.,* 2023).

I.2.2.3 Cadmium occurrence in agricultural soils

Cd occurs naturally in soil. The amount of Cd in soil (excluding local contamination) are typically between 0.10 and 1 mg/kg dry weight (DW) and are a function of soil parent material, location, and land use (Alloway, 2013; Hooda, 2010). A recent study in south-west region of Cameroon shown that soil Cd concentration range from less than 11,24 mg/kg to 899,20 mg/kg in cocoa farms (Manga *et al.,* 2020). Cd is primarily derived from zinc minerals, such as sphalerite (ZnS) (Hooda, 2010). Cd concentrations in agricultural soils are increasing due to anthropogenic activities such as the use of phosphate fertilizers, application of sewage sludge to soils, and air pollution (Achkir *et al.,* 2023; Roberts, 2014). Cd remains in the upper soil layer for approximately several hundred years. Therefore, in classical agricultural systems, Cd flux from the topsoil is small compared to the Cd content in the soil (McLaughlin *et al.,* 2021). Cd is distributed in different soil compartments. Soil solutions and soil solid phases (weakly adsorbed to clay, poorly soluble precipitates, strongly adsorbed to Fe, Mn, Al oxides, adsorbed by organic substances) (Bouida *et al.,* 2022). Cd concentrations in crops depend on soil availability. Soil Cd availability increases with increasing total soil Cd, increasing soil acidity, decreasing soil organic matter, and in some cases with zinc deficiency and chloride salinity (Filipović *et al.,* 2018; Z. Yan *et al.,* 2023). In summary, the following factors can increase Cd concentrations in soil: young volcanic soils, such as the one in south-western Cameroon and near rivers, acidic soils ($pH \leq 6.5$) and low organic matter, artificial fertilizers and pesticides as well as air pollution.

Table II: Cadmium fertilizer content

Fertilizer Type	Cadmium Concentration (mg/kg)	Source
Single Super Phosphate (SSP)	10-20	(Satarug *et al.,* 2003)
Triple Super Phosphate (TSP)	5-15	(Niño-Savala *et al.,* 2019)
Diammonium Phosphate (DAP)	2-10	(Lugon-Moulin *et al.,* 2006)
Ammonium Phosphate (AP)	1-5	(Lugon-Moulin *et al.,* 2006)
Rock Phosphate (RP)	20-50	(Lugon-Moulin *et al.,* 2006)
Bone Meal (BM)	1-5	(Garcia & Rosentrater, 2008)

I.3 Plant cadmium accumulation

Cd is a component that is not fundamental for plants and is profoundly harmful. Its poisonous quality is considered to be 2 to 20 times higher than that of other overwhelming metals (Balali-Mood *et al.,* 2021) and, agreeing to Påhlsson (1989), it is the fourth most harmful metal to vascular plants. In any case, helplessness to Cd varyies between species and indeed cultivars of the same species (Perrier *et al.,* 2016; Shahid *et al.,* 2017). While a small number of plants can tolerate and/or accumulate high levels of this metal, all plants, including these tolerant varieties, exhibit symptoms of toxicity (Bohra *et al.,* 2015). Cadmium absorption and accumulation in plant is mediated by three physiological processes including (1) root Cd uptake from the soil solution, (2) root to shoot xylem translocation, and (3) shoot to seed phloem translocation (Clemens *et al.,* 2002).

I.3.1 Root cadmium uptake and transport

Cadmium accumulation in plant organs begins with uptake by the roots and then moves to various organs (Sterckeman & Thomine, 2020). Cadmium uptake and entry into the apoplasm is facilitated by cell membrane negativity. Again and again, passive uptake of Cd in roots is important and ideally controlled by potential electrochemical differences between the root cell

cytoplasm and apoplasm (Sterckeman & Thomine, 2020). The high value of this potential difference allows Cd^{2+} to be transferred to the cytoplasm even when the activity of Cd in the apoplasm is low (Figure 5). However, Cd more likely enters cells via energy-dependent active influx using protein transporters (McLaughlin *et al.,* 2021; Sterckeman & Thomine, 2020). The most known transporters belong to zinc iron regulatory protein (ZIP) or natural resistance-associated macrophage protein (NRAMP) (McLaughlin *et al.,* 2021; Ullah *et al.,* 2018). These proteins transport divalent transition metals such as Fe(II), Zn, Mn, and Cd (Clemens & Ma, 2016). In Arabidopsis, AtIRT1 (derived from the ZIP family) was identified as a key transporter of His-Cd uptake into roots, especially under iron-deficient conditions (Vert *et al.,* 2002). Under iron-deficient conditions, AtNRAMP1 was shown to be involved in His-Cd uptake in Arabidopsis. In rice, several transporters were found to be associated with Cd uptake (OsIRT1, OsIRT2, OsNRAMP1, OsNRAMP5) (McLaughlin *et al.,* 2021). More specifically, the OsNRAMP5-Mn transporter has been proven to be a major pathway for Cd in rice roots (Sasaki *et al.,* 2012). However, it must also be remembered that the rate of Cd uptake is much faster than the uptake of Cd into the soil by water inflow. As a result, a diffusive Cd concentration gradient occurs in the mycorhizosphere (Smolders *et al.,* 2020). Several models predict transport-controlled uptake by plants and negligible influence of root ion uptake dynamics on Cd uptake from soil (Lin *et al.,* 2016; Perriguey *et al.,* 2008). After Cd is taken up by the epidermis or integument, it is transported through the cortex, endothelium, and pericytes (Clemens & Ma, 2016). Cadmium moves through either symplastic (intracellular) or apoplastic (extracellular) routes. This flow can be impeded by sequestration within the vacuole of the root cell. This transport from the cytosol to the vacuole may increase the Cd tolerance in plants (Sterckeman & Thomine, 2020).

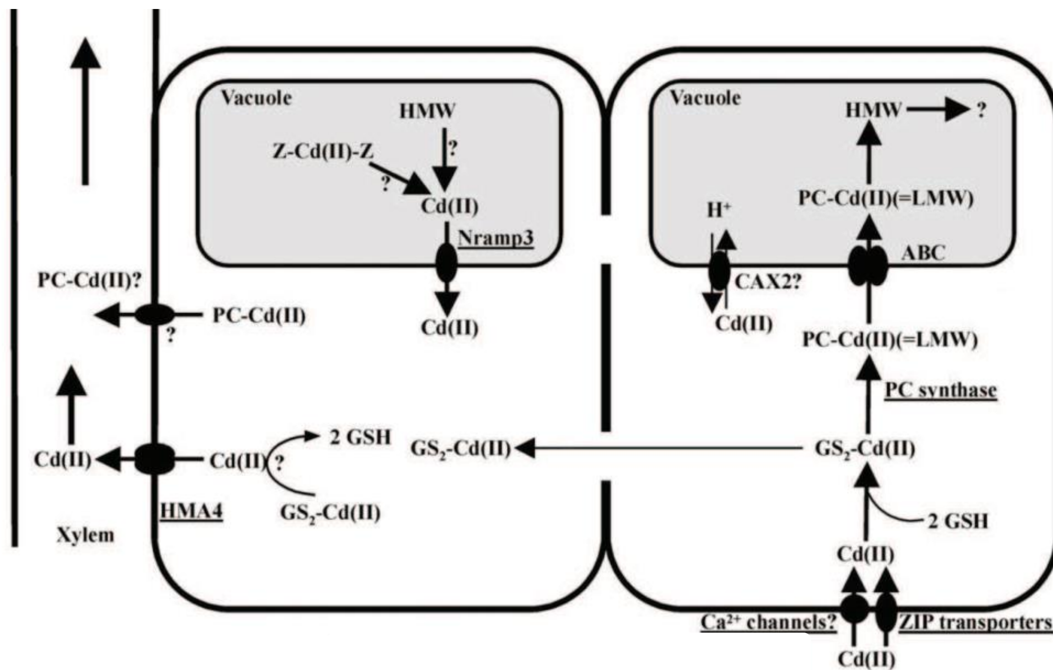


Figure 5: Process involved in the absorption, sequestration and translocation of cadmium at the root cell level (Clemens, 2006).

This diagram shows two root cells. The first cell (right) borders the rhizosphere and the second cell (left) borders the xylem. The two cells are symptomatically connected to each other via plasmodesm. Cd^{2+} ions are taken up into plant cells probably via Fe^{2+} and/or Zn^{2+} transporters of the ZIP family and Ca^{2+} channels. In the cytosol, Cd^{2+} ions are chelated by the first ligand, such as glutathione (GSH). This chelation allows the formation of the GS2-Cd(II) complex. These complexes interact to induce the expression of phytochelatin synthase (PCS), which activates phytochelatin synthesis (PC). The PC-Cd(II) complex (LMW = low molecular weight) is transported into the vacuole via an ABC transporter (yet unknown). Within the vacuole, LMWs aggregate with each other to form high molecular weight complexes (HMWs). The metabolic fate of the HMW complex is still unknown. A second possible pathway for vacuolar sequestration is via Cd^{2+}/H^+ anticarriers. The two candidate proteins are CAX2 and HMA3 from A. thaliana. A portion of the HMW complex or an unidentified complex (Z-Cd(II)-Z) may be remobilized to the cytosol, possibly via proteins such as AtNramp3. Symplastic transition requires the availability of high-affinity mobile ligands (GS2-Cd(II), XCd(II)). Pumps located in the plasma membrane of cells adjacent to the xylem are responsible for charging the xylem. One of these pumps in Arabidopsis is AtHMA4. Flow of the PC- Cd(II) complex is also suggested (Clemens, 2006).

I.3.2 Cadmium translocation from root to shoot

Cadmium that is still mobile in the roots is loaded into the xylem and exported to the shoot. Because Cd transport occurs against its electrochemical gradient, xylem loading requires an active transport system. In Arabidopsis, AtHMA4 and AtHMA2 have been shown to import Cd into the xylem (Mills *et al.,* 2003; Verret *et al.,* 2004; Wong *et al.,* 2009). In rice, knocking out the homolog OsHMA1 reduced root-to-shoot translocation and reduced grain content (Ueno *et al.,* 2011). Therefore, the transfer of Cd to the shoot is determined by three processes: i) sequestration in the root cell vacuole, ii) transport within the symplast to the root stone, and iii) loading into the xylem. A review made by Sterckeman & Thomine (2020) highlight that in most plants, Cd accumulates more in roots than in shoots. Nevertheless, when grown in low-Cd soils content, some plants had higher shoot Cd concentrations than root Cd concentrations. However, when plants were grown in higher Cd conditions, root concentrations exceeded shoot Cd concentrations (John, 1973). Therefore, internal translocation depends on the plant species and its Cd exposure. Subsequent transport through the xylem occurs by transpiration (Song *et al.,* 2023). Cadmium can migrate as a free ion, form complexes with organic and inorganic bases, or adsorb to the cell walls of xylem vessels (Sterckeman & Thomine, 2020). Cadmium ions can then move through the xylem cell wall to the stem and leaf cell walls, again driven by the transpiration flux. Once in the leaf/stem apoplast, Cd is transported to the cytosol via membrane transporters (Zhang *et al.,* 2023). In the cytosol, Cd is stored in vacuoles. In Arabidopsis, NcHMA3 was shown to be involved in Cd sequestration in leaf cell vacuoles (Ning *et al.,* 2023).

I.3.3 Phloem and xylem cadmium transportation to storage organs

Some of the Cd in the leaves is redistributed through the phloem to other plant organs such as reproductive tissues. Phloem may also be involved in Cd transport before reaching the leaves through xylem-to-phloem exchange in the stem (Clemens & Ma, 2016; Sterckeman & Thomine, 2020). In rice, most of the Cd is exported to transpiration organs such as leaves. During grain development, Cd is obtained from two sources. One is direct transfer from roots (xylem root) and the other is remobilization from plant organs that store mineral nutrients during vegetative plant growth (phloem root) (Yan *et al.,* 2023). In wheat, it was shown that half of the grain Cd comes from Cd taken up during filling, and the other half comes from remobilized Cd (Yan *et al.,* 2019). In rice, it is estimated that 91–100% of the Cd accumulated within the grain originates from the phloem (Tanaka *et al.,* 2007). In potato, a low-transpiration

(low xylem flow) crop, Cd was shown to be rapidly distributed to all tissues throughout the phloem (Hu *et al.,* 2019). This suggests that Cd has high mobility in both xylem and phloem (Figure 6).

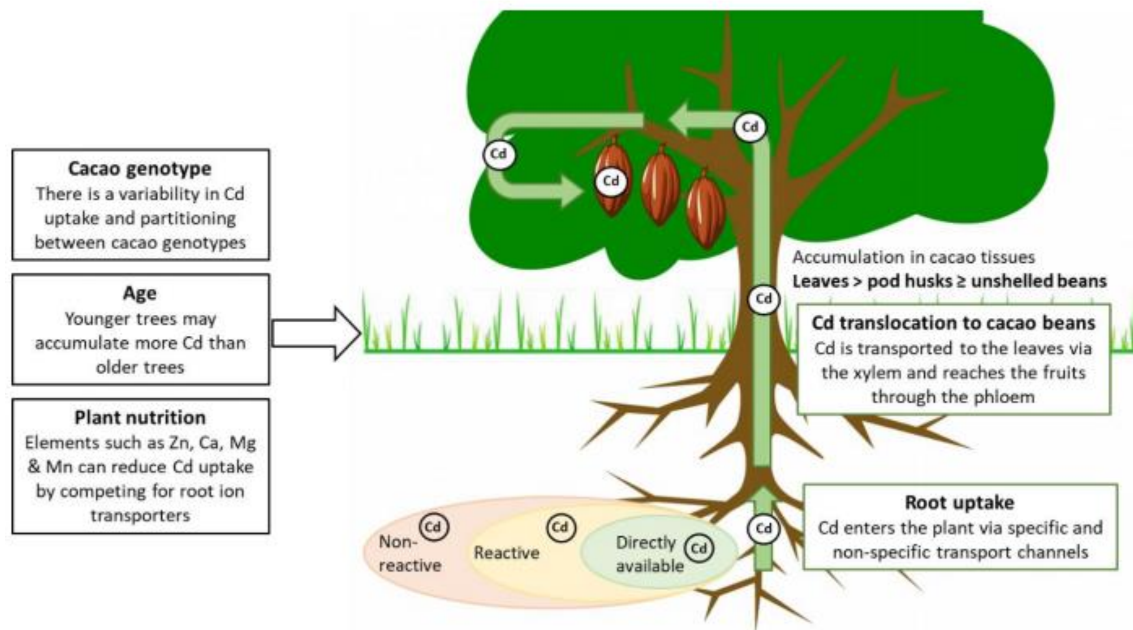


Figure 6: Transport and partitioning of cadmium within the cocoa tree (Meter *et al.,* 2019).

I.3.4 Cadmium speciation inside plants

At different stages of Cd accumulation in plants, Cd binds to functional groups of biomolecules (Sterckeman & Thomine, 2020). The speciation or chemical form of a metal is an important parameter controlling its bioavailability and toxicity (Aucour *et al.,* 2023). In this manuscript, we distinguish between S-ligands (such as glutathione, phytochelatins, and cysteine-rich polypeptides) and O ligands (organic bases such as carboxylates and phosphoryl-containing ligands). The chemical properties of Cd result in a strong affinity for soft sulfur-donating groups such as thiolate (R-S-). These thiolate groups are derived from the amino acid cysteine, which is a constituent of peptides such as glutathione, phytochelatins, and metallothioneins (Poole, 2015). Glutathione (GSH) and phytochelatin (PC) are peptides that have been shown to be involved in Cd detoxification in many plant speciesn (Sterckeman & Thomine, 2020). Glutathione is essential to all plant cells. When the metal is in excess, PC is synthesized from GSH. Synthesis of PC upon Cd exposure has been demonstrated in several plant species. In some species, phytochelatins have been shown to increase plant tolerance to Cd toxicity

(Sterckeman & Thomine, 2020). Another form of S-ligands are metallothioneins (MTs) and other cysteine-rich polypeptides. MT is a low molecular weight cysteine-rich protein (Cobbett & Goldsbrough, 2002). They play an important role in metal homeostasis and tolerance in plants. In the group of O ligands, carboxylates have been shown to contribute to her-Cd complexation in root and shoot tissues (Sterckeman & Thomine, 2020). Proven ligands are citrate, malate, and oxalate. Complexation of Cd with carboxylates is more important in epidermal cells where vacuoles occupy more than 99% of the symplast volume (Tian *et al.,* 2017). Another link between Cd and O ligands is through cell wall polysaccharides (Isaure *et al.,* 2006). At present, it is completely unclear how the nutritional and toxic status of plants affects the binding of Cd to biomolecules. The ratio of S to O ligands has been shown to increase with Cd exposure and decrease with Cd accumulation properties (Isaure *et al.,* 2015). Nevertheless, both S and O ligands have been observed in systems with high Cd concentrations and Cd was mainly bound to S-ligands in roots and O-ligands in shoots (Pongrac *et al.,* 2018).

I.3.5 Effects of cadmium toxicity on plant growth and metabolism

Some studies have shown that the presence of Cd in the medium above a certain limit can lead to the appearance of toxic symptoms with inhibition of weight growth of plants (Aslam *et al.,* 2023). This inhibition affects aerial organs to a greater extent than root organs (Dias *et al.,* 2013; Djebali *et al.,* 2005) and also affects biomass with significant changes in leaf anatomy, structure, and ultrastructure (Djebali *et al.,* 2005). Ekmekçi *et al.* (2008) showed that the dry matter content of roots and leaves of *Zea mays* decreased significantly as the Cd concentration in the medium increased. Cd can cause oxidative damage by increasing cellular concentrations of reactive oxygen species and reducing cellular antioxidant capacity (Jawad *et al.,* 2020). Excess Cd disrupts several physiological metabolic processes in plants, such as photosynthesis, water and mineral uptake, evapotranspiration or respiration (Aslam *et al.,* 2023; Shahid *et al.,* 2017). This causes mineral deficiencies, dehydration and oxidation of cellular components, leading to albinism, root browning, stunted growth, and ultimately plant death (Shiyu *et al.,* 2020; Zimmermann & Zentgraf, 2005). The preferred targets of Cd are thioltransferase-type antioxidant enzymes that contain two cysteine residues in the active site (Wätjen & Beyersmann, 2004). Thioredoxin and thioredoxin transferase, which is involved in photosynthetic activity, and glutaredoxin, which is involved in the detoxification of reactive oxygen species (ROS) such as hydrogen peroxide. (H_2O_2) or glutathione reductase, as well as functions to reduce oxidized glutathione and recycle it into the cell's oxidation-fighting

mechanisms (Chrestensen *et al.,* 2000). Cadmium toxicity may be related to its binding to protein sulfhydryl groups. This binding leads to inhibition of enzyme activity, thus inducing disruption of the cellular redox state and/or generation of ROS, especially H_2O_2 (Cho & Seo, 2005; Genchi *et al.,* 2020). It has also been suggested that iron/sulfur-centered synthetic processes are inhibited, leading to disruption of iron metabolism and increased free radical production (Chrestensen *et al.,* 2000). In upper eukaryotes, Cd has an inhibitory effect on the protein p53 ("p53-like" in plants), which modulates the response to genotoxic substances, especially the induction of repair systems. Inhibition of p53 by metals will increase its mutagenic potential (Filipič, 2012; Méplan *et al.,* 1999). Studies have shown that Cd is a potent inhibitor of DNA repair systems such as excision/repair systems, nucleotides, and mismatches Since most enzymes of DNA repair systems contain a zinc atom that is essential for their activity, it is thought that the mechanism involved is the replacement of zinc with Cd (Roldán-Arjona & Ariza, 2009).

I.3.6 Effects of cadmium toxicity on the oxidative stress

Reactive oxygen species (ROS) are normally created by normal cellular activities such as photorespiration, but their levels rise during cadmic stress (Figure 7), causing oxidative damage to membrane lipids, proteins, and nucleic acids (Anjum *et al.,* 2015; Desikan *et al.,* 2005; Pérez-Chaca *et al.,* 2014). The most well-known of these reactive forms are superoxide ion ($O^{.-2}$), hydrogen peroxide (H_2O_2), hydroxyl radical (OH), and singlet oxygen ($^1O_2$) (Desikan *et al.,* 2005). Plants use antioxidant enzymes and non-enzymatic components to defend themselves from the harmful action of these ROS (Ali *et al.,* 2019). Cadmium exposure can increase the level of MDA in plants, indicating that Cd induces oxidative stress and membrane damage. In fact, MDA is a product of lipid peroxidation, which reflects the degree of oxidative damage caused by reactive oxygen species (ROS) in plant cells. However, MDA can also act as a signaling molecule to activate defence responses, such as antioxidant enzyme activities, phytochelatin synthesis, and gene expression (Morkunas & Ratajczak, 2014).

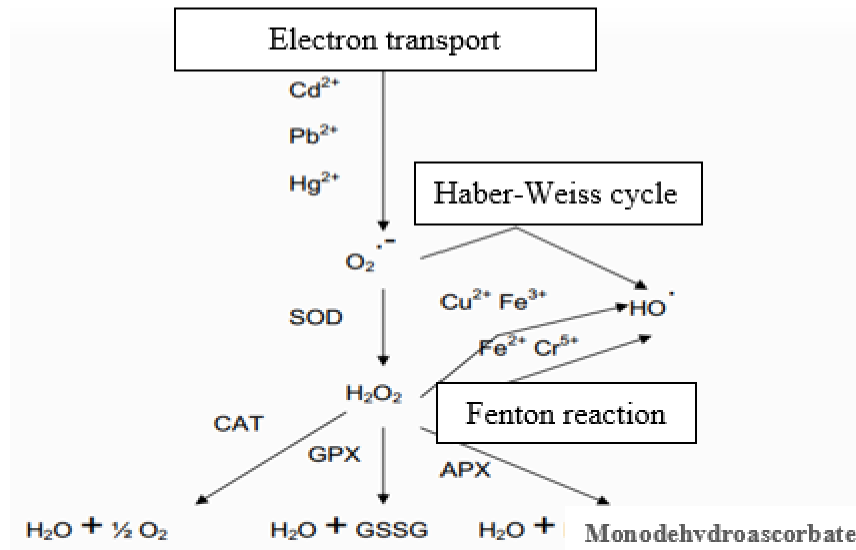


Figure 7 Production of reactive oxygen species (ROS) in plants following exposure to heavy metals (Benavides *et al.,* 2005)

I.3.7 Plant defence against cadmium toxicity

Cadmium is a heavy metal that can cause serious damage to plants, affecting their growth, development, and productivity. Plants have developed various defence mechanisms to cope with Cd stress, such as chelation, sequestration, antioxidation, and signalling. Globally, exclusion strategies and tolerance strategies are the two types of mechanisms that lead to heavy metal tolerance (Hall, 2002; Narayanan & Ma, 2023). Exclusion mechanisms limit metal penetration, preventing it from entering plant tissues. Metal can be accumulated, stored, and immobilized by plants with tolerance mechanisms. The first defence system operates at the root level and includes the chelation and sequestration processes due to their roles in Cd absorption, distribution, and accumulation, as well as Cd immobilization by parietal compounds: pectic sites and histidyl groups (Leita *et al.,* 1996). Tolerance may also involve plasmalemma, either by lowering absorption or by increasing the outflow of ingested heavy metals. The number of cases of exclusion in higher plants is fairly limited; nonetheless, a lower rate of arsenic uptake was observed in an arsenic-tolerant Holcus lanatus genotype (Hall, 2002). Active metal efflux as an intracellular metal management method was before, only been established in bacteria and mammalian cells but today some authors highlight the fact that it also exists in certain plant. In fact, they shown that P1B-type ATPase AtHMA4 transports Cd plays a role in detoxification of high Cd level intoxication by operating as a Cd efflux pump at the plant plasma cells (Kim *et al.,* 2007; Mills *et al.,* 2005). Cadmium compartmentalization

can also occur at the leaf level. Cd accumulation preferentially in the trichomes of *B. junceaet tobacco* leaves has been found (Salt *et al.,* 1995b; Choi *et al.,* 2001). Numerous investigations on the role of phytochelatins in metal chelation, particularly Cd chelation, in relation to Cd tolerance have been described by Clemens (2006), Seregin & Kozhevnikova (2023), Sethi (2023). Using Brassica juncea, Haag-Kerwer *et al*. (1999) demonstrate that Cd buildup is accompanied by a rapid increase of phytochelatin production, which is theoretically adequate to safeguard the plant's physiological activities. Plants use antioxidant enzymes and non-enzymatic components to protect themselves from the destructive action of these ROS (Yu *et al.,* 2013). Among antioxidant enzymes, superoxide dismutase, found in chloroplasts and mitochondria, converts $O_2^{\cdot-}$ to H_2O_2; the latter is directly eliminated by catalase in peroxisomes, but also by guaiacol peroxidase, found in the cytosol, vacuole, and wall: H_2O_2 is also eliminated by the action of the ascorbate-glutathione ring, which is catalyzed by ascorbate peroxidase (APX), monodehydroascorbate reductase (MDAR), dehydroascorbate reductase (DHAR), and glutathione reductase (GR) (Bela *et al.,* 2015; Gupta *et al.,* 2022). In plants, the ascorbate-glutathione cycle can occur in the cytosol as well as in all organelles that require ROS detoxification: Numerous studies have found that heavy metals increase antioxidant activity (Bhaduri & Fulekar, 2012; Chauhan *et al.,* 2022). Certain antioxidant enzyme activities can be inhibited above a certain Cd concentration (Ekmekçi *et al.,* 2008).

On the other hand, organic molecules such as prolin and polyamines are among the non-enzymatic components involved in ROS removal. Plants accumulate amino acids, particularly prolin, as one of their defence mechanisms against the toxic effects of heavy metals (Zhao *et al.,* 2023). Under Cd stress, this compatible solute increased in sunflower, silenus, beans, and soybeans (Siripornadulsil *et al.,* 2002; F. Zhang *et al.,* 2000; Zhao *et al.,* 2023). Prolin is thought to eliminate or reduce the production of toxic oxygen species such as singlet oxygen ($^1O_2$) and hydroxyl radical (OH) in addition to its recognized role as an osmoticum (prolin & Pardha Saradhi, 2002). On the other hand, Soluble sugars are important metabolites that provide energy and carbon skeletons for plant growth and development. They can also act as osmolytes to maintain cell turgor and water potential under Cd stress (Keunen *et al.,* 2013). Moreover, soluble sugars can modulate the redox state of plant cells by scavenging ROS or regulating the activity of antioxidant enzymes (Couée *et al.,* 2006). Some sugars, such as trehalose and raffinose, can also protect membrane integrity and protein stability under Cd stress (Keunen *et al.,* 2013). Furthermore, soluble sugars can serve as signaling molecules to regulate the expression of stress-responsive genes and the synthesis of other defence

compounds, such as phenolics (Morkunas & Ratajczak, 2014). Phenolic compounds also play an important role in plant defence against Cd stress. They are a large group of secondary metabolites that have diverse functions in plant defence against Cd toxicityn (Goncharuk & Zagoskina, 2023). Phenolics can chelate Cd ions and reduce their availability and toxicity in plant cells (Mongkhonsin *et al.,* 2016). They can also enhance the lignification of cell walls and the suberization of endodermis to restrict Cd translocation and accumulation (Le Thi *et al.,* 2020; Podazza *et al.,* 2012). In addition, phenolics can act as antioxidants to scavenge ROS and protect plant cells from oxidative damage. Some phenolics, such as salicylic acid and flavonoids, can also modulate the signaling pathways involved in plant defence responses, such as phytohormone balance, gene expression, and systemic acquired resistance (Kumar *et al.,* 2020).

I.4 Effects of cadmium on soil microbiota

Cadmium (Cd) is recognized for its pronounced toxicity to terrestrial microorganisms, with even moderate contamination levels causing significant disruptions in soil microbial community composition and diversity (You *et al.,* 2024). For example, exposure to 2.5 mg/kg Cd led to a marked decrease in the relative abundance of most microbial taxa, while certain phylum like firmicutes and actinobacteriota increased, and *Bacillus* genus (Firmicutes) emerged as a biomarker of contamination (You *et al.,* 2024). Cd's toxic effects arise from its ability to denature proteins and compromise cell membrane integrity, which impairs the growth, morphology, and metabolic functions of soil microorganisms (Leita et al., 1995). Functional analyses show that lipid metabolism in soil microbes is noticeably decreased under Cd pollution, indicating a broader metabolic disruption (Zhao *et al.,* 2022). Additionally, dissolved organic nitrogen drops by 27%, and microbial respiration rates decline by 12% under Cd exposure (Filipović *et al.,* 2020). Numerous studies confirm that soil bacterial biomass and diversity decline with increasing metal contamination. For instance, in a tropical agricultural soil, the proportion of proteobacteria increased from 37.4% in uncontaminated soil to 50.5% in Cd-contaminated soil, while Actinobacteria dropped significantly, and the overall community structure shifted ($P<0.0001$) (Qui et *al.,* 2021). These shifts are accompanied by the loss of many native (autochthonous) microbial species not adapted to Cd stress, as well as the emergence of heavy metal resistance genes (such as czcCBA efflux system and cation diffusion facilitators) in the microbial resistome (Salam *et al.,* 2020). Cd also impacts soil fungal communities, reducing fungal biomass, diversity, and activity (Iradukunda *et al.,* 2021).

Microbial processes essential for nutrient cycling, such as nitrogen fixation, nitrification, denitrification, ammonification, and mineralization, are all negatively affected. For example, Cd reduces nitrification rates by 17%, β-1,4-glucosidase activity by 21%, and urease activity by 16% (Zhao *et al.,* 2022). The abundance of genes involved in acetyl-CoA synthesis, organic nitrogen mineralization, nitrogen fixation, and nitrous reduction is especially sensitive to Cd, leading to losses in soil carbon and nitrogen pools and increased N_2O emission potential (Zhao *et al.,* 2022).Cd inhibits nitrogen-fixing bacteria by interfering with nitrogenase enzymes or iron metabolism and disrupts nitrifying bacteria by damaging membrane integrity or inhibiting ammonia monooxygenase activity (Zulfiqar *et al*., 2022).

I.5 Factors that upper the plant cadmium uptake

Cadmium has a high mobility in soils and is easily taken up by plants when compared to other heavy metals (Gramlich *et al.,* 2018). Cadmium bioavailability and retention in soils are influenced by a variety of soil properties (Figure 8). Soil composition and texture have a strong influence on Cd retention and transfer to plants. Soil Cd concentrations differ depending on the type of rock present, with sedimentary rocks exhibiting higher Cd levels than igneous and metamorphic rocks (Kubier *et al.,* 2019). The availability of Cd to plants is influenced by soil pH. A higher soil pH causes less Cd accumulation in crops because it stimulates Cd adsorption to soil particles, resulting in less availability. The optimal soil pH for cocoa tree growth ranges from 5.0 to 7.5 (Gramlich *et al.,* 2018; Meter *et al.,* 2019; Zug *et al.,* 2019). Furthermore, the cation exchange capacity (CEC), defined as a soil's total ability to retain exchangeable cations, influences the bioavailable Cd concentration of soils. A high CEC indicates that soil particles have a high ability to retain cations, resulting in lower Cd bioavailability. A rising soil pH corresponds to a rising CEC (Meter *et al.,* 2019). The presence and abundance of beneficial microorganisms such as AMF can also influence Cd bioavailability (Cakmak *et al.,* 2023). In the review of Oliveira *et al*. (2022) The main factors related to Cd uptake and accumulation by the cocoa tree were summarise as the farm geographical location, soil pH, soil Cd bioavailability, cocoa genotype, agronomic factors such as pesticides use and phosphate fertilizers as well as Cd interaction with other minerals such as Mn and Zn in the soil. All these fators are summarise in Figure 8 below.

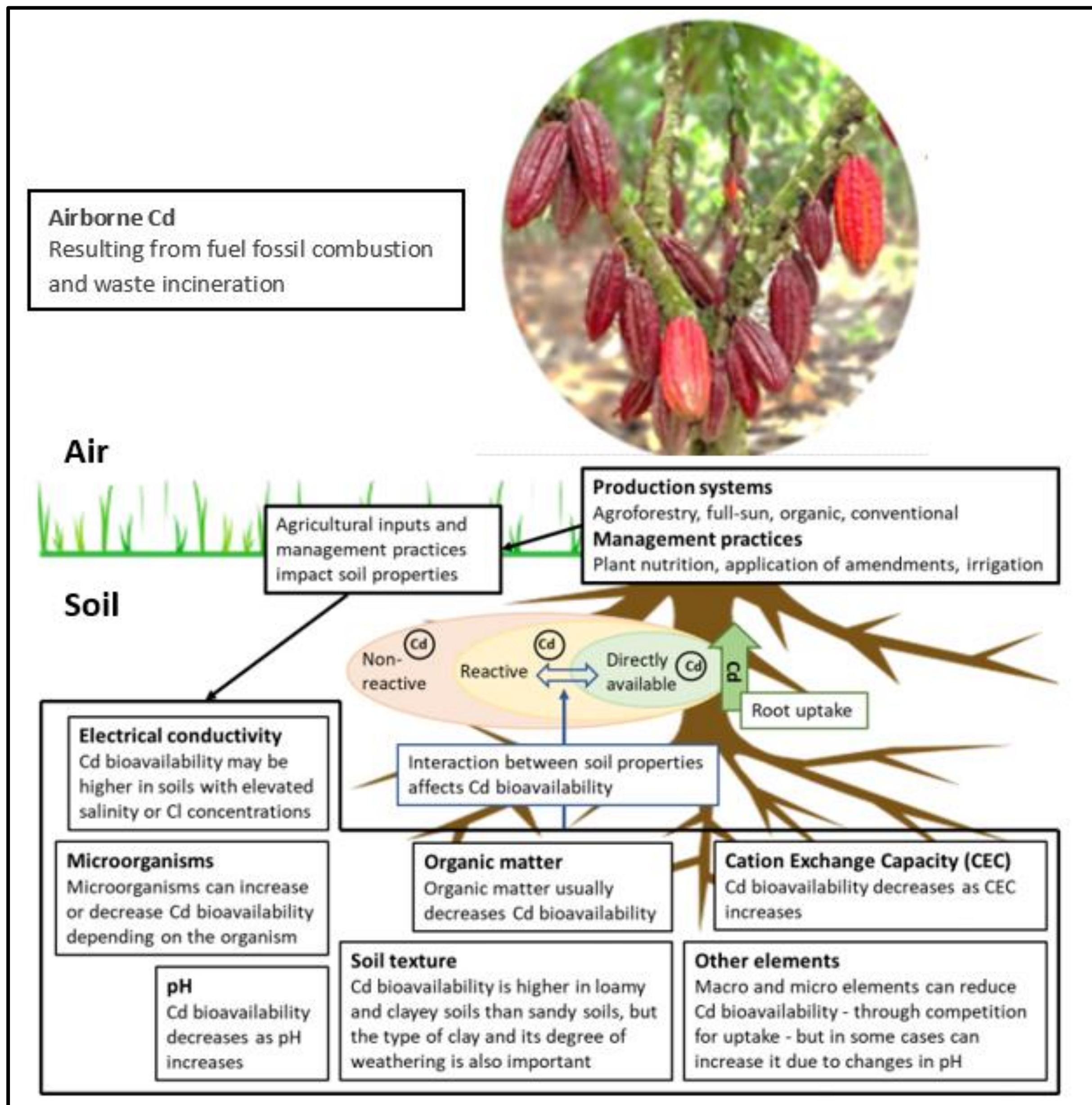


Figure 8 Factors influencing cadmium bioavailability and uptake by root stock or shoot in cocoa. Inspired by Meter *et al*. (2019)

I.6 Legislation regarding cadmium in cocoa

The risks associated with the consumption of food products containing Cd on as early as 1972 led the WHO to set limits on the quantities of Cd that an individual could consume daily without endangering his health. Thus, the tolerable daily intake (TDI) has been set at 1 µg Cd kg-1 body weight (FAO & WHO, 1972). This value was revised downwards in 2009 by the European Food Safety Agency (EFSA), which based on a TDI of 0.36 µg Cd kg-1 of body weight (BW) (EFSA, 2009). In a 2012 report, EFSA states that Europeans' dietary exposure to Cd comes mainly from seeds and derived products (27%), vegetables and vegetable products

(16%) and tubers or roots (13%) (EFSA, 2012). In this report, chocolate and related products contribute 4% for adults and more than 9% for children to dietary exposure to Cd. These evaluations have resulted in the strict regulation of the Cd in cocoa products. The dietary admissions limit of WHO and EFSA are utilized to infer nourishment limits. For the EU this can be the 'Commission Control No 1881/2006', the system EU enactment which sets most extreme levels for chemical contaminants in foodstuffs (EU, 2014). The World Exchange Organization (WTO) recognizes the benchmarks of the Codex Alimentarius (or 'Food Code'), based on the WHO/FAO limits, to ensure the consumers. The guidelines of the Codex Alimentarius can in this way be translated as the universal Cd guidelines for exchange in foodstuff (Codex Alimentarius, 2018). The European Union looked into in 2014 existing most extreme levels of Cd in foodstuff (EC No. 488/2014) (EU, 2014). The review focused especially at the protection of infants and young children. Chocolate was targeted by these new maximum levels, since the consumption of chocolate was shown to be a substantial contribution to the intake of Cd by children. Three maximum levels in chocolate were set (Table III) and entered into force on 1 January 2019. The strictest levels are imposed on chocolates that are most consumed by children (like milk chocolate) (Alves *et al.,* 2018). Similar regulations have been, and are expected to be, implemented worldwide, e.g. in Australia and New Zealand (FSANZ, 2017), Russia (Riker, 2017) and the countries within the Southern Common Market (Vanderschueren & Pulleman, 2021). In July 2018 the commission of the Codex Alimentarius has set as well limits for Cd in different types of chocolate (Table III). There are no official Cd limits in ready-for-sale beans (Codex Alimentarius, 2018). That is because the risk assessments have used the final product on the market (chocolate) as the basis. This situation is clearly different compared to staple crops such as rice and wheat for which the limits are on the raw materials and, hence, the effects of the limits on trade are straightforward. For cocoa, the limits of Table III can be recalculated to bean limits but that requires assumptions that can vary. Consequently, in practice, cocoa buyers insist on a relatively low Cd content of the cocoa bean with informal bean limits ranging between 0.5 and 1.1 µg Cd/kg bean (Meter *et al.,* 2019).

Table III: Maximum cadmium concentrations (mg kg-1 wet weight) on specific cocoa and chocolate products (Codex Alimentarius, 2018; EFSA, 2012).

Description	Regulation	mg Cd kg^{-1}
Milk chocolate with less than 30% total dry cocoa solids	EC No. 488/2014	0.10
Chocolate with more than 50% total dry cocoa solids; milk **Chocolate with more than 30% total dry cocoa solids**	EC No. 488/2014	0.30
Chocolate with more than 50% total dry cocoa solids	EC No. 488/2014	0.80
Cocoa powder sold to the final consumer or as an ingredient in sweetened cocoa powder sold to the final consumer (drinking chocolate)	EC No. 488/2014	0.60
Chocolate with 50% to 70% total dry cocoa solids	Codex Alimentarius, 2018	0.80
Chocolate with more than 70% total dry cocoa solids	Codex Alimentarius, 2018	0.90

I.7 Strategies for monitoring cadmium accumulation and toxicity

The current Cd-cocoa regulations apply to the final product, not the cocoa beans. Therefore, mitigation strategies can be applied at all stages of the production process, from tree to chocolate bar. Mitigation practices at different production steps will likely have to be combined to achieve a final product that complies with the new EU regulations. For soil Cd content, remediation of contaminated soils is the primary method of monitoring the toxicity of heavy metals (Upadhyaya *et al.,* 2023). It can be carried out on site, off site or in situ. In the first two

cases, it is a question of excavating the soil and then washing using chemical agents (reducers or chelators) and/or physical processes, such as flotation techniques (Dermont *et al.,* 2010). This type of remediation requires the implementation of a heavy and expensive construction site and has the disadvantages of destructuring the soil, depleting it of essential elements (Fe and Ca) and using chelators that are often non-biodegradable, such as EDTA (Udovic & Lestan, 2010). Today, studies are oriented towards methods without earth excavation. These include physico-chemical methods (essentially electrochemical) such as electrokinetic remediation which has limitations, related to the formation of OH- ions that will form hydroxides generally insoluble with cations and thus gradually plug the spaces between the particles and reduce the diffusion flow (Virkutyte *et al.,* 2002), phytoremediation which is slow, Cumbersome with the risk that when the leaves fall, the metals concentrated in them are put back into circulation (Van Nevel *et al.,* 2007). Others Mitigation practices can be based on soil amendments to reduce Cd uptake into the plant; plant-based strategies, i.e. selection of cultivars with reduced translocation of Cd to the cocoa beans; or postharvest processing (Grant *et al.,* 2008). Agronomic practices have also been suggested to influence Cd concentrations in cocoa but additional research on their potential effects is required.

I.7.1 Genotype selection

Varietal selection is a process of choosing the best varieties of crops that can tolerate or resist certain environmental stresses, such as heavy metal contamination (Paulin & Eskes, 1995). The history of use of varietal selection to combat heavy metal accumulation in crops can be traced back to the 1970s, when researchers started to screen and breed plants that can grow in metal-contaminated soils. For instance, in 2022, Gupta *et al.* reported in a review that some cultivars of corn, soybean, and wheat had lower Cd uptake than others when grown in Cd-rich soils. They suggested that varietal selection could be a feasible strategy to reduce Cd exposure from food crops. Similarly, other investigation found that some varieties of rice, spinach, pea soybeen and cabbage had lower Cd concentrations than others when grown high heavy metal contaminated levels (Barman *et al.,* 2020; Hasan *et al.,* 2009; Xu *et al.,* 2022). They recommended that varietal selection could be a practical way to minimize Cd intake from plant. Since then, many studies have been conducted to identify and develop crop varieties that have low heavy metal accumulation or high heavy metal tolerance. For example, in 2021 Arévalo-Hernández *et al.* screened some cocoa genotype from Latin America for their ability to accumulate Cd. They found a total of 11 cocoa genotypes could be proposed as low Cd-

accumulating genotypes that Based on their lower Cd concentration and that these differences were mainly due to genetic factors. They concluded that varietal selection could be an effective tool to reduce Cd contamination cocoa beans. Earlier in 2018, Lewis *et al.,* results point out the potential of use a varietal strategy to mitigate Cd accumulation within cocoa beans either through the use of low Cd accumulating rootstocks in grafting or through breeding. They found that some genotypes had higher biomass production and lower Cd accumulation than others under Cd toxicity. They suggested that genotype selection could be a useful method to improve cocoa productivity and quality in Cd-affected areas.

Varietal selection has several advantages over other methods of mitigating heavy metal accumulation in crops, such as soil amendments, phytoremediation, and genetic engineering. First, varietal selection is relatively simple and inexpensive, as it does not require any special equipment or chemicals. Second, varietal selection is environmentally friendly, as it does not introduce any foreign genes or substances into the soil or plants (Devi *et al.,* 2019; Heffner *et al.,* 2010). Third, varietal selection is socially acceptable, as it does not raise any ethical or legal issues regarding the safety or ownership of the crops (Devi *et al.,* 2019; Singh *et al.,* 2014). However, varietal selection also has some limitations and challenges that need to be addressed. First, varietal selection may not be effective for all crops and all heavy metals, as different crops may have different mechanisms of uptake, translocation, and detoxification of heavy metals. Second, varietal selection may not be sufficient for highly contaminated soils, as even the most tolerant or resistant varieties may still have unacceptable levels of heavy metals in their edible parts (Mathews & Campbell, 2000). Third, varietal selection may face some difficulties in implementation and adoption, as it requires extensive screening and breeding programs, reliable testing methods, and farmer awareness and cooperation (Devi *et al.,* 2019; Gaille, 2017).

I.7.2 Genotypic variability in cadmium accumulation: a review of recent studies on cocoa genotypes

Cadmium accumulation in cocoa plants varies significantly across different genotypes, offering potential strategies for reducing Cd levels in cocoa beans. For instance, a study by Galvis *et al.* in 2023 found that among nine cocoa rootstock genotypes, *EET61* showed the highest Cd concentration in shoots, while *PA46* exhibited the lowest, highlighting the importance of genotypic variation in managing Cd accumulation. Similarly, Engbersen *et al.* in 2019 after a

study conducted in 661 northern Honduras noted that the *POUND7* genotype demonstrated a lower predisposition to incorporate Cd compared to other cultivars *(SPA9* and *IMC67*), underscoring the role of genetic factors in Cd management. In another study focusing on fine-aroma cocoa genotypes (*INDES38, JSM2, INDES39, JSM3* and *JMB1*) grown under hydroponic conditions, significant genotypic variation in Cd uptake and accumulation was observed, suggesting that selecting appropriate genotypes could mitigate Cd levels in cocoa products (Meléndez-Mori *et al.,* 2023). Additionally, Lewis et al. in 2018 reported a variation of up to 13 times in Cd concentration among 100 evaluated cocoa genotypes. Further emphasizing the genetic basis of Cd accumulation in cocoa. a study conducted in Peru by Arévalo-Gardini *et al.,* 2017 found that certain genotypes like *CCN51* and *ICS95* demonstrated a higher propensity to Cd accumulation compared to native genotypes. The genetic variability among cocoa genotypes offers a promising avenue for managing Cd accumulation in cocoa beans. By selecting genotypes with inherently low Cd accumulation and understanding the genetic basis of Cd uptake and allocation, it is possible to develop effective strategies for reducing Cd levels in cocoa products. This approach not only addresses commercial concerns but also contributes to improving the health safety of cocoa products. However, a such study on local cocoa genotype regarding Cd accumulation remain poorly documented in Cameroon.

I.7.3 Biological control

Biological control is defined as "the directed, precise management of common ecosystem components to protect plants from stress (Van Driesche & Bellows, 2012). It is the intentional use of introduced or resident living organisms other than disease-resistant host plants to suppress the activities and populations of one or more plant pathogens (Heimpel & Mills, 2017). Biological control of plant stress is slow and yields few immediate profits, but it can be long-lasting, inexpensive, and non-harmful to life. Biocontrol systems do not eliminate pathogens or diseases, but rather restore them to natural balance (Dinango *et al.,* 2022; Huffaker, 2012; Rochal *et al.,* 2021). A wide range of living organism have been implicated as biocontrol agents for a variety of plant diseases. These organisms, known as Biocontrol Agents (BCA) or Bioagents, include yeasts, amoebae, viruses, bacteria, fungi, and actinomycetes (Waage & Greathead, 1988). Microorganisms have a significant impact on the heavy metal phytoavailability because they influence the root growth and morphology, plant physiology and development, metal fractionation and uptake by the root (Seshadri *et al.,* 2015; Veerapagu *et al.,* 2023). Mycorrhizal fungi is cited to play an important role (Tan *et al.,* 2022). Soil

microorganisms have various strategies to cope with Cd stress by chelating, sequestering, effluxing or detoxifying Cd ions; scavenging reactive oxygen species; protecting cellular structures and functions; regulating gene expression; and forming symbiotic associations with plants. Soil organic amendments can enhance the remediation of Cd-contaminated soils by improving the soil physicochemical properties and microbial properties (Abeed *et al.,* 2022; Mathivanan *et al.,* 2021).

I.7.4 Arbuscular Mycorrhizal Fungi (AMF): a heavy metal toxicity alleviator

I.7.4.1 Overview of mycorrhizae

Mycorrhizae were first described as a symbiotic association between plant roots and fungi by a German plant pathologist in 1855. During this symbiosis, a chemical element and nutrient exchange occurs in which the plant provides carbon to the heterotrophic fungus while the latter transmits different nutrients to it and protects it from certain diseases and toxic substances (Corradi & Bonfante, 2012). Endomycorrhizae and ectomycorrhizae are the two major groups currently recognized, distinguished by the ability or inability of their mycelium to penetrate the roots. Ectomycorrhizae are symbiotic fungi that surround the roots with mycelium in the form of sleeves rather than penetrate them (Brundrett, 2004). Arbuscular endomycorrhizae (AMF) are distinguished by non-septated (non-partitioned) hyphae that are restricted to epidermal root cells (Brundrett, 2004). Early taxonomic classifications of AMF were largely based on spore morphology and placed species within the order *Glomerales* of the phylum *Glomeromycota*. Advances in molecular phylogenetics, especially analyses of SSU and LSU rRNA genes, tubulin sequences, and ITS regions, have led to a significant reorganisation of this phylum (Schüßler & Walker, 2011). Currently, the phylum *Glomeromycota* is divided into five orders: *Glomerales*, *Diversisporales*, *Archaeosporales*, *Paraglomerales*, and *Ambisporales* (Catalogue of Life Secretariat, 2025). These orders encompass 13 families, 49 genera, and approximately 360 described species worldwide, reflecting a much greater diversity than previously recognised.

Within the order *Glomerales*, the families *Glomeraceae* and *Claroideoglomeraceae* are prominent. Several species formerly classified under the genus *Glomus* have been reassigned to genera such as *Funneliformis* and *Rhizophagus*. Other orders include *Diversisporales*, which contains families like *Diversisporaceae* and *Acaulosporaceae*, and *Archaeosporales*, which

includes the family *Archaeosporaceae*. The order *Paraglomerales* is represented by the family *Paraglomeraceae*. The phylum Glomeromycota and its subdivisions are shown in Table IV base on Catalogue of Life Secretariat (2025).

Table IV : Current Classification of the phylum Glomeromycota (Catalogue of Life Secretariat, 2025)

Order	Family	Genera
Diversisporales	Diversisporaceae	*Tricispora*, *Otospora*, *Diversispora*, *Corymbiglomus*, *Redeckera*
	Acaulosporaceae	*Acaulospora*
	Sacculosporaceae	*Sacculospora*
	Pacisporaceae	*Pacispora*
	Gigasporaceae	*Scutellospora*, *Gigaspora*, *Intraornatospora*, *Paradentiscutata*, *Dentiscutata*, *Centraspora*, *Racocetra*
Glomerales	Claroideoglomeraceae	*Claroideoglomus*
	Glomeraceae	*Glomus*, *Funneliformis*, *Septoglomus*, *Rhizophagus*, *Sclerocystis*
Archaeosporales	Ambisporaceae	*Ambispora*
	Geosiphonaceae	*Geosiphon*
	Archaeosporaceae	*Archaeospora*
Paraglomerales	Paraglomeraceae	*Paraglomus*
Ambisporales	Ambisporaceae	*Ambispora* (Note: Some taxonomic treatments merge this family with Archaeosporales)

I.7.4.2 Importance of AMF in agriculture

Because of their role as natural biofertilizers and bioprotectant, AMF have been advocated as the biological means for managing sustainable agriculture (Delaeter *et al.,* 2024; Igiehon & Babalola, 2017; Lone *et al.,* 2017). They are also used as bio-protectants against fungal,

bacterial, and nematode pathogens and drought stress (Adamou et al., 2023; Eke et al., 2016; Ebbisa, 2022; Mbogning *et al.,* 2024; Nwaga *et al.,* 2010; Nwaga *et al.,* 2011). In root systems, AMF form mutualistic symbioses. The mycorrhizal fungus improves the host plant's ability to absorb mineral nutrients (especially phosphate) and water from the soil in exchange for carbon sources from the host plant (Ngakou *et al.,* 2007; Ngonkeu 2003; Yang *et al.,* 2023). AMF can also improve plant growth by promoting soil aggregation through the growth and excretion of external hyphae. AMF fungal hyphae, for instance, can affect host plant performance and thus influence plant diversity and ecosystem stability (Yang *et al.,* 2014). Furthermore, there is mounting evidence that AMF can reduce disease incidence and the number of propagules of several soil-borne pathogens as Macrophomina, Phytophthora, Pythium, and Fusarium (Adamou *et al.,* 2023; Ahmed *et al.,* 2023; Eke *et al.,* 2016, Mbogning *et al.,* 2024) resulting in increased plant growth and productivity (Eke *et al.,* 2016). More over AMF has been reported Mbassi *et al.* (2020) to reduce the cassava cyanide concentration and as well as to improved it nutritional properties, highlighting therefore their capacity to improve not only the production but also the crop quality.

I.7.4.3 The importance of AMF in enhancing cocoa productivity and quality

Research in Cameroon shows a diverse community of AMF associated with cocoa, influenced by soil properties, altitude, and agroforestry practices (Ketchiemo *et al.,* 2024; Snoeck *et al.,* 2010). Well-managed agroforestry systems harbour more diverse AMF populations, essential for soil fertility and plant health. Key AMF genera identified include *Glomus*, *Gigaspora*, and *Acaulospora* (Ketchiemo *et al.,* 2024). Different AMF species vary in colonization efficiency and nutrient transfer, impacting cocoa growth and resilience. Studies confirm that AMF inoculation improves cocoa seedling growth in nurseries, increasing height, biomass, and root development by enhancing phosphorus and nitrogen uptake (Djenatou et al., 2020; Ntsoli et al.). This reduces fertilizer needs and supports sustainable farming. AMF also enhance disease resistance, notably against black pod rot. Tchameni *et al.* (2011, 2012) as well as Nana *et al.* (2016) demonstrated that AMF-colonized seedlings have lower disease severity due to increased production of defence compounds. Beyond biotic stress mitigation, AMF significantly contribute to abiotic stress tolerance, especially drought, which increasingly threatens cocoa production. Agele *et al*. (2018) and Kwashie *et al.* (2023) found that AMF inoculation improves water uptake, leaf water status, and root architecture, aiding drought resilience. AMF also produce glomalin, a glycoprotein that improves soil structure and

supports root growth and microbial activity, vital for sustainable cocoa systems (Fokom *et al.,* 2012).

Importantly, recent research highlights that AMF can influence cocoa quality and aroma, key factors for market value and consumer preference. AMF enhance nutrient uptake and plant metabolism, which can positively affect the biochemical composition of cocoa beans, including compounds responsible for flavour and aroma (Delgado-Ospina *et al.,* 2021). Studies have shown that AMF inoculation improves phosphorus content in leaves and shoots, which correlates with better bean development and potentially richer flavour profiles (Delgado-Ospina *et al.,* 2021). Moreover, AMF can reduce the presence of harmful fungi during fermentation and drying stages, indirectly preserving bean quality by limiting mycotoxin contamination and spoilage (Delgado-Ospina *et al.,* 2021). These benefits suggest that AMF not only improve plant health but also contribute to the sensory and commercial quality of cocoa products. Agroforestry systems with shade trees and intercropping foster favourable conditions for AMF diversity and abundance. Research by Smock *et al.* (2010) and Kumah *et al.* (2025) shows that shaded cocoa farms harbour richer AMF communities than monocultures, enhancing nutrient cycling and ecosystem stability. Intercropping with legumes further boosts AMF diversity and soil fertility. In a broader context, beneficial soil microbes, including AMF, are critical for restoring soil fertility in humid tropical zones like Cameroon’s forest region. Studies on slash-and-burn agriculture in Sub-Saharan Africa highlight how microbial communities improve nutrient cycling, organic matter decomposition, and soil structure, supporting crop productivity in nutrient-depleting systems (Nwaga *et al.,* 2010). These findings align with cocoa cultivation, where AMF inoculation enhances nutrient uptake and soil resilience, forming part of strategies to rehabilitate degraded soils and sustain agriculture.

Despite benefits, AMF adoption in Cameroon faces challenges such as limited farmer awareness, inoculum availability, and environmental variability affecting efficacy. Coordinated research, extension, and policy efforts are needed to develop microbial consortia, optimize plant-fungal compatibility, and improve inoculum delivery. Supporting policies and farmer education are essential to scale AMF use sustainably.

I.7.4.4 Effects of AMF on the plant under stress

I.7.4.4.1 AMF and biotic damages

AMF can affect plant growth and resistance to biotic stress, which is the negative impact of living organisms such as pathogens, herbivores, and competitors on plant health and productivity (Fiorilli *et al.,* 2024). The mycorrhization provides host plants with active protection against pathogens, primarily fungi and nematodes that attack plant roots (Eke *et al.,* 2016; Fiorilli *et al.,* 2024). Several mechanisms account for this protection. First, the nutritional benefits of mycorrhization allow host plants to be more vigorous and resist pathogen attacks (Ahmed *et al.,* 2023; Eke *et al.,* 2016). Second, infection of the host plant's roots with mycorrhizal fungi has been shown to precondition plants to deal with pathogens and other pests like grazers. Indeed, mycorrhization induces a state of active immunisation in the plant, allowing it to be more effective in these responses to pathogen attacks (Enebe & Erasmus, 2023). According to Gough *et al*. (2020), AMF can enhance the resistance or tolerance of a plant to root-lesion nematodes (*Pratylenchus spp.*), which are migratory endoparasites that feed and move through the root cortex, causing necrotic lesions and yield losses. Another study investigated the effects of AMF on plant growth and herbivore infestation under contrasting levels of soil water and nutrients, found that AMF inoculation reduced the abundance of the foliar herbivore *Chrysolina aeruginosa* on plants that had been grown on the low nutrient soil, but not on high nutrient soil (Wang *et al.,* 2023). However, the effects of AMF on plant biotic stress are not always consistent and may depend on various factors such as the AMF species and plant, the availability of soil water and nutrients, and the type and intensity of biotic stress (Jamiołkowska *et al.,* 2018).

I.7.4.4.2 AMF and abiotic stress

Abiotic stress is the negative action of non-living factors on the living organisms in a particular environment (Zhu, 2016). These abiotic factors can be drought (water stress), excessive watering (water logging), extreme temperatures (cold, frost and heat), salinity and mineral toxicity as heavy metal toxicity (Gong *et al.,* 2013). Several studies suggest that mycorrhizae symbionts play an important role in plant adaptation to heavy metal-rich soils, such as mining soils (Silva-Castro *et al.,* 2023; Trocio & Paguntalan, 2023). Mycorrhizal plants grow better in drought (Abdalla *et al.,* 2023), salinity (Razvi *et al.,* 2023), and on media contaminated with metallic trace elements, radio-elements (de Boulois *et al.,* 2008; Silva-Castro *et al.,* 2023), and persistent organic pollutants (Lenoir *et al.,* 2016), implying that mycorrhization protects against abiotic stress. Under heavy metal pollution, AMF inoculation can improve the mineral nutrition of plants and directly affects the absorption and accumulation of heavy metals in

plants (Silva-Castro *et al.,* 2023). Thus, AMF can enhance plant tolerance to heavy metals, which is usually associated with an increase in the production of osmotic regulatory substances, plant hormones, and antioxidants (Zulfiqar *et al.,* 2022). AMF can improve plant resistance to Cd toxicity by regulating certain morphological, physiological, biochemical, and molecular mechanisms of host plants (Eke *et al.,* 2015; Tchameni et al., 2012). Such effects of AMF include the regulation of active metabolism, development, oxidase production, functional traits, and the activity of heavy metal resistance genes and heavy metal transport protein genes (Diagne *et al.,* 2020). The AMF also influence the compartmentation in vacuole, efflux from cells, and decrease enrichment of Cd as well as an increase in the antioxidant capacity of plants (Huang *et al.,* 2017).

I.7.4.4.3 Effects of AMF on cadmium stress and accumulation

An advantage conferred on host plants by mycorrhization is active protection against the absorption and toxicity of heavy metals. Indeed, Redon *et al*. (2009) and Bissonnette *et al*. (2010) showed that the colonization of plants by AMF limited the accumulation of Cd only in the root part. In addition, some AMF have been shown to have the ability to sequester metals in their vacuoles and walls, thus preserving plant tissues. Similarly, Janoušková & Pavlíková (2010) demonstrated that AMF were able to reduce the adverse effects of Cd on plant growth through a phytostabilization process. Thus, AMF would also protect plants by accumulating and making Cd less bioavailable. Mitigation of Cd accumulation and stress in plants can be the results of several mechanisms, such as:

⇒ **Enhancing nutrient uptake and phytostabilization of Cd in the soil**:

AMF can increase the availability and absorption of P and other essential nutrients, such as nitrogen, potassium, calcium, magnesium, and zinc, by extending their hyphae into the soil and forming a network of mycelia. This can improve plant growth and health under heavy metal stress. AMF can also immobilize heavy metals in the soil by binding them to their cell walls, organic acids, and glomalin, a glycoprotein produced by AMF (Agarwal *et al.,* 2017; Fokom *et al.,* 2012; Purin & Rillig, 2008). This can reduce the mobility and bioavailability of heavy metals and prevent their accumulation in plant tissues (Gough *et al.,* 2020).

⇒ **Absorbing and detoxifying heavy metals in plant roots**:

AMF can take up heavy metals from the soil and transport them to their intraradicial structures, such as vesicles, arbuscules, and spores. This can reduce the concentration of heavy metals in the root cortex and protect the plant cells from oxidative damage. AMF can also activate the antioxidant system of plants by increasing the activity and expression of enzymes, such as superoxide dismutase, catalase, and peroxidase, and molecules, such as glutathione, ascorbate, and phytochelatins, that can scavenge ROS and chelate heavy metals (Han *et al.,* 2023).

⇒ **Regulating the expression of genes involved in stress response and tolerance**:

AMF can modulate the expression of genes that are related to plant growth, development, metabolism, and defence under heavy metal stress. For example, AMF can upregulate the expression of 14-3-3 proteins, which are involved in signal transduction, cell cycle regulation, and stress adaptation. 14-3-3 proteins can interact with various target proteins and influence their activity, localization, and stability. 14-3-3 proteins can also respond to different abiotic stresses, such as low P and drought, and have a positive correlation with the antioxidant system, osmotic regulation, and P metabolism (Han *et al.,* 2023).

However, the effects of AMF on roots under Cd stress may vary depending on the type, concentration, and duration of heavy metal exposure, the species and strain of AMF, the host plant species and cultivar, and the environmental conditions (Lin *et al.,* 2023). Therefore, it is important to select the appropriate combination of AMF and plants for the remediation of heavy metal-contaminated soils and the improvement of plant productivity and quality. Table V below summarizes the different means used by AMF to reduce Cd-related toxicity (Amir & Ducousso, 2010).

Table V: Modes of action of AMFs during tolerance to heavy metals (Amir et al., 2010)

Mechanism	Mode of action	Engaged molecules	Source
Extracellular Inactivation	Exudation of complexing agents through hyphae	Citric acid, malic acid, oxalic acid, phenolic acid, glomalin	Cairney & Meharg (2003); Hinsinger *et al.* (2009)
	Association with bacteria	Chelating aggregates (Zn, Pb, Mn)	Cabala *et al*. (2009); Chen *et al. (*2019)
	Exudation of redox enzymes	SOD, GPX, GSH PX	Vallino *et al. (*2009)
Cell-walls fixation	cell-wall Structure with the metals fixing sites	Glucans, chitines et Galactosamines polymers, petits peptides et protéines, glomalin	Agarwal *et al.* (2017); Purin & Rillig (2008)
Intracellular inactivation	Increase in Heavy Metal Effluxes	Proteins transporters or permeases	Hildebrandt *et al.* (2007); Ouziad *et al.* (2005)
	Intracellular Compartmentation: vacuoles, vesicles, spores	Chelators and transporters: same molecules as before, fungal and vegetable metallottihines	Ferrol *et al.* (2009); González-Guerrero *et al.* (2009)
Oxidative stress response	Synthesis of oxidative stress resistance molecules (enzymatic and non-enzymatic pathways)	Glutathione, Vitamin C, E and B6, Catalase, Superoxide Dismutase, Thioreductase	Ferrol *et al.* (2009); Wang *et al. (*2023)

I.7.4.4.4 Glomalin and cadmium stress resistance

AMF produce a protein called glomalin-related soil protein (GRSP), which aids in stabilizing soil aggregates and sequestering carbon. Glomalin's advantages for fixing heavy metals, enhancing soil quality, and sequestering carbon have drawn a lot of attention (Fokom *et al.,* 2012; Hossain, 2021; Rago *et al.,* 2023). The ability of plants to withstand the harmful metal (Cd), which can have an impact on plant growth and health, is another function of globulin. The following details regarding glomalin and Cd stress resistance are based on certain online search results: The amount of glomalin produced by AMF varies depending on the Cd levels and soil conditions (Fokom *et al.,* 2012; Gerami *et al.,* 2021). AMF root colonization and glomalin production are generally inhibited by elevated Cd concentrations. Cadmium can be bound by glomalin, which lessens both its availability and toxicity to plants (Gao *et al.,* 2021). Additionally, under Cd stress, glomalin promotes the uptake of nutrients by plants, particularly phosphorus, which can improve plant growth and metabolism (Fokom *et al.,* 2012; Guo *et al.,* 2023). The ability of various AMF species to produce glomalin and withstand Cd stress varies. According to certain research, *Rhizophagus intraradices* is less efficient than *Funneliformis mosseae* and *Claroideoglomus etunicatum* at complexing Cd and generating glomalin (Adeyemi *et al.,* 2021). Additionally, glomalin can increase prolin and glycine betaine activity under stress (Jajoo & Mathur, 2021). In plants, glomalin may function as a stress-response molecule or a signaling molecule. Therefore, glomalin is a valuable soil protein that can improve the plant and soil health under heavy metal stress (Hossain, 2021). Glomalin can be extracted from laboratory cultures of AMF and from soils, and its quantification and characterization can provide insights into the functioning and application of AMF in biostabilization of polluted soils (Irving *et al.,* 2021).

I.7.4.4.5 Cadmium accumulation in cocoa and the role of AMF

Recent research provides contrasting insights into the effects of AMF on Cd accumulation and translocation in cocoa. In a study by Ramtahal et al. (2012), a commercial AMF bio-fertilizer significantly increased Cd accumulation in cocoa leaves and stems compared to non-inoculated plants. The study attributed this to enhanced Cd absorption by fungal mycelia spreading around the roots, emphasizing the potential variability in AMF effects depending on the fungal strain and plant genotype. Conversely, Pérez et al. (2019) demonstrated that native AMF species, including *Glomus macrocarpum* and *Rhizoglomus intraradices*, reduced Cd concentrations in all plant organs of the *IMC 67* cocoa genotype under varying Cd doses (0–24 $mg \cdot kg^{-1}$),

highlighting their role as effective Cd immobilizers. Similarly, Vallejos-Torres et al. (2022) observed that AMF consortia from Peruvian Amazon regions, dominated by families such as *Claroideoglomeraceae* and *Glomeraceae*, reduced Cd uptake in cocoa stems and soils by up to 30%. However, when combined with compost amendments, these AMF further decreased Cd absorption by 40%, showcasing synergistic effects. Luis-Alaya et al. (2023) identified additional AMF families (*Acaulosporaceae*, *Diversisporaceae*) in San Martín, Peru, which contributed to phytostabilization of Cd through glomalin production, though hyperaccumulator soils (>50 $mg \cdot kg^{-1}$) impaired colonization rates. A study on agroforestry systems by Vallejos-Torres et al. (2023) found that AMF colonization was higher in these systems compared to monocultures, resulting in lower bean Cd levels due to enhanced fungal diversity (*Glomeraceae*, *Acaulosporaceae*). Meanwhile, *Glomus versiforme* has been noted for reducing root Cd by 45% in Trinidadian cocoa, highlighting species-specific effects. Despite these promising findings, gaps remain among which: how AMF propagation hosts direct the behaviour of AMF in the direction of increasing or decreasing Cd accumulation. These studies collectively highlight the dual potential of AMF to either mitigate or exacerbate Cd accumulation depending on fungal strain, host plant genotype, and environmental conditions, necessitating targeted research for optimizing their application in cocoa production.

CHAPTER II:

MATERIALS AND METHODS

CHAPTER II: MATERIALS AND METHODS

II.1 Materials

II.1.1 AM fungal strains and inoculum production

II.1.1.1 AM fungal strains

The Soil Microbiology Laboratory and Biotechnology Centre at the University of Yaoundé I provided the AM fungus strains *Glomus intraradices* (Gli), *Glomus hoi* (Glh), *Gigaspora margarita* (Gim), and *Scutellospora dipurpurescens* (Scd) used in this investigation (Figure 9). They came from a heavily metal-polluted farmland (Ngonkeu, 2009). In the literature, these species have been used to mitigate heavy metal toxicity. Their identities are detailed in the works of Ngonkeu (2003) and Nwaga *et al*. (2004).

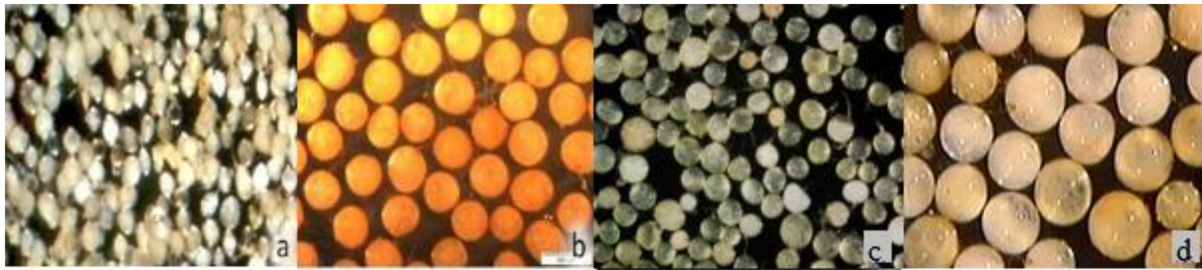


Figure 9: AM Fungal spores used: (a) *Glomus intraradices*; (b) *Glomus hoi*; (c) *Scutellospora dispurpurescens*; (d) *Gigaspora margarita* (Ngonkeu, 2003)

II.1.1.2 AMF inoculums

In order to produce the AM fungal inoculum, an equivalent quantity of spores from the strains Gli, Glh, Gim, and Scd were concurrently introduced into the substrate. The substrate consisted of a combination of sterile river sand and agricultural soil in a ratio of 2:1 (weight/weight). The spores were introduced using the seed-bed method, as described by Ngonkeu et al. (2003). In order to get the treatments listed below, three different propagation hosts and host systems were seeded.

MFP = AMF inoculum propagated with Leek (*Allium Porum*)

MFSG = AMF inoculum propagated with the combination of sorghum (*Sorghum bicolour*) and Groundnut (*Arachis hypogaea* L)

MFSC = AMF inoculum propagated with the combination of sorghum (*Sorghum bicolour)* and Cowpea (*Vigna unguiculata*).

The AMF were propagated in 5-liter pots in the greenhouse at the Biocontrol Agents Unit, Laboratory for Phytobiochemistry and Medicinal Plant Studies, University of Yaoundé I. Four months after sowing, soil from each pots were collected, spore concentration were quantifying after extraction and microscopically observation. Then the inoculum from each treatment was prepared a calibrates to have a final concentration of 100 spores per gram of substrate (Figure 10).

Figure 10: Mycorrhizal inoculums: FM1 = MFSG; FM2 = MFP; FM3 = MFSC (Ghomsi, 2024)

II.1.2 Sources of cadmium and planting material

The cocoa seeds of 16 cocoa hybrids (genotypes): *SNK10*IMC67*, *SNK13*ICS95, UPA143*SNK37, IMC67*SNK109, BBK1418*MO20, ICS1*SNK37, T79/501*SNK13, SNK16*T60/1174, T79/501*SNK109, UPA143*SNK64, T60/887*SNK64, T79/501*SNK16, SNK64*T40/1170, T60/1174*UPA134, SCA12*ICS40, SNK16*UPA143,* produced by hand pollination. They were acquired from the experimental farm of the National Cocoa Research Company (SODECAO) at Nkoemvone (Latitude 2°90'N, Longitude 11°20'E), South Region, Cameroon.

Cadmium sulfate octahydrate ($3CdSO_4.8H_2O$, MW: 769.51, purity ≥ 99%) utilised in this research was purchased from HIMEDIA Chemical, India. The Cd solution was made in deionized water so as to have a stock solution of 5 mg/mL by dispersing a suitable quantity of powder in deionized water.

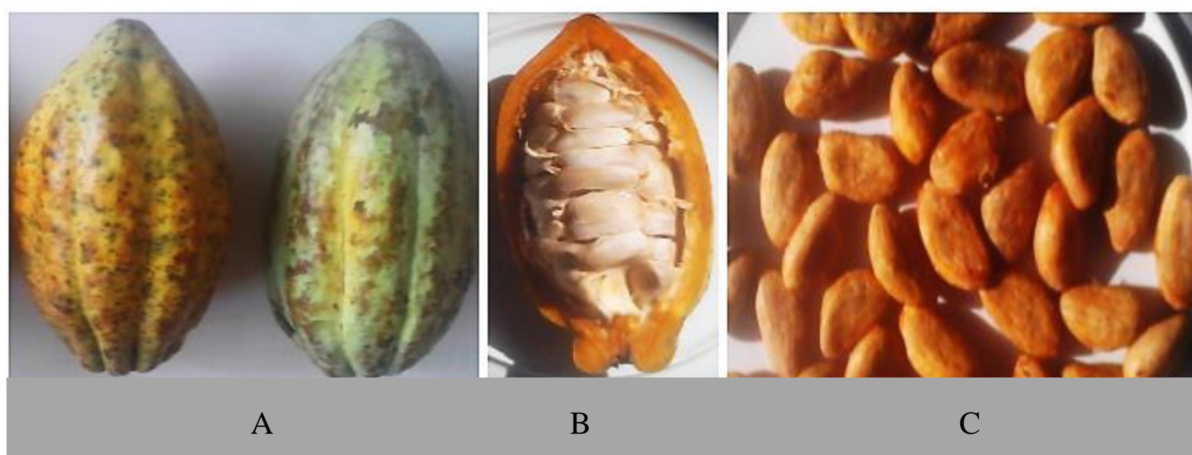


Figure 11: Cocoa pod and beans (*SNK16*UPA143)* used as planting material (Ghomsi, 2024)

A; (mature pods), B; dishusked pods and C; dried seeds ready for sowing

II.1.3 Characteristics of substrates used in pot experiments

The soil substrate with Rhodic kandiudult profile (U.S. soil taxonomy) was used [pH (water): 5.89; average chemical and elemental composition: CH_3COONa: 0.52 mM; Ca: 3.97 Cmol/kg; Mg: 0.92 Cmol/kg; K: 0.28 Cmol/kg; Na: 0.023 Cmol/kg; P: 1.54 ppm; Al: 0.43 Cmol/kg; Mn: 0.02 Cmol/kg; Clay: 51.9%; C/ N: 11.17, Cd: 0.00 µg/g]. Prior to the experiments, the soil was air-dried and passed through a 4 mm sieve, then mixed (3:1, v/v) with river sand and autoclaved twice at 121°C for 1h.

II.2 Methods

II.2.1 Evaluation of the cocoa seedling genotype sensitivity to cadmium stress and accumulation profile

II.2.1.1 Germination of cocoa seeds

Prior to the growth of *T. cacao,* the seeds from each cocoa hybrid genotype were pre-germinated separately according to the methodology reported by Tchameni *et al*. (2012). The beans from a cocoa hybrid were carefully cleaned with distilled water and germinated in tanks containing sterilized sand for 10 days (Figure 11 and Figure 12). The trays were placed on shelves. in the nursery and watered daily with 250 mL of water per pot.

Figure 12: Pregermination of cocoa seeds in trays phase of beans (Ngonkeu 2009)

II.2.1.2 Sowing, seedlings intoxication and experimental design

The seedlings were grown in 5-litre pots containing a homogeneous growth substrate. The substrate, which was autoclaved beforehand (121 °C, 2 hours twice), was introduced at a quantity of 2.5 kg per pot before the transplantation of pre-germinated seeds. To simulate cadmium contamination, four weeks after sowing, a specific treatment was applied to reach a final concentration of 200 mg of cadmium per kilogram of soil. This value is included in the concentration range identified in the soils of south-west Cameroon (11.24 to 899.20 mg/kg). Each treatment, including the control and the cadmium treatment, was replicated 10 times per hybrid, resulting in a total of 160 pots (16 hybrids × 2 treatments × 10 replicates). A completely random block design was set up, and seedlings were watered regularly.

⇒ **Treatments:**

Control (Untreated): No cadmium was added to the substrate, serving as a reference to compare the hybrids' response.

Cadmium Treatment: The substrate was amended with a solution of cadmium sulphate ($CdSO_4$), prepared from a stock solution of 5 mg/mL. The amount of solution to be added was calculated to achieve the final concentration of 200 mg/kg of soil.

⇒ **Preparation of the Cadmium Treatment:**

The cadmium sulphate solution was diluted for precise use (5 mg/mL). The volume of solution to incorporate into each pot was determined based on the substrate volume (2.5 kg per pot) and the desired concentration. The amount of cadmium sulphate needed for each pot was calculated using the following formula:

$$\text{Volume of solution} = \frac{\text{Desired concentration (mg/kg)} * \text{Weight of substrate (kg)}}{\text{Stock solution concentration (mg/mL)}} \quad (1)$$

Since the substrate weight per pot was 2.5 kg, the volume of solution to be added to each pot was calculated to reach the target concentration.

II.2.1.3 Assessment of agronomic parameters of cocoa seedlings

Cocoa seedlings were carefully uprooted 95 days after transplantation. The shoot and root lengths (SL and RL) were measured. After 72 hours of oven drying at 60 °C, the shoot dry weight (SDW) and root dry weight (RDW) were monitored until they reached constant weight.

The tolerance index (TI) was calculated to express the ability of the plant to grow in the Cd contaminated environment, according to Wilkins, (1978):

$$\text{TI} = \frac{\text{Dry weight of Cd intoxicated plant}}{\text{Dry weight of control plant}} * 100 \quad (2)$$

II.2.1.3.1 Assessment of the effects of cadmium intoxication on photosynthetic pigments content of cocoa genotypes

The photosynthetic pigments were extracted according to Arnon (1949). Cocoa seedling leaf samples (100 mg) were ground using a mortar and pestle in 5 mL of 80% acetone, then filtered through No. 2 Whatman filter paper. Using the UV-visible spectrophotometer (Tecan Infinite M200), filtrate absorption was measured at three-wave lengths: 470 nm, 646 nm, and 663 nm. The amounts of pigments were calculated according to Lichtenthaler & Wellburn (1983) simultaneous equations:

$$\text{Chlorophyll a } (\mu g/mL) = 12.21\ A_{663} - 281\ A_{646} \quad (3)$$

$$\text{Chlorophyll b } (\mu g/mL) = 20.13\ A_{646} - 5.03\ A_{663} \quad (4)$$

$$\text{Carotenoids } (\mu g/mL) = \frac{1000\ A470 - 3.27[\text{Chl a}] - 104\ [\text{Chl b}]}{227} \qquad (5)$$

Total pigments = chlorophyll a + chlorophyll b + carotenoids (6)

II.2.1.3.2 Assessment of cadmium uptake and accumulation by cocoa genotypes

⇒ **Sample mineralization**

The mineralization of the samples was performed using the aqua regia process and a DK6 digester, as described by VELP Scientifica (Dufour & Migon, 2017). Root tissues were oven-dried (60 °C, 72 hours), crushed to a fine powder, and sieved (mesh size: 0.1 mm). The flasks were thereafter filled with 3 g of dry matter, followed by the addition of 21 mL of HCl and 7 mL of HNO_3. 10 mL of 0.5N nitric acid was equally added to the upper glass absorber and positioned above the refrigerator. After cooling, the contents of the absorbers and the flasks were thoroughly mixed. Afterward, the mixture was filtered and recovered in a 100 mL volumetric flask for Cd analysis.

⇒ **ICP-AES analyses**

Quantitative Cd estimation in the samples was assessed following US EPA 6010b-Inductively Coupled Plasma Atomic Emission Spectrometry (US EPA, 2019). The digests were assayed with optimum spectra 8000 and the Cd emission spectra was measured at 228.8 nm and expressed in terms of mg Cd/kg dry biomass (1).

$$\text{TCd } (\mu g.g-1) = \frac{(\text{Sc x Fd x Ve})}{\text{DB}} \qquad (7)$$

Where: TCd = Total Cd content; Sc = sample concentration (µg/L); Fd = Dilution factor; Ve = Extraction volume (l) and DB = Dry biomass (kg).

⇒ **Bio-concentration Factor (BCF), Translocation Factor (TF) and accumulation factor (AF) Calculation**

The Cd bio-concentration factor (BCF) by root system and aerial part (stem, leaves and secondary stems) was calculated according to (Zayed *et al.,* 1998), as follows:

$$BCF = \frac{\text{Cd concentration in the whole plant (mg.kg}-1)}{\text{Cd concentration in the soil subtrate (mg.kg}-1)} * 100 \qquad (8)$$

The translocation factor (TF) was calculated to evaluate the capability of plant to accumulate the metal, absorbed by roots, in the shoots (Padmavathiamma & Li, 2007):

$$TF = \frac{\text{Cd concentration in shoots (mg.kg−1)}}{\text{Cd concentration in the roots (mg.kg−1)}} * 100 \quad (9)$$

The accumulation factor (AF) of heavy metals were calculated according (Yanqun *et al.,* 2005) as follows:

$$AF = \frac{\text{Cd in shoot}}{\text{Cd in the whole plant}} * 100 \quad (9)$$

II.2.2 Evaluation of the effects of AMF on cadmium accumulation and toxicity in cocoa seedlings.

II.2.2.1 Plant material and growth conditions

The substrate (2.5 kg) was filled in 25-cm-diameter plastic pots and inoculated through the seed-bed technique (Tchameni *et al.,* 2012). Briefly, 10 g of each AMF inoculum were introduced as seed-bed. One pre-germinated seedling was placed into each pot. Two weeks after transplanting, 40 mL of distilled water or Cd stock solution (5 mg/mL) were added to the pots so as to make a final concentration of 200 mg/kg of soil per pot. Sterile soil without AMF or Cd served as a negative control. Ten replications were prepared per treatment. The overall pots were arranged in a randomized block design in the greenhouse (Figure 13). The leaching water from the pots was recovered using a leaching recovery system and re-cycled into the appropriate treatments. The treatments used were as follows:

Control = treatment without Cd or AMF (negative control),
Cd = Cd treatment alone (positive control),
Cd_MF_{SG} = Cd treatment and inoculation with the mycorrhizal formulation propagated with sorghum and groundnut (MF_{SG}),
Cd_MF_{P} = Cd treatment and inoculation with the mycorrhizal formulation propagated with Leek (MF_{p}),
Cd_MF_{SC} = Cd treatment and inoculation with the mycorrhizal formulation propagated with sorghum and cowpea (MF_{SC}).

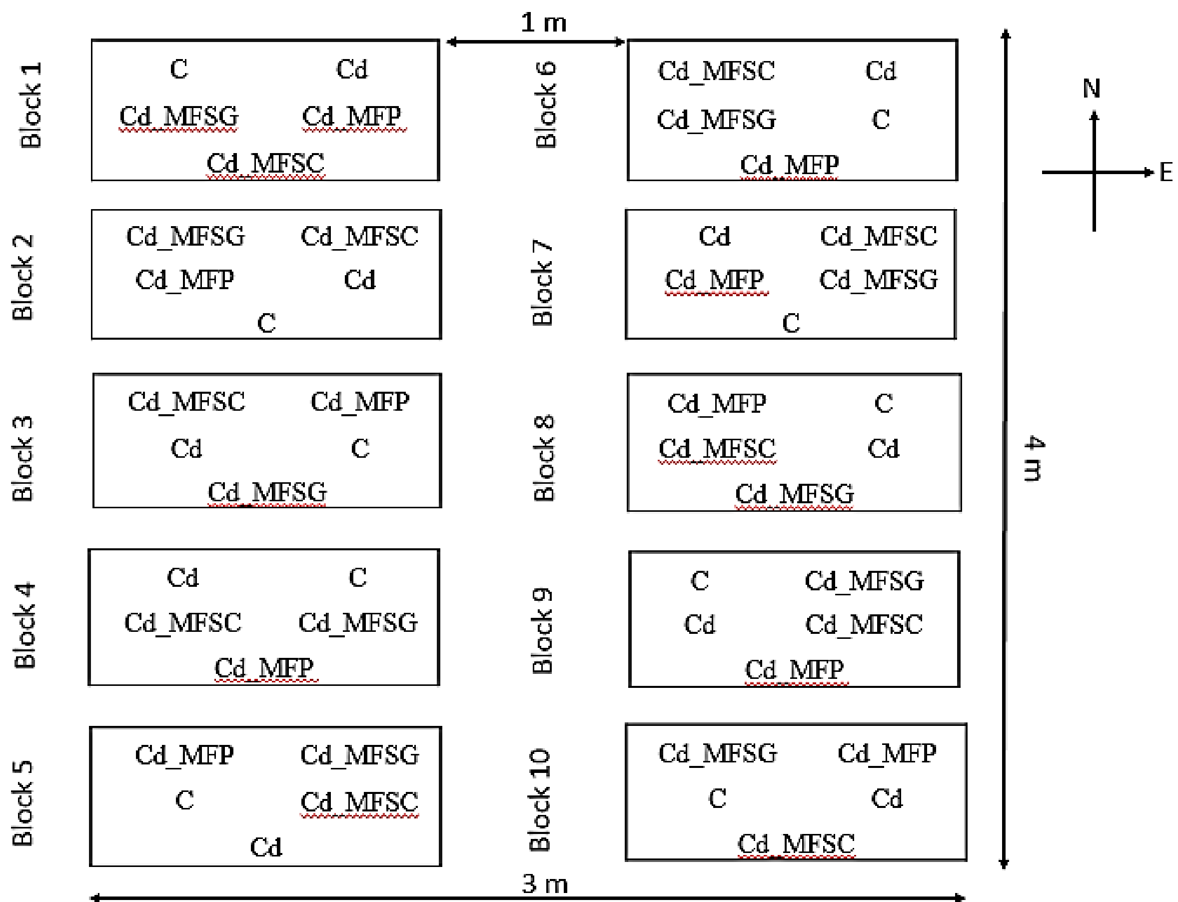


Figure 13 : Experimental design 2; Layout of the randomised complete block design use the evaluation of cadmium intoxication and AMF inoculation on cocoa seedlings

The experiment is arranged in 10 blocks distributed in two columns (4 m wide each) separated by a 1 m alley. Each block contains five treatments randomly assigned to plots: Control (no Cd or AMF), Cd alone (positive control), Cd with mycorrhizal formulation propagated with sorghum and groundnut (Cd_MFSG), Cd with mycorrhizal formulation propagated with Leek (Cd_MFP), and Cd with mycorrhizal formulation propagated with sorghum and cowpea (Cd_MFSC). This design controls for spatial variability and allows comparison of treatment effects under uniform field conditions.

II.2.2.2 Harvest and data collection

Ninety-five days after transplantation (DAT), cocoa seedlings were carefully uprooted, and the shoot (SL) and root (RL) lengths as well as shoot dry weight (SDW) and root dry weights (RDW) were havested after 72 hours' oven drying (60 °C) until constant weight. The photosynthetic pigments were extracted, and absorbance was measured according Arnon (1949). The amounts of pigments were calculated according to Lichtenthaler & Wellburn's (1983) simultaneous equations, as described in section II.2.1.3.1 page 46.

⇒ **Assessment of cocoa AMF root colonization**

To test each AMF formulation's capability to colonize cocoa seedling roots, fine roots were taken after uprooting and the root system was rinsed with tap water to remove residual debris. Thereafter, root samples were cut and preserved in 50% ethylic alcohol until processed. Root subsamples (0.5–1 cm in length) were discoloured in 10% potassium hydroxide (KOH) and dyed with 0.1% methylene blue reagent in the laboratory for mycorrhizal colouration (Kormanik & McGraw, 1982). Excess dye was washed away with distilled water, and mycorrhiza was observed using a light microscope (Huma Scope) at 40X. The colonization of AM fungus into the interior of the roots was then determined and expressed in percentages using the gridline intersect method described by Giovannetti & Mosse (1980). Observed strctures included vesicles, mycelia, spore and arbuscular growth.

⇒ **Evaluation of the effects of AMF on cocoa seedling cadmium uptake and translocation**

Fresh root and shoot subsamples from each treatment were rinsed thoroughly with deionized water and oven-dried for 24 hours at 70 °C. Dried materials were ground to powder, mineralized using a DK6 digester, and the Cd concentration was determined using ICP-OES at optimal spectra of 8000 at 228.8 nm, as stated in experiment 1. Cd concentration was determined for each treatment and reported in mg Cd/g dry sample. Bio-concentration factor (BCF), translocation factor (TF), tolerance index (TI), and accumulation factor (AF) were calculated according to the equations described early in the first experiment.

⇒ **Assessment of the effects of cadmium intoxication and AMF inoculation on mycorrhizosphere pH and conductivity**

The mobility and bioavailability of Cd in soils vary according to the physical and chemical properties of the environment, including aeration, pH, and conductivity (ROSS, 1994). Soil pH and electrical conductivity were measured in a soil-to-water suspension with a ratio of 1:2.5. The pH measurement was performed by the electrometric method using a Vernier pH (PH-BTA) (CEAEQ, 2010b) and the measurement of electrical conductivity was performed by Richard's method using a conductivity meter (HANNA DIST 30) (CEAEQ, 2010a).

⇒ **Assessment of the effects of cadmium intoxication and AMF inoculation on glomalin related soil proteins (GRSP) production**

After the experiment, the soil properties of all treatments were determined using standard laboratory protocols. GRSP extraction was performed on each sample using a method slightly modified from Wright *et al*. (1998). Briefly, 8 mL of sodium citrate buffer (50 mM, pH 8.0) was added to about 1 g of soil, and then the solution was placed in an autoclave (121 °C, 60 min) for repeated extractions until the supernatant lost its typical red-brown colour. Between each cycle, the supernatant was collected by centrifugation (8,000 *g*, 10 min). Before measurement, the mixed supernatant was centrifuged again (10,000 *g*, 10 min), and the protein concentration in the extract representing GRSP was measured using the Bradford (1976) method. Bovine serum albumin was used as a standard.

⇒ **Assessment of the effects of cadmium intoxication and AMF inoculation on mycorrhizosphere microbiota abundance**

The potential of the studied AMF formulations to restore the soil microbiota as a result of Cd toxicity mitigation was assessed by monitoring the soil's total aerobic mesophilic flora (TAMF) (Vieira & Nahas, 2005). In brief, 10 grams of fresh composite soil substrate were collected from each treatment and carefully cleaned of any extraneous materials. Each sample were mixed well in 90 mL of a 0.1% (w/v) sodium pyrophosphate solution. Samples were then diluted in sterile distilled water (up to 10–5), and an aliquot (0.1 mL) of the resulting solutions was seeded by flooding onto solidified nutrient agar plates. After 48 hours of incubation at 27 °C, the colony-forming units (CFU) were counted, compared with non-mycorrhizal samples, and expressed as CFU/g of soil.

II.2.3 Biochemical changes in cocoa seedling as influenced by AMF inoculation and cadmium stress

II.2.3.1 Assessment of the effects of cadmium intoxication and AMF inoculation on metal-chelating capacity and antioxidant capacity of cocoa seedling methanolic extracts

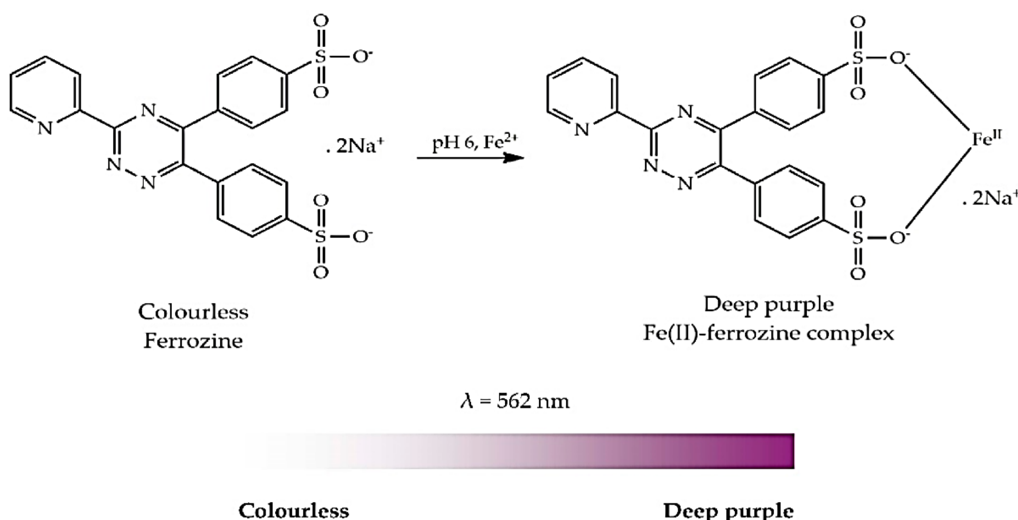
II.2.3.1.1 Assessment of the effects of cadmium intoxication and AMF inoculation on metal-chelating capacity of cocoa seedling methanolic extracts

Metal chelating is generally regarded as the most potent and common antioxidant method. Metal chelation often leads to the inactivation of Cd and its subsequent inability to harm plants.

The metal-chelating capacity of the root extract was determined by the previously described ferrous ion chelating assay described by Hsu *et al*. (2003).

Principle

The iron-chelating activity is determined by measuring the absorbance of the iron (II)-ferrozine complex. This combination generated a red chromophore with a maximum absorbance of 562 nm. The chelator agents can grab ferrous ions before ferrozine. The equation for the reaction is as follows:



Formation of a chelating complex with ferrous iron (Fe^{2+})

⇒ **Procedure**

Cocoa seedling roots (2 g) were ground in a mortar and extracted with 10 mL of methanol-HCl (4:1) for two days. The filtered extract (120 µL) was mixed with 50 µL of 2 mM $FeCl_2$ and 200 µL of 5 mM ferrozine. After the vortexing, the mixture was incubated for 10 minutes at room temperature, and then the absorbance was measured at 562 nm. The metal-chelating capacity was calculated by using the following equation with EDTA as the control:

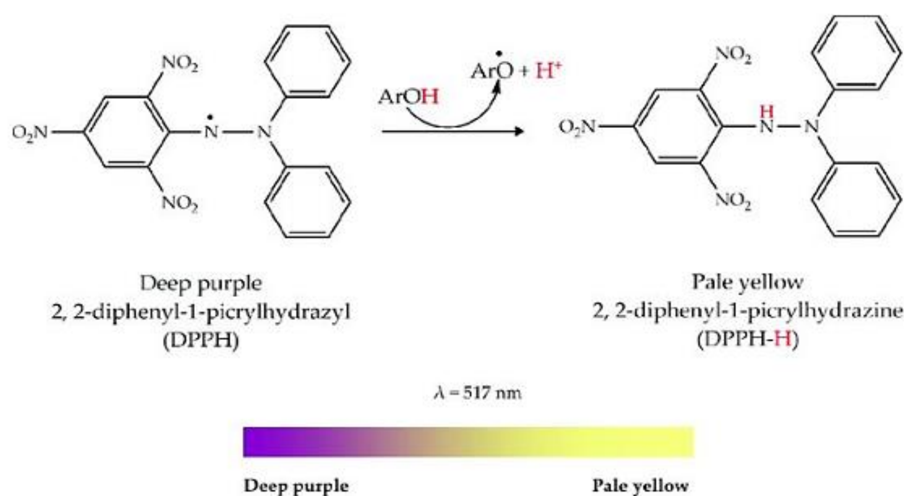
$$\text{Chelating capacity (\%)}=[1-\frac{(\text{A562 nm, sample})}{(\text{A562 nm, control})}] * 100 \quad (10)$$

II.2.3.1.2 Assessment of the effects of cadmium intoxication and AMF inoculation on antioxidant activity in methanolic extracts of cocoa seedling

Antioxidant activity is defined as the ability of antioxidants to scavenge free radicals. The antioxidant properties of the coca seedlings suggest their ability to biosynthesise antioxidant compounds upon Cd intoxication. (Ahmad *et al.,* 2010). The antioxidant activity was tested *in vitro* using the free radical 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) (Ferreira *et al.,* 2007).

⇒ **Principle**

The DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate) free radical method is an antioxidant assay based on electron transfer that results in a violet solution in ethanol. This free radical, which is stable at ambient temperature, is reduced in the presence of an antioxidant molecule, resulting in a colourless ethanol solution that absorbs at 517 nm. The equation for the reaction is as follows:



Reduction of DPPH radical by an antioxidant

⇒ **Procedure**

Briefly, 0.5 mL of a 0.25 mM DPPH radical solution was added to 1 mL of previously obtained methanolic extracts. The combination was shaken and left at room temperature for half an hour in the dark before being measured spectrophotometrically at 517 nm. The inhibition percentage was calculated by comparing it to the control, and the standard was gallic acid. The scavenging activity was determined using the equation shown below.

$$SCA = \frac{\mathrm{Ao} - \mathrm{A1}}{\mathrm{Ao}} * 100 \qquad (11)$$

Where SCA is DPPH scavenging activity, Ao is the absorbance of the control reaction and A1 is the absorbance of the sample

II.2.3.2 Evaluation of the effects of cadmium intoxication and AMF inoculation on some host stress-related biomolecules.

Prolin, other free amino acids, and sugars work together to detoxify heavy metals, reduce cellular damage, and assure the plant's survival under harsh conditions. Together, these chemicals help reduce the harmful effects of heavy metal stress by regulating multiple pathways and allowing plants to survive and grow in contaminated environments (Dar *et al.,* 2016; Khan *et al.,* 2020).

II.2.3.2.1 Extraction of prolin, free amino acid and solubles sugars

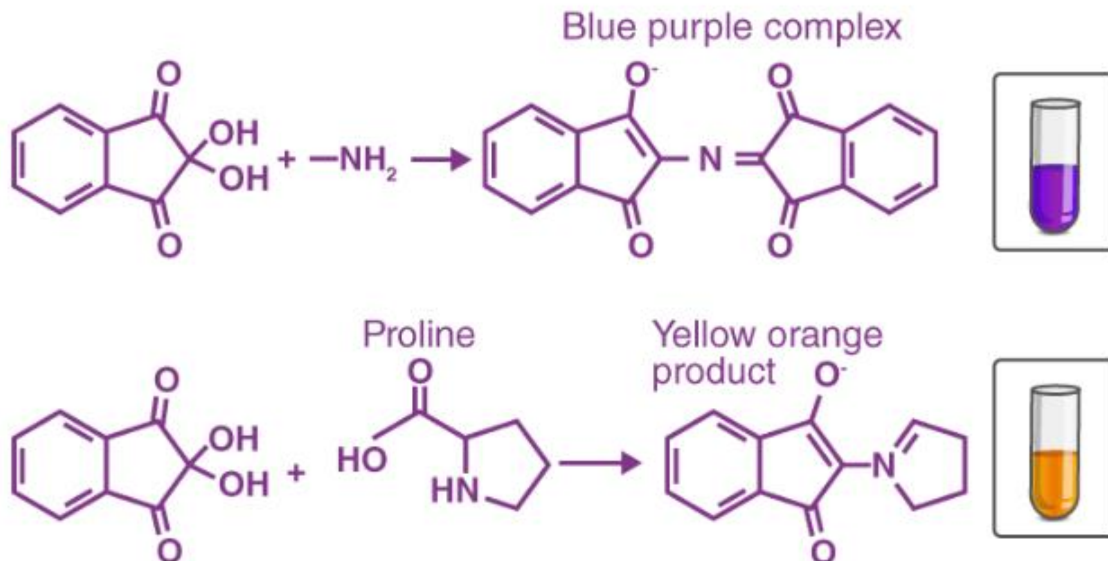
Prolin, total free amino acids, and soluble sugars were extracted using the method described by Cross *et al*. (2006). In concrete terms, 500 mg of cocoa seedling root tissue were heated twice in 5 mL of 80% ethanol and centrifuged at 14,000 x g for 5 min. The supernatant was then collected and used as an ethanolic extract for the various assays.

II.2.3.2.2 Assessment of the effects of cadmium intoxication and AMF inoculation on cocoa seedlings prolin and total Free Amino Acid content

The quantification of prolin and total free amimo acid was carried out according to the ninhydrin base method, as described respectively by Bates *et al*. (1973) and Yemm *et al*. (1955) respectively.

⇒ **Principle of Ninhydrin assay**

The ninhydrin reaction is essentially a redox reaction. Here, ninhydrin acts as an oxidizing agent and itself gets reduced. Ninhydrin reacts with the free amino acid's amino group in the test sample and oxidizes the compound, resulting in deamination. In this reaction, two gases get released. These are ammonia (NH_3) and carbon dioxide (CO_2). Besides the gasses, we obtain an aldehyde and hydrindantin, which are formed by the reduction of ninhydrin. Now, the released ammonia further reacts with the ninhydrin, giving rise to di-ketohydrin, which forms a coloured complex. This coloured complex is what we call Ruhemann's purple, and this gives our solution the deep blue colouration. A yellow complex is formed when amino acids, such as prolin, are present in the analyte.



Reaction of free amino acids or prolin with ninhydrin

⇒ **Procedure**

a) **Assessment of the effects of cadmium intoxication and AMF inoculation on cocoa seedlings total Free Amino Acid content**

One milliliter of the ethanolic extract (page 54) was pipetted into a test tube. One drop of methyl red indicator was applied. The material was neutralized using 1 mL of 0.1 N sodium hydroxide. To this, 1 mL of ninhydrin reagent was added and carefully mixed. The test tube's contents were heated in a boiling water bath for 20 minutes. Five milliliters of diluent solution were added and boiled in a water bath for ten minutes. The tubes were cooled with running water, and the contents were well mixed. Blanks were made without extract. The absorbance was measured at 570 nm using a UV spectrophotometer (Hitachi U-2900). The absorbance measurements were converted to mg/g dry weight (DW) tissue using a glycine reference curve.

b) **Assessment of the effects of cadmium intoxication and AMF inoculation on the cocoa seedlings prolin content**

In a test tube, 2 mL of ethanolic extract (page 54) were mixed with 2 mL of acid ninhydrin reagent and 2 mL of glacial acetic acid. The mixture was incubated in a water bath for one hour at 100 degrees Celsius. The tubes were placed in an ice bath to stop the reaction. Then, 4 mL of toluene was added to each test tube and aggressively mixed with a test tube before being swirled for 10–20 seconds. The toluene containing the chromophore was separated from the

aqueous phase using a separating funnel, and the absorbance was measured at 520 nm in a UV spectrophotometer (Hitachi U-2900) with an adequate blank. The prolin content was determined using a prolin-based standard curve, and the results are represented as mg g-1FW.

II.2.3.2.3 Assessment of the effects of cadmium intoxication and AMF inoculation on the cocoa seedlings soluble sugar content

The total soluble sugar contents of cocoa seedling roots were estimated using anthrone reagent reactions as described by Ahsen *et al*. (2019).

⇒ **Principle:**

Carbohydrates are dehydrated by concentrated H_2SO_4 to form furfural. The active form of the reagent is anthranol, the enol tautomer of anthrone, which reacts by condensing with the carbohydrate furfural derivative to give a green colour in dilute solutions and a blue colour in concentrated solutions, which is determined colourimetrically. The blue-green solution reaches its maximum absorption at 630 nm. The equation for the reaction is as follows:

I **Dehydration---**product is a furfural

Monosaccharide ⟶ Furfural

II **Reaction of furfural with anthrone**

Furfural + Anthrone reagent ⟶ Blue green complex

Reaction of carbohydrates with anthrone under acidic conditions

⇒ **Procedure**

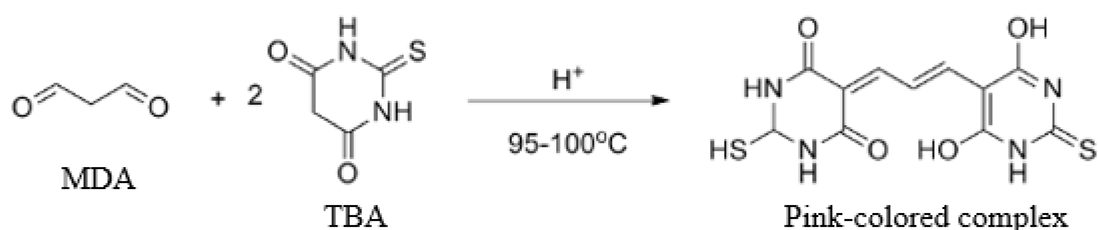
100 mg of dried and powdered samples were weighed. Then, extraction was performed by using 10 mL of 95% ethanol. Extracts were first shaken for 15 minutes on a shaker with 360 rpm and were then centrifuged for 15 minutes at 3500 rpm. Then 3 mL anthrone reagent were added to the top phase of centrifuged samples. Finally, after applying boiling water temperature for 10 minutes, the amount of light absorption was recorded at the wavelength of 630 nm. The amount of soluble sugar in the sample was calculated by using a standard graph prepared made with pure glucose was used in this experiment.

II.2.3.2.4 Assessment of the effects of cadmium intoxication and AMF inoculation on the cocoa seedlings malondialdehyde concentration.

Oxidative damage in root cells was assessed as the concentration of total 2-thiobarbituric acid (TBA) reactive components and expressed as equivalents of malondialdehyde (MDA) according to Cakmak & Horst (1991) method.

⇒ **Principle:**

The assay is based on a condensation reaction of two molecules of TBA with one molecule of MDA at high temperature, which forms a pink-coloured complex with an absorption maximum at 532 nm in a UV spectrophotometer. The equation for the reaction is as follows:



Formation of a coloured complex between MDA and thiobarbituric acid (TBA)

⇒ **Procedure**

In total, 100 mg of root tissue were ground into a fine powder using a mortar and pestle with liquid nitrogen. The fine powder was homogenized in 2 mL of 0.1% (w/v) trichloracetic acid (TCA) solution in an ice bath. The homogenate was centrifuged at 12,000 × g for 15 min at 4 °C. After centrifugation, 0.5 mL of the supernatant was reacted with 1.5 mL of TBA solution (0.5% in 20% TCA) for 30 min in a boiling water bath at 95 °C. After this period, the reaction was stopped in an ice bath. The samples were centrifuged at 9,000 × g for 10 min, and the specific absorbance was measured at 532 nm. The nonspecific absorbance was estimated at 600 nm and subtracted from the specific absorbance value. An extinction coefficient of 155 $mM^{-1}cm^{-1}$ (Heath and Packer 1968) was used to calculate the MDA concentration, which was expressed as micromoles per kilogram of fresh weight.

II.2.3.2.5 Extraction of phenolic compounds released in on cocoa seedlings following cadmium intoxication and AMF inoculation

The extraction of phenolic compounds was performed as previously described (Tchameni *et al.,* 2011). Briefly, five grams of typical, dried *T. cacao* root samples were macerated in 70%

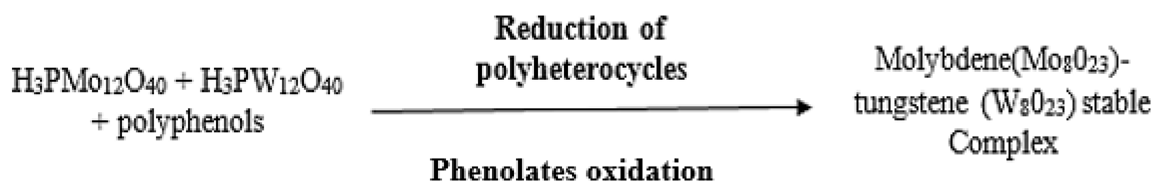
methanol for one hour at room temperature and thereafter filtered (Whatman No. 1) and evaporated at 40 °C using a rotary evaporator (BUCHI 001). To remove the pigment, the resultant methanolic extract was again suspended in 70 mL of distilled water. Depigmentation was done by successive immersion in 50 mL of 40% ammonium sulphate [$(NH_4)_2 SO_4$], 1.5 mL of 80% phosphoric acid (H_3PO_4), and 50 mL of diethyl ether. The mixture was then extracted several times with 500 mL ethyl acetate. Five grams of anhydrous magnesium sulphate ($MgSO_4$) was later on added to the organic fractions and filtered after 5 min using Whatman No. 1 filter paper. Ultimately, the phenolic extracts were gotten after subsequent evaporation under a vacuum at 40°C. using a Rotary evaporator.

II.2.3.2.6 Assessment of the effects of cadmium intoxication and AMF inoculation on the cocoa seedlings total phenolic compounds

The total phenol content was determined using the Folin-Ciocalteu test, essentially as described by Siddhuraju *et al*. (2002).

⇒ **Principle**

In the presence of polyphenols, the Folin-Ciocalteu reagent (a mixture of phosphomolybdic (H3PMo12O40) and phosphotungstic (H3PW12O40) acids) is reduced to a blue complex of tungsten and molybdenum. This complex, whose colouring intensity is proportional to the concentration of phenolic compounds, has a maximum absorption at around 725 nm. The equation for the reaction is as follows:



Reaction of phenolic compounds with the Folin–Ciocalteu reagent

⇒ **Procedure**

Three mL of distilled water, 250 µL of Folin-Ciocalteu reagent, and 750 µL of sodium carbonate 70% were combined with 50 µL of the extract made above in water. The liquid was vortexed completely, then incubated for eight minutes at room temperature. The reaction mixture was then mixed with 950 µL of distilled water and left to incubate at room temperature

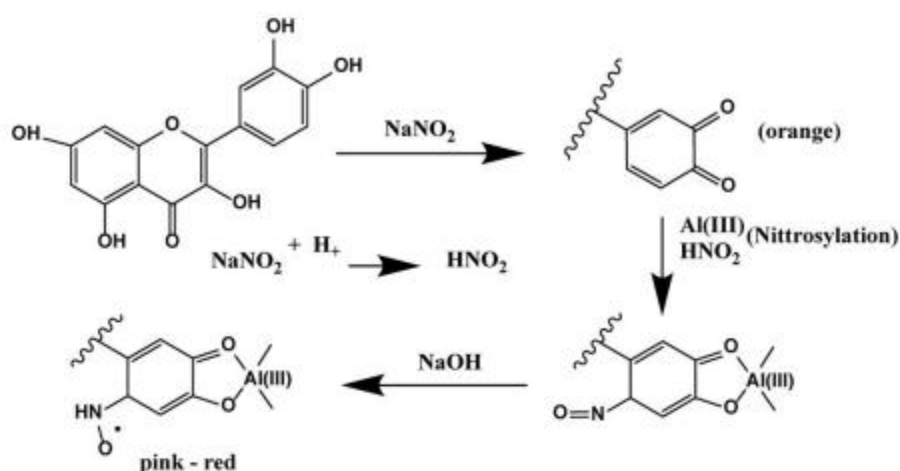
for two hours. A UV-visible spectrophotometer (UV 160, Shimadzu, Japan) performed absorbance read-outs at 725 nm against a blank. The total phenol contents of each treatment were expressed in terms of chloragenic acid equivalents (µg Chl Ac/g dry sample) using a calibration curve ($R^2 = 0.99$) plotted from pure chlorogenic acid.

II.2.3.2.7 Assessment of the effects of cadmium intoxication and AMF inoculation on the cocoa seedlings total flavonoid content

The aluminum chloride protocol described by Enujiugha (2010) was used to quantify total flavonoid content in cocoa seeding from each treatment.

⇒ **Principle**

In the presence of NaNO2, aluminum chloride forms acid-stable complexes with the C-4 keto group and either the C-3 or C-5 hydroxyl groups of flavones and flavonols, according to the basic principle of the aluminum chloride colourimetric method. The equation for the reaction is as follows:



Complex formation between flavonoids and ferric chloride ($FeCl_3$)

⇒ **Procedure**

Essentially, 1.25 mL of distilled water was added to 0.25 mL of each phenolic extract of young *T. cacao* seedling root in triplicate. Then, 75µL of 50% $NaNO_3$ was added and allowed to stand for 6 minutes; thereafter, 150µL of aluminum chloride (10%) was added. After 5 minutes of incubation at room temperature, 0.5 mL of NaOH (1M) was added. The reaction mixture was then made into 2.5 mL by adding distilled water. A different set of test tubes received the same

treatment, but the phenolic extract was replaced by the chlorogenic acid standard at different concentrations (10 to 1000 µg/mL). After homogenization, the absorbance was measured at 510 nm. The flavonoid contents of each treatment were expressed in terms of quercetin equivalent (Qeq) (µg Qeq/g dry sample) using a calibration curve ($R^2 = 0.99$) plotted from pure quercetin.

II.2.3.3 Assessment of the effects of cadmium intoxication and AMF inoculation on the cocoa seedlings activities of selected oxidizing and chelating enzymes

The absorption of Cd triggers the production of reactive oxygen species (ROS). The rise in superoxide dismutase (SOD), catalase, ascorbate peroxidase (APX), guaiacol peroxidase (GPX), phenylalanine ammonialyase (PAL), polyphenol oxidase (PPO) and fumarase activities serve as stress markers.

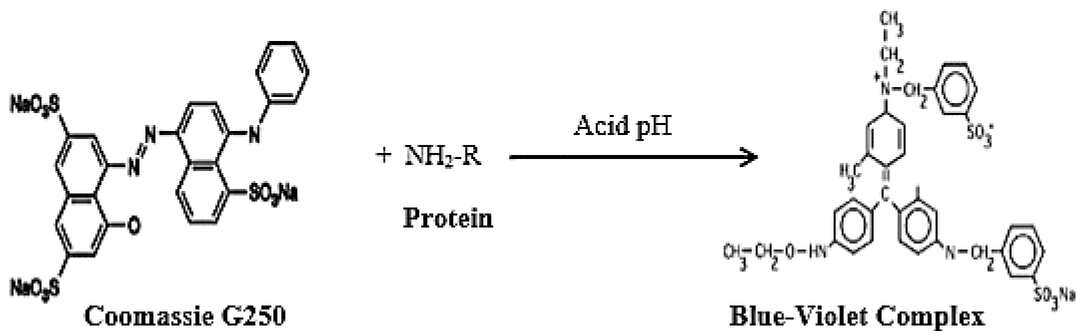
Extraction of these enzymes were done following the method describe by Elavarthi & Martin (2010). Brefly, Fresh root samples (0.5 g) were pulverised in a pre-chilled mortar and pestle with 5 mL of cold 50 mM potassium phosphate buffer (pH 7.0) containing 2 mM ascorbic acid, 1% (w/v) soluble polyvinyl polypyrrolidone (PVPP), and 1 mM EDTA. Supernatants were collected and utilised for protein quantification as well as SOD, GPX, PAL, PPO, APX, catalase, and fumarase activities after filtering (Millipore, Mitex 0.5 mm) and centrifugation at 12000 g for 20 minutes.

II.2.3.3.1 Assessment of the effects of cadmium intoxication and AMF inoculation on the cocoa seedlings total soluble protein content

To determine protein content, the Bradford protein assay (Bradford, 1976) was used.

- ⇒ **Principle**

Coomassie blue G250 forms a chromogenic complex of blue colouration with a maximum absorption at 595 nm when the side chains of basic amino acids (lysine, arginine, and histidine), aromatic amino acids, and the free amine functions of the polypeptide chain are present. The equation for the reaction is as follows:



Formation of a protein–dye complex with Coomassie Brilliant Blue G-250

⇒ **Procedure**

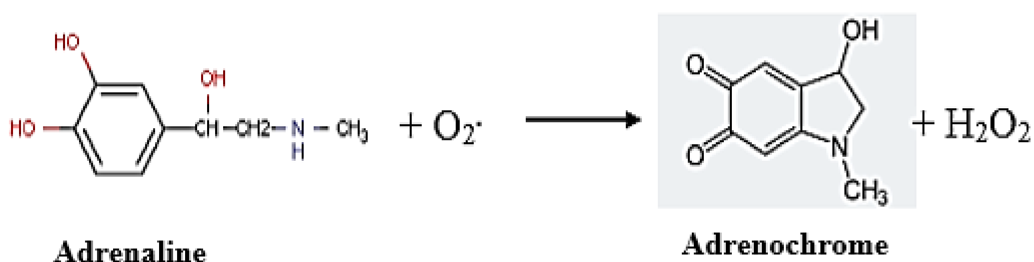
For the protein assay, 100 µL of the previously obtained protein extract (supernatant) was collected, and 0.5 mL of phosphate buffer, distilled water, and 2 mL of Bradford's reagent were added for a final volume of 3 mL. Calibration was performed by replacing the extract with increasing concentrations of BSA (Bovine Serum Albumin), and optical densities were read at 595 nm after 10 minutes of incubation at 25 °C.

II.2.3.3.2 Assessment of the effects of cadmium intoxication and AMF inoculation on the cocoa seedlings superoxide dismutase activity (SOD EC 1.15.1.1)

The SOD activity was measured using the technique outlined by Misra & Fridovich (1972).

⇒ **Principle**

When the superoxide anion O2•- is present, adrenaline (epinephrine) spontaneously oxidises to adrenochrome, a coloured molecule that absorbs at 480 nm. This reaction is inhibited by SOD, whose purpose is to decrease the O2• anion (Polle *et al.,* 1989).



Oxidation of adrenaline into adrenochrome

⇒ **Procedure**

To 166 µL of carbonate buffer (0.05 M, pH 10.2) and methionine (130 mM), 134 µL of protein extract were added. The reaction started upon the addition of 200 mL of 0.3 mM epinephrine.

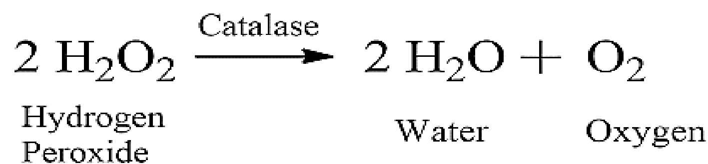
After 20 and 80 seconds, the optical density (OD) was measured at 480 nm. The activity of SOD was defined as the amount of SOD necessary to prevent epinephrine oxidation to adrenochrome by 50%.

II.2.3.3.3 Assessment of the effects of cadmium intoxication and AMF inoculation on the cocoa seedlings catalase activity (EC 1.11.1.6)

The capacity of catalase contents in the enzymatic extract to reduce H_2O_2 in water molecules was evaluated according to the method described by Aebi (1984).

⇒ **Principle**

Catalase degrades hydrogen peroxide (H2O2) into water and oxygen. The disappearance of hydrogen peroxide can be measured by spectrophotometry at 290 nm. This is made according to the reaction:



Decomposition of hydrogen peroxide by Catalase

⇒ **Procedure**

The reaction consisted of a mixture of 100 µL potassium phosphate buffer (50 mM; PH 7), 30 µL enzyme extract, and 2 µL H_2O_2 (0.3%). The reduction of H_2O_2 (molar extinction coefficient of H_2O_2 (36 $M^{-1}cm^{-1}$)) was measured at 240 nman the specific activity of catalase calculated using the following formula:

$$SA = \frac{\Delta\, A.Vt}{\varepsilon\, .\ \Delta\, t.L.Ve.P} \quad (12)$$

where:

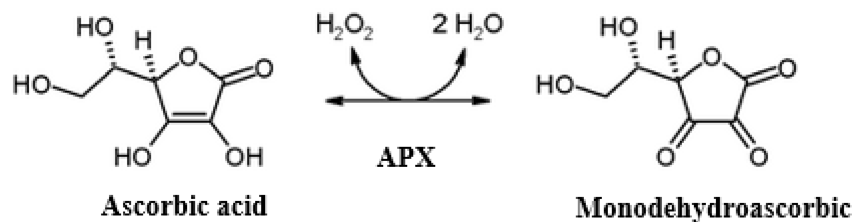
SA: Specific enzymatic activity in µmol/min/mg of protein, ε: Molar extinction coefficient in $\mu M^{-1} \cdot cm^{-1}$, ΔA: Average change in absorbance, Vt: Total volume of the reaction mixture in mL, Ve: Volume of the enzyme extract in mL, L: Path length of the cuvette in cm, P: Protein content in mg, and Δt: Reading time in minutes.

II.2.3.3.4 Assessment of the effects of cadmium intoxication and AMF inoculation on the cocoa seedlings Ascorbate peroxidase activity (APX EC 1.11.1.11)

The APX specific activity was determined according to the Nakano & Asada (1981) method.

⇒ **Principle**

The reaction is based on ascorbate peroxidase's ability to reduce hydrogen peroxide to water using ascorbic acid as a substrate and form the oxidised form of monodehydroascorbic acid. The disappearance of hydrogen peroxide can be measured by spectrophotometry at 290 nm. This is made according to the reaction:



Reduction of hydrogen peroxide by ascorbate peroxidase (APX)

⇒ **Procedure**

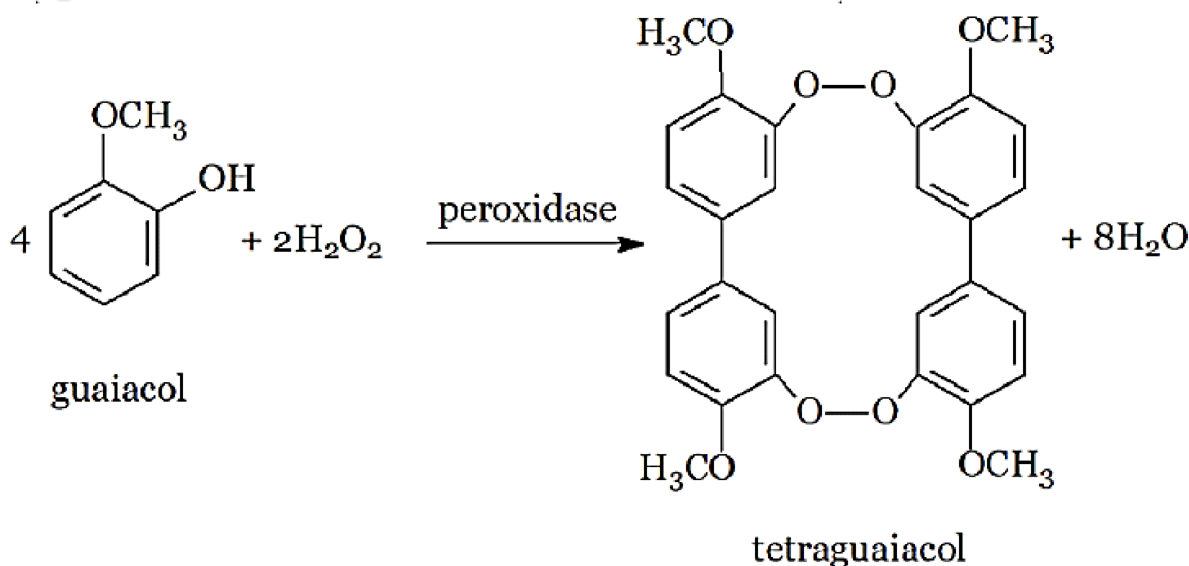
The reaction mixture consisted of 50 mM potassium phosphate buffer (pH 6.8), 1 mM H_2O_2, and 0.8 mM ascorbate in a volume of 195 µl. The reaction started after the addition of 5 µl of the crude enzyme extract. The APX activity was measured by the rate of ascorbate oxidation at 290 nm for 1 min at 27 °C. According to the formula describe by equation (11), APX specific activity was calculated using the molar extinction coefficient of ascorbate at 290 nm (ε = 2.8 mM^{-1} cm^{-1}) and expressed in µmol $mL^{-1}min^{-1}$.

II.2.3.3.5 Assessment of the effects of cadmium intoxication and AMF inoculation on the cocoa seedlings guaiacol peroxidase activity (GPX, EC 1.11.17)

For GPX, the approach reported by Fielding & Hall (1978) was employed.

⇒ **Principle**

This method is based on the polymerization of guaiacol to tetraguaiacol in the presence of hydrogen peroxide catalyse by GPX. The tetraguaiacol compound with an absorption wavelength of 470 nm. The reaction equation is as follows:



Reduction of hydrogen peroxide by guaiacol peroxidase (GPX)

⇒ **Procedure**

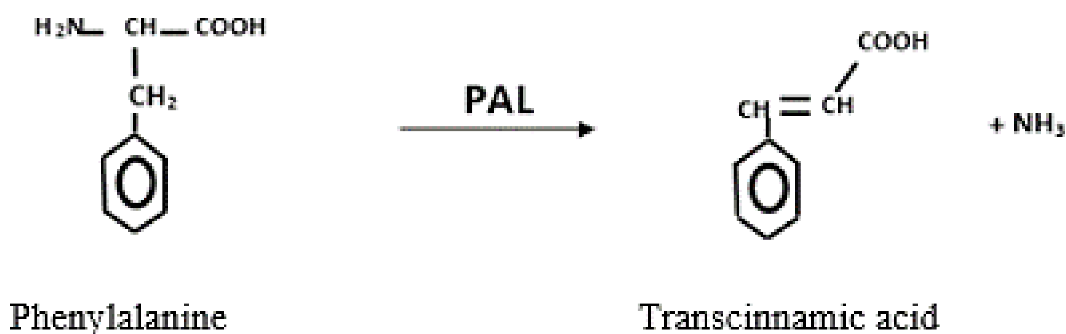
A solution of 3 mL, containing 100 µL of enzymatic extract, 50 µL of 0.3% H_2O_2, and 2850 µL of phosphate-guaiacol buffer (50 mM phosphate buffer, 8 mM guaiacol, pH = 7.20), was prepared. After 1 minute of incubation at 27 °C with hydrogen peroxide, 0.5 mL of 2N hydrochloric acid was added to halt the reaction. The measurement of the optical density (OD) of the produced tetraguaiacol was made at 470 nm (UV 160, Shimadzu, Japan). According to the formula describe equation (11), GPX specific activity was calculated using the molar extinction coefficient of tetraguaiacol ($\varepsilon = 26.6\ mM^{-1}\ cm^{-1}$) and expressed in µmol $mL^{-1}min^{-1}$.

II.2.3.3.6 Assessment of the effects of cadmium intoxication and AMF inoculation on the cocoa seedlings phenylalanine amonialyase activity (PAL EC 4.3. 1.24)

To test the phenylalanine amonialyase specific activity, the Whetten & Sederoff (1992) technique was used.

⇒ **Principle**

In the presence of Phenylalanine amonialyase (PAL), Phenylalanine is deaminated to trans-cinnamic acid capable of absorbing light at a wavelength of 290 nm. The equation for the reaction is as follows:



Deamination of phenylalanine by phenylalanine ammonialyase (PAL)

⇒ **Procedure**

On the laboratory bench, a mixture of 500 L of 50 mM sodium phosphate buffer (pH 8.8), 100 mL of enzyme extract, and 600 L of 1 mM L-Phenylalanine was incubated for 1 hour at 27 °C. The reaction was then stopped by adding 2 mL of 2 N hydrochloric acid. The optical density (OD) readout of the produced trans-cinnamic acid was measured at 290 nm (UV 160, Shimadzu, Japan). According to the formula describe equation (11), PAL specific activity was calculated using the molar extinction coefficient of transcinamic acid ($\varepsilon = 9.63\ mM^{-1}\ cm^{-1}$) and expressed in $\mu mol\ mL^{-1} min^{-1}$.

II.2.3.3.7 Assessment of the effects of cadmium intoxication and AMF inoculation on the cocoa seedlings polyphenol oxidase activity (PPO EC 1.10. 3.1)

The capacity of polyphenol oxidase contents in the enzymatic extract to generate O-quinone by oxidising phenolic compounds was assayed according to the method described by Vamos-Vigyazo & Nadudvari-Markus (1983).

⇒ **Principle**

The method is based on the oxidation of phenolic compounds to o-quinones by the enzyme polyphenol oxidase (PPO). The o-quinones produced have the ability to absorb visible light at a wavelength of 540nm. This can be schematized as follows:

Oxidation of cathecol into o-quinone by Polyphenol Oxidase (PPO)

⇒ **Procedure**

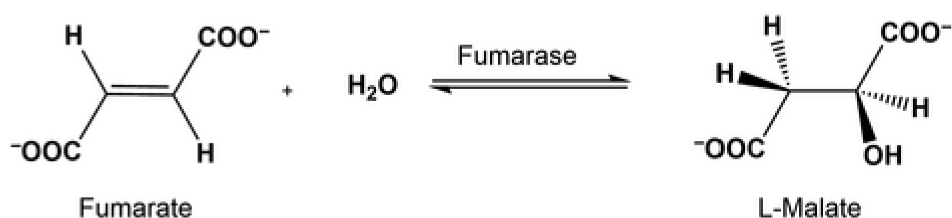
The reaction consisted of a mixture of 100µL potassium phosphate buffer (50 mM; PH 7), 25µL of catechol (0,024 M), and 25µL enzyme extract. The production of O-quinone was measured by spectrophometry at 540 nm after 5 minutes of incubation at 27 °C. Furthermore, according to the formula describe equation (11) before, PPO specific activity was calculated using the molar extinction coefficient of O-quinone (ε = 1.15 $mM^{-1}cm^{-1}$) and expressed in µmol $mL^{-1}min^{-1}$.

II.2.3.3.8 Assessment of the effects of cadmium intoxication and AMF inoculation on the cocoa seedlings fumarase activity (EC 4.2.1.2)

The activity of fumarase was measured using the method given by Hill & Bradshaw (1969).

⇒ **Principle**

In the presence of fumarase, malate is metabolized to fumarate, which absorbs UV at 250 nm.



<u>**Dehydration of malate into fumarate by Fumarase**</u>

⇒ **Procedure**

Enzyme extracts (25 µL) were added to a reaction mixture with 915 µL of phosphate buffer (50 mM pH 7.2), 10 µL of EDTA, and 50 µL of 500 mM malate. After 5 minutes of incubation at 27 °C, 2 mL of 2 N hydrochloric acid was added to break up the reaction. The absorbance of fumaric acid was measured at 250 nm (UV 160, Shimadzu, Japan). According to the formula describe equation (11), fumarse specific activity was calculated using the molar extinction coefficient of funaric acid (ε = 2.44 mM^{-1} cm^{-1}) and expressed in µmol $mL^{-1}min^{-1}$

II.3 Statistical analysis

Data from the trials were subjected to the analysis of variance (ANOVA) using the R statistical software (4.0.4 version). The distances between averages were measured using the Scott-Knott multiple comparison-based test. The principal components analysis (PCA) was utilised (Origin

Prolab. version 9.02) to compute the relationship between the agronomic, biochemical, and absorptive abilities of the cocoa seedlings under the influence of Cd and/or mycorrhiza.

CHAPTER III:

RESULTS AND DISCUSSION

CHAPTER III: RESULTS AND DISCUSSION

III.1 Cadmium accumulation profile of selected cocoa genotypes

III.1.1 Results

III.1.1.1 Effects of cadmium intoxication on the agronomic parameters of cocoa seedlings genotype

III.1.1.1.1 Effects of cadmium intoxication on the cocoa seedling growth parameters

Soil Cd pollution resulted in a substantial reduction in all growth parameters in most cocoa genotypes when compared to the control treatment (Tables VI and VII). Significant root and shoot growth decreases were seen in plants emerging from Cd-contaminated treatments. We found losses in root length ranging from -2.10% to -41.61% and in shoot length ranging from -2.42% to -41.55%. Hybrids *BBK1418*MO20* for root length (-41.61%) and *T79/501*SNK16* for shoot length (-41.55%) showed the biggest declines. Hybrids *SNK64*T40/1170* for root (-2.10%) and *T60/1174*UPA134* for shoot (-2.42%) produced the smallest length reductions. Dry weight losses ranged from -9.12% to -35.41% of shoot, and -4.53% to -65.57% of shoot, respectively. The hybrids SNK16*UPA143 for shoot (-35.41%) and *SNK13*ICS95* for root (-65.57%) showed the most significant declines. The smallest percentage losses were seen with hybrids *SNK16*UPA143* for shoot (-9.12%) and *SNK13*ICS95* for root (-4.53%). Regardless of the Cd tolerance index, hybrid *BBK1418*MO20* had the highest tolerance capacity, with TI of 109.22%. The hybrids *SNK16*UPA143* and *SNK13*ICS95* were the most affected by Cd stress, with TI values of 65.45% and 68.80%, respectively (Figure 14).

Table VI : Root and shoot lengths of cocoa genotypes as influenced by cadmium intoxication

Cocoa genotype	RL			SL		
	Control (cm)	Cd (cm)	Significance (control vs Cd)	Control (cm)	Cd (cm)	Significance (control vs Cd)
SNK10*IMC67	20.63 ± 1.25^{b}	20.13 ± 0.85^{b}	*P>0.05*	29.63 ± 1.93^{d}	23.00 ± 1.08^{d}	*P<0.0001*
SNK13*ICS95	22.00 ± 0.82^{c}	19.00 ± 0.41^{b}	*P<0.5*	25.25 ± 0.96^{b}	20.38 ± 1.70^{c}	*P<0.01*
UPA143*SNK37	22.25 ± 0.96^{c}	20.00 ± 1.15^{b}	*P<0.5*	23.75 ± 1.04^{b}	17.38 ± 2.06^{b}	*P<0.0001*
IMC67*SNK109	21.25 ± 0.85^{b}	19.30 ± 0.83^{a}	*P<0.05*	22.88 ± 1.55^{b}	21.63 ± 1.49^{d}	*P>0.05*
BBK1418*MO20	33.25 ± 4.19^{d}	20.00 ± 0.82^{b}	*P<0.0001*	22.5 ± 0.91^{b}	19.38 ± 1.11^{c}	*P>0.05*
ICS1*SNK37	19.75 ± 0.65^{a}	17.13 ± 0.85^{a}	*P<0.05*	23.13 ± 0.85^{b}	18.38 ± 1.25^{c}	*P<0.01*
T79/501*SNK13	19.00 ± 0.82^{a}	19.63 ± 0.48^{b}	*P>0.05*	26.75 ± 1.71^{c}	21.75 ± 1.55^{d}	*P<0.001*
SNK16*T60/1174	20.75 ± 0.65^{b}	19.13 ± 0.25^{b}	*P<0.05*	32.00 ± 0.82^{e}	27.38 ± 1.11^{e}	*P<0.01*

RL = root length, SL = shoot length. Values are means of 8 replicates and expressed in terms of Mean ± SD. For each parameter, values in each column affected by the same letter are not significantly different between treatments, as given by the Scott-Knott *post-hoc* test at the corresponding *P-value*.

Table VI (continuation)

Cocoa genotype	RL			SL		
	Control (cm)	Cd (cm)	Significance (control vs Cd)	Control (cm)	Cd (cm)	Significance (control vs Cd)
T79/501*SNK109	17.63 ± 0.75[a]	19.88 ± 0.85[b]	*P<0.05*	18.50 ± 1.29[a]	13.00 ± 0.82[a]	*P<0.0001*
UPA143*SNK37	18.63 ± 0.48[a]	18.88 ± 0.63[a]	*P<0.05*	24.00 ± 1.83[b]	20.50 ± 0.91[c]	*P>0.05*
T60/887*SNK64	20.75 ± 1.71[b]	17.88 ± 0.25[a]	*P<0.05*	21.13 ± 1.31[a]	13.75 ± 1.71[a]	*P<0.0001*
T79/501*SNK16	17.75 ± 0.65[a]	16.75 ± 1.50[a]	*P>0.05*	23.25 ± 1,26[b]	13.60 ± 1.25[a]	*P<0.0001*
SNK64*T40/1170	17.88 ± 0.85[a]	17.50 ± 1.47[a]	*P>0.05*	22.50 ± 1.29[b]	17.88 ± 2.32[b]	*P<0.01*
T60/1174*UPA134	18.00 ± 0.82[a]	19.13 ± 0.63[b]	*P>0.05*	20.63 ± 1.11[a]	20.13 ± 0.25[c]	*P>0.05*
SCA12*ICS40	18.98 ± 1.03[a]	17.75 ± 1.71[a]	*P>0.05*	19.75 ± 1.71[a]	15.25 ± 1.71[a]	*P<0.01*
SNK16*UPA143	23.38 ± 1.04[c]	18.78 ± 1.82[b]	*P<0.001*	28.38 ± 1.33[c]	20.60 ± 0.66[c]	*P<0.0001*
P-value	*<0.0003*	*<0.0001*		*<0.0001*	*<0.0001*	

RL = root length, SL = shoot length. Values are means of 8 replicates and expressed in terms of Mean ± SD. For each parameter, values in each column affected by the same letter are not significantly different between treatments, as given by the Scott-Knott *post-hoc* test at the corresponding *P-value*.

Table VII : Root and shoot dry weight of cocoa genotypes as influenced by Cd intoxication

Cocoa genotype	SDW			RDW		
	Control (g)	Cd (g)	Significance (Control vs Cd)	Control (g)	Cd (g)	Significance (Control vs Cd)
SNK10*IMC67	2.55 ± 0.07^{e}	2.29 ± 0.08^{g}	*P>0.05*	0.84 ± 0.02^{c}	0.60 ± 0.10^{d}	*P<0.01*
SNK13*ICS95	1.83 ± 0.09^{c}	1.39 ± 0.03^{c}	*P<0.0001*	0.68 ± 0.03^{b}	0.24 ± 0.04^{a}	*P<0.0001*
UPA143*SNK37	2.66 ± 0.19^{f}	2.05 ± 0.11^{f}	*P<0.0001*	0.79 ± 0.08^{c}	0.67 ± 0.02^{d}	*P>0.05*
IMC67*SNK109	1.94 ± 0.11^{c}	1.65 ± 0.11^{d}	*P>0.05*	0.78 ± 0.02^{c}	0.70 ± 0.03^{e}	*P>0.05*
BBK1418*MO20	2.52 ± 0.07^{e}	2.62 ± 0.08^{h}	*P>0.05*	0.67 ± 0.06^{b}	0.47 ± 0.05^{c}	*P<0.05*
ICS1*SNK37	2.07 ± 0.15^{d}	1.85 ± 0.14^{e}	*P>0.05*	0.63 ± 0.02^{b}	0.53 ± 0.02^{c}	*P>0.05*
T79/501*SNK13	2.82 ± 0.13^{f}	2.12 ± 0.07^{f}	*P<0.0001*	0.62 ± 0.07^{b}	0.78 ± 0.07^{e}	*P>0.05*
SNK16*T60/1174	2.46 ± 0.11^{e}	1.92 ± 0.12^{e}	*P<0.0001*	0.66 ± 0.15^{b}	0.63 ± 0.08^{d}	*P>0.05*
T79/501*SNK109	1.52 ± 0.05^{b}	1.27 ± 0.07^{b}	*P>0.05*	0.75 ± 0.04^{c}	0.45 ± 0.09^{c}	*P<0.0001*

RDW = root dry weigh, SDW = shoot dry weigh. Values are means of 8 replicates and expressed in terms of Mean ± SD. For each parameter, values in each column affected by the same letter are not significantly different between treatments, as given by the Scott-Knott *post-hoc* test at the corresponding *P-value*.

Table VII (continuation)

Cocoa genotype	SDW			RDW		
	Control (g)	Cd (g)	Significance (Control vs Cd)	Control (g)	Cd (g)	Significance (Control vs Cd)
UPA143*SNK64	2.83 ± 0.09[f]	2.57 ± 0.12[h]	*P>0.05*	0.60 ± 0.08[b]	0.46 ± 0.05[c]	*P>0.05*
T60/887*SNK64	2.80 ± 0.11[f]	2.46 ± 0.13[g]	*P<0.01*	0.80 ± 0.08[c]	0.33 ± 0.03[b]	*P<0.0001*
T79/501*SNK16	1.76 ± 0.14[c]	1.42 ± 0.05[c]	*P<0.01*	0.60 ± 0.06[b]	0.24 ± 0.03[a]	*P<0.0001*
SNK64*T40/1170	2.16 ± 0.08[d]	1.75 ± 0.10[d]	*P<0.001*	0.44 ± 0.03[a]	0.43 ± 0.03[c]	*P>0.05*
T60/1174*UPA134	2.45 ± 0.08[e]	1.95 ± 0.07[e]	*P<0.0001*	0.83 ± 0.05[c]	0.75 ± 0.02[e]	*P>0.05*
SCA12*ICS40	1.19 ± 0.09[a]	0.95 ± 0.07[b]	*P<0.001*	0.57 ± 0.04[b]	0.46 ± 0.01[c]	*P>0.05*
SNK16*UPA143	4.53 ± 0.20[g]	2.92 ± 0.08[i]	*P<0.0001*	0.46 ± 0.03[a]	0.35 ± 0.04[b]	*P>0.05*
P-value	*<0.0001*	*<0.0001*		*<0.0001*	*<0.0001*	

RDW = root dry weigh, SDW = shoot dry weigh. Values are means of 8 replicates and expressed in terms of Mean ± SD. For each parameter, values in each column affected by the same letter are not significantly different between treatments, as given by the Scott-Knott *post-hoc* test at the corresponding *P-value*

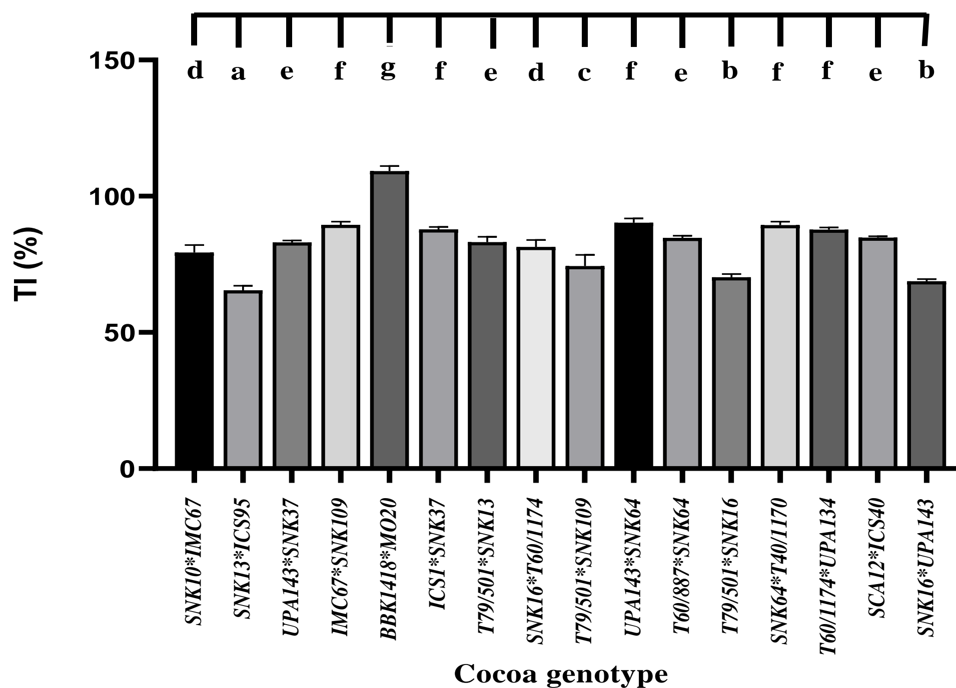


Figure 14: Cadmium tolerance index (TI) of cocoa seedling genotypes following cadmium intoxication

Values are means of 8 replicates and expressed in terms of Mean ± SD. Bar charts bearing the same superscript indicate a non-significant difference at *P<0.0001*.

III.1.1.1.2 Effects of cadmium intoxication on photosynthetic pigments content

Tables VIII and IX indicate the relative differences in chlorophyll pigment content vs. the negative control. According to these data, Cd exposure significantly reduced the mean amounts of chlorophyll a and carotenoids in the leaves of nine (56.25%) of the genotypes studied (*P<0.0001*). The *ICS1*SNK37* cocoa hybrid saw the highest loss, with a -20.86% decrease in chlorophyll a and -33.66% in carotenoids. Seven genotypes (43.76%) had a significant increase in chlorophyll b content: *SNK13*ICS95, BBK1418*MO20, T79/501*SNK13, T79/501*SNK109, T60/887*SNK64, SCA12*ICS40,* and *SNK16*UPA143.* The genotype *T60/887*SNK64* had the most significant increase (254.89%). Similarly, five hybrids demonstrated an increase in carotenoid content: *SNK13*ICS95, BBK1418*MO20, UPA143*SNK64, T79/501*SNK16,* and *SNK16*UPA143.* However, the majority of these hybrids exhibit an increase in chlorophyll B production. The most important combination was *T60/887*SNK64*, which boosted Chl b by 254.49%.

Table VIII: Cocoa seedling photosynthetic pigment content following Cadmium intoxication

Cocoa genotype	Chl a			Chl b		
	Control (µg.mg-1 FW)	Cd (µg.mg-1 FW)	Significance Control/Cd	Control (µg.mg-1 FW)	Cd (µg.mg-1 FW)	Significance Control/Cd
SNK10*IMC67	10.90 ± 0.15^{e}	9.54 ± 0.54^{b}	*P<0.0001*	5.07 ± 0.50^{e}	3.72 ± 0.31^{c}	*P<0.0001*
SNK13*ICS95	8.02 ± 0.05^{a}	9.49 ± 0.97^{b}	*P<0.0001*	2.64 ± 0.02^{a}	2.87 ± 0.05^{b}	*P>0.05*
UPA143*SNK37	10.88 ± 0.03^{e}	10.52 ± 0.09^{d}	*P>0.05*	3.81 ± 0.09^{b}	3.75 ± 0.01^{c}	*P>0.05*
IMC67*SNK109	10.36 ± 0.10^{h}	10.06 ± 0.38^{d}	*P<0.0001*	4.07 ± 0.18^{d}	3.83 ± 0.29^{c}	*P<0.001*
BBK1418*MO20	10.68 ± 0.16^{d}	11.67 ± 0.08^{e}	*P<0.0001*	3.88 ± 0.11^{b}	4.77 ± 0.04^{d}	*P<0.001*
ICS1*SNK37	9.37 ± 0.03^{c}	7.41 ± 0.06^{a}	*P<0.0001*	2.68 ± 0.05^{a}	1.99 ± 0.01^{a}	*P<0.01*
T79/501*SNK13	11.10 ± 0.18^{f}	12.15 ± 0.07^{f}	*P<0.0001*	3.83 ± 0.16^{b}	7.88 ± 0.27^{g}	*P<0.0001*
SNK16*T60/1174	11.27 ± 0.03^{g}	9.99 ± 0.08^{c}	*P<0.0001*	3.96 ± 0.01^{b}	3.04 ± 0.06^{b}	*P<0.001*
T79/501*SNK109	11.35 ± 0.03^{g}	11.99 ± 0.01^{f}	*P<0.01*	4.25 ± 0.13^{c}	8.72 ± 0.11^{h}	*P<0.0001*

Chl a = Chlorophyll a, Chl b = chlorophyll b, FW = fresh weigh. Values are means of 3 replicates and expressed in terms of Mean ± SD. For each parameter, values in each column affected by the same letter are not significantly different between treatments, as given by the Scott-Knott *post-hoc* test at the corresponding *P-value*.

Table VIII (continuation)

Cocoa genotype	Chl a			Chl b		
	Control (µg.mg-1 FW)	Cd (µg.mg-1 FW)	Significance Control/Cd	Control (µg.mg-1 FW)	Cd (µg.mg-1 FW)	Significance Control/Cd
UPA143*SNK64	12.06 ± 0,06^{j}	12.03 ± 0.03^{f}	*P>0.05*	7.96 ± 0.09^{g}	7.74 ± 0.10^{g}	*P>0.05*
T60/887*SNK64	8.99 ± 0.06^{b}	12.13 ± 0.12^{f}	*P<0.0001*	2.45 ± 0.02^{a}	8.67 ± 0.22^{h}	*P<0.0001*
T79/501*SNK16	12.00 ± 0.01^{j}	11.50 ± 0.01^{e}	*P>0.05*	5.13 ± 0.01^{e}	3.88 ± 0.02^{c}	*P<0.0001*
SNK64*T40/1170	12.13 ± 0.03^{j}	11.89 ± 0.08^{f}	*P>0.05*	7.14 ± 0.14^{f}	9,56 ± 0.14^{i}	*P<0.0001*
T60/1174*UPA134	12.17 ± 0.08^{j}	11.71 ± 0.08^{e}	*P>0.05*	6.90 ± 0.02^{f}	11.86 ± 0.39^{j}	*P<0.0001*
SCA12*ICS40	11.80 ± 0.22^{i}	12.18 ± 0.27^{f}	*P>0.05*	4.20 ± 0.44^{c}	6.92 ± 0.84^{f}	*P<0.0001*
SNK16*UPA143	10.76 ± 0.03^{d}	11.98 ± 0.03^{f}	*P<0.0001*	4.61 ± 0.19^{d}	5,51 ± 0,10^{e}	*P<0.001*
P-value	*<0.0001*	*<0.0001*		*<0.0001*	*<0.0001*	

Chl a = Chlorophyll a, Chl b = chlorophyll b, FW = fresh weigh. Values are means of 3 replicates and expressed in terms of Mean ± SD. For each parameter, values in each column affected by the same letter are not significantly different between treatments, as given by the Scott-Knott *post-hoc* test at the corresponding *P-value*.

Table IIX : Change in carotenoids and total pigment content in cocoa seedling following cadmium intoxication

Cocoa genotype	Carotenoids			Total pigment		
	Control (µg.mg-1 FW)	Cd (µg.mg-1 FW)	Significance control / Cd	Control (µg.mg-1 FW)	Cd (µg.mg-1 FW)	Significance control /Cd
SNK10*IMC67	4,74 ± 0,02[f]	4,52 ± 0,37[d]	*P>0.05*	13.06 ± 0.49[e]	11,50 ± 0,57[c]	*P<0.0001*
SNK13*ICS95	3,83 ± 0,06[b]	4,29 ± 0,30[d]	*P<0.001*	9.72 ± 0.07[a]	10,51 ± 0,57[b]	*P>0.05*
UPA143*SNK37	4,84 ± 0,08[g]	4.83 ± 0.02[e]	*P>0.05*	11.90 ± 0.16[d]	11,83 ± 0,03[c]	*P>0.05*
IMC67*SNK109	4,53 ± 0,09[f]	4.59 ± 0.18[e]	*P>0.05*	12.71 ± 0.23[e]	11.41 ± 0.38[c]	*P<0.01*
BBK1418*MO20	4.49 ± 0.07[e]	4.81 ± 0.01[e]	*P>0.05*	11.63 ± 0.17[c]	12.84 ± 0.02[d]	*P<0.0001*
ICS1*SNK37	4.33 ± 0.02[d]	3.43 ± 0.01[a]	*P<0.0001*	10.26 ± 0.07[a]	8.68 ± 0.01[a]	*P<0.0001*
T79/501*SNK13	4.84 ± 0.04[g]	4.65 ± 0.01[e]	*P>0.05*	11.93 ± 0.20[d]	15.78 ± 0.21[d]	*P<0.0001*
SNK16*T60/1174	4.74 ± 0.14[f]	3.85 ± 0.10[c]	*P<0.0001*	11.95 ± 0.10[d]	10.14 ± 0.07[b]	*P<0.0001*

FW = fresh weigh. Values are means of 3 replicates and expressed in terms of Mean ± SD. For each parameter, values in each column affected by the same letter are not significantly different between treatments, as given by the Scott-Knott *post-hoc* test at the corresponding *P-value*.

Table IX (continuation)

Cocoa genotype	Carotenoids			Total pigment		
	Control (µg.mg-1 FW)	Cd (µg.mg-1 FW)	Significance control / Cd	Control (µg.mg-1 FW)	Cd (µg.mg-1 FW)	Significance control /Cd
T79/501*SNK109	5.13 ± 0.05[i]	3.40 ± 0.02[a]	*P<0.0001*	12.63 ± 0,09[b]	15.37 ± 0.07[d]	*P<0.0001*
UPA143*SNK64	4.10 ± 0.07[c]	4.51 ± 0.01[d]	*P<0.01*	15.31 ± 0.13[g]	15.50 ± 0.06[d]	*P>0.05*
T60/887*SNK64	5.65 ± 0.03[k]	4.50 ± 0.06[d]	*P<0.0001*	11.35 ± 0.04[c]	16.43 ± 0.23[e]	*P<0.0001*
T79/501*SNK16	3.42 ± 0.05[a]	5.42 ± 0.02[g]	*P<0.0001*	11.81 ± 0.02[d]	12.55 ± 0.01[d]	*P>0.05*
SNK64*T40/1170	4.33 ± 0.03[d]	3.65 ± 0.03[b]	*P<0.0001*	14.72 ± 0.12[f]	16.47 ± 0.14[e]	*P<0.0001*
T60/1174*UPA134	4.71 ± 0.05[f]	3.92 ± 0.10[c]	*P<0.0001*	14.86 ± 0.07[f]	19.04 ± 0.16[f]	*P<0.0001*
SCA12*ICS40	5.37 ± 0.21[j]	4.98 ± 0.12[f]	*P<0.05*	12.82 ± 0.58[e]	15.15 ± 0.48[d]	*P<0.0001*
SNK16*UPA143	4.99 ± 0.11[h]	5.06 ± 0.14[f]	*P>0.05*	20.36 ± 0.09[h]	22.55 ± 0.19[g]	*P<0.0001*
P-value	*<0.0001*	*<0.0001*		*<0.0001*	*<0.0001*	

FW = fresh weigh. Values are means of 3 replicates and expressed in terms of Mean ± SD. For each parameter, values in each column affected by the same letter are not significantly different between treatments, as given by the Scott-Knott *post-hoc* test at the corresponding *P-value*.

III.1.1.1.3 Principal component and cluster analysis depicted cocoa genotype and growth parameters

The separation efficacy of critical elements in cocoa seedling growth was investigated using principal components analysis (PCA) for growth characteristics (figure 15). The PCA test demonstrated a positive association between growth parameter-related aspects and Cd treatment, showing that Cd may effectively impede cocoa seedling development. Whereas genotypes *SNK10*IMC67, SNK13*ICS95, BBK1418*MO20, ICS1*SNK37, T79/501*SNK109, UPA143*SNK64, T60/887*SNK64*, and *T79/501*SNK16* were strongly impacted at the level of the root length, genotype *SNK16*T60/1174* was more influenced at the level of the shoot length. Cadmium uptake has a detrimental effect on the genotypes: *UPA143*SNK37, IMC67*SNK109, T79/501*SNK13, SNK64*T40/1170, T60/1174*UPA134, SCA12*ICS40,* and *SNK16*UPA143*. According to hierarchical cluster analysis, there are four main cocoa genotype groups: Cluster III has the highest sensitivity, while Cluster IV contains the most resistant genotypes (figure 16).

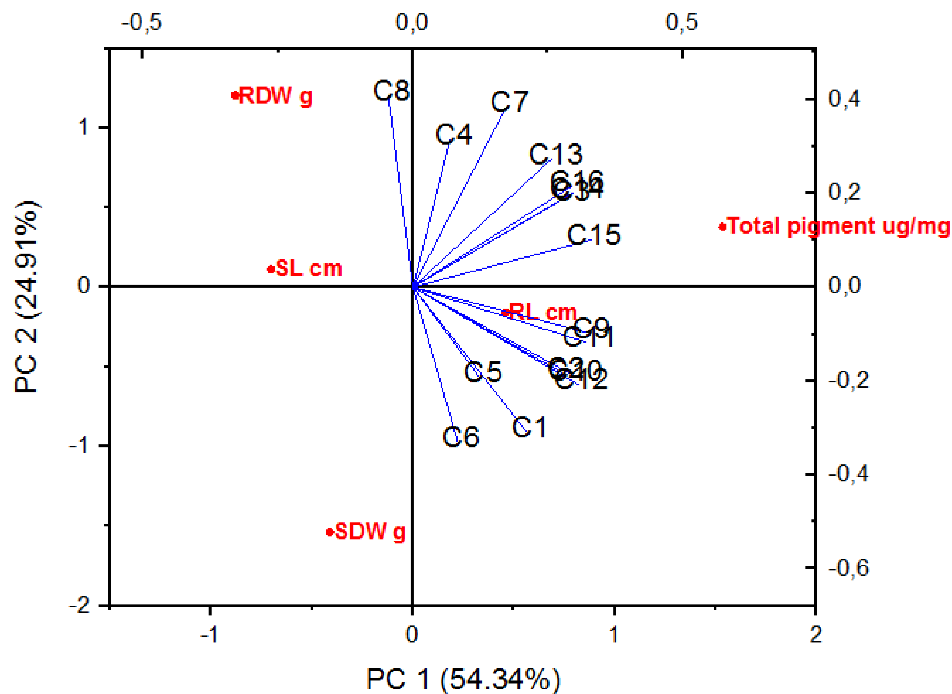


Figure 15: Principal component analysis elucidating the correlations between the cocoa genotypes and growth traits

Cocoa hybrids (genotypes): *(SNK10*IMC67* (C1), *SNK13*ICS95* (C2), *UPA143*SNK37* (C3), *IMC67*SNK109* (C4), *BBK1418*MO20* (C5), *ICS1*SNK37* (C6), *T79/501*SNK13* (C7), *SNK16*T60/1174* (C8), *T79/501*SNK109 (C9), UPA143*SNK64 (C10), T60/887*SNK64 (C11), T79/501*SNK16 (C12), SNK64*T40/1170* (C13), *T60/1174*UPA134* (14), *SCA12*ICS40* (C15), *SNK16*UPA143* (16). RL = root length, SL = shoot length, RDW = root dry weigh, SDW = shoot dry weigh.

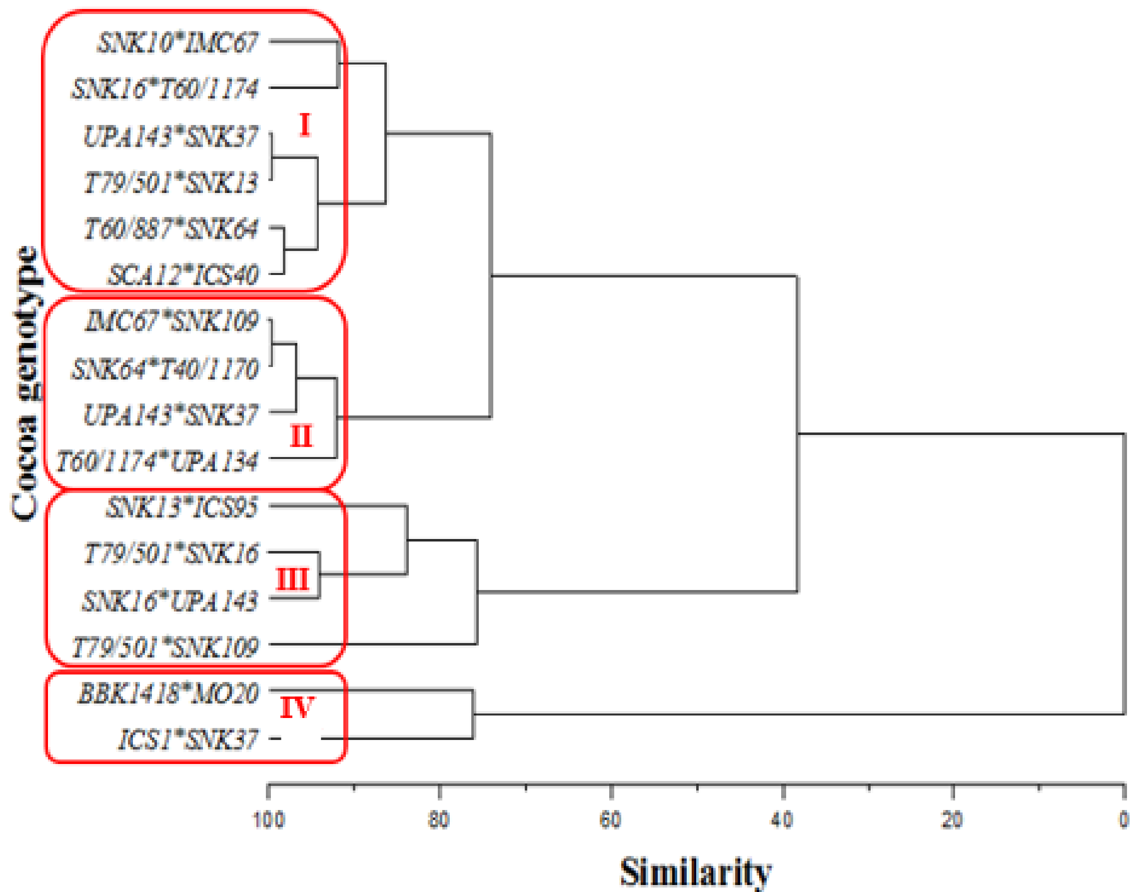


Figure 16: Dendogram of Cocoa genotype sensitivity using similarity based on growth parameter of cocoa genotype under cadmium stress conditions.

I = cluster of the genotypes with medium sensitivitivity, II= cluster of the genotypes with low medium low medium sensitivity, Cluster III= cluster of the genotypes with highest sensitivity, IV= cluster of the most resistant genotypes

III.1.1.2 Cadmium uptake and accumulation in cocoa seedlings per genotype

The predisposition of cocoa varieties to the development of Cd in the roots and leaves is demonstrated in Table X. The study finds that cocoa hybrids have the greatest amounts of Cd in their roots and leaves. The *SNK16*UPA143* hybrid has the lowest root values, whereas the *ICS1*SNK37* hybrid has the largest root concentrations. Most cocoa genotype studied displayed considerable variability in Cd levels, with the exception of *SNK13*ICS95* and *SNK16*T60/1174* hybrids. Shoot cd content was not substantially different amongst hybrids. Cadmium accumulation followed the same pattern: roots > shoots. Bioconcentration parameters differed greatly amongst genotypes, with the exception of groups like *SNK10*IMC67, SNK16*T60/1174,* and *SCA12*ICS40*. The study of Cd translocation indicated

a considerable variation amongst the hybrids, with the greatest translocation factor recorded in the *SNK16*UPA143* hybrid (96.55%). The *SNK10*IMC67* and *SNK16*UPA143* hybrids recorded the maximum accumulation factor (42.58% and 41.98%), whereas the *T79/501*SNK13* hybrid recorded the lowest value (16.16%). The *SNK16*UPA143* hybrid demonstrated the strongest accumulation propensity, with the highest translocation factor (96.55%) and accumulation factor (41.98%).

Table X : Cadmium uptake and accumulation in cocoa seedling hybrids following Cd intoxication

Cocoa genotype	Cd shoot (mg/kg)	Cd root (mg/kg)	TF (%)	BCF (%)	AF (%)
SNK10*IMC67	230.51 ± 3.38^{i}	310,85 ± 1.79^{d}	74.16 ± 0.52^{m}	270.68 ± 0.89^{e}	42.58 ± 0.14^{m}
SNK13*ICS95	209.37 ± 1.89^{f}	427.38 ± 1.79^{i}	48,99 ± 0.25^{f}	318.37 ± 0.90^{h}	32.88 ± 0.09^{f}
UPA143*SNK37	206.68 ± 2.68^{f}	360.70 ± 1.54^{g}	57,30 ± 0.30^{h}	283.69 ± 0.77^{f}	36.43 ± 0.10^{h}
IMC67*SNK109	205.83 ± 2.65^{e}	301.58 ± 2.64^{c}	58.61 ± 0.63^{i}	277.98 ± 1.32^{b}	37.21 ± 0.20^{i}
BBK1418*MO20	161.17 ± 2.68^{d}	375.12 ± 2.20^{h}	42.97 ± 0.31^{d}	268.15 ± 1.10^{d}	30.05 ± 0.12^{d}
ICS1*SNK37	213.42 ± 0.71^{g}	616.73 ± 1.04^{n}	34.61 ± 0.07^{c}	415.08 ± 0.52^{k}	25.71 ± 0.03^{c}
T79/501*SNK13	141.93 ± 2.76^{b}	736.12 ± 1,93^{o}	19.28 ± 0.06^{a}	439.02 ± 0.96^{m}	16.16 ± 0.04^{a}
SNK16*T60/1174	113.42 ± 3.22^{a}	425.64 ± 1,38^{i}	26.65 ± 0.11^{b}	269.53 ± 0.69^{e}	21.04 ± 0.05^{b}
T79/501*SNK109	206.10 ± 3.22^{f}	329.09 ± 1.14^{f}	62.63 ± 0.27^{j}	267.60 ± 0.57^{d}	38.51 ± 0.08^{j}
UPA143*SNK64	155.41 ± 1.73^{c}	449.24 ± 1.97^{k}	34.59 ± 0.19^{c}	302.32 ± 0.99^{g}	25.70 ± 0.08^{c}
T60/887*SNK64	297.55 ± 1.49^{j}	553.27 ±1.05^{m}	53.78 ± 0.13^{g}	425.41 ± 0,53^{l}	34.97 ± 0.04^{g}
T79/501*SNK16	207.31 ± 1.40^{f}	467.71 ± 1.10^{l}	44.32 ± 0.13^{e}	337.51 ± 0,55^{j}	30.71 ± 0.05^{e}
SNK64*T40/1170	233.61 ± 2.49^{i}	431.78 ± 1.48^{j}	54.11 ± 0.23^{g}	332.69 ± 0.74^{i}	35.11 ± 0.08^{g}
T60/1174*UPA134	205.15 ± 1.88^{f}	283.55 ± 1.21^{b}	72.35 ± 0.38^{l}	244.35 ± 0.60^{c}	41.98 ± 0.10^{l}
SCA12*ICS40	222.82 ± 2.53^{h}	315.60 ± 1.38^{e}	70.60 ± 0.38^{k}	269.21 ± 0.69^{e}	41.38 ±0.11^{k}
SNK16*UPA143	161.86 ± 3.17^{a}	167.66 ± 1.43^{a}	96.55 ± 1.01^{n}	164.76 ± 0.72^{a}	49.12 ± 0.21^{n}
P-value	*<0.0001*	*<0.0001*	*<0.0001*	*<0.0001*	*<0.0001*

BCF = bioconcentration factor, TF = translocation factor, AF = accumulation factor. Values are means of 3 replicates and expressed in terms of Mean ± SD. For each parameter, values in each column affected by the same letter are not significantly different between treatments, as given by the Scott-Knott *post-hoc* test at the corresponding *P-value*.

III.1.1.2.1 Interaction between cocoa genotype and cadmium accumulating parameter through PCA

The PCA association with Cd accumulation in cocoa seedlings demonstrated a favourable correlation with the principal components (PC) and separation efficiency for key parameters (figure 17). The PCA test discovered 99.78% of the total variance, with PC1 and PC2 accounting for 96.77% and 3.19%, respectively. A grouping study demonstrated that Cd treatment had a positive connection with Cd accumulation features, with genotypes *SNK10*IMC67, UPA143*SNK64, IMC67*SNK109, T79/501*SNK109, T60/887*SNK64, SNK64*T40/1170, T60/1174*UPA134, SCA12*ICS40,* and *SNK16*UPA143* demonstrate more effective Cd accumulation in shoots and BCF. Cd buildup in roots was more prominent in genotypes: *SNK13*ICS95, BBK1418*MO20, ICS1*SNK37, T79/501*SNK13, SNK16*T60/1174, UPA143*SNK64* and *T79/501*SNK16*. Hierarchical cluster analysis of Cd accumulation split cocoa genotypes into three broad categories. Cluster 1 is made up of the genotypes of cocoa that are most likely to accumulate Cd, whereas Cluster 3 is made up of those that are least likely to do so (figure 18).

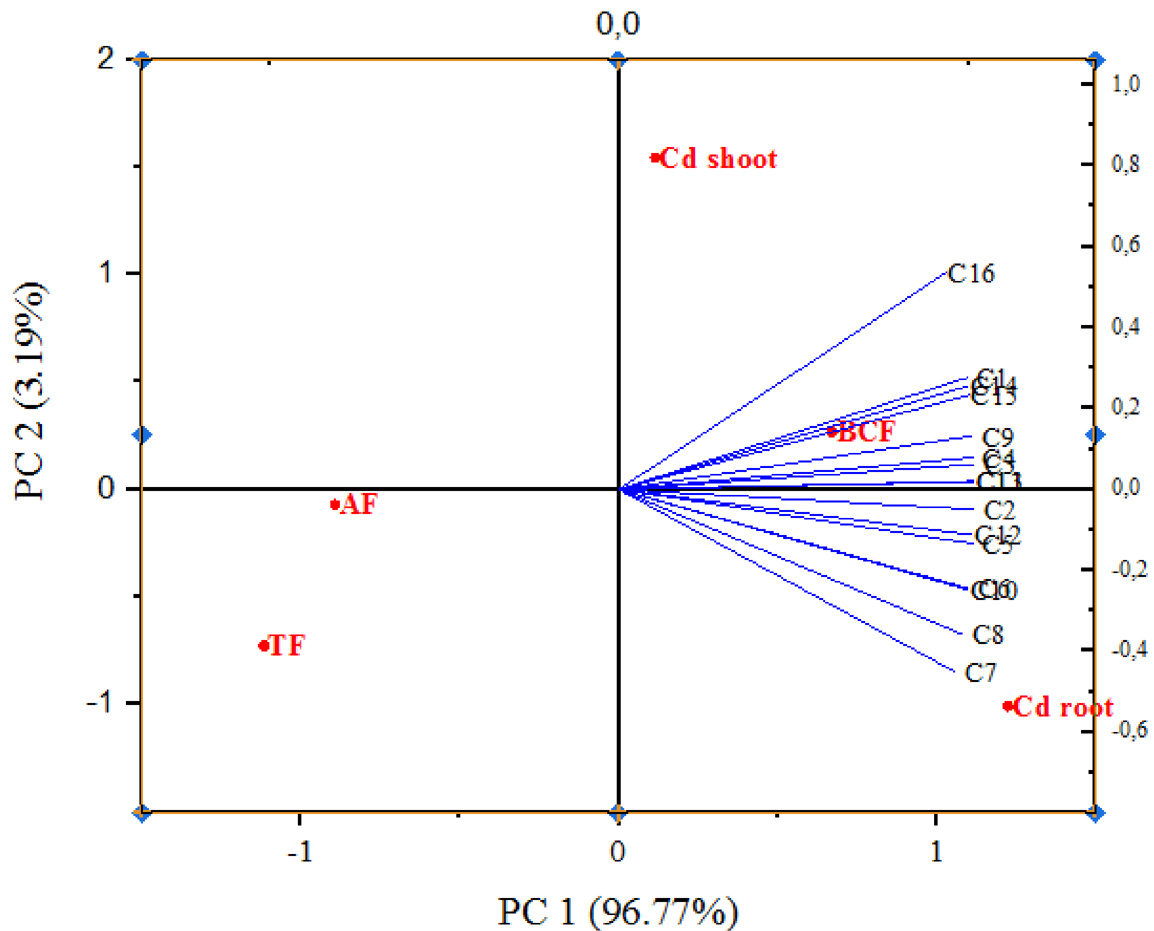


Figure 17: Principal component analysis highlights the correlations between the analysed accumulation variables and cocoa genotypes.

Cocoa hybrids (genotypes): *(SNK10*IMC67* (C1), *SNK13*ICS95* (C2), *UPA143*SNK37* (C3), *IMC67*SNK109* (C4), *BBK1418*MO20* (C5), *ICS1*SNK37* (C6), *T79/501*SNK13* (C7), *SNK16*T60/1174* (C8), *T79/501*SNK109* (C9), *UPA143*SNK64* (C10), *T60/887*SNK64* (C11), *T79/501*SNK16* (C12), *SNK64*T40/1170* (C13), *T60/1174*UPA134* (14), *SCA12*ICS40* (C15), *SNK16*UPA143* (16). BCF = bioconcentration factor, TF = translocation factor, AF = accumulation factor.

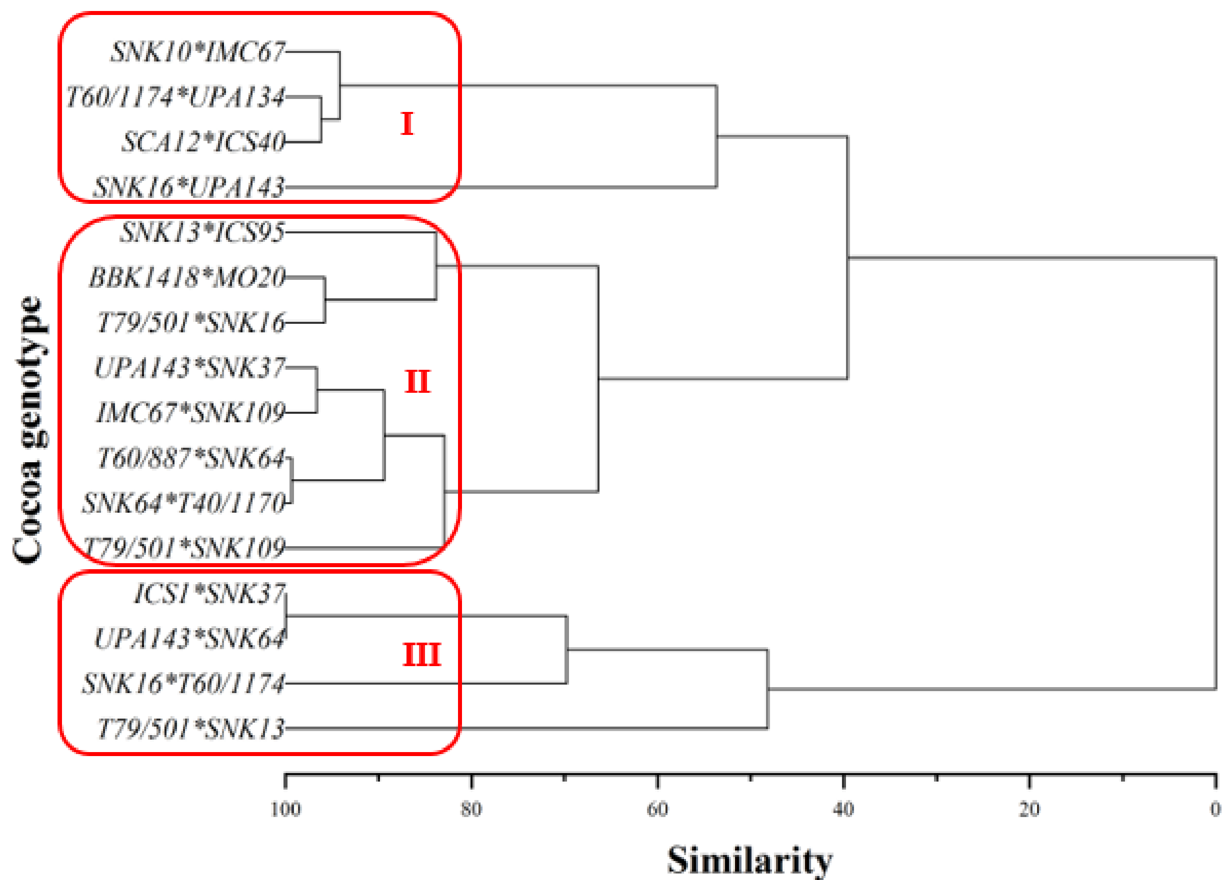


Figure 18: Dendogram of accumulating potential using similarity based on accumulating parameter of cocoa genotype under cadmium stress conditions

I = cluster of the genotypes with low accumulation profile, II= cluster of the genotypes with medium accumulation profile, Cluster III= cluster of the genotypes with highest accumulation profile

III.1.2 Cadmium accumulation profile of selected cocoa genotypes: discussion

Health protection groups worldwide have long been concerned about the prevalence of toxic heavy metals in agricultural and agro-industrial goods. Recently, significant attention has been placed on Cd due to its presence in internationally traded cocoa beans, which might pose risks to consumers of cocoa products (Codex Alimentarus, 2018). New regulations implemented in 2019 require cocoa-producing nations to adopt practices that prevent the accumulation of this metal. Using low-accumulating cocoa genotypes has emerged as a sustainable and promising strategy to address this issue. Given the importance of cocoa for producing countries and especially for the Cameroonian economy, categorizing cocoa genotypes in Cameroon's seed fields according to their responsiveness and propensity to accumulate Cd has become an urgent need.

In this investigation, seedlings from sixteen different cocoa genotypes from clonal farms in Cameroon were subjected to a greenhouse experiment under Cd stress. The results clearly indicate that the presence of Cd typically negatively affects the development of cocoa plants. These declines are caused by several factors, including increased production of reactive oxygen species (ROS) that damage macromolecules and cell membranes, inhibition of glycolysis-mediated cell division and elongation (Dalla Vecchia *et al.,* 2005), and reduced mitotic division of meristematic cells (Abbas *et al.,* 2018). It also reduces nutrient uptake and photosynthetic efficiency, thereby decreasing the capacity to produce photosynthetic energy and increasing ROS production (Haider *et al.,* 2021).

These findings align with a study by Hayat *et al*. (2021), demonstrating that high Cd concentrations reduced the dry root and shoot biomass as well as the length of *Cajanus cajan* L. Attributed to a considerable drop in chlorophyll a and b content, corroborating the fluctuations in chlorophyll pigment concentration under Cd stress observed in the current study. These results indicate that varietal specificity should be considered, as different cocoa genotypes exhibit various responses to Cd stress. Once ingested, Cd induces physiobiochemical changes with detrimental effects on pigment and protein content, nutrient absorption, antioxidant defence systems, and eventually chlorophyll synthesis (Qadir *et al.,* 2014). cadmium accumulation in roots adversely affects root morpho-physiological processes, leading to a smaller decrease in aboveground biomass. To protect its physiological and metabolic processes in photosynthetic tissues and minimize Cd mobility in the shoot system, the plant accumulates more Cd in the root cell walls and vacuoles (Li *et al.,* 2020; Yuan & Huang, 2016). However, low Cd transfer to the shoots is observed, especially in tolerant genotypes.

Our findings on Cd concentration in various plant organs indicate that Cd is more concentrated in the roots of cocoa plants as compare in the shoot. Comparisons between Cd-tolerant and sensitive genotypes reveal that tolerant genotypes accumulate more Cd in their roots under Cd stress. Plants often employ chelation mechanisms to mitigate Cd's harmful effects, involving compartmentalization or sequestration in vacuoles, leading to varying accumulation levels depending on plant species and variety (Parrotta *et al.,* 2015). Also, different cocoa genotypes tested had significantly varied Cd amounts in roots and leaves, bioconcentration factors (BCF), translocation factors (TF), and accumulation factors (AF). For instance, TF values ranged from 19.28% (*T79/501*SNK13)* to 96.55% *(SNK16*UPA143),* and BCF values ranged from 164.76% (*SNK16*UPA143)* to 439.02% *(T79/501*SNK13*).

Some studies have found variability in Cd accumulation by various cocoa genotypes under the same Cd stress environment (Earl, 2021; Zug *et al.,* 2019). Wadeid *et al*. (2022) conducted a meta-analysis of soil-plant data from across the cocoa belt to determine the causes of Cd accumulation in cocoa beans, observed that different cocoa genotypes exhibited variable degrees of bioconcentration of Cd in their beans. These differences are attributed to various plant genotypes possessing different alleles or variants of metal-related genes, which influence their metal affinity, specificity, activity, and location. Certain plant genotypes may have more effective metal transporters that exclude or limit metal absorption from the soil or translocate metals from roots to shoots (Paape *et al.,* 2022). Other genotypes may contain more potent metal chelators that bind and detoxify metals in the cytosol or vacuole, such as phytochelatins (PCs) or metallothioneins (MTs) (Jablonkai, 2022; Zhang *et al.,* 2022). Additionally, some genotypes may have sensitive metal regulators that modify the expression of metal-related genes according to metal availability and stress level (Moore *et al.,* 2020). These genetic variations result in different physiological responses and adaptations to heavy metal stress among plant genotypes within the same species (Feki *et al.,* 2021; Kumar & Aery, 2016; Paape *et al.,* 2022).

Partial Conclusion I

This section of the study, which focuses on assessing the sensitivity of various cocoa seedling genotypes to Cd stress and their Cd accumulation profile, leads to the following conclusions:

- Cadmium negatively impacts the growth and chlorophyll production of the majority of sixteen cocoa hybrids investigated. However, sensitivity varies significantly depending on the hybrid. The genotype *SNK13*ICS95* shows the highest sensitivity, whereas *BBK1418*MO20* has the highest hardness.
- All tested cocoa genotypes absorb, translocate, and store Cd in both the root and shoot, although concentrations vary greatly depending on the variety. The genotype *SNK16*UPA143* shows the highest accumulation propensity, whereas *T79/501*SNK13* has the lowest.

The results support the hypothesis that a more detailed study of the variety and level of accumulation can provide valuable information to guide farmers on varietal choices. Moreover, it draws researchers' attention to accumulating varieties that can serve as models to study the ability of microbial agents, such as mycorrhizae, to reduce or even prevent the accumulation of this toxic metal.

III.2 AMF alleviated cadmium accumulation in cocoa seedlings

III.2.1 Results

III.2.1.1 Mycorrhizal root colonization

Despite the intoxication with Cd, *T. cacao* plants successfully developed the symbiotic relationship with all the AMF inoculum tested (Figure 19). The AM consortium MFSC performed best (RCF = 66.52 %), followed by MFSG (RCF = 65.83 %). Notably, the mycorrhizal formulation MFP was the least infectious (RCF = 55.27 %).

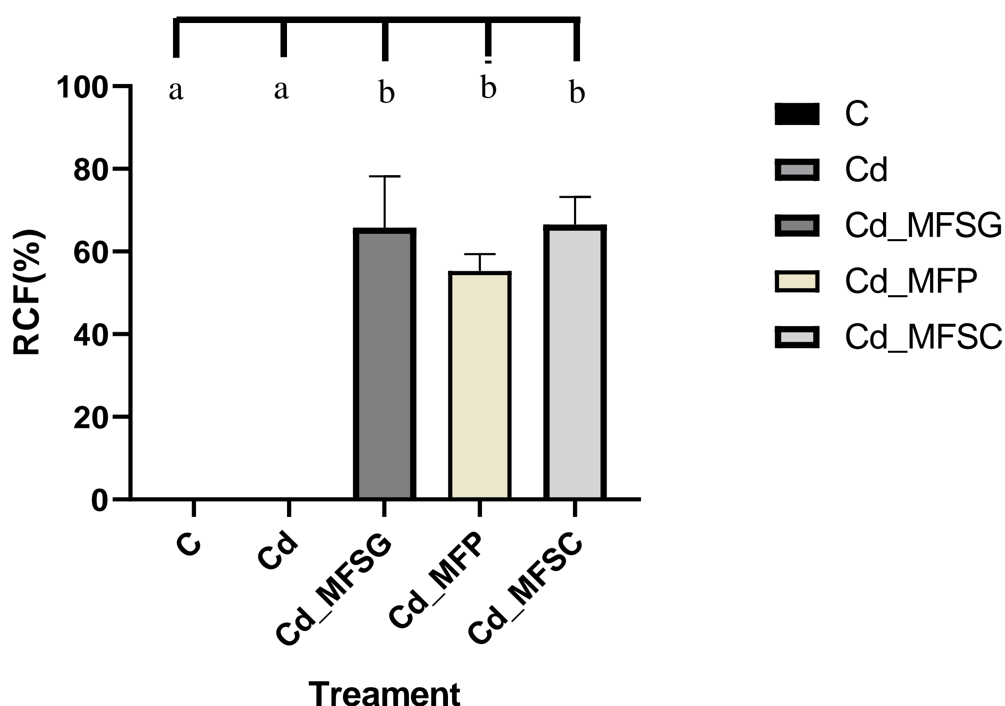


Figure 19: *T. cacao* mycorrhizal root colonisation following cadmium intoxication and AMF inoculation.

RCF = root colonisation frequency, C= unintoxicated and uninfected seedlings, Cd = Soil amended with Cd; Cd_MFSG= Cd amended and inoculation with AMF propagated with sorghum and groundnut (MFSG), Cd_MFP = Cd amended and inoculation with AMF propagated with leek (MFP); Cd_MFSC = Cd amended and inoculation AMF propagated with sorghum and cowpea (MFSC)., Values are means of 3 replicates and expressed in terms of Mean ± SD. Bar charts bearing the same superscript indicate a non-significant difference at *P<0.0001.*

III.2.1.2 Mycorrhizal inoculation alleviates cadmium uptake in cocoa seedlings

In contrast to control seedlings, Cd was identified in cocoa seedling amended soil, resulting in its absorption. The ICP-AES analysis found Cd in tissues after amendment into the soil. Notably, Cd concentration was higher in the root system (197.55 µg/g RDW). The combination of Cd and AM fungus resulted in much lower Cd absorption, ranging from -11.45% to -100%.

The root-to-shoot transmission was also investigated, with the greatest TF found in Cd-intoxicated non-mycorrhizal plantlets (TF = 96%). Mycorrhizal inoculation reduced root-to-shoot transmission, with TF levels varying from 44.79 to 100%. The Cd_MFSG formulation performed best, with significant Cd sequestration into root systems (TF = 9%) (Table XI). In contrast to control seedlings, Cd was identified in cocoa seedling soil amendment, resulting in its absorption.

Table XI: *T. cacao* L. root colonization frequency (RCF) and accumulation parameters in seedlings following cadmium intoxication and AMF inoculation

Treatment	Parameter				
	Cd Shoot ($mg.kg^{-1}$)	Cd Root ($mg.kg^{-1}$)	TF	BCF (%)	AF (%)
Control	0 ± 0.00 ^{a}	0 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0,00 ± 0,00^{a}
Cd	161 ± 0.11^{d}	168.12 ± 0.20^{d}	96.00 ± 4.00^{d}	164.67 ± 0.42^{e}	48,95 ±0,15^{e}
Cd_MFSG	17.78 ± 0.32^{b}	197.55 ± 0.43^{e}	9.00 ± 3.00^{b}	107.66 ± 0.36^{b}	8,26 ± 0,09^{b}
Cd_MFP	79.03 ± 0.15^{c}	148.87 ± 0.66^{c}	53.67 ± 5.69^{c}	113.90 ± 0.32^{d}	34,70± 0,08^{c}
Cd_MFSC	104.27 ± 0.32^{e}	114.15 ± 0.34^{b}	90.96 ± 7.92^{d}	109.20 ± 0.18^{c}	47,74 ± 0,08^{d}
P-value	*<0.0001*	*<0.0001*	*<0.0001*	*<0.0001*	*<0.0001*

Cd = Soil amended with Cd, Cd_MFSG= Cd amendment and inoculation with AMF propagated with sorghum and groundnut (MFSG), Cd_MFP = Cd amendment and inoculation with AMF propagated with leek (MFP); Cd_MFSC= Cd treatment and inoculation AMF propagated with sorghum and cowpea (MFSC). Cd Root = Cd concentration in root, Cd Shoot = Cd concentration in shoot, BCF = bioconcentration factor, TF = translocation factor, AF = accumulation factor. Values are means of 3 replicates and expressed in terms of Mean ± SD. For each parameter, values in each column affected by the same letter are not significantly different between treatments, as given by the Scott-Knott *post-hoc* test at the corresponding *P-value*.

III.3.2.4 Principal component and cluster analysis depicted cocoa genotype and cadmium accumulation parameters

As a result of the PCA related to Cd accumulation, case projections on a factor plane with two principal components (PC) were carried out (Figure 20). The PCA test indicated that PC1 and PC2 were responsible for 79.9% and 18.8% of the total variation in Cd accumulation, respectively. Grouping analyses revealed that the Cd and Cd_MFSG treatments were significantly associated with factors related to Cd shoot accumulation, accumulation factor, and translocation factor, respectively. Cd_MFSC treatment exhibited a high association with Cd accumulation in the root and bioconcentration factor, but Cd_FMP treatment impacted all parameters without preference.

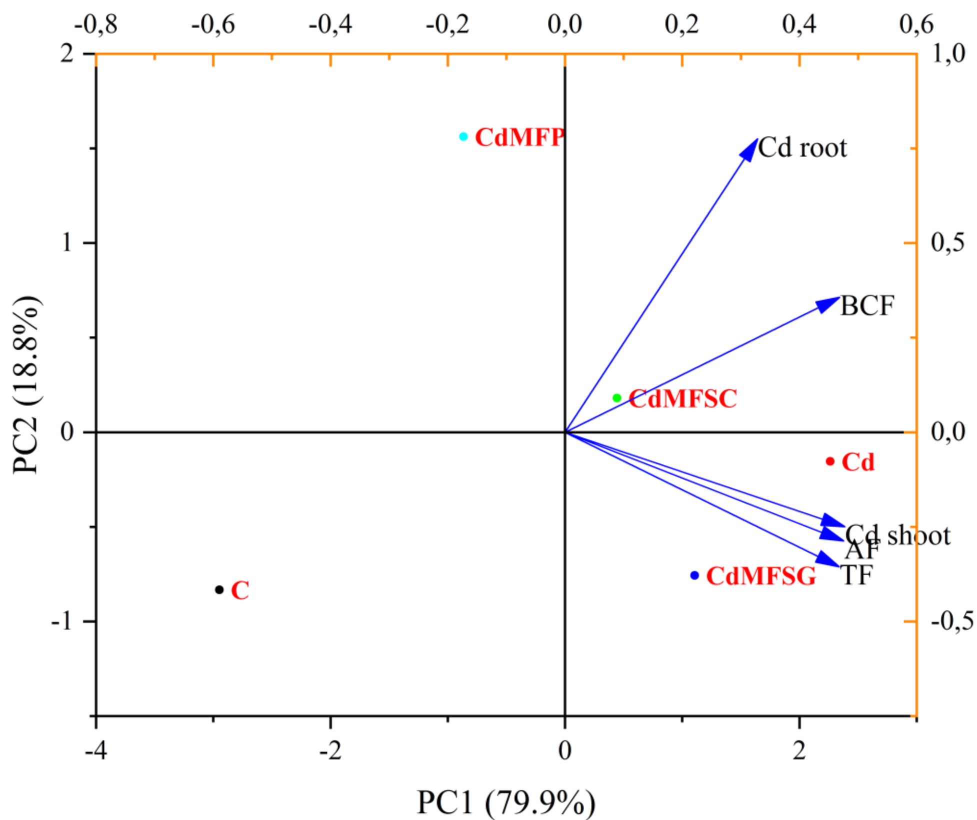


Figure 20: The principal component analysis highlights the links between accumulating factors and the distinct AM fungus inoculum.

Cd = Soil amended with Cd, Cd_MFSG= Cd amendment and inoculation with AMF propagated with sorghum and groundnut (MFSG), Cd_MFP = Cd amendment and inoculation with AMF propagated with leek (MFP);

Cd_MFSC= Cd treatment and inoculation AMF propagated with sorghum and cowpea (MFSC). BCF = bioconcentration factor, TF = translocation factor, AF = accumulation factor, PC = principal components.

III.2.1.3 Effects of cadmium intoxication and AMF inoculation on cocoa seedlings growth

Growth parameters of *T. cacao* were significantly affected by inoculation with AM fungus and/or Cd poisoning (Figures 21 and 22). The addition of Cd to the soil resulted in a severe decrease in overall growth metrics. In comparison to non-intoxicated plantlets, the root system shrank by -27.45% and -28.6%, while shoot length and shoot dry weight decreased by -23.45% and -26.8%, respectively (P<0.0008). Following inoculation with the different AM fungal inoculums, the tendency was reversed. Treatments Cd_MFP, Cd_MFSG, and Cd_MFSC increased SL by 35.44%, 49.01%, and 49.20%; correspondingly, these treatments increased shoot dry weight by 47.92%, 47.60%, and 68.49%. Mycorrhizal seedlings showed a substantial increase in root development in response to the same observations. Cd_MFSG, Cd_MFP, and Cd_MFSC culminated at up to 48.24%, 47.05%, and 184.41% in root dry weight, respectively, and 23.82%, 46.16%, and 56.03% in root length, respectively, in comparison to the inebriated and non-inoculated seedlings. However, AM fungus treatment, independent of the Cd tolerance index (TI), indicates a higher capacity for cocoa tolerance (67.86%) than Cd treatment alone. The TI values range from 100.98% to 110.99%. With a tolerance score of 110.99%, treatment Cd_MFSC was the best (Figure 23).

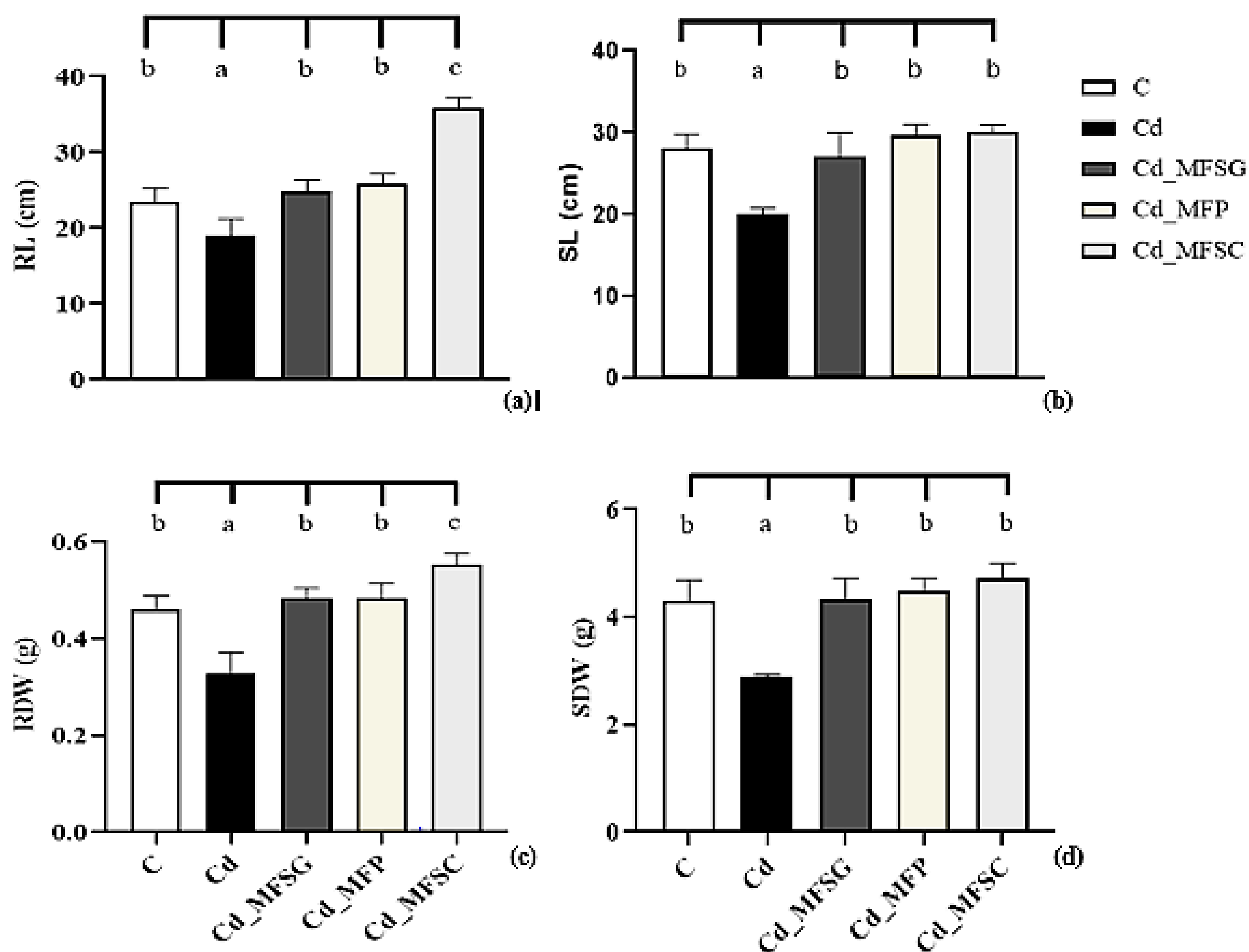


Figure 21: Effects of cadmium intoxication and AMF inoculation on cocoa seedlings growth parameters*:* (a) = root length (RL), (b) = shoot length (SL), (c) = root dry weight (RDW), (d) = shoot dry weight (SDW),

C= unintoxicated and uninfected seedlings, Cd = Soil amended with Cd; Cd_MFSG= Cd amended and inoculation with AMF propagated with sorghum and groundnut (MFSG), Cd_MFP = Cd amended and inoculation with AMF propagated with leek (MFP); Cd_MFSC = Cd amended and inoculation AMF propagated with sorghum and cowpea (MFSC)., Values are means of 3 replicates and expressed in terms of Mean ± SD. Bar charts bearing the same superscript indicate a non-significant difference at *P<0.0008*

Figure 22: *T. cacao* seedling as influenced by cadmium intoxication and AMF inoculation (Ghomsi, 2024)

C= unintoxicated and uninfected total seedlings, Cd = Soil treated with Cd; Cd_MFSG = Cd intoxication and inoculation with AMF propagated with sorghum and groundnut (MFSG), Cd_MFP = Cd intoxication and inoculation with AMF propagated with Leek (MFP); Cd_MFSC = Cd treatment and inoculation AMF propagated with sorghum and cowpea (MFSC).

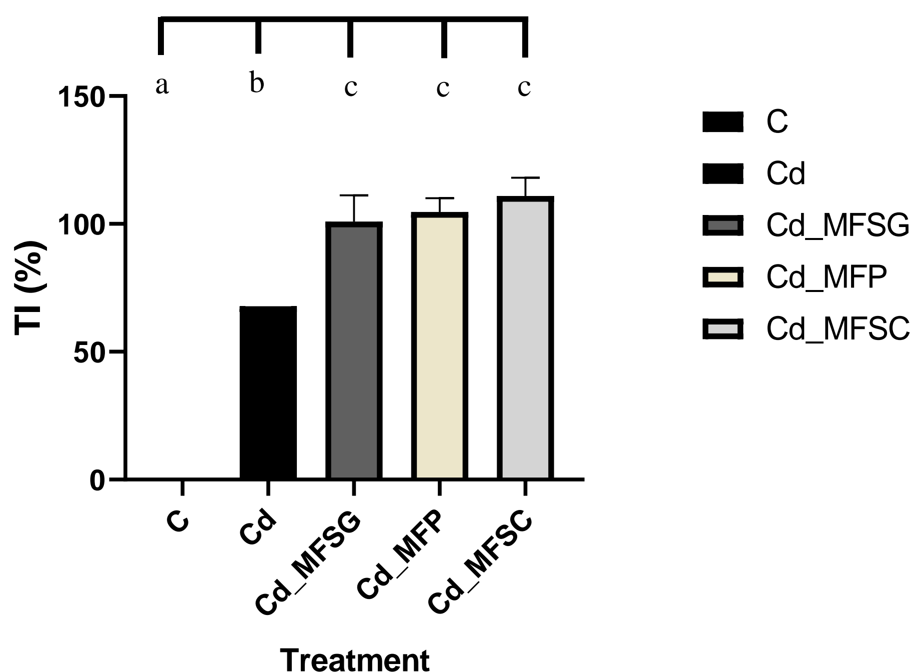


Figure 23: Variation of *T.cacao* tolerance index (TI) as affected by cadmium intoxication and AMF inoculation

C= unintoxicated and uninfected seedlings, Cd = Soil amended with Cd; Cd_MFSG= Cd amended and inoculation with AMF propagated with sorghum and groundnut (MFSG), Cd_MFP = Cd amended and inoculation with AMF propagated with Leek (MFP); Cd_MFSC = Cd amended and inoculation AMF propagated with sorghum and cowpea (MFSC). Values are means of 3 replicates and expressed in terms of Mean ± SD. Bar charts bearing the same superscript indicate a non-significant difference at $P<0.0001$.

III.2.1.4 Effects of cadmium intoxication and AMF inoculation on pigments content

Compared to the control group, treatment with Cd resulted in higher average levels of Chl a, Chl b, carotenoids, and total pigments in cocoa seedlings. Inoculation with MFSG, MFP, or MFSC resulted in a decrease of -12.30%, -7.46%, and -18.91% of Chl a, respectively, for each upgraded inoculum. These mycorrhizal treatments increased Chl b by +51.32%, +10.90%, and +73.16%, respectively. However, MFSG, MFP, and MFSC treatments reduced carotenoids by -36.60%, -11.71%, and -24.10%, respectively, compared to Cd-treated seedlings alone ($P<0.0001$). Cocoa seedlings treated with MFSC showed the highest photosynthetic pigment production (24.15 µg/mg FW) (Table XII).

Table XII: Effects of cadmium intoxication and AMF inoculation on cocoa seedlings photosynthetic pigment content

Pigments	Treatments					
	Control	Cd	Cd_MFSG	Cd_MFP	Cd_MFSC	*P-value*
Chl a ($\mu g.mg^{-1}$ FW)	9.91 ± 0.08^{a}	11.97 ± 0.01^{d}	10.50 ± 0.02^{b}	11.08 ± 0.01^{c}	9.71 ± 0.10^{a}	*<0.0001*
Chl b ($\mu g.mg^{-1}$ FW)	3.82 ± 0.56^{a}	5.52 ± 0.07^{b}	8.35 ± 0.15^{d}	6.12 ± 0.10^{c}	9.56 ± 0.05^{e}	*<0.0001*
Carotenoids ($\mu g.mg^{-1}$ FW)	5.72 ± 0.20^{c}	6.44 ±0.46^{d}	4.08 ± 0.08^{a}	5.68 ±0.25^{c}	4.88 ± 0.19^{b}	*<0.0001*
Total Pigment ($\mu g.mg^{-1}$ FW)	19.45 ± 0.20^{a}	23.92 ± 0.46^{c}	22.93 ± 0.08^{b}	22.88 ± 0.25^{b}	24.15 ± 0.19^{c}	*<0.0001*

Cd = Soil amended with Cd, Cd_MFSG= Cd amendment and inoculation with AMF propagated with sorghum and groundnut (MFSG), Cd_MFP = Cd amendment and inoculation with AMF propagated with Leek (MFP); Cd_MFSC = Cd treatment and inoculation AMF propagated with sorghum and cowpea (MFSC), Chl a = chlorophyll a, Chl b = chlorophyll b, FW: fresh weight. Values are means of 3 replicates and expressed in terms of Mean ± SD. For each parameter, values in each row affected by the same letter are not significantly different between treatments, as given by the Scott-Knott *post-hoc* test at $p<0.0001$.

III.2.1.5 Effects of Cadmium intoxication and AMF inoculation on rhizosphere parameters

⇒ **Effects of cadmium intoxication and AMF inoculation on cocoa seedlings mycorhizosphere acidity and conductivity**

Table XIII illustrates how mycorrhizal inoculation affects the pH and conductivity of cocoa seedlings' rhizosphere. The presence of Cd appears to have induced a considerable rise in pH (+10.82%) and a decrease in conductivity (-52.20%) when compared to the control group. Treatment with our diverse inoculums resulted in pH decreases ranging from -2.27% to -7.93% when compared to Cd treatment alone, but they were considerably greater than the control. However, when compared to Cd treatment alone, conductivity increased by 8.07% to 59.79%.

The Cd_FMSC treatment resulted in the greatest drop in pH (-7.93%), whereas their greater conductivities were reported with the Cd_FMSG therapy (+59.79%), albeit much lower than the control. The findings show that all AM fungus consortiums secrete glomalin under Cd stress conditions, with concentrations ranging from 0.26 to 0.46 mg/kg. Glomalin production varied significantly among AM fungus consortiums, with MFSG producing the most (0.46 µg.g-1 soil).

Table XIII: Variations of mycorrhizosphere acidity, conductivity, and glomalin as affected by cadmium intoxication and AMF inoculation

Parameters	Treatments					
	Control	**Cd**	**Cd_MFSG**	**Cd_MFP**	**Cd_MFSC**	***P-value***
pH	6.22 ± 0.03^{a}	6.90 ± 0.03	6.73 ± 0.03^{c}	6.74 ± 0.03^{c}	6.35 ± 0.02^{b}	*<0.0001*
Conductivity µs (Siemens)	247.16 ± 1.06^{c}	118.13 ± 0.58^{a}	188.76 ± 0.62^{e}	127.67 ± 0.61^{b}	157.89 ± 0.57^{d}	*<0.0001*
Glomalin (µg.g-1 soil)	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.48 ± 0.03^{d}	0.41 ± 0.02^{c}	0.26 ± 0.01^{b}	*<0.0001*

Cd = Soil amended with Cd, Cd_MFSG= Cd amendment and inoculation with AMF propagated with sorghum and groundnut (MFSG), Cd_MFP = Cd amendment and inoculation with AMF propagated with Leek (MFP); Cd_MFSC = Cd treatment and inoculation AMF propagated with sorghum and cowpea (MFSC), Values are means of 3 replicates expressed as Mean ± SD. For each parameter, values in each row affected by the same letter are not significantly different between treatments, as given by the Scott-Knott *post-hoc* test at $p<0.0001$

⇒ **Effects of cadmium intoxication and AMF inoculation on cocoa seedlings on mycorhyzosphere microbiota**

Soil pollution with Cd resulted in a considerable decline (-66.73%) in soil total aerobic mesophyll microbiota compared to the normal control substrate (P<0.0001). Interestingly, soil application of AMF stimulated soil colonization by up to 301.7% to 926%, as referred to Cd-contaminated soil. The inoculum MFSC demonstrated the highest colonization rate (925.98%), whereas the inoculum MFSG was the least effective (301.7%) (Figure 24).

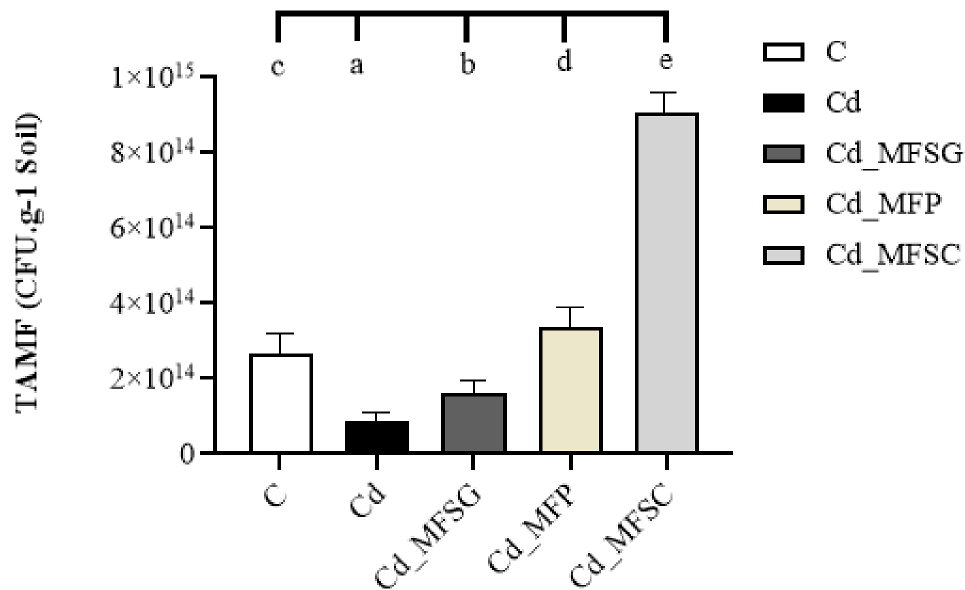


Figure 24: Mycorhizosphere microbial abundance (TAMF) as affected by cadmium intoxication and AMF inoculation

C = unintoxicated and uninfected seedlings, Cd = Soil amended with Cd; Cd_MFSG= Cd amended and inoculation with AMF propagated with sorghum and groundnut (MFSG), Cd_MFP = Cd amended and inoculation with AMF propagated with Leek (MFP); Cd_MFSC = Cd amended and inoculation AMF propagated with sorghum and cowpea (MFSC), CFU = colony forming unit, TAMF = total aerobic mesophyll microbiota. Values are means of 3 replicates and expressed in terms of Mean ± SD. Bar charts bearing the same superscript indicate a non-significant difference between treatments at $P<0.0001$

III.3.2.4 Principal component analysis of AMF treatment and mycorhizosphere parameters

As a consequence of the PCA-associated mycorhizosphere characteristics, case projections on a factor plane with the two principal components (PC) were performed (Figure 25), and the separation efficiency for the key factors was determined. The PCA test found 83.1% of the overall variance, with PC1 and PC2 accounting for 44.7% and 38.4%, respectively. Grouping analysis revealed that Cd_FMSG treatment had a favourable correlation with glomalin, TAMF, and RCF. Treatments with Cd_MFP and Cd_MFSC had a greater impact on soil pH. The soil conductivity, on the other hand, was more closely associated with the control treatment, whereas the Cd treatment influenced all of these factors.

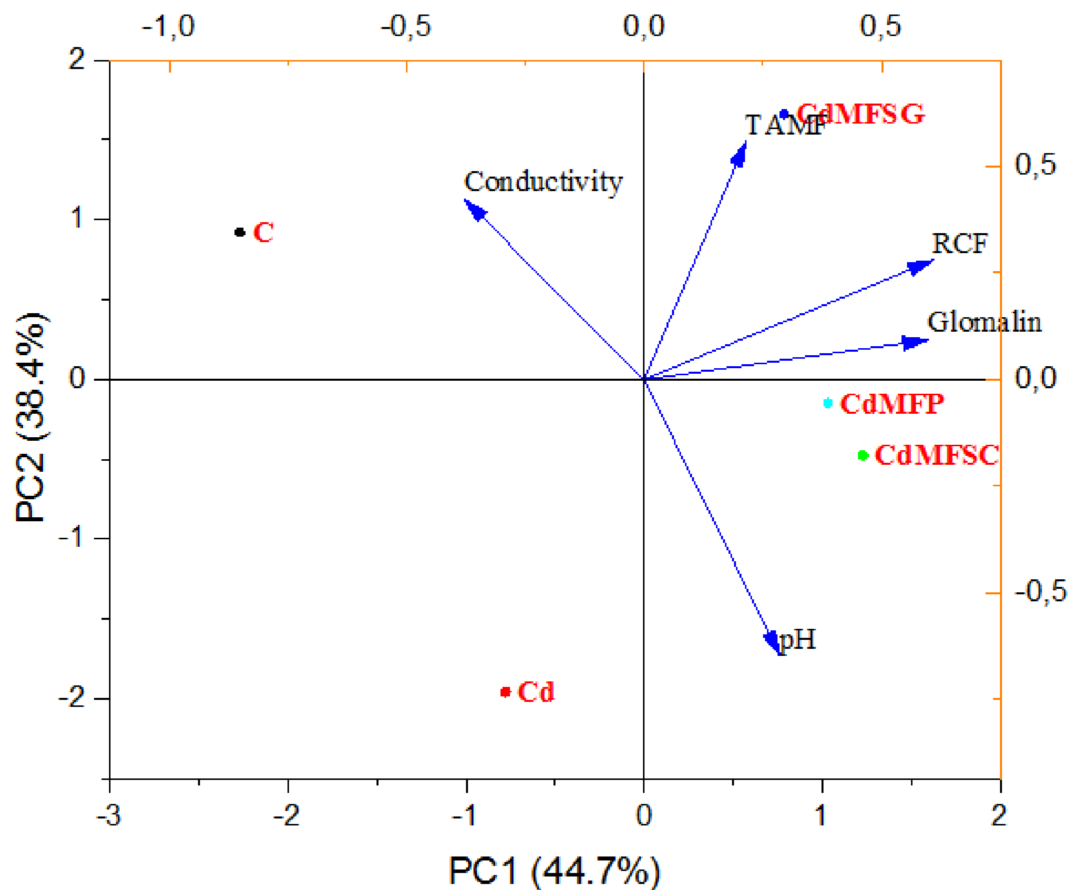


Figure 25: Principal component analysis highlighting the relationships between mycorhizosphere parameters and the various AMF inoculums.

C = unintoxicated and uninfected seedlings, Cd = Soil amended with Cd; Cd_MFSG= Cd amended and inoculation with AMF propagated with sorghum and groundnut (MFSG), Cd_MFP = Cd amended and inoculation with AMF propagated with Leek (MFP); Cd_MFSC = Cd amended and inoculation AMF propagated with sorghum and cowpea (MFSC), TAMF = total aerobic mesophilic flora, RCF = root colonisation frequency, CFU = colony forming unit.

III.2.2 Evaluation of cadmium accumulation profile of selected cocoa genotypes: discussion

Mycorrhizal symbiosis between plant roots is common in natural environments. This relationship involves the exchange of photosynthates and mineral nutrients, enhancing plant growth and resistance to various biotic and abiotic stresses (Eke *et al.,* 2016). Additionally, plant extraction and absorption from subsoil solutions are almost equivalent due to the similar physicochemical properties of heavy metals and soil mineral nutrients (Liu *et al.,* 2022; Sahraoui, 2013). The positive correlation between soil Cd concentration and absorbed

percentage in this study, though expected, provides insight into the potential for Cd accumulation in cocoa plants, as certain species may be resistant.

The findings of the present study provide significant insights into the crucial role that AMF play in alleviating Cd toxicity in cocoa seedlings, illustrating their potential as a viable strategy to overcome crop metal accumulation in environments contaminated with heavy metals. The effective colonization of cocoa roots by various inoculums of AMF, particularly those designated as MFSC and MFSG, even under conditions of Cd stress, highlights the remarkable resilience of these mycorrhizal associations. This observation aligns with a growing body of research that indicates AMF can significantly mitigate environmental stresses through multiple mechanisms, such as enhancing nutrient uptake, promoting root development, and establishing protective barriers that limit the bioavailability and absorption of toxic metals (Nie et al., 2024; Sun et al., 2024). Specifically, the study demonstrated that the inoculation of cocoa seedlings with mycorrhizal fungi led to a marked reduction in the accumulation of Cd, as well as a significant decrease in root-to-shoot translocation of this heavy metal. This is particularly critical, as higher concentrations of Cd in the shoot can lead to detrimental physiological effects, including stunted growth and altered metabolic processes, both of which can compromise plant health and agricultural yield (Garg & Bhandari, 2014; Li et al., 2023). The reduction in Cd translocation corroborates the findings of Huang *et al.,* (2017), which suggested that AMF possess the ability to reduce metal uptake and translocation to above-ground plant parts, thereby protecting the plant from toxicological impacts. For instance, Janoušková *et al*. (2006) found that *G. intraradices* extra-radical hyphae could retain thirty times more Cd than traditional root systems while maintaining equivalent output.

Moreover, the study highlights substantial improvements in various growth metrics associated with AM fungal inoculation, including enhanced shoot length, increased root dry weight, and superior overall biomass accumulation in the mycorrhizal seedlings when compared to non-mycorrhizal counterparts under Cd stress. These findings follow those of Bano & Ashfaq (2013), who extensively documented the growth-promoting effects of mycorrhizal fungi in heavy metal-stressed environments. The physiological assessments carried out during the study further revealed that AMF positively influenced chlorophyll b production, a crucial pigment associated with photosynthesis, while concomitantly reducing the levels of carotenoids, which suggest an adaptive response of the plants to optimize their photosynthetic mechanism under Cd-induced stress conditions. These physiological adjustments are essential for maintaining

photosynthetic efficiency and overall plant vitality in metal-contaminated soils (Bertrand et al., 2001).

In addition to enhancing plant growth and physiological performance, the study provides compelling evidence regarding the impact of AMF on the surrounding soil environment. Notably, the presence of mycorrhizal fungi was associated with significant alterations in rhizosphere properties. The observed effects on pH and conductivity indicate that these fungi participate actively in soil chemical dynamics. Such alterations can create a more favourable microenvironment for plant root expansion and microbial diversity, ultimately supporting improved soil health (Santoyo *et al.,* 2017). In concordance with this, the staggering increase in total aerobic mesophyll microbiota of the mycorhizosphere following AM inoculation strongly implies that mycorrhizal treatments foster a more conducive environment for beneficial soil microorganisms, further corroborating the role of AMF in soil health restoration in Cd-contaminated environments. These results are similar to those of Li *et al.* (2023), who found that the rhizosphere bacterial population drops under heavy metal stress, but the presence of AMF significantly enhances the richness and diversity of the bacterial population. Furthermore, the quantification of glomalin production revealed that AMF not only contribute to improved plant growth but also enhance the structural stability of soils, free radical scavenging activity, and soil metal chelating capacity, restricting therefore the Cd bioavailability and its absorption by roots through the secretion of this important glycoprotein. This was put in evidence in this study were an inoculum (MFSG) producing the highest amount of glomalin was the one inducing the less cadmium translocation factor and accumulation factor. In 2004, Gonzalez-Chavez *et al*. Highlight the importance of glomalin in remediating soil contamination and observed that this protein could stabilize toxic elements reduce their availability and decrease the risk of toxicity to soil microorganisms and plants in contaminated sites. This was corroborated not only by this work but also by various studies showing that the glomalin produced by AMF can make plants more tolerant to metals by reducing their toxicity and availability in soil and plant tissues (Gujre *et al.,* 2021; Qiu *et al.,* 2022; Zhou *et al.,* 2023). The variation among AMF found globally may be due to plant propagation hosts creating unique microenvironments that favour certain fungal species, modifying the richness and composition of fungal communities in tissues and rhizosphere (Broeckling *et al.,* 2008; X.-F. Huang *et al.,* 2014). For example, plant species richness and evenness may increase fungal colonization and diversity, while plant spatial aggregation may decrease it (Boer *et al.,* 2005; Lopes *et al.,* 2021). Plant hosts can also affect fungal behaviour and functionality by altering

metabolism, signalling pathways, and gene expression. Plant hormones can impact fungal proliferation and branching, while defensive chemicals may trigger stress responses or detoxification processes (Marcianò *et al.,* 2021).

The integration of these findings suggests that AMF play a multifaceted role in promoting plant resilience against heavy metal toxicity while simultaneously fostering beneficial soil ecological interactions. Therefore, the findings expand our understanding of sustainable cocoa cultivation practices, particularly in areas where soil contamination is prevalent. By integrating AMF into agroecological systems, cocoa growers could potentially mitigate heavy metal stress effects, improving plant health, safety productivity, and soil quality. Further, the interplay between different fungal formulations and Cd tolerance opens avenues for research into the optimization of specific AMF for diverse agricultural applications.

Partial Conclusion II

This section of the study, focusing on the effects of AMF on Cd stress and accumulation in cocoa seedlings with the highest accumulation propensity, leads to the following conclusions:

- All AMF inoculums have reduced the negative effects of Cd toxicity on cocoa seedling growth.
- In cocoa seedlings, all AMF inoculums have reduced Cd uptake and translocation in shoot, with the Cd_MFSG treatment showing the best performance, resulting in substantial Cd sequestration into root systems.
- All AMF inoculums produced glomalin and positively influenced mycorhizosphere paramaters.

III.3 Biochemical modifications in cocoa seedlings following cadmium intoxication and AMF inoculation

III.3.1 Results

III.3.1.1 Metal-chelating ability and antioxidant capacity

III.3.1.1.1 Changes in metal-chelating ability of cocoa seedlings extract following cadmium intoxication and AMF inoculation

The metal-chelating ability of cocoa seedlings following poisoning with Cd and treatment with AMF is illustrated in Figure 26 below. Following Cd intoxication, cocoa seedlings' metal chelating activity increased significantly (+52.36). Nevertheless, the application of AMF inoculum led to an important raise in this parameter as compared to the Cd treatment alone. These values range from +95.13% to +107.94%. Treatment Cd_MFP reveals the biggest variation (+107.94%).

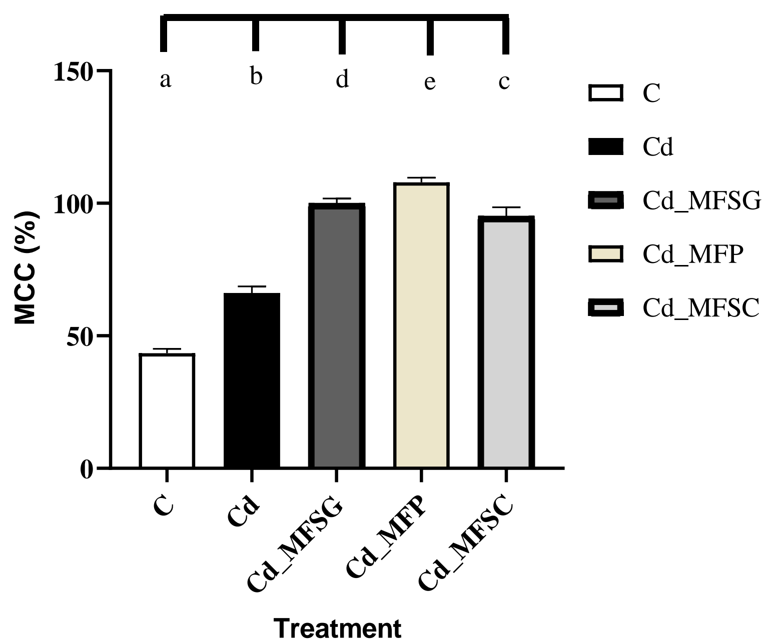


Figure 26: Metal-chelating capacity (MCC) of cocoa seedlings following cadmium intoxication and AMF inoculation

C = unintoxicated and uninfected seedlings, Cd = Soil amended with Cd; Cd_MFSG= Cd amended and inoculation with AMF propagated with sorghum and groundnut (MFSG), Cd_MFP = Cd amended and inoculation with AMF propagated with Leek (MFP); Cd_MFSC = Cd amended and inoculation AMF propagated with sorghum and cowpea (MFSC), CFU = colony forming unit. Values are means of 3 replicates and expressed in terms of Mean ± SD. Bar charts bearing the same superscript indicate a non-significant difference between treatments at *P<0.0001*

III.3.1.1.2 Change in antioxidant activity of cocoa seedling extracts following Cadmium intoxication and AMF inoculation

The antioxidant capacity of cocoa seedling extracts following Cd intoxication and treatment by AMF was evaluated using DPPH scavenging activity and displayed in Figure 27 below. The data obtained throughout the experiment clearly demonstrated that the free radical scavenging activity greatly increased when cocoa seedlings were exposed to Cd stress. An increase of +1.89% in the DPPH scavenging ability of cocoa seedling extracts was found after exposure to Cd stress. However, treatment with mycorrhizal formulations did not significantly affect this activity as compared to Cd treatment alone. Nonetheless, inoculum MFP led to a reduction in antioxidant activity compared to that found in the control treatment.

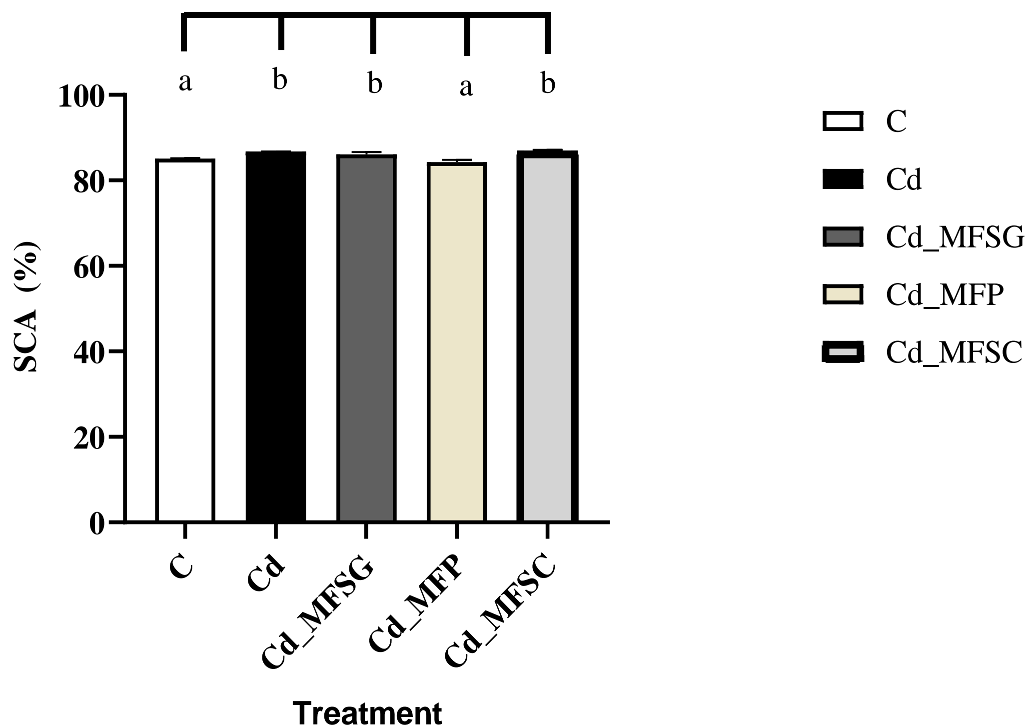


Figure 27: Scavenging activity (SCA) of cocoa seedling extracts following Cd intoxication and AMF inoculation

C = unintoxicated and uninfected seedlings, Cd = Soil amended with Cd; Cd_MFSG= Cd amended and inoculation with AMF propagated with sorghum and groundnut (MFSG), Cd_MFP = Cd amended and inoculation with AMF propagated with Leek (MFP); Cd_MFSC = Cd amended and inoculation AMF propagated with sorghum and cowpea (MFSC). Values are means of 3 replicates and expressed in terms of Mean ± SD. Bar charts bearing the same superscript indicate a non-significant difference between treatments at *P = 0.0159*

III.3.1.2 Effects of cadmium intoxication and AMF inoculation on some host stress-related biomolecules.

III.3.1.2.1 Changes cocoa seedlings soluble sugars

The results achieved (Figure 28) showed that the sugar content was affected by all treatments. Substrate poisoning with Cd resulted in a significant (*P<0.0001*) increase in sugar content (+67.09%). However, all mycorrhizal treatments significantly reduced the sugar content. The treatments Cd_MFSG and Cd-MFSC displayed the greatest decreases, with respective reductions of -67.08% and -68.14% as compared to the Cd treatment alone.

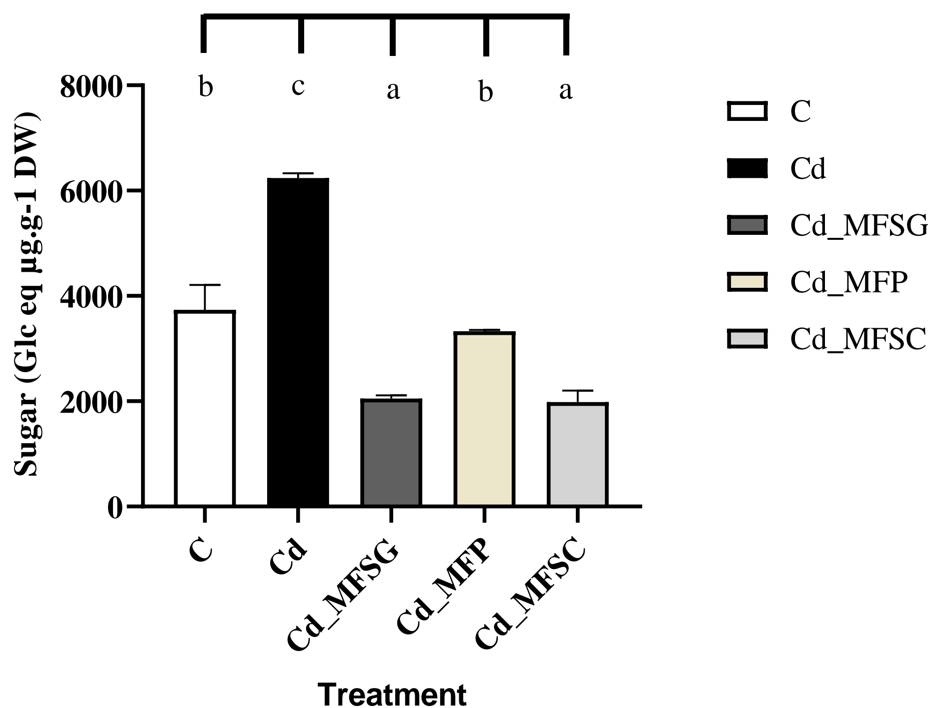


Figure 28: Variation differences in cocoa soluble sugars content as affected by cadmium intoxication and AMF inoculation

C = unintoxicated and uninfected seedlings, Cd = Soil amended with Cd; Cd_MFSG= Cd amended and inoculation with AMF propagated with sorghum and groundnut (MFSG), Cd_MFP = Cd amended and inoculation with AMF propagated with Leek (MFP); Cd_MFSC = Cd amended and inoculation AMF propagated with sorghum and cowpea (MFSC), Glc = glucose. Values are means of 3 replicates and expressed in terms of Mean ± SD. Bar charts bearing the same superscript indicate a non-significant difference between treatments at *P<0.0001*

III.3.1.2.2 Changes in total free amino acids content

The results achieved (figure 29) showed that the presence of Cd in the substrate had highly significant effect (P<0.0001) on the free amino acids content. A significant increase of +226.08% of this parameter was noticed upon amendment with Cd. However, the level of free

amino acids decreased drastically in the presence of mycorrhizal consortium compared to intoxicated and non-treated seedlings by -74.94% for Cd_MFSG, -86.61% for Cd_FMP, and -83.30% for Cd_FMSC.

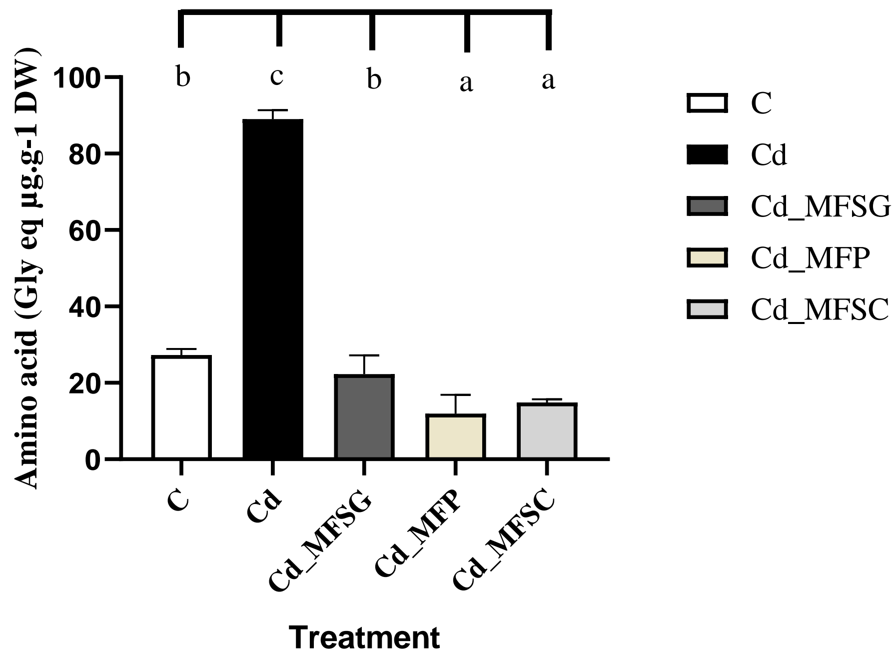


Figure 29: Variation differences in total free amino acids in cocoa as affected by cadmium intoxication and AMF inoculation

C = unintoxicated and uninfected seedlings, Cd = Soil amended with Cd; Cd_MFSG= Cd amended and inoculation with AMF propagated with sorghum and groundnut (MFSG), Cd_MFP = Cd amended and inoculation with AMF propagated with Leek (MFP); Cd_MFSC = Cd amended and inoculation AMF propagated with sorghum and cowpea (MFSC), Gly = glycine. Values are means of 3 replicates and expressed in terms of Mean ± SD. Bar charts bearing the same superscript indicate a non-significant difference between treatments at $P<0.0001$.

III.3.1.2.3 Changes in cocoa seedlings prolin content

The results obtained (Figure 30) showed that the presence of Cd in the substrate had highly significant effect ($P<0.0001$) on the prolin content. A significant increase of +490.36% of this parameter was noticed upon amendment with Cd. However, application of the mycorrhizal consortium led to a significant reduction in prolin content compared to intoxicated and non-treated seedlings (-90.59% for Cd_MFSG, -94.37% for Cd_FMP, and -95.15% for Cd_FMSC). No significant difference was observed between the mycorrhizal treatments, but their values were significantly low compared to the control.

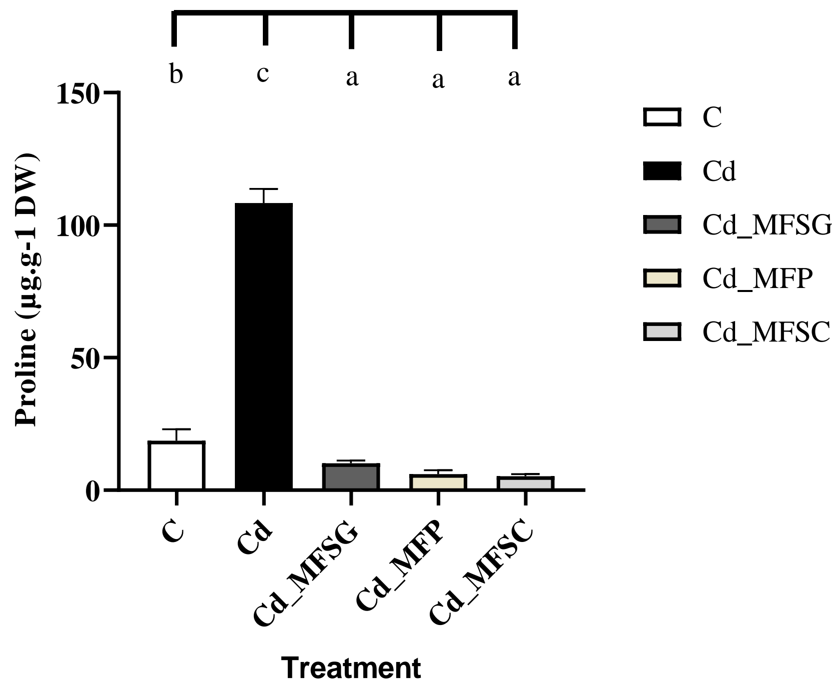


Figure 30: Variation differences in cocoa seedlings prolin content between treatment following cadmium intoxication and AMF inoculation

C = unintoxicated and uninfected seedlings, Cd = Soil amended with Cd; Cd_MFSG= Cd amended and inoculation with AMF propagated with sorghum and groundnut (MFSG), Cd_MFP = Cd amended and inoculation with AMF propagated with Leek (MFP); Cd_MFSC = Cd amended and inoculation AMF propagated with sorghum and cowpea (MFSC). Values are means of 3 replicates and expressed in terms of Mean ± SD. Bar charts bearing the same superscript indicate a non-significant difference at $P<0.0001$.

III.3.1.2.4 Changes in cocoa seedlings malondialdehyde (MDA) content

The changes induced by AMF on the MDA content of cocoa seedlings are shown in Figure 31. The results revealed a significant increase ($P=0.0038$) in MDA content after exposure to Cd stress (+59.07%). Also, upon application of AMF, a significant reduction of this parameter was observed except for the Cd-MFP treatment, which value remained unchanged. The treatments Cd-MFSG and Cd-MFSC induced MDA content reductions of -26.75% and -26.34% respectively, when compared to the Cd treatment.

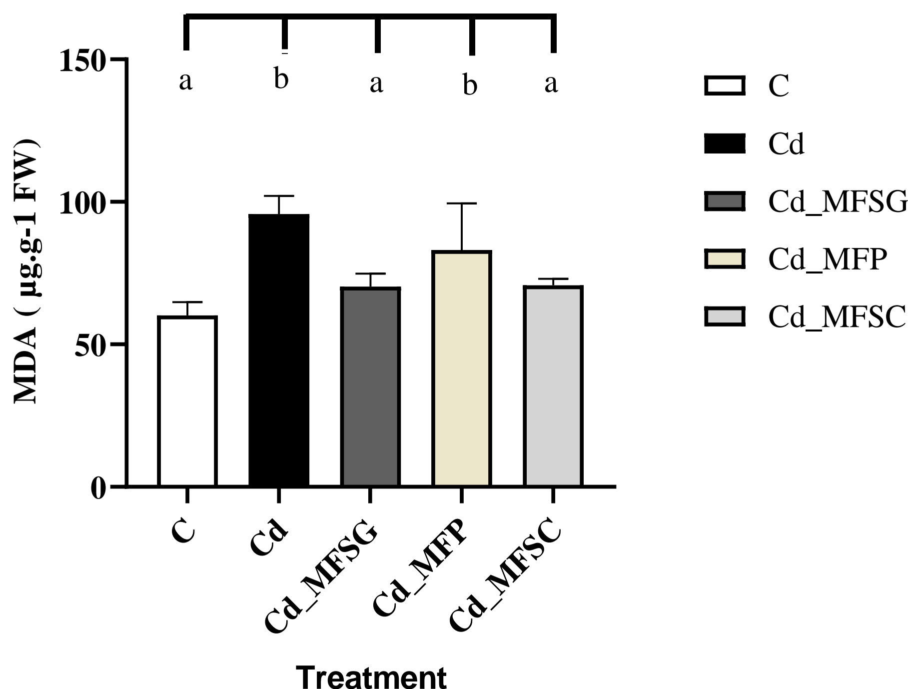


Figure 31: Variation differences in cocoa seedlings malondialdehyde (MDA) content between treatment following cadmium intoxication and AMF inoculation

C = unintoxicated and uninfected seedlings, Cd = Soil amended with Cd; Cd_MFSG= Cd amended and inoculation with AMF propagated with sorghum and groundnut (MFSG), Cd_MFP = Cd amended and inoculation with AMF propagated with Leek (MFP); Cd_MFSC = Cd amended and inoculation AMF propagated with sorghum and cowpea (MFSC), CFU = colony forming unit. Values are means of 3 replicates and expressed in terms of Mean ± SD. Bar charts bearing the same superscript indicate a non-significant difference at *P=0.0038*.

III.3.1.2.5 Changes in phenolic compounds synthesis

Phenols act as metal chelators through hydroxyl and carboxyl groups, as well as lipid peroxidation inhibitors by trapping alkoxyl radicals (Vidal *et al.,* 2020). The results obtained showed no significant change in total phenol content in Cd treatment as compared to the control group (*P<0.0001*). However, treatment with MFSG and MFSP significantly reduced phenolic concentration with -5.89% and -17.46% decreases for each one While MFSC treatment leads to an increase of 7.67% as compared to the Cd treatment alone. Elsewhere, a significant increase in flavonoid content was noted after exposure to Cd. However, inoculums MFSC, MFSG, and MFP significantly reduced the flavonoid content with respective decreases of -13.30%, -36.15%, and -57.74% compared to Cd-intoxicated seedlings. Results clearly show a decrease in total flavonoid compounds upon mycorrhization (Table XIV).

Table XIV: Total phenolic compounds and flavonoids in cocoa seedlings following Cd and AMF inoculation.

Treatment	Phenolic compound ($\mu g.kg^{-1}$ DW)	
	Total soluble phenols (Chl AcEq)	**Soluble flavonoids (QEq)**
Control	10170 ± 113^{c}	5370 ± 112^{c}
Cd	10020 ± 130^{c}	6390 ± 142^{d}
Cd_MFSG	9430 ± 140^{b}	4080 ± 125^{b}
Cd_MFP	8270 ± 187^{a}	2700 ± 117^{a}
Cd_MFSC	10790 ± 311^{d}	5540 ± 114^{c}
P-value	*<0.0001*	*<0.0001*

Cd = Soil amended with Cd, Cd_MFSG= Cd amendment and inoculation with AMF propagated with sorghum and groundnut (MFSG), Cd_MFP = Cd amendment and inoculation with AMF propagated with Leek (MFP); Cd_MFSC = Cd treatment and inoculation AMF propagated with sorghum and cowpea (MFSC), Chl AcEq = chloragenic acid equivalent, QEq = quercetine equivalent. Values are means of 3 replicates expressed as Mean ± SD. For each parameter, values in each row affected by the same letter are not significantly different between treatments, as given by the Scott-Knott *post-hoc* test at the given *P-value*

III.3.1.3 Principal component analysis depicted interaction between AMF and plants metabolites

The PCA analysis identified 86.7% of the total variation, and PC1 and PC2 accounted for 58.6% and 28.1%, respectively as indicated in Figure 32. Grouping analyses indicated that Cd treatment affect preferentially prolin free amino acid, sugars and MDA. Treatments Cd_FMSG and Cd_FMP acted more on scavenging ability (SCA) as well as control treatment. The Cd_FMSG treatment displayed a strong influence on proteins content and metal chelating capacity.

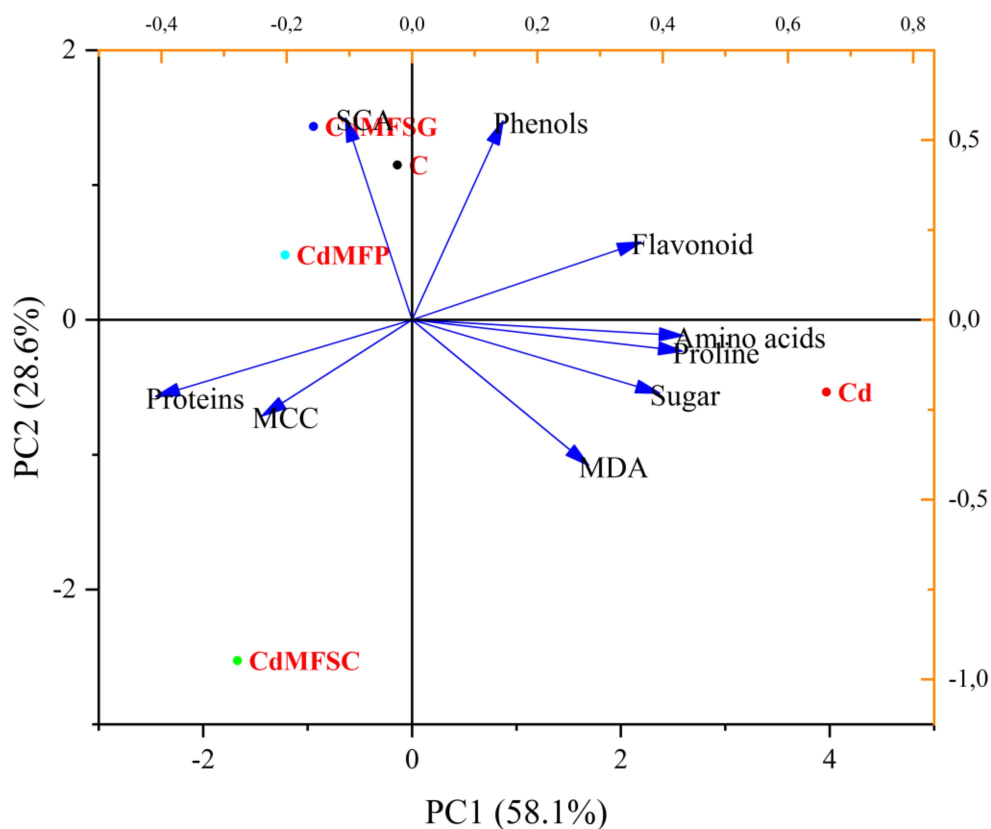


Figure 32: Principal component analysis highlighting the relationships between plant metabolites and the various AMF formulations.

C = unintoxicated and uninfected seedlings, Cd = Soil amended with Cd; Cd_MFSG= Cd amended and inoculation with AMF propagated with sorghum and groundnut (MFSG), Cd_MFP = Cd amended and inoculation with AMF propagated with Leek (MFP); Cd_MFSC = Cd amended and inoculation AMF propagated with sorghum and cowpea (MFSC), MDA = malondialdehyde, SCA = scavenging activity, MCC = metal chelating capacity.

III.3.1.4 Mycorrhization altered the activity of selected destressing enzymes and soluble protein in *T. cacao* seedlings.

The data indicated significantly higher activities of SOD, GPX, APX, PPO, PAL, catalase and fumarase when compared to control (stress-free seedlings), suggesting that the plantlets' inherent defence mechanism was boosted (Table XV). Regardless of the enzyme, mycorrhization resulted in a significant drop in the specific activities of SOD, GPX, PPO, PAL, catalase and fumarase enzymes, attaining -43.04%, -99.74%, -76.30%, -21.73%, -71.48% and -77.77%, respectively with the MFSG treatment as compared to Cd treatment. However, with

APX, reductions of -82.00% and -85.02% where recorded with MFSG and MFSC treatments whereas an increase of +103.27% was achieved with MFP treatment.

Table XV: Specific activities of SOD, APX, GPX, Catalase, PPO, PAL, and Fumarase in Cocoa seedlings following cadmium intoxication and AMF inoculation

Enzymes	Enzymes activity per treatment					
	Control	**Cd**	**Cd_MFSG**	**Cd_MFP**	**Cd_MFSC**	***P-value***
Protein (mg.g^{-1} FW)	3.68 ± 0.09^{b}	3.23 ± 0.13^{a}	3.91 ± 0.10^{c}	4.14 ± 0.12^{c}	3.73 ± 0.13^{b}	*<0.0001*
SOD (Log SOD units.mg^{-1} Pr)	4.47 ± 0.01^{a}	4.66 ± 0.02^{b}	4.41 ± 0.10^{a}	4.49 ± 0.07^{a}	4.49 ± 0.04^{a}	*0.0014*
GPX (µmol.min^{-1}.mg^{-1} Pr)	0.13 ± 0.03^{a}	42.77 ± 5.18^{c}	0.11 ± 0.02^{a}	31.45 ± 6.74^{b}	50.83 ± 3.41^{c}	*<0.0001*
Catalase (µmol.min^{-1}.mg^{-1} Pr)	3.71 ± 0.19^{a}	15.99 ± 3.24^{b}	5.00 ± 0.38^{a}	55.22 ± 2.75^{c}	4,56 ± 0.78^{b}	*<0.0001*
APX (µmol.min^{-1}.mg^{-1} Pr)	8.79 ± 1.13^{a}	36.48 ± 7.73^{b}	6.57 ± 2.17^{a}	74.16 ± 2.41^{b}	5.46 ± 0.69^{a}	*<0.0001*
PPO (µmol.min^{-1}.mg^{-1} Pr)	0.287 ± 0.021^{a}	1.236 ± 0.031^{b}	1.132 ± 0.093^{b}	0.293 ± 0.047^{b}	1.069 ± 0.111^{b}	*<0.0001*
PAL (µmol.min^{-1}.mg^{-1} Pr)	0.48 ± 0.03^{a}	0.82 ± 0.04^{d}	0.62 ± 0.05^{b}	0.63 ± 0.06^{b}	0.70 ± 0.18^{c}	*0.0013*
Fumarase (µmol.min^{-1}.mg^{-1} Pr)	0.06 ± 0.02^{a}	0.27 ± 0.01^{b}	0.07 ± 0.02^{a}	0.05 ± 0.02^{a}	0.06 ± 0.01^{a}	*<0.0001*

Cd = Soil amended with Cd, Cd_MFSG= Cd amendment and inoculation with AMF propagated with sorghum and groundnut (MFSG), Cd_MFP = Cd amendment and inoculation with AMF propagated with Leek (MFP); Cd_MFSC = Cd treatment and inoculation AMF propagated with sorghum and cowpea (MFSC), PAL = phenylalanine amonialyase, PPO = polyphenol oxidase, SOD, superoxide dismutase, GPX = guaiacol peroxidase, APX = ascorbate peroxidase, Pr = protein, FW: fresh weight. Values are means of 3 replicates expressed as Mean ± SD. For each parameter, values in each row affected by the same letter are not significantly different between treatments, as given by the Scott-Knott *oc* test at the give*post-h*n *P-value*

III.3.1.5 Principal component depicted variations of AMF treatment and biochemical parameters

As a consequence of the PCA, case projections on a factor plane with the two principal components (PC) were done (Figure 33), and the separation efficiency for the key factors was evaluated. The PCA test detected 85.3% of the overall variation. PC1 and PC2 accounted for 52.0% and 33.3%, respectively. Grouping analysis revealed that PPO, SOD, and fumarase activities were more influenced by Cd and Cd_FMSG treatments. All the characteristics that were taken into consideration showed that the Cd_FMSC and Cd_FMP treatments, as well as the control treatment, acted in the same way.

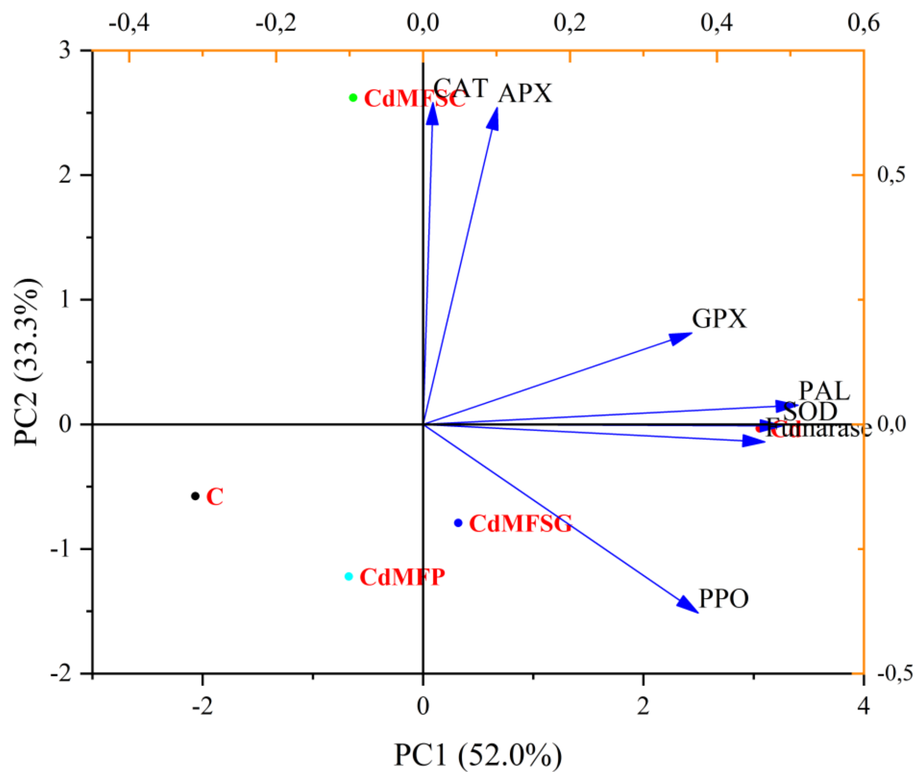


Figure 33: Principal component analysis highlighting the relationships between plant metabolites and the various AMF inoculums.

C = unintoxicated and uninfected seedlings, Cd = Soil amended with Cd; Cd_MFSG= Cd amended and inoculation with AMF propagated with sorghum and groundnut (MFSG), Cd_MFP = Cd amended and inoculation with AMF propagated with Leek (MFP); Cd_MFSC = Cd amended and inoculation AMF propagated with sorghum and cowpea (MFSC PAL = phenylalanine amonialyase, PPO = polyphenol oxidase, SOD, superoxide dismutase, GPX = guaiacol peroxidase, APX = ascorbate peroxidase, CAT = catalase.

III.3.1.6 Correlation between parameters following cadmium intoxication and AMF inoculation

As a result of the correlation analysis, the result showed a positive correlation between Cd concentration in shoot and activities of PAL (R= 1), PPO (R= 0.7), catalase (R= 0.5), SOD (R= 0.7), GPX (R= 0.8) as well as MDA (R= 0.9) and soil pH (R= 0.7). The same observations were made between these parameters and TF as well as AF. Also, flavonoids presented a positive correlation with PPO (R= 0.90) and SOD (R= 0.82) activities. Moreover, soil microbial biomass (TAMF) and mycorrhizal colonisation (RCF) significantly correlated with growth parameters. However, a significant negative correlation was established between primary metabolites (free soluble sugar, amino acid and prolin) and growth parameters (Figure 34).

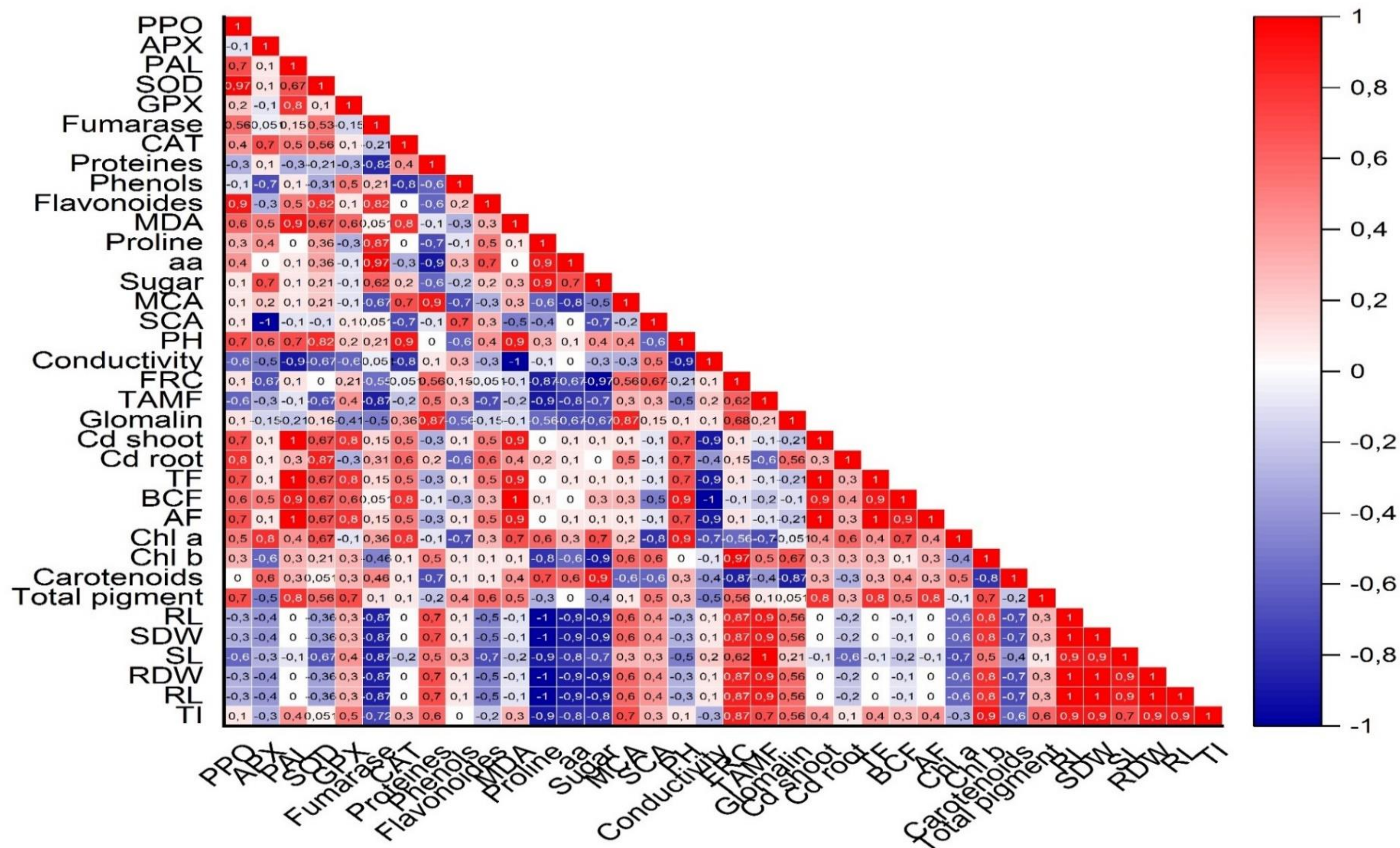


Figure 34: Correlation coefficients between the investigated parameters following Cd intoxication and AMF inoculation

PAL = phenylalanine amonialyase, PPO = polyphenol oxidase, SOD, superoxide dismutase, GPX = guaiacol peroxidase, APX = ascorbate peroxidase, CAT = catalase, Chl a = chlorophyll a, Chl b = chlorophyll b, MDA= malondialdehyde, aa= amino acid, SCA= DPPH scavenging activity, MCA- metal chelating capacity, RL = root length, SL = shoot length, BCF = bioconcentration factor, TF = translocation factor, AF = accumulation factor, TI= translocation index, TAMF= soil total aerobic mesophilic flora.

III.3.2 Biochemical modifications in cocoa seedlings following AMF inoculation and cadmium exposition: discussion

Cadmium is a non-essential heavy metal that causes toxicity and oxidative stress in cocoa plants. It can disrupt several physiological and biochemical processes, including photosynthesis, mineral nutrition, antioxidant defence, and gene expression (Oliveira *et al.,* 2022; Shahid *et al.,* 2017). Plants have evolved various strategies to cope with Cd stress, such as reducing Cd absorption, sequestering Cd into vacuoles, chelating Cd with phytochelatins or metallothioneins, and activating various antioxidants and metabolites (Narayanan & Ma, 2023).

The results of this study reveal that Cd stress increases DPPH radical scavenging activity and metal chelating capacity in cocoa seedlings, indicating enhanced antioxidant and metal chelating activities. This is associated with a significant rise in sugar concentration following Cd exposure, highlighting their importance in the plant's defence mechanism. Sugars serve as energy sources, osmolytes, signalling molecules, and precursors of other metabolites, supporting the synthesis of antioxidants and other defensive molecules during Cd stress (Sami *et al.,* 2016). They help regulate osmotic potential and water status in plant cells and influence the expression of genes involved in stress response and adaptation (Saddhe *et al.,* 2021). Prolin, a key metabolite involved in Cd stress tolerance, acts as an osmolyte, metal chelator, free radical scavenger, and signalling molecule. It protects cellular membranes from lipid peroxidation, maintains cellular redox balance, stabilizes protein structures and functions, and alters the expression of stress-responsive genes (Siripornadulsil *et al.,* 2002; Verslues & Sharma, 2010). An increase in prolin and free amino acids following Cd exposure, consistent with Gerami *et al*. (2021b), who reported enhanced prolin and soluble sugar synthesis in sorghum after Cd poisoning. Phenolic substances, including phenolic acids, flavonoids, lignin, and tannins, also play a role in Cd stress tolerance. They function as antioxidants, metal chelators, and modulators of plant growth and development. Phenolic compounds scavenge reactive oxygen species (ROS) generated by Cd stress, bind Cd ions, reduce their availability and toxicity, and modulate enzyme activity involved in plant defence (Le Thi *et al.,* 2020). Going in the same line an increase in flavonoids in cocoa seedlings exposed to Cd stress were recorded but the amount decrease significantly in the presence of AMF which reduce Cd absorption and therefore the stress in cause of increase in flavonoids production. Also, Malondialdehyde (MDA), a marker of oxidative stress induced by Cd, results from lipid

peroxidation and can disrupt cellular membranes, proteins, and nucleic acids, leading to further ROS generation and oxidative damage (Nair *et al.,* 1986). The high MDA levels in intoxicated cocoa seedlings reflect the extent of oxidative stress and damage caused by Cd. Similar findings were reported by Li *et al*. (2013) in *Pistia stratiotes* L. treated with Cd. Also, the increased of antioxidant activity in cocoa seedlings in response to Cd intoxication observed in this study confirm that Cd intoxication leads to an increase of ROS production. In fact, detoxification of ROS or Cd ions in plants involves various enzymes. An observation of a significant increase in activity of enzymes like superoxide dismutase (SOD), catalase (CAT), guaiacol peroxidase (GPX), ascorbate peroxidase (APX), polyphenol oxidase (PPO), phenylalanine ammonialyase (PAL), and fumarase following Cd exposure. These enzymes catalyze processes that transform ROS into less harmful compounds or chelate Cd ions with organic acid derivatives, enhancing Cd tolerance in plants (Labidi *et al.,* 2021; Manquián-Cerda *et al.,* 2018; Yousefi *et al.,* 2018). AMF therapy significantly reduced these biochemical markers, conferring biotic and abiotic stress tolerance by regulating plants' physiological and molecular responses (Gerami *et al.,* 2021). AM fungal inoculation minimises Cd accumulation in plant tissues by reducing root-to-shoot translocation or enhancing sequestration into fungal structures, boosting plants' antioxidant systems, and altering antioxidant enzyme activities under Cd stress (Zulfiqar *et al.,* 2022). It also modifies the production of various metabolites, such as amino acids, organic acids, and phytohormones (Gerami *et al.,* 2021). This supports the hypothesis that AMF improve Cd tolerance by secreting organic acids and glomalin, which chelate and sequester Cd in the soil, reducing its bioavailability (Adeleke *et al.,* 2017; Malekzadeh *et al.,* 2016).

The varying responses of AMF inoculums could be due to the activation of fungal silent genes mediated by different propagation hosts. Co-cultivation with ecosystem microbes can knock out silent gene clusters and identify novel compounds (Brakhage & Schroeckh, 2011). Host plants can downregulate microbial genes through silencing RNAs or transgenic foldback RNA transcription (Baulcombe, 2015). Host plants can also cause gene silencing in associated organisms (Hartmann *et al.,* 2020). Phytohormones secreted in root exudates can induce mycorrhizae development and enhance AM fungal efficiency, varying with the host (Liao *et al.,* 2018). Root exudates influence the expression of metabolic, transport, regulatory, and stress-responsive genes in rhizosphere microbes, highlighting plant-microbe interactions (Ma *et al.,* 2022; Mavrodi *et al.,* 2021).

Partial Conclusion III

This section of the study, focusing on the biochemical changes in cocoa seedlings following AM fungal inoculation and Cd exposure, leads to the following conclusions:

- AMF inoculums improved metal chelation and globally maintain ROS scavenging activity in cocoa seedlings.
- AM fungal inoculums regulated metabolite content in cocoa seedlings.
- AM fungal inoculums modulated the activities of distressing enzymes affected by Cd stress.

GENERAL CONCLUSION

AND

PERSPECTIVES

GENERAL CONCLUSION AND PERSPECTIVES

General conclusion

Following completion of this study that aimed at investigating the effects of AMF on Cd accumulation by cocoa seedling genotypes with high accumulating profile, the following concluding remarks can be highlighted:

- Cocoa genotypes were found to be affected differentially by Cd stress, and their accumulation varied depending on the varieties investigated.
- All AMF inoculants elicited an alleviation of Cd stress as well as a reduction in the translocation of Cd and their accumulation in shoot. The most efficient inoculum was MFSG with a reduction of translocation rate of 90 %.
- All AMF inoculants elicited an overall regulation of stress resistance biochemical markers incidence.

Future perspectives

Following this initial demonstration of the beneficial traits of AMF in alleviating Cd pressure incidence in cocoa seedlings, we anticipate to perform the following specific aims:

- To realize a comparative study of the transcriptome of cocoa varieties with contrasting Cd absorption profiles in order to identify the genes responsible for the observed variations on growth and Cd accumulation.
- To decrypt the effect of the best AMF on the expression of genes identified in clones with high accumulation profiles.
- To evaluate the effect of AMF inoculants on the accumulation of Cd in cocoa beans.

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APPENDICES

APPENDICES

Appendice 1: Tolerence index (TI) Amomg Cocoa genotype

Cooa genotype	***SNK10*IMC67***	***SNK13*ICS95***	***UPA143*SNK64***	***IMC67*SNK109***	***BBK1418*MO20***	***ICS1*SNK37***
TI (%)	79.37 ± 2.72^{d}	65.45 ± 1.70^{a}	83.08 ± 0.68^{e}	89,53 ± 1,12^{f}	109.22 ± 1.89^{h}	87.87 ± 0.88^{f}
Cooa genotype	***T79/501*SNK13***	***SNK16*T60/1174***	***T79/501*SNK109***	***UPA143*SNK64***	***T60/887*SNK64***	***T79/501*SNK16***
TI (%)	83,19 ± 1.88^{e}	81.43 ± 2.51^{d}	74.32 ± 4.10^{c}	90.29 ± 1.59^{f}	84.35 ± 0.80^{e}	70.28 ± 1.12^{b}
Cooa genotype	***SNK64*T40/1170***	***T60/1174*UPA134***	***SCA12*ICS40***	***SNK16*UPA143***		
TI (%)	89.45 ± 1.22^{f}	87.74 ± 0.77^{f}	84.79 ± 0.49^{e}	68.80 ± 0.81^{b}		

In comparison to the control conditions, the negative values represented decreasing percentages of the investigated parameters. Letters in the same row in red and the others in the same colon in black with different mea nings specify statistically significant differences ($p \leq 0.01$) using RScott Knott multiple range test. The values are presented by mean ±standard error. The values shown are the averages of eight separate measurements.

Appendice 2 : *T. cacao* growth parameter following Cd intoxication and AMF treatments.

Growth parameters	Treatments				
	Control	Cd	Cd_MFSG	Cd_MFP	Cd_MFSC
RDW	0.46 ± 0.03^{b}	0.33 ± 0.04^{a}	0.48 ± 0.02^{b}	0.49 ± 0.04^{c}	0.55 ± 0.02^{c}
RL	23.50 ± 1.27^{b}	19.00 ± 2.65^{a}	24.92 ± 1.06^{b}	25.87 ± 1.91^{b}	35.83 ± 1.01^{c}
SDW	4.30 ± 0.47^{b}	2.89 ± 0.05^{a}	4.32 ± 0.49^{b}	4.49 ± 0.29^{b}	4.73 ± 0.33^{b}
SL	28.50 ± 1.74^{b}	20.23 ± 0.81^{a}	26.63 ± 3.26^{b}	30.33 ± 0.21^{b}	30.37 ± 0.91^{b}
TI (%)	/	67.86 ± 0.00^{a}	100.98 ± 10.19^{b}	104.69 ± 5.40^{c}	110.99 ± 7.04^{c}

Cd = Soil amended with Cd, Cd_MFSG= Cd amendment and inoculation with AMF propagated with sorghum and groundnut (MFSG), Cd_MFP = Cd amendment and inoculation with AMF propagated with Leek (MFP); Cd_MFSC= Cd treatment and inoculation AMF propagated with sorghum and cowpea (MFSC). Values are means of 3 replicates and expressed in terms of Mean ± SD. Mean values in each column, superscripted with the same letter (s) are not significantly different, as given by the Scott-Knott *post-hoc* test at $P<0.0001$.

Appendice 3: Plant metabolites content following Cd intoxication and AMF treatments.

Plant metabolite	Treatments				
	Control	Cd	Cd_MFSG	Cd_MFP	Cd_MFSC
MDA (µg.g-1 FW)	60,22 ± 4.60[a]	95,79 ± 6.32[b]	70,32 ± 4.49[a]	83,10 ± 6.36[b]	70,71 ± 2.24[a]
Prolin ($\mu mol.min^{-1}.mg^{-1}$ Pr)	18.34 ± 4.34[b]	108.31 ± 5.38[c]	10.19 ± 1.01[a]	6.09 ± 0.1.44[a]	5.25 ± 0.79[a]
Free amini acid (Gly eq µg.g-1 DW)	27.30 ± 1.59[b]	89.02 ± 2.31[c]	22.30 ± 4.92[b]	11.92 ± 4.93[a]	14.87 ± 0.83[a]
Sugar (Glc eq µg.g-1 DW)	3734.98 ± 471.39[b]	6240.80 ± 88.51[c]	2053.99 ± 58.87[a]	3328.91 ± 24.11[b]	1987.24 ± 217.33[a]

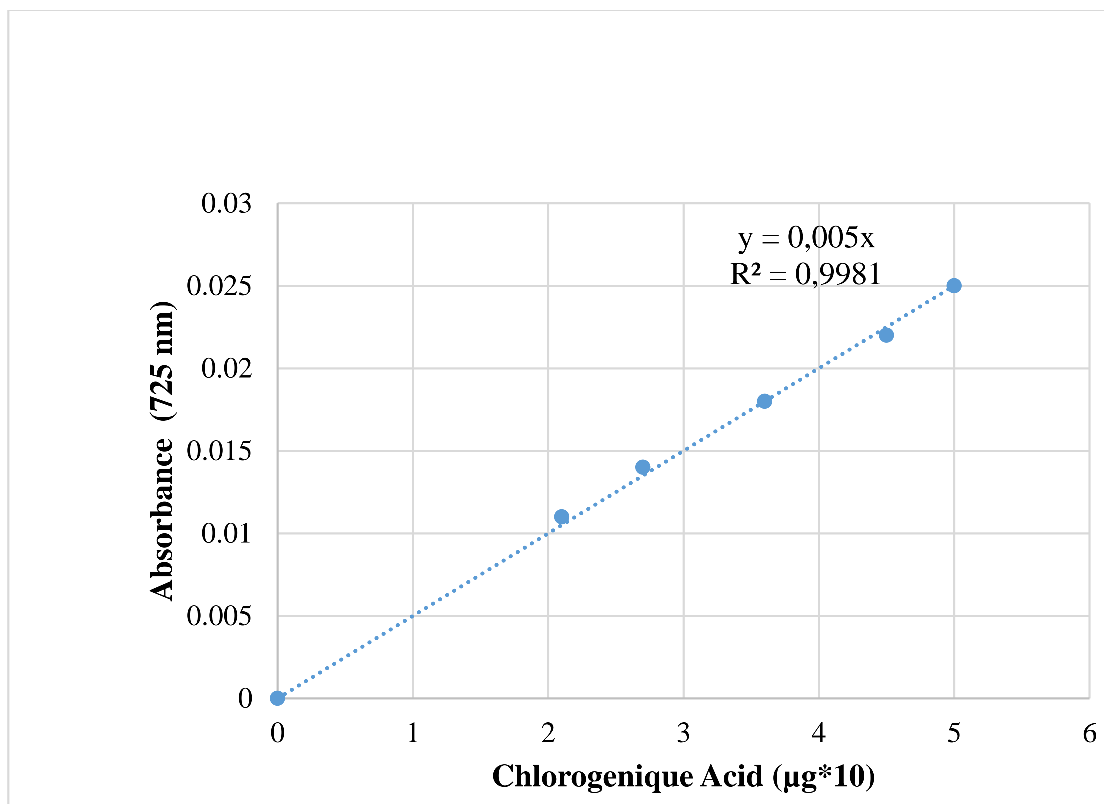
Cd = Soil amended with Cd, Cd_MFSG= Cd amendment and inoculation with AMF propagated with sorghum and groundnut (MFSG), Cd_MFP = Cd amendment and inoculation with AMF propagated with Leek (MFP); Cd_MFSC= Cd treatment and inoculation AMF propagated with sorghum and cowpea (MFSC). Values are means of 3 replicates and expressed in terms of Mean ± SD. Mean values in each column, superscripted with the same letter (s) are not significantly different, as given by the Scott-Knott *post-hoc* test at $P<0.0001$.

Appendice 4: Some activities of cocoa extract following Cd intoxication and AMF treatments

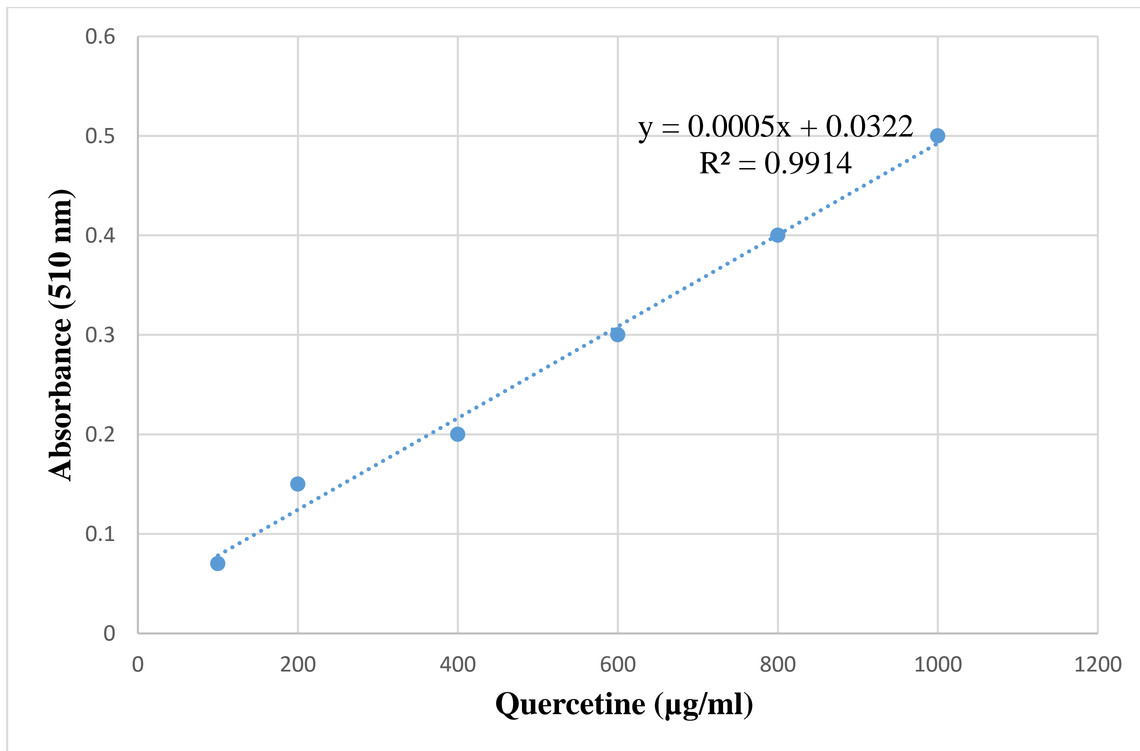
Plant extract capacity	Treatments				
	Control	**Cd**	**Cd_MFSG**	**Cd_MFP**	**Cd_MFSC**
MCC (%)	43.43 ± 1.61^{a}	66.18 ± 2.49^{b}	99.93 ± 1.85^{d}	107.94 ± 1.72^{e}	95.13 ± 3.41^{c}
SCA (%)	85.96 ± 1.48^{a}	85.08 ± 0.59^{b}	86.75 ± 0.62^{b}	84.28 ± 0.51^{a}	86.77 ± 0.41^{b}

Cd = Soil amended with Cd, Cd_MFSG= Cd amendment and inoculation with AMF propagated with sorghum and groundnut (MFSG), Cd_MFP = Cd amendment and inoculation with AMF propagated with Leek (MFP); Cd_MFSC= Cd treatment and inoculation AMF propagated with sorghum and cowpea (MFSC). Values are means of 3 replicates and expressed in terms of Mean ± SD. Mean values in each column, superscripted with the same letter (s) are not significantly different, as given by the Scott-Knott *post-hoc* test at $P<0.0001$

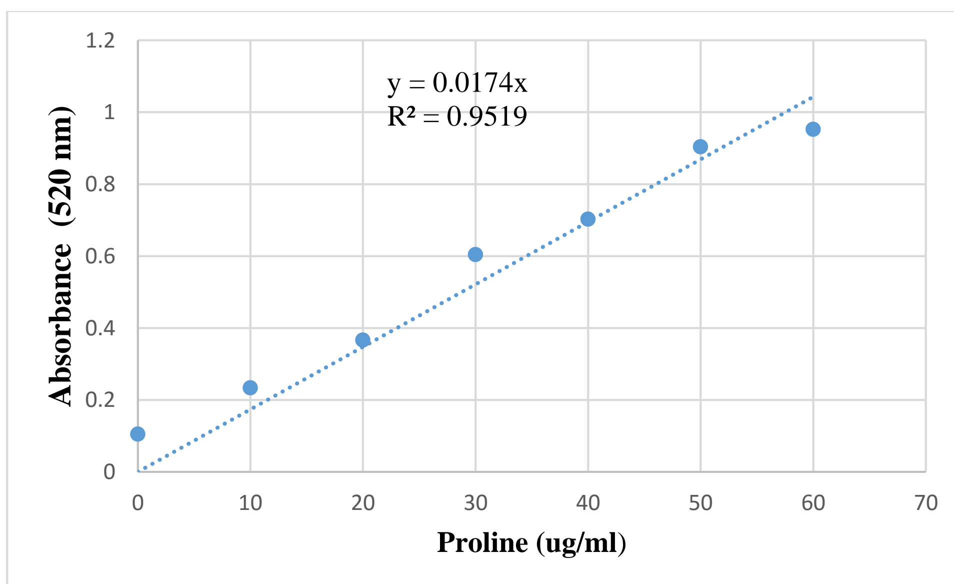
Appendice 5: Chloragenic acid satandard cuve for phenols estimation



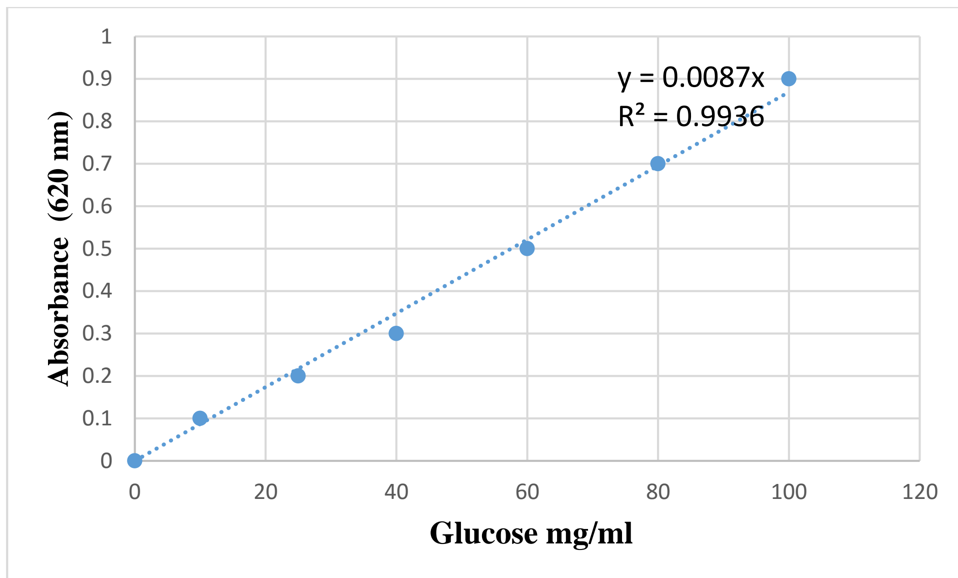
Appendice 6: Quercetine satandard cuve for flavonoids estimation



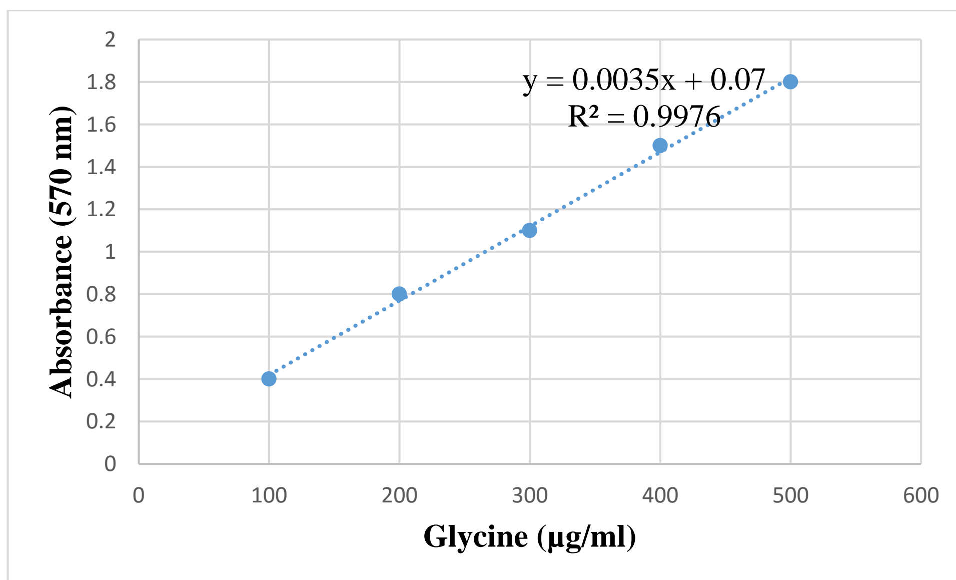
Appendice 7: Prolin satandard cuve for prolin estimation



Appendice 8: Glucose satandard cuve for sugar estimation



Appendice 9: Glycine satandard cuve for free amino acid estimation



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