The background image is a landscape photograph of a river basin. In the foreground, there is a field of dry, brownish vegetation. A bright green strip, possibly a grassy path or a small stream, runs diagonally across the middle ground. Beyond this, there are terraced fields in various shades of brown and green. In the far background, there are rolling hills and mountains under a clear sky.

MOBILITY OF GEOGENIC CHROMIUM IN ASOPOS RIVER BASIN

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TECHNICAL UNIVERSITY OF CRETE

SCHOOL OF ENVIRONMENTAL ENGINEERING

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ENVIRONMENTAL AND SANITARY ENGINEERING

HYDROGEOCHEMICAL ENGINEERING AND
REMEDICATION OF SOILS LABORATORY

Ph.D. Thesis

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BASIN**

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ABSTRACT

The present thesis investigates the mobility and controlling mechanisms of chromium (Cr) release from soils obtained from an area of wide spread geogenic contamination. The objectives of this study were (1) to assess the geochemical characterization and classification of soils and river sediments with respect to the origin of Cr along Asopos river, (2) to assess the processes affecting the mobility of Cr(VI) in soils and sediments along Asopos river and to elucidate the mechanisms of Cr release from soils and (3) to develop a methodology to assess the impact of geogenic origin Cr(VI) uptake by agricultural products (specifically carrots), and the risk of human consumption of carrots grown in Asopos River basin in Greece.

In Chapter 1 a comprehensive review on Cr and its presence in the environment, and specifically in soil and plant system is given. Moreover, the study area and the framework of analysis of this thesis are drawn.

Chapter 2 presents the published article in the Journal of Hazardous Materials entitled: *Characterization and mobility of geogenic chromium in soils and river bed sediments of Asopos basin*. A field and laboratory study was conducted to assess the origin and mobility of Cr(VI) in Asopos basin. Sampling was designed in such way as to capture the spatial variability of Cr occurring in sediments and soils in different lithological units in the area. Physicochemical and geochemical characterization of surface agricultural soils obtained from river terraces and river bed sediments was conducted in order to determine the natural background of chromium. Lithologies with strong calcareous, siliceous and ultramafic components were identified using principal component analysis. Laboratory mobility studies quantified the rates of Cr sorption and release from soils and their capacity to adsorb Cr. Heavy metal analysis and local geology study support the hypothesis that the main source of Cr is of geogenic origin. Cr distribution in Asopos river bed was influenced from the eroded products derived from extensive areas with ultramafic rocks the last 5 Ma. The mobility studies showed that leaching process was very fast and sorption capacity was significant and capable to retain chromium in case of waste release in the river. Finally the mobility of Cr release is limited due to existing attenuation capacity controlled by ferric oxides coatings on the soil and sediments.

Chapter 3 presents the submitted article entitled: *Identifying the controlling mechanism of geogenic origin chromium release in soils*. A laboratory study was conducted to assess the mobility and mechanisms of Cr release from soils obtained from Asopos river basin. The agricultural soil sample used in this study was taken from the Schimatari area in Asopos river basin. In order to refine the isolation of minerals contained in the soil, two types of separation analysis were conducted. First, a size fractionation with hydrocyclone and second, a weight fractionation with heavy liquids. The separated fractions were characterized using chemical, mineralogical and surface analysis. The results provided consistent evidence that the heavy fraction of the soil is related directly to the mobile fraction of Cr. At acidic pHs, the clay-sized fraction also plays an additional important role in the mobility of Cr, due to the fact that this fraction has high surface area and Cr reactivity index. In addition, pH-edge leaching studies showed a high correlation between Cr–Ni, Cr–Mn and Cr–Y released from the soil which also suggests that the mobility of Cr is controlled by chromite weathering which is the case observed in Asopos river basin.

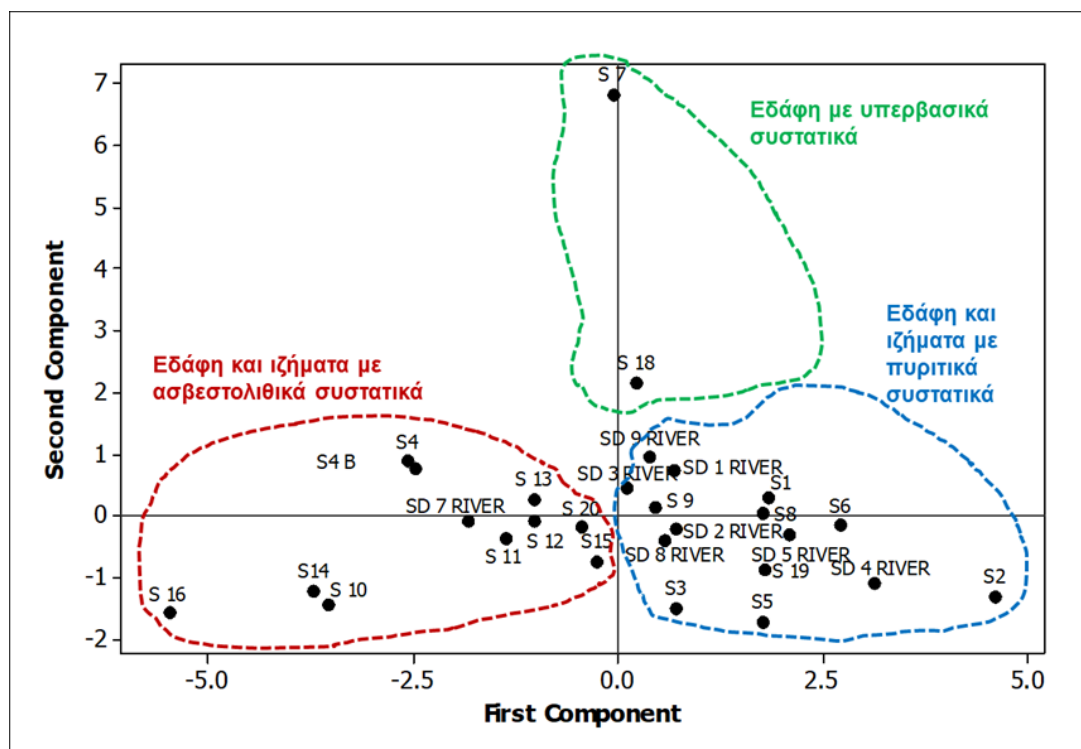
Chapter 4 presents the published article in the Environmental Research entitled: *Assessing the impact of geogenic chromium uptake by carrots (*Daucus carota*) grown in Asopos river basin*. A methodology was developed to assess the impact of geogenic origin Cr(VI) uptake by carrots, and the risk of human consumption of carrots grown in Asopos river basin. A field scale experiment was conducted with carrots cultivated in treatment plots, with and without compost amendment, in order to assess the impact of carbon in the mobility and uptake of Cr by plants. The results suggested that there is a trend for Cr mobilization and uptake in the surface and the leaves of the carrots cultivated in the treatment plot with the higher carbon addition, but not in the core of the carrots. Limited mobility of Cr(VI) in the soil-plant-water system is presented due to the affinity of Cr to be retained in the solid phase and be uptaken by plants. Cr(VI) tolerant bacterial strains were isolated from the carrots. These endophytic bacteria, present in all parts of the plant, were able to reduce Cr(VI) to Cr(III) form to levels below the detection limit. Finally, a site-specific risk assessment analysis suggested no adverse effects to human health due to the consumption of carrots. These findings are of particular importance since they confirm that carrots grown in soils with geogenic origin Cr does not pose any adverse risk for human consumption, but could also have the beneficial effect of the micronutrient Cr(III).

In Chapter 5 the results of the present study are summarized and conclusions are drawn. The overall impact of this thesis was aimed to develop an integrated approach to outline an area with wide spread geogenic origin Cr contamination, and to give answers to questions concerning Cr existence in groundwater, soils and agricultural products. The general findings showed Cr concentrations in groundwater of Asopos basin below the drinking water standards. Increased geogenic Cr(VI) concentrations are only observed in areas directly affected by ophiolite weathering. Chromite weathering is the controlling mechanism of Cr release in soils of Asopos basin. The high adsorption and immobilization capacity of soils and sediments are important suggesting significant Cr retardation in case of wastes release with high Cr concentration and specifically for wastes with high acidity. Finally, there is a case of Cr uptake by agricultural products, however, it is not important due to the direct reduction of Cr(VI) by the endophytic bacteria. The human health risk assessment showed that the population is unlikely to experience adverse effects due to the consumption of these products.

ΕΚΤΕΤΑΜΕΝΗ ΠΕΡΙΛΗΨΗ

Στην Ελλάδα, οι κυριότερες εμφανίσεις οφιολιθικών πετρωμάτων των Ελληνίδων διακρίνονται σε τέσσερις οφιολιθικές ζώνες. Τα βουνά της Πίνδου καλύπτονται με υπερβασικά πετρώματα, τα οποία διαβρώνονται και κομμάτια αυτών έχουν συνδυαστεί με Τριτογενείς και Τεταρτογενείς αποθέσεις που βρίσκονται στις κοιλάδες. Από τη διάβρωση αυτών των πετρωμάτων προήλθαν υπερβασικά εδάφη τα οποία παρουσιάζουν ρύπανση χρωμίου γηγενούς προέλευσης. Η περιοχή μελέτης της παρούσης διατριβής εντοπίζεται στην κεντρική Ελλάδα και συγκεκριμένα στη λεκάνη απορροής του ποταμού Ασωπού, όπου οι αναφερόμενες συγκεντρώσεις χρωμίου και νικελίου στα υπόγεια νερά, τα γεωργικά εδάφη και τα αγροτικά προϊόντα οφείλονται στην ύπαρξη και διάβρωση των υπερβασικών πετρωμάτων. Επιπλέον, η ευρύτερη περιοχή του ποταμού Ασωπού, αποτελεί μια μοναδική περίπτωση συνδυασμού ανθρωπογενούς και γηγενούς προέλευσης ρύπανσης από χρώμιο. Παρόλο που η ανθρωπογενούς προέλευσης ρύπανση χρωμίου έχει μελετηθεί εκτεταμένα λόγω της αποκατάστασης ρυπασμένων βιομηχανικών περιοχών σε όλο τον κόσμο, υπήρξε ένα κενό στη γνώση σχετικά με τη ρύπανση και κινητικότητα του γηγενούς χρωμίου κυρίως στα εδάφη και τα ιζήματα των ποταμών. Συγκεκριμένα, υπήρχε ανάγκη να αναπτυχθούν μεθοδολογίες και μια ολοκληρωμένη προσέγγιση για τη σκιαγράφηση μιας περιοχής που παρουσιάζει εκτεταμένη ρύπανση χρωμίου γηγενούς προέλευσης, και να αναπτυχθεί ένα πλαίσιο ανάλυσης για την κατανόηση της προέλευσης και της κινητικότητας του γηγενούς χρωμίου στα εδάφη της λεκάνης απορροής του ποταμού Ασωπού. Η παρούσα διατριβή αποσκοπούσε στην κάλυψη αυτής της ανάγκης. Οι στόχοι ήταν οι εξής: (1) να αξιολογηθεί ο γεωχημικός χαρακτηρισμός και η ταξινόμηση των εδαφών και των ιζημάτων του ποταμού σε σχέση με την προέλευση του χρωμίου κατά μήκος του ποταμού Ασωπού, (2) να αξιολογηθούν οι διαδικασίες που επηρεάζουν την κινητικότητα του εξασθενούς χρωμίου στα εδάφη και τα ιζήματα και να αποσαφηνισθούν οι μηχανισμοί απελευθέρωσης χρωμίου από τα εδάφη και (3) να αναπτυχθεί μια μεθοδολογία για την εκτίμηση των επιπτώσεων της πρόσληψης εξασθενούς χρωμίου από αγροτικά προϊόντα (συγκεκριμένα καρότα), και του κινδύνου στην ανθρώπινη υγεία λόγω της κατανάλωσης καρότων που καλλιεργούνται στη λεκάνη απορροής του Ασωπού ποταμού.

Για να επιτευχθεί αυτό, ακολουθήθηκε μια προσέγγιση τριών φάσεων:



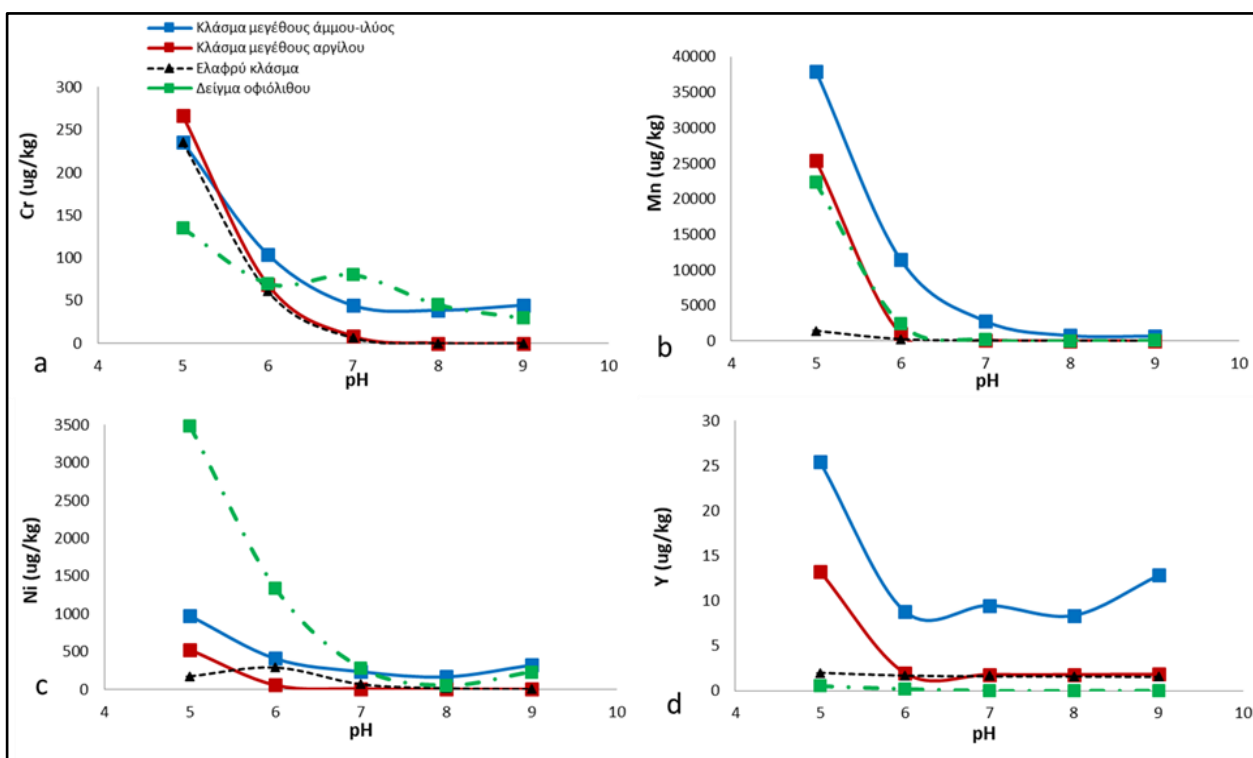
Σχήμα 1. Ομάδες που προέκυψαν από την ανάλυση κύριων συνιστωσών στα εδάφη και τα ιζήματα του ποταμού.

Στην πρώτη φάση σχεδιάστηκε και διεξήχθη μια έρευνα παρακολούθησης και δειγματοληψίας πεδίου και συλλέχθηκαν δείγματα εδαφών, επιφανειακών ιζημάτων του ποταμού και ιζημάτων πυρήνα. Επιπλέον αναλύθηκαν τα φυσικοχημικά χαρακτηριστικά των δειγμάτων αυτών. Τα φυσικοχημικά χαρακτηριστικά χρησιμοποιήθηκαν σε ανάλυση κύριων συνιστωσών ώστε να ταξινομηθούν τα δείγματα σύμφωνα με τα γεωχημικά χαρακτηριστικά τους. Η ανάλυση των βαρέων μετάλλων και η μελέτη της τοπικής γεωλογίας υποστήριξαν την υπόθεση ότι η κύρια πηγή χρωμίου είναι γηγενούς προέλευσης. Τα στοιχεία που υποστηρίζαν τη γηγενή προέλευση του χρωμίου, ήταν οι θετικές και υψηλές συσχετίσεις μεταξύ των συγκεντρώσεων χρωμίου και νικελίου στα εδάφη και τα ιζήματα, και η ομοιόμορφη κατανομή του χρωμίου και νικελίου ανάντη και κατάντη του βιομηχανικού πάρκου στην περιοχή των Οινοφύτων. Τα εδάφη της λεκάνης απορροής του ποταμού Ασωπού παρουσίασαν υψηλή συγκέντρωση χρωμίου ανεξάρτητα από τις διάφορες λιθολογίες που χαρακτηρίστηκαν από ασβεστολιθικά, πυριτικά και υπερβασικά συστατικά (Σχήμα 1). Οι μελέτες κινητικότητας που πραγματοποιήθηκαν στο εργαστήριο ανέδειξαν τα ποσοστά της προσρόφησης και εκχύλισης του χρωμίου από τα εδάφη, και την ικανότητά τους να προσροφούν χρώμιο. Εφαρμόστηκε μοντελοποίηση της

προσρόφησης του χρωμίου στο έδαφος για να διευκρινιστεί ο μηχανισμός προσρόφησης. Η συγκέντρωση ισορροπίας του χρωμίου 1-2 $\mu\text{g/L}$ που προέκυψε από τα εργαστηριακά πειράματα αποδείχθηκε ότι είναι συμβατή με τις συγκεντρώσεις του χρωμίου που συναντώνται στα υπόγεια νερά της περιοχής. Τα αποτελέσματα της προσρόφησης έδειξαν ότι τα εδάφη της λεκάνης απορροής του ποταμού Ασωπού έχουν σημαντική ικανότητα προσρόφησης.

Στη δεύτερη φάση, χρησιμοποιήθηκε ένα αντιπροσωπευτικό γεωργικό δείγμα εδάφους της λεκάνης απορροής του ποταμού Ασωπού, με σκοπό να αξιολογηθεί η κινητικότητα του χρωμίου και το γηγενές συστατικό της ρύπανσης, απομονώνοντας τα μεταλλικά στοιχεία που περιέχονται στο έδαφος. Η μεθοδολογία σχεδιάστηκε αρχικά για να διαχωριστεί το δείγμα εδάφους σε δευτερεύοντα κλάσματα, και έπειτα για να χαρακτηριστούν τα κλάσματα αυτά. Αρχικά πραγματοποιήθηκε μια κλασματοποίηση με υδροκυκλώνα με βάση το μέγεθος των κόκκων του εδάφους και έπειτα, μια κλασματοποίηση με βαρύ υγρό με βάση το βάρος των κόκκων του εδάφους. Τα κλάσματα που προήλθαν από το διαχωρισμό, χαρακτηρίστηκαν χρησιμοποιώντας χημική, ορυκτολογική και ανάλυση επιφάνειας. Η παρούσα φάση περιελάμβανε επίσης μελέτες εκχύλισης στο κάθε κλάσμα, για να μελετηθεί η απελευθέρωση χρωμίου ως συνάρτηση του pH, και για να προσδιοριστεί ο μηχανισμός που ελέγχει την κινητικότητα του χρωμίου. Τα αποτελέσματα έδειξαν ότι το κλάσμα μεγέθους – αργίλου που προήλθε από το διαχωρισμό με υδροκυκλώνα αποτελεί το 55% του συνολικού δείγματος εδάφους. Το ελαφρύ και το βαρύ κλάσμα που προήλθαν από το διαχωρισμό με βαρύ υγρό αποτελούν το 44.4% και 0.6% του συνολικού, αντίστοιχα. Οι μελέτες στην επιφάνεια των κλασμάτων έδειξαν ότι υπάρχει εξασθενές χρώμιο (περίπου 30-40% του ολικού χρωμίου) στην επιφάνεια το οποίο μπορεί πιθανόν να εκχυλιστεί. Όσον αφορά στην κινητικότητα, αυτή σχετίζεται άμεσα με το βαρύ κλάσμα του εδάφους, το οποίο περιέχει χρωμίτη (6.9%), αιματίτη (6.7%) και γκαιτίτη (10.1%) ως κύρια ορυκτά, σύμφωνα με την ορυκτολογική ανάλυση. Σε όξινα pH (5 και 6), το κλάσμα μεγέθους - αργίλου παίζει επιπρόσθετα σημαντικό ρόλο στην κινητικότητα του χρωμίου και ελέγχει την εκχύλιση του, λόγω του υψηλού δυναμικού αντίδρασης. Το κύριο συμπέρασμα αυτής της μελέτης είναι ότι η διάβρωση του χρωμίτη είναι ο μηχανισμός που ελέγχει την εκχύλιση του χρωμίου. Οι μελέτες εκχύλισης συναρτήσεως του pH έδειξαν ότι η συμπεριφορά της απελευθέρωσης χρωμίου σε δείγμα οφιόλιθου συσχετίζεται με τα άλλα κλάσματα του εδάφους, υποδηλώνοντας ότι η κινητικότητα

του χρωμίου σχετίζεται με τη διάβρωση του οφιόλιθου. Ο εμπλουτισμός των κλασμάτων του εδάφους με χρώμιο, νικέλιο, μαγγάνιο και ύτριο είναι το αποτέλεσμα της διάβρωσης και μεταφοράς των υπερβασικών πετρωμάτων της περιοχής του Αυλώνα (Σχήμα 2). Οι υψηλές συσχετίσεις μεταξύ χρωμίου - νικελίου, χρωμίου - μαγγανίου και χρωμίου - υτρίου στα κλάσματα τους εδάφους επιβεβαιώνουν ότι το έδαφος προήλθε από τα προϊόντα της διάβρωσης (που μεταφέρθηκαν μηχανικά ως κλάσματα που φέρουν χρώμιο) των υπερβασικών πετρωμάτων της λεκάνης του Ασωπού. Τα αποτελέσματα του πειράματος εκχύλισης συναρτήσεως του pH είναι συμβατά με τις συγκεντρώσεις του χρωμίου στο πεδίο έπειτα από αλλαγή της κλίμακας χρησιμοποιώντας τυπικές φυσικές παραμέτρους των εδαφών.

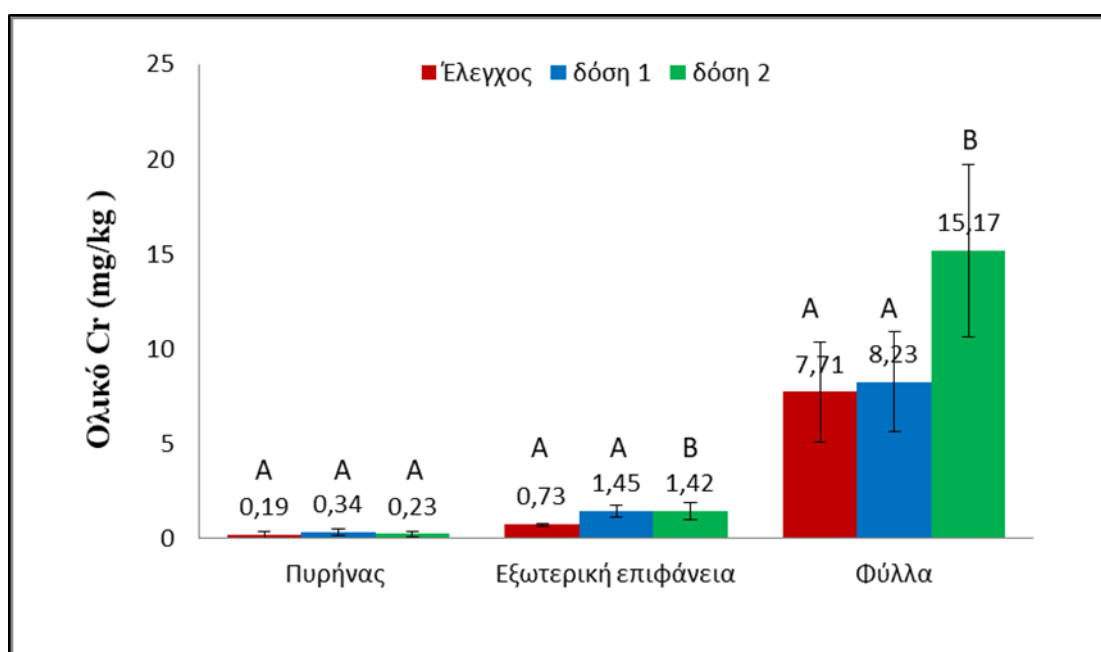


Σχήμα 2. Συγκέντρωση του Cr (a), Mn (b), Ni (c), Y (d) που εκχυλίστηκε από τα διαφορετικά κλάσματα του εδάφους και από δείγμα πέτρωματος οφιόλιθου ως συνάρτηση του pH.

Η τελική φάση αυτής της μελέτης στόχευε στον προσδιορισμό της βιοδιαθεσιμότητας της πρόσληψης χρωμίου από τα καρότα, της τύχης του εξασθενούς χρωμίου μέσα στο φυτό και της περίπτωσης εμφάνισης κινδύνου στην ανθρώπινη υγεία λόγω της κατανάλωσης καρότων, με τη χρήση τυποποιημένων μεθοδολογιών της ΕΡΑ. Η επιλογή του συγκεκριμένου φυτού (καρότο) έγινε διότι αποτελεί ένα από τα κύρια προϊόντα που καλλιεργούνται στην περιοχή του Σχηματαρίου της λεκάνης απορροής

του Ασωπού ποταμού. Επίσης υποτέθηκε ότι θα μπορούσε να έχει την υψηλότερη πρόσληψη και το δυναμικό συσσώρευσης. Προκειμένου να εκτιμηθεί ο κίνδυνος στην ανθρώπινη υγεία λόγω της κατανάλωσης καρότων τα οποία καλλιεργούνται σε μια περιοχή η οποία παρουσιάζει ρύπανση χρωμίου γηγενούς προέλευσης: προσδιορίστηκε η πρόσληψη εξασθενούς χρωμίου από τα καρότα που καλλιεργούνται στον αγρό κάτω από πραγματικές συνθήκες, προσδιορίστηκε η τύχη του εξασθενούς χρωμίου μέσα στο φυτό και η δυνατότητα αναγωγής του από τα ενδοφυτικά βακτήρια, και τέλος, διεξήχθη ανάλυση επικινδυνότητας στην ανθρώπινη υγεία λόγω της κατανάλωσης καρότων χρησιμοποιώντας τα δύο προηγούμενα στάδια.

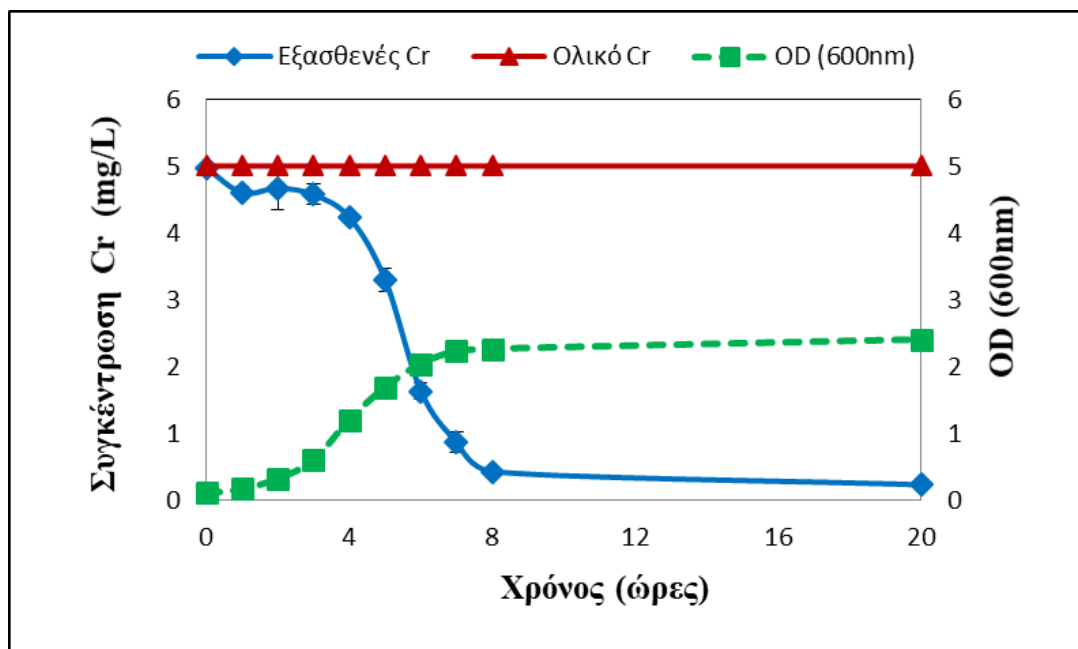
Τα αποτελέσματα του πειράματος σε αγροτεμάχιο της περιοχής του Σχηματαρίου έδειξαν ότι υπάρχει τάση για κινητικότητα και πρόσληψη του χρωμίου στην εξωτερική επιφάνεια και τα φύλλα των καρότων που καλλιεργήθηκαν στα τμήματα με υψηλότερη συγκέντρωση άνθρακα. Τα αποτελέσματα δεν έδειξαν ότι υπάρχει αντίστοιχη τάση κινητικότητας και στους πυρήνες των καρότων (βρώσιμο τμήμα του καρότου) (Σχήμα 3).



Σχήμα 3. Συγκέντρωση ολικού χρωμίου στα μέρη του καρότου (πυρήνας, εξωτερική επιφάνεια, φύλλα) για τη διαχείριση "έλεγχος" και τις διαχειρίσεις με προσθήκη κομπόστ ("δόση 1" και "δόση 2").

Τα αποτελέσματα της μελέτης των ενδοφυτικών βακτηρίων έδειξαν ότι τα καρότα έχουν την ικανότητα μέσω της κοινότητας των ενδοφυτικών βακτηρίων να ανάγουν το

εξασθενές χρώμιο σε τρισθενές (Σχήμα 4). Τα ενδοφυτικά βακτήρια που αποτελούνται από ανθεκτικά σε εξασθενές χρώμιο στελέχη και βρίσκονται σε όλα τα μέρη του φυτού (πυρήνας, εξωτερική επιφάνεια, φύλλα), μπορούν να αναπτυχθούν σε μέσο 1 mg/L εξασθενούς χρωμίου και να ανάγουν το εξασθενές χρώμιο σε τρισθενές. Τα αποτελέσματα αυτά μας οδήγησαν στη διερεύνηση δύο ενδοφυτικών βακτηρίων που απομονώθηκαν από το φύλλο και τριών από τη ρίζα (πυρήνας) τα οποία βρέθηκαν ότι είναι ικανά να αναπτυχθούν σε μέσο 3 mg/L εξασθενούς χρωμίου, και επτά από την επιφάνεια που είναι ικανά να αναπτυχθούν σε μέσο με 5 mg/L εξασθενούς χρωμίου. Επιπροσθέτως όλα αυτά τα στελέχη βρέθηκε ότι μπορούν να μειώσουν την αρχική συγκέντρωση εξασθενούς χρωμίου (3 mg/L και/ ή 5 mg/L) σε επίπεδα κάτω από το όριο ανίχνευσης σε 20 μόλις ώρες. Επιπλέον παρατηρήθηκε ότι η κυτταρική ανάπτυξη και η μείωση του εξασθενούς χρωμίου ξεκίνησαν ταυτόχρονα για όλα τα στελέχη (Σχήμα 4). Η εκτίμηση του ρυθμού αντίδρασης και η ημίσεια ζωή των στελεχών των ενδοφυτικών βακτηρίων που απομονώθηκαν από τον πυρήνα και την επιφάνεια των καρότων, υποδηλώνει πολύ γρήγορους ρυθμούς μετασχηματισμού για το εξασθενές χρώμιο.



Σχήμα 4. Συγκέντρωση ολικού χρωμίου, αναγωγή εξασθενούς χρωμίου και καμπύλη ανάπτυξης του ενδοφυτικού βακτηρίου της ρίζας (πυρήνα), στέλεχος R1 σε αρχική συγκέντρωση εξασθενούς χρωμίου 5 mg/L, pH 7,0 και 30 ° C.

Όσον αφορά την εκτίμηση του συσσωρευμένου μη καρκινογόνου ρίσκου, ο δείκτης επικινδυνότητας βρέθηκε να είναι $6.73 \cdot 10^{-3}$, υποδεικνύοντας ότι δεν υπάρχουν δυσμενείς επιπτώσεις στην ανθρώπινη υγεία λόγω της κατανάλωσης καρότων. Το καρκινογόνο ρίσκο εκτιμήθηκε στο 0.077 - 0.24 στο 1 εκατομμύριο. Τα αποτελέσματα αυτά έχουν ιδιαίτερη σημασία, δεδομένου ότι επιβεβαιώνουν ότι προϊόντα που καλλιεργούνται σε εδάφη με γηγενούς προέλευσης χρώμιο δεν παρουσιάζουν δυσμενείς επιπτώσεις στην ανθρώπινη υγεία, αλλά θα μπορούσαν επίσης να έχουν και ευεργετικά αποτελέσματα λόγω των μικροθρεπτικών συστατικών του τρισθενούς χρωμίου.

Ο συνολικός αντίκτυπος αυτής της διατριβής αποσκοπούσε στην ανάπτυξη μιας ολοκληρωμένης προσέγγισης για τη σκιαγράφηση μιας περιοχής που παρουσιάζει εκτεταμένη ρύπανση χρωμίου γηγενούς προέλευσης, καθώς και στο να δώσει απαντήσεις σε ερωτήματα σχετικά με την ύπαρξη χρωμίου στα υπόγεια νερά, τα εδάφη και τα γεωργικά προϊόντα της λεκάνης απορροής του ποταμού Ασωπού. Τα γενικά συμπεράσματα έδειξαν συγκεντρώσεις χρωμίου στα υπόγεια νερά κάτω από τα επιτρεπτά όρια για το πόσιμο νερό. Αυξημένες συγκεντρώσεις γηγενούς εξασθενούς χρωμίου παρατηρούνται μόνο στις περιοχές της λεκάνης που επηρεάζονται άμεσα από τη διάβρωση των υπερβασικών πετρωμάτων. Η διάβρωση του χρωμίτη είναι μάλιστα η διαδικασία που ελέγχει το μηχανισμό της κινητικότητας του χρωμίου στα εδάφη της λεκάνης του Ασωπού. Η σημαντική ικανότητα προσρόφησης και ακινητοποίησης που παρουσιάζουν τα εδάφη και τα ιζήματα της περιοχής υποδηλώνει επιβράδυνση/δέσμευση του χρωμίου σε περίπτωση απόρριψης αποβλήτων με υψηλή συγκέντρωση χρωμίου και ειδικά για απόβλητα υψηλής οξύτητας. Τέλος, υπάρχει περίπτωση πρόσληψης χρωμίου από τα αγροτικά προϊόντα, ωστόσο είναι αμελητέας σημασίας λόγω της άμεσης αναγωγής του εξασθενούς χρωμίου από τα ενδοφυτικά βακτήρια που υπάρχουν στα φυτά. Η ανάλυση επικινδυνότητας έδειξε ότι δεν υπάρχουν δυσμενείς επιπτώσεις στην ανθρώπινη υγεία λόγω της κατανάλωσης καρότων.

LIST OF ACRONYMS

Abbreviation	Meaning
Cr	Chromium
Cr(III)	Trivalent Chromium
Cr(VI)	Hexavalent Chromium
CDI	Chronic Daily Intake
CRI	Cr Reactivity Index
EC	Electrical Conductivity
hc	hydrocyclone
HI	Hazard Index
hl	heavy liquid
HQ	Hazard Quotient
ICP-MS	Inductively Coupled Plasma Mass Spectroscopy
LADD	Lifetime Average Daily Dose
Ni	Nickel
ORP	Oxidation-Reduction Potential
PCA	Principal Components Analysis
RfD	Chronic Reference Dose
TN	Total Nitrogen
TOC	Total Organic Carbon
U.S. EPA	United States Environmental Protection Agency
XPS	X-Ray Photoelectron Spectroscopy
XRF	X-Ray Fluorescence
XRD	X-ray Diffraction

Table of Contents

CHAPTER 1. INTRODUCTION	31
1.1 General information about chromium	32
1.2 Chromium in the environment.....	32
1.2.1 Chromium species	32
1.2.2 Redox, precipitation and sorption reactions of chromium.....	34
1.2.3 Maximum allowable levels of chromium in the environment.....	35
1.2.4 Chromium sources.....	36
1.2.5 Natural sources of chromium - Geogenic chromium	36
1.3 Bioavailability, speciation and mobility of chromium in soils.....	39
1.4 Chromium in soil-plant system	40
1.5 Human health risk assessment.....	42
1.6 The study area of Asopos river basin and framework of analysis	43
References	47
CHAPTER 2. CHARACTERIZATION AND MOBILITY OG GEOGENIC CHROMIUM IN SOILS AND RIVER BED SEDIMENTS OF ASOPOS BASIN	55
2.1 Abstract	56
2.2 Introduction	56
2.3 Materials and methods.....	58
2.3.1 Site description	58
2.3.2 Experimental design	61
2.4 Results and discussion.....	63
2.4.1 Physicochemical characterization of soils and sediments	63
2.4.2 Geochemical analysis and classification of soils and sediments	65
2.4.3 Mobility studies	70
2.5 Conclusions	73
References	74
CHAPTER 3. IDENTIFYING THE CONTROLLING MECHANISM OF GEOGENIC ORIGIN CHROMIUM RELEASE IN SOILS	76
3.1 Abstract	77
3.2 Introduction	77
3.3 Materials and methods.....	79
3.3.1 Site description and sampling.....	79
3.3.2 Experimental design	80
3.4 Results and discussion.....	82
3.4.1 Mineral and chemical characterization of the separated fractions.....	82
3.4.2 Surface characterization of the separated fractions	86

3.4.3 Mobility of chromium and other metals at different pH values	88
3.5 Conclusions	91
References	93
CHAPTER 4. ASSESSING THE IMPACT OF GEOGENIC CHROMIUM UPTAKE BY CARROTS (<i>DAUCUS CAROTA</i>) GROWN IN ASOPOS RIVER BASIN.....	97
4.1 Abstract	98
4.2 Introduction	98
4.3 Material and methods	100
4.3.1 Design of analysis.....	100
4.3.2 Field scale experiment.....	101
4.3.3 Endophytic bacteria experiments.....	102
4.3.4 Risk assessment methodology	104
4.4 Results and discussion.....	106
4.4.1 Cr concentrations in carrots cultivated in compost amended soils.....	106
4.4.2 Endophytic bacteria.....	108
4.4.3 Risk assessment	112
4.5 Conclusions	113
References	115
CHAPTER 5. SUMMARY-CONCLUSIONS	119
PUBLICATIONS RELATED TO THIS THESIS	126
PUBLICATIONS IN SCIENTIFIC JOURNALS	127
CONFERENCE PRESENTATIONS	127
APPENDIX – STABILIZATION OF HEXAVALENT CHROMIUM IN CONTAMINATED SEDIMENT USING ZERO VALENT IRON TECHNOLOGIES	129
A.1 Materials and methods.....	130
A.1.1 Sampling.....	130
A.1.2 Physicochemical analysis	130
A.1.3 Experimental design	131
A.1.4 Statistical analysis.....	132
A.2 Results and discussion.....	132
A.2.1 Physicochemical characterization.....	132
A.2.2 Trace elements stabilization	133
A.2.3 Correlations	135
A.3 Conclusions	136
References	138

Table of Figures

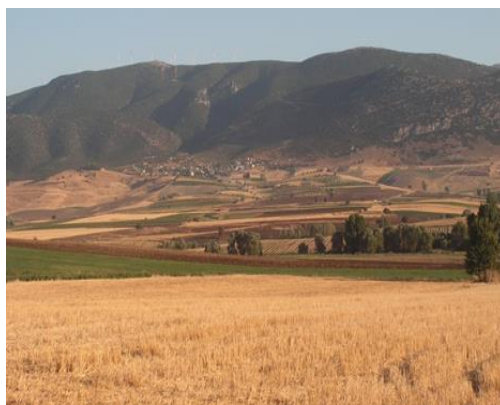
Figure 1.1. Eh versus pH diagram for chromium (Rai et al., 1987)	34
Figure 1.2. A north polar projection showing the distribution of serpentinites and ophiolites (in red) (Oze et al., 2007).	37
Figure 1.3. The main ophiolite outcrops of the Hellenides are distinguished in four ophiolite belts corresponding to the oceanic terranes of H2, H4, H6 and H8 (Papanikolaou, 2009).	44
Figure 2.1. Geology map of the study area with the main lithological units. Red circles depict soil and river sediment sampling sites and black circle depicts the industrial park at the Oinophyta area.	60
Figure 2.2. Cr distribution of industrial park upstream and downstream surface river bed and core sediments. There are no sd2 and sd9 core samples because of rocky texture sediments.	64
Figure 2.3. Correlation diagram of Ni and Cr content in soils and river bed sediments by a) HNO ₃ digestion and b) XRF analysis respectively.	65
Figure 2.4. Groups resulting from PCA in soils and river bed sediments.	66
Figure 2.5. CRI and total chromium correlation in calcareous, siliceous and ultramafic soils and river bed sediments.	68
Figure 2.6. Boxplot showing the Cr/Fe ratio for the three groups of soils and river bed sediments. Values of Fe concentration were divided by 10 ⁴ . The top of the box is the third quartile, the bottom of the box is the first quartile, the internal horizontal line indicates the median, and the whiskers extend to the maximum and minimum data value.	69
Figure 2.7. Leaching kinetic experiment and adsorption isotherm test using the samples from the surface river bed sediments sd7 (calcareous) and sd8 (siliceous). Ce is the chromium concentration in equilibrium in solution and and qe is Cr(VI) concentration sorbed onto soil.	71
Figure 3.1. Schematic diagram of the separation procedure.	81
Figure 3.2. Total Cr, Ni and Fe concentration for the different fractions of soil using EPA3051A and EPA3052 measuring methods.	85
Figure 3.3. XP Deconvoluted Spectra of (A) Fe2p and (B) Cr2p of the clay-sized (hc) (a), heavy (hl) (b) and light (hl) (c) fraction of soil.	87
Figure 3.4. Concentration of Cr (a), Mn (b), Ni (c), Y (d) desorbed from the different fractions of soil and from an ophiolite rock sample as a function of pH. Error bars correspond to the standard error of the triplicates.	89
Figure 3.5. Correlation diagram of Cr with Ni, Mn, Y content in sand/silt-sized (hc) and clay-sized (hc) fraction of soil.	92
Figure 4.1. Total chromium concentration in the parts of carrots (core, surface, leaves) for the control and compost treatments (“dose 1” and “dose 2”). Error bars correspond to standard deviation of the replicates. The data with different letters (A, B) in the same carrot part indicate significant differences at the $\alpha=0.05$ level using ANOVA. e.g. for the surface of the carrots, “dose 2”compost treatment presents significant differences compared with the other two, while “dose 1” compost treatment and control do not differ significantly with each other.	108
Figure 4.2 . Total chromium concentration, Cr(VI) reduction and growth curves of the root (core) endophytic bacterium, strain R1 at 5 mg/L Cr(VI) initial concentration, pH 7.0 and 30°C. Error bars correspond to standard deviation of the triplicates.	110
Figure 4.3. Total chromium concentration, Cr(VI) reduction and growth curves of the root (surface) endophytic bacterium, strain RS1 at 5 mg/L Cr(VI) initial concentration, pH 7.0 and 30°C. Error bars correspond to standard deviation of the triplicates.	111

Figure 4.4. Total chromium concentration, Cr(VI) reduction and growth curves of the leaf endophytic bacterium, strain L1 at 3 mg/L Cr(VI) initial concentration, pH 7.0 and 30°C. Error bars correspond to standard deviation of the triplicates.	112
Figure 5.1. Groups resulting from PCA in soils and river bed sediments.	121
Figure 5.2. Concentration of Cr (a), Mn (b), Ni (c), Y (d) desorbed from the different fractions of soil and from an ophiolite rock sample as a function of pH.....	123
Figure 5.3. Total chromium concentration in the parts of carrots (core, surface, leaves) for the control and compost treatments (“dose 1” and “dose 2”)......	124
Figure 5.4. Total chromium concentration, Cr(VI) reduction and growth curves of the root (core) endophytic bacterium, strain R1 at 5 mg/L Cr(VI) initial concentration, pH 7.0 and 30°C.	124
Figure A.1. (a) Cr, (b) As, (c) Ni, (d)Fe and (e) Mg concentrations in solution at different Connelly’s ZVI, EHC® and DARAMEND® to soil ratios. Error bars represent the standard deviation of three replicates.....	135

List of Tables

Table 2.1. Average chemical characteristics of the 3 groups of data. Parentheses show standard deviation. The data with different letters (a-c) in the same row indicate significant differences at the $\alpha = 0.05$ level using ANOVA.....	67
Table 3.1. Quantitative separation of the bulk soil. “–” shows that this element is not detected in the related fraction.....	83
Table 3.2. Mineralogical analysis for the bulk soil and for all derived fractions. “–” shows that these minerals are not detected in the related fraction.....	84
Table 3.3. Atomic ratio (%) of Cr/Fe and intensity ratio of Cr(VI) to the total intensity.....	87
Table 4.1. Lag phase, reaction rate (k) and half-life (τ) for the root (core and surface) endophytic bacteria and leaf endophytic bacteria.....	110
Table 4.2. Risk assessment values for chromium.....	113
Table A.1. Chemical characteristics of river sediment sample	133

CHAPTER 1. INTRODUCTION



1.1 General information about chromium

Chromium (Cr) was first discovered in the mineral crocoite (PbCrO_4) in 1798 by the French chemist Vauquelin. The element was named chromium after the Greek word chroma, meaning color, due to its strong influence on mineral pigmentation (Burns and Burns, 1975). Cr is a transition, silver color hard metal, in the sixth group of the periodic table, has atomic number 24, molecular weight 51.1 and density 7.19 g/cm. Cr is the 7th most abundant element (Nriagu, 1988), 21st most abundant metal (Economou-Eliopoulos et al., 2013) of the Earth's crust and the 2nd most abundant inorganic contaminant in groundwater at many hazardous waste sites as reported by the National Academy of Sciences Committee on Groundwater Cleanup (NAS, 1994). Cr is one of the 18 core hazardous air pollutants (HAPs), 33 urban air toxicants, 188 HAPs (U.S. EPA), and has been ranked 7th among the top 20 hazardous substances by the Agency for Toxic Substances and Disease Registry (Oh et al., 2007). Moreover, it is ranked 5th among the heavy metals in the Comprehensive Environmental Response, Compensation, and Liability Act (Ma et al., 2007), and it is also categorized as no.1 carcinogen according to the International Agency for Research on Cancer (IARC, 1987) and the National Toxicology Program. Therefore, this metal requires detailed understanding and in-depth monitoring in the environment.

1.2 Chromium in the environment

1.2.1 Chromium species

Cr has a complex electronic and valence shell chemistry owing to its high potential to easily convert from one oxidation state to another (Prado et al., 2016). Cr has several oxidation states (-2 to +6), but hexavalent chromate (Cr(VI)) and trivalent chromite (Cr(III)) forms are the most common and stable in the natural environment (Ashraf et al., 2017).

The majority of Cr on the earth exists as Cr(III) which is an immobile cation that complexes strongly with organic matter and adsorbs on oxides and silicates even at low pHs ($\text{pH} > 4$) (Rai and Zachara, 1988; Rai et al., 1989). Moreover, the chemical mobility of Cr(III) is limited due to the formation of highly stable hydroxides (Rai and Zachara,

1988; Rai et al., 1989). Cr(III) has the highest octahedral site preference energy of any trace element and it isomorphically substitutes into octahedral sites in oxides, (oxy)hydroxides, sulfides, and silicates.

Cr(VI) is a strongly oxidizing and weakly adsorbing oxyanion in the form of chromate ($\text{H}_x\text{CrO}_4^{2-x}$) (Rai and Zachara, 1988; Rai et al., 1989). Consequently, Cr(VI) is chemically mobile and bioavailable over a wide range of pH (Rai and Zachara, 1988) and is also more mobile in soils (Lackovic et al., 1997; Nikolaidis et al., 1999). Cr(VI) is tetrahedrally coordinated and behaves similarly to sulfate in minerals such as jarosite (Baron and Palmer, 1996).

Both the species of Cr differ greatly with respect to their sorption and bioavailability in soil, absorption and translocation to aerial parts and toxicity inside plants (Choppala et al., 2016). Additionally, these forms (Cr(III) and Cr(VI)) have different chemical, epidemiological and toxicological features; they are separately regulated by Environmental Protection Agency (U.S. EPA), which presents a distinctive feature of Cr among the heavy metals. Cr(III), being necessary for lipid and sugar metabolism (Bai et al., 2015), is an essential trace element for human and animal health (Eskin, 2016), however, it is not required by the plants (Shanker et al., 2005). The recommended human intake for Cr(III) is 50–200 µg/day. In contrast, Cr(VI) is a highly toxic species and it produces adverse carcinogenic and mutagenic effects in cells (IARC, 1990; Lewicki et al., 2014; Vincent, 2013). Specifically, inhalation of Cr(VI) is known to cause irritation in the nose, including symptoms such as nosebleeds, ulcers and perforations in the nasal septum (Katz and Salem, 1993). Ingesting considerable quantities of Cr(VI) can result in stomach ulcers, convulsions, kidney and liver damage, and death (Katz and Salem, 1993). Skin contact with certain Cr(VI) compounds causes allergic contact dermatitis and irritant dermatitis (Katz and Salem, 1993). Overall, the Environmental Protection Agency (U.S. EPA), the Occupational Safety and Health Administration (OSHA), the World Health Organization (WHO), and the Department of Health and Human Services (DHHS) have determined that Cr(VI) compounds can increase the risk of lung cancer primarily by inhalation as well as other forms of cancer caused by ingestion.

1.2.2 Redox, precipitation and sorption reactions of chromium

Cr can be oxidized or reduced from one form to the other in the environment, depending upon concentrations of the reactants, pH of the media, temperature, light intensity, and other entities involved in the reaction (surfaces/sorbents, complexing agents, bacteria, oxidizers/reducers, etc.). Manganese oxides are a natural soil component that can convert Cr(III) to Cr(VI), while V^{2+} , Fe^{2+} , S^{2-} , HNO_2 , HSO_3^- , and some organic species can reduce Cr(VI) to Cr(III) (Kimbrough et al., 1999). Since water is one of the reactants involved in many Cr oxidation reactions, along with Cr(III) and the oxidizing agent, the conversion from Cr(III) to Cr(VI) rarely takes place in dry systems. Cr(III) is an octahedral hydrolysable molecule, which is usually positively charged in most environmental settings. It has a Cr atom surrounded by six other molecules that can be either water molecules or hydroxides, depending upon the pH of the medium (Fig. 1.1).

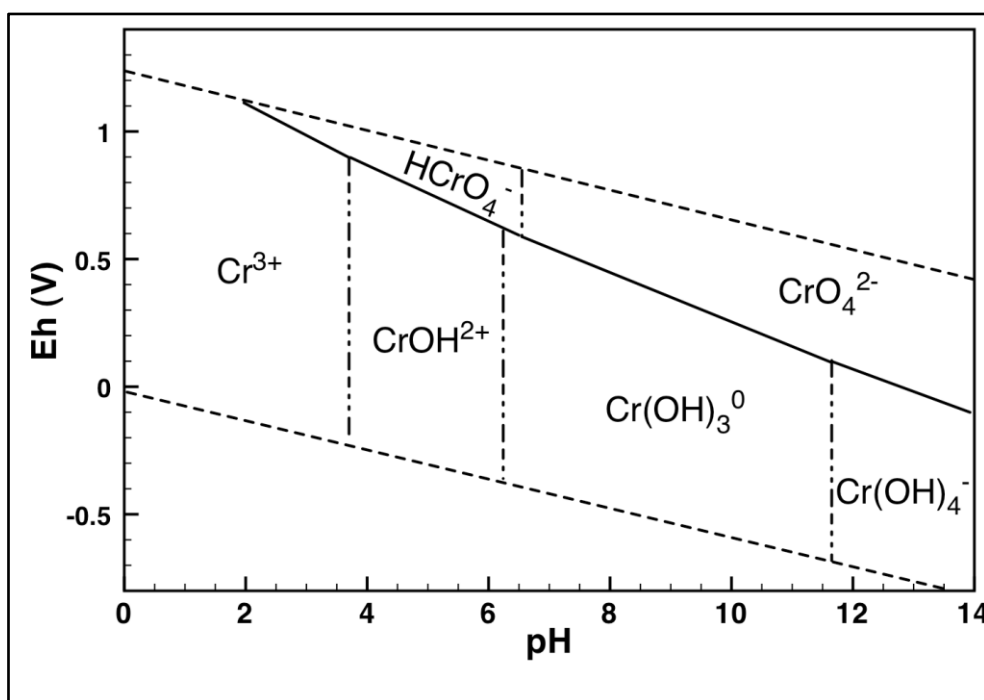


Figure 1.1. Eh versus pH diagram for chromium (Rai et al., 1987)

Cr(III) species have a low solubility index and are not soluble at most environmental conditions. Aqueous Cr(III) can form different hydroxide precipitates depending upon the concentration and the pH of the medium. Although Cr(VI) is soluble at most environmental conditions, it can form salts with divalent cations, such as Sr^{2+} , Zn^{2+} , Pb^{2+} , and Cu^{2+} . Therefore, these Cr(VI) precipitates can provide a continual source of

chromate ions in the environment.

Cr tends to tightly sorb on soil components. For example, Cr(III) binds as an inner sphere complex with goethite and hydrous ferric oxide (Charlet and Manceau, 1992), silica (Fendorf et al., 1994), and birnessite (Fendorf et al., 1992). Similarly, Cr(VI) is bound to goethite as an inner sphere complex (Fendorf et al., 1997). The effect of aging on sorption of partitioning of Cr was illustrated by Asikainen and Nikolaidis (1994). The results of sequential extraction experiments revealed that longer contact times (decades) between Cr and glaciated soils resulted in stronger partitioning.

1.2.3 Maximum allowable levels of chromium in the environment

Cr is found in all phases of the environment, including soil, water and air. The acceptable level in soil for the protection of environmental and human health has been estimated at 64 mg/kg (CCME, 2015). The maximum allowable levels of total Cr in agricultural soils of Italy and Poland, Czech Republic, Austria, Canada, Serbia are 150, 100-200, 100, 64, and 100 mg/kg, respectively (Ding et al., 2014). The Italian regulation defined a maximum allowable concentration of 2 mg/kg for Cr(VI) in soils for private and residential use.

The threshold limits for Cr(III) concentration in sea water, fresh water, and irrigation water are 50, 8, and 5 µg/L, while for Cr(VI) these values are 1, 1, and 8 µg/L, respectively (Zayed and Terry, 2003). In different countries diverse regulations have been adopted that limit the content of total Cr and Cr(VI) in water and in air. In the case of Europe, EC Directive 98/83/EC (1998) established maximum amounts of total Cr and Cr(VI) in drinking water of 50 and 20 µg/L, respectively. The U.S. EPA limited the maximum contaminant level for total Cr in water to 100 µg/L, but the Office for Environmental Health Hazards Assessment of California established a public goal of 0.02 µg/L for Cr(VI) as a protection level against the undesirable toxic effects of this pollutant (U.S. EPA, 2011). Furthermore, in working ambient air NIOSH recommends that airborne exposure to all Cr(VI) compounds be limited to a concentration of 0.2 µg Cr(VI)/ m³ for an 8 -h time- weighted average exposure during a working week of 40 hours (NIOSH, 2013).

1.2.4 Chromium sources

Environmental contamination of Cr has gained major consideration because of its high levels in the water and soil as a result of natural and anthropogenic activities (Ashraf et al., 2017). The highest proportion of this element in the environment is generated from anthropogenic sources, such as industrial and agricultural activities. Cr annual mine production increased between 2001 and 2015 from 12,000 thousand metric tons to 27,000 thousand metric tons (USGS, 2016). Cr is widely used for several applications in the metallurgy industry, in the chemical production of paints and pigments, in synthetic fertilizers and crop protection products, in leather tanning activities, in textile dyes and mordants and in pulp and paper production (Namiesnik and Rabjczyk, 2015). However an also appreciable amount of Cr released into the environment by geogenic and natural sources including weathering processes on rocks that are rich in this element, emanations from volcanos, and soil erosion.

1.2.5 Natural sources of chromium - Geogenic chromium

In the Earth's crust, Cr is concentrated (>200 mg/kg) in ultramafic rocks and serpentinites of ophiolite complexes that constitute $\approx 1\%$ of the terrestrial landscape. They are common along the the Circum-Pacific and Mediterranean region (Fig. 1.2) (Oze et al., 2007).

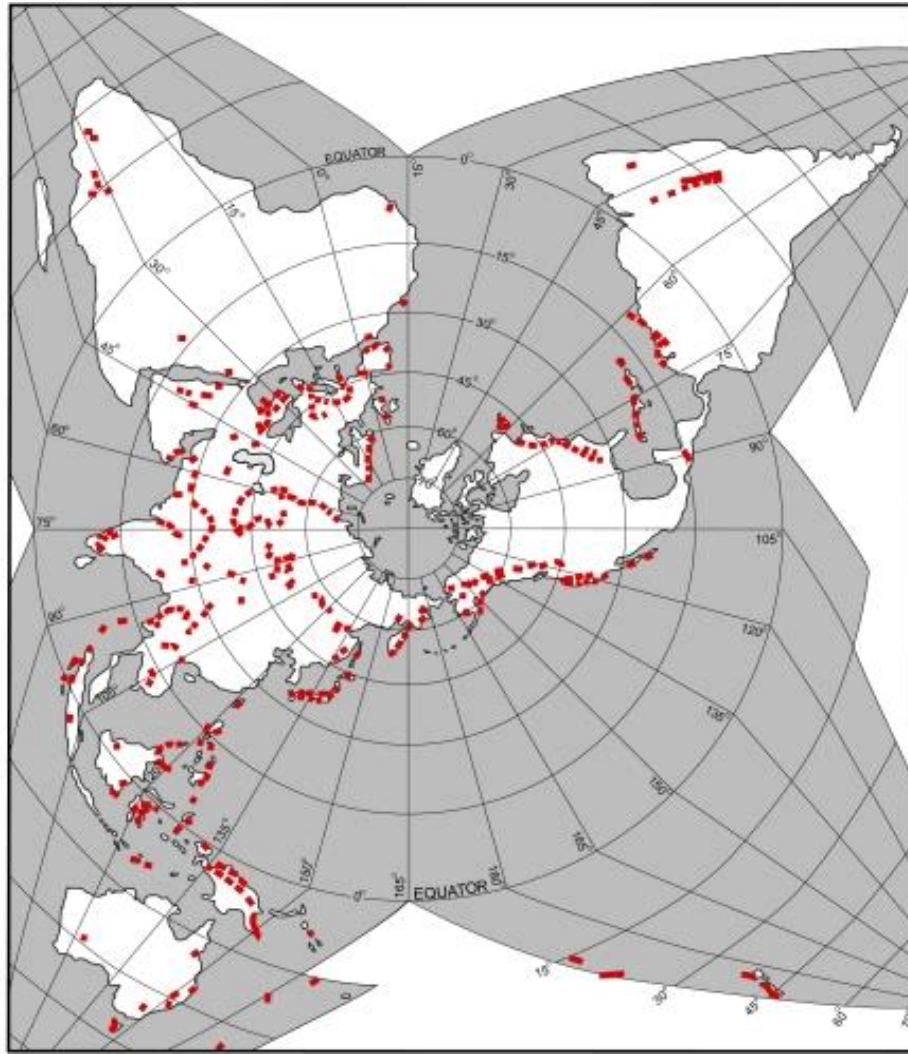


Figure 1.2. A north polar projection showing the distribution of serpentinites and ophiolites (in red) (Oze et al., 2007).

Naturally occurring in soils, Cr concentration ranges from 10 to 50 mg/kg, while in ultramafic soils, Cr varies within 634 to 125000 mg/kg (Adriano, 1986). Due to the wide distribution and occurrence of ultramafic rocks, Cr concentrations in soils are highly variable and strongly influenced by the geochemistry and mineralogy of the parent material (Shanker et al., 2005) and climatic conditions, as well as other factors including topography, biota and time (Lee et al., 2001; Oze et al., 2004; Kierczak et al., 2007).

Serpentine soils are the soils derived from ultramafic rocks or serpentinites (Oze et al., 2004), are green to grayish-green “broken rocks”, comprised of thin layers, which can easily break up and fragment in small particles. Serpentine soils are known for their elevated metal concentrations including Cr, Ni, Mn and Co (Rajapaksha et al., 2012;

Vithanage et al., 2014) and these soils provide an opportunity to assess the dynamics of potential metal interactions.

Cr and nickel (Ni) in soils are primarily derived from the weathering of minerals contained in the ultramafic rocks like chromite, olivines, pyroxenes, etc. (Morrison et al., 2009). A two-stage mechanism of chromite weathering was proposed by Cooper (2002): a slow hydrolysis of Cr(III) to Cr(OH)₃, followed by a slow oxidation step to Cr(VI) by Mn oxides. During this process, Cr derived from pyroxene, olivine and chromites weathering, is integrated into minerals, i.e. iron (Fe) oxides or smectite (Garnier, 2009). The primary mineralogical component of Cr in serpentinite is chromite and Cr-rich magnetite with lower amounts contained in chlorite, augite, enstatite, Fe hydroxides, and clay (Mills et al., 2011; Becquer et al., 2003; Oze et al., 2004). Cr can remain in minerals, co-precipitated with manganese (Mn), aluminum (Al), and Fe oxides, which are generally adsorbed on soil particles, and complexed with soil organic compounds (Hsu et al., 2015). The difference in the geochemical characterization of the Cr host minerals is the Cr solubility in acids like HNO₃. According to Morrison et al. (2009), Cr in chromite and other Cr-rich spinels are mainly recalcitrant in acid etching, whereas Cr in pyroxenes, olivines, serpentine, chlorite-chromium mixtures or in clays contain a higher proportion of acid-soluble forms.

During the last years, geogenic origin Cr contamination has been found to be wide spread in diverse areas around the world such as in Mexico, California, Zimbabwe and Italy, thus it has stimulated significant scientific interest (Robles-Camacho and Armienta, 2000; Cooper, 2002; Fantoni et al., 2002; Oze et al., 2004; Garnier et al., 2009; Morrison et al., 2009; Mills et al., 2011; Morrison et al., 2015). Specifically, the Sacramento Valley comprises the northern half of California's Central Valley and is bordered by mountain ranges with extensive ultramafic outcrops (Morrison et al., 2009; Oze et al., 2007). Weathering of these outcrops has resulted in soils and vadose zone sediments of the Southern Sacramento Valley that are consistently elevated in Cr (up to 42 mmol/kg) compared to the geometric mean (0.71 mmol/kg) for surface soils in the conterminous U.S. (Mills et al., 2011; Morrison et al., 2009). Moreover, in Zimbabwe there are significant areas of ultramafic soils associated with the 530 km long Great Dyke. This is a layered complex comprising about 70% ultramafic rocks, mainly serpentinitized dunite and harzburgite. Cr toxicity has been suspected in ultramafic soils for many years and strongly implicated in Zimbabwean soils (Cooper,

2002). In Italy, ultramafic rocks are present in the La Spezia province and high Cr concentrations in groundwater were detected (Fantoni et al., 2002). Additionally, the Sierra de Guanajuato ultramafic units and their alteration products were identified as having the largest potential to be sources of Cr contamination in the waters to the East of León Valley, in central Mexico (Robles-Camacho and Armienta, 2000).

1.3 Bioavailability, speciation and mobility of chromium in soils

The fate and behaviour of metals in soils are determined by their presence in various chemical forms and their ability to bind with different soil components. The cycling of substances in the natural environment is a complex process, thus when measuring the total content of metals is not adequate to determine their mobility and bioavailability (Quevauviller, 2003; Wang et al., 2009). Speciation studies are significant for assessing the mobility and bioavailability processes of metals in soils due to the possibility of differentiation of the chemical forms in which metals can occur in soils (Jamalia et al., 2009; Rauret, 1998).

Soil pH, organic matter and redox potential (Eh) can influence remarkably the geochemical behavior of Cr by affecting its chemical speciation (Amin and Kassem, 2012; Choppala et al., 2016; Xiao et al., 2015). Reduction processes prevail at low Eh whereas oxidation processes dominate at high Eh (Frohne et al., 2015). Reduction processes increase pH by consuming protons while oxidation processes decrease pH by producing protons. Reduced soil conditions cause conversion of toxic Cr (VI) into less toxic Cr (III), as well as immobilization and precipitation of Cr (III) (Xiao et al., 2015). This process is being used as a key step in treatment of Cr-contaminated soils (Pettine, 2000). Cr (III) has low solubility only at pH < 5.5 (Kabata-Pendias, 2010). Above that pH, Cr(III) almost completely precipitates, and therefore its compounds are regarded highly stable in soil. Alternatively, Cr (VI) is highly unstable in soils and remains mobilized in both acidic and alkaline soil pH (Kabata-Pendias, 2010). Moreover, reduction of Cr (VI) to Cr (III) species using various organic amendments (plant biomass, seaweed, black carbon, biosolid compost, farm yard manure, pig manure, and poultry manure) is commonly used in remediation and soil reclamation technique (Shahid et al., 2015).

The processes of Cr release from soils and adsorption to soils has been the subject of

significant body of research and the understanding of the mechanisms affecting Cr fate in aquifers (oxidation, reduction, adsorption and desorption) is of paramount importance (Hellerich and Nikolaidis, 2005). Sorption and desorption reactions are relevant to the mobility of Cr (Rai et al., 1989). Transport of chromate is mainly controlled by adsorption on the surfaces of Fe oxides such as hematite (Johnston and Chrysoschoou, 2014), amorphous aluminum and organic complexes, and Cr(III) can be adsorbed onto silicacious components (Bartlett and James, 1988). The maximum Cr adsorption capacity was assessed for the bulk samples and their clay and Fe-free clay fractions of four subtropical soils differing in mineralogy and found that Cr adsorption was the highest in the soil with the highest organic matter content and increased with increasing pH (Bavaresco et al., 2017). Furthermore, Cr, Ni, and vanadium occurred predominantly in the fraction bound with Fe and Mn oxides and in the residual fraction, thus showed low mobility (Jeske and Gworek, 2012). Generally, assessment of contaminant mobility is a necessary tool in order to establish either risk-based or mobility-based, site-specific cleanup levels for remediation (Nikolaidis and Shen, 2000).

1.4 Chromium in soil-plant system

Cr uptake by plants has gained considerable attention worldwide because of its role as an essential element in human metabolism, but also due to its carcinogenic effects. Therefore, optimal level of Cr in human nutrition has become extremely topical especially in risk assessment and remediation studies. In plants, particularly crops, Cr at low concentrations (0.05 – 1 mg/L) was found to promote growth and increase yield, but there is no evidence that plays a role in their physiological function (Oliveira, 2012). On the other hand, Sridhar et al. (2007) has shown that Cr can accumulate in plants, reducing their growth, altering mineral nutrition and causing oxidative stress via formation of reactive oxygen species (Raj et al., 2011).

The ability of plants to uptake Cr depends on plant type and Cr species (Shukla et al., 2007). Cr(III) had different effects on cress and cucumber shoots: low toxicity was found in cucumber seeds exposed to a concentration of Cr of 300 mg/kg in soils, while biostimulating effects were recorded in cress plants exposed to up to 100 mg/kg of Cr (Baderna et al., 2015). Cr(VI) is harmful to many plants for concentrations ranging from

0.16 mg/L (lettuce) to 75 mg/L (sweet orange) (Barcelo and Poschenrieder, 1997, Pawlisz et al., 1997, Poschenrieder et al., 1987). Dai et al. (2009) have suggested the determination of a soil environmental quality standard for farmland based on the effects of Cr on crop growth and Cr accumulation in the edible part.

Cr(III) uptake in plants is a passive process and does not require any energy by the plants (Shanker et al., 2005)., whereas Cr(VI) uptake is an active process and is performed by carriers of essential elements such as sulphate (Hossner et al., 1998, Oliveira, 2012). Among heavy metals, Cr is reported to be least mobile element in the plant roots (Shukla et al., 2007). Concentration of Cr in roots is sometimes 100 times higher than the shoots (Shanker et al., 2005). Cr(VI) absorbed by the root is reduced to Cr(III) and retained mostly by the roots (James and Bartlett, 1984). Studies on uptake by onions (Srivastava et al., 1994), maize and groundnut plants (Mishra et al., 1995) showed that the uptake of Cr was higher when it was supplied by irrigation water. A significant amount of Cr was absorbed by the plant roots which played an important role in modifying the state of Cr to a bioavailable form. The amount of Cr translocated to grain of paddy was less compared to the above three crops (Mishra et al., 1997). Studies on chamomile plants presented similar results regarding Cr accumulation in roots (Kovacik et al., 2014).

The presence of plants in wetland vegetated systems enhances the reduction of Cr(VI) in sediments by modifying the governing biogeochemical cycle (Zazo et al., 2008). Furthermore, bacterial mediated reduction of Cr(VI) to Cr(III) is a widely - known and accepted technology for the remediation of contaminated soils and groundwaters (Hellerich and Nikolaidis, 2005). Similarly, endophytic bacteria (defined as the bacteria that are present in the plant internal tissues without imposing any negative effect) isolated from many plant species have shown to be able to reduce Cr(VI) (Kuklinsky et al., 2004). Experiments conducted using *J. acutus* showed that isolated endophytic bacteria were found to be Cr(VI) tolerant and have a high potential to reduce Cr(VI) to Cr(III) (Dimitroula et al., 2015). The microbial reduction of Cr(VI) to Cr(III), which is a useful process, takes place intra-cellularly (Narayani and Shetty, 2013) and reduces Cr toxicity.

Carrot (*Daucus carota*) is a root crop which is rich in bioactive compounds such as carotenoids and dietary fibers with appreciable levels of other functional components with significant health-promoting properties (Sharma et al., 2012). Carrots have been

suggested as biomarkers for toxicity assessment in terrestrial ecosystems (U.S. EPA, 1996). A greenhouse experiment conducted in Chinese soils showed that, the carrot yield significantly decreased in soils with Cr addition, and the Cr content of carrot grown in soils with pH>8.0 exceeded the maximum allowable level (0.5 mg/kg), according to the Chinese General Standard for Contaminants in Foods (Ding et al., 2014).

1.5 Human health risk assessment

Contamination of food chain with heavy metals is considered as one of the major environmental pathways of human exposure leading to potential health risk, therefore food safety has recently gained substantial consideration worldwide (Xiong et al., 2016). As far as vegetables, they play an important role in our daily diet and constitute essential diet components. Vegetables can take up heavy metals by absorbing them from contaminated soils, as well as from deposits on parts of the vegetables exposed to the air from polluted environments (Wang et al., 2005). Vegetables may accumulate metals in concentrations greater than the maximum permissible limits (Mombo et al., 2016).

Chronic intakes of heavy metals have damaging effects on human beings and other animals. For example, Cr, Cu and Zn can cause non-carcinogenic hazardous such as neurologic involvement, headache and liver disease, when they exceed their safe threshold values (US EPA, 2000). There is also evidence that chronic exposure to low doses of cancer-causing heavy metals may cause many types of cancer. For example, Park et al. (2004) found increased lifetime risk of lung cancer death resulted from occupational exposure to dusts and mists containing Cr(VI).

During past 2-3 decades, numerous studies focused on the cultivation of vegetables on Cr and other heavy metals contaminated soils, and estimated the associated possible health risks (Noli and Tsamos, 2016). Consumption of food contaminated with Cr is considered to be the major pathway (>90%) of human exposure compared to inhalation and dermal contact (Xiong et al., 2014). This is well-defined by numerous Cr-induced clinical disorders in human exposed to Cr via vegetable consumption such as respiratory, carcinogenic, renal, hepatic, gastrointestinal, cardiovascular,

hematological, reproductive and developmental, genotoxic and mutagenic (ATSDR, 2013).

Regardless of the studies mentioned above, precise estimation of the availability of Cr in plants as well as the associated health risks still remains a challenge, since the analytical methods for Cr speciation in foodstuffs are not yet fully clarified. The key objective is to assess the oral genotoxicity of Cr(VI) from foodstuffs to reduce the risk to human health from this pathway (Proctor et al., 2002). It is likely that any ingested Cr(VI) will be reduced to Cr(III) in the acidic conditions of the stomach (Milacic and Stupar, 1994), and any Cr(VI) transferred from soil to plant will also be reduced. However, there are other sources of Cr(VI) introduced during the manufacture and processing of foodstuffs (e.g. stainless steel vats) that justify the need to undertake Cr speciation. It is therefore vital to understand the limitations and interferences of each speciation technique (Hamilton et al., 2018).

Nowadays, the magnitude of the risks involved with the consumption of vegetables cultivated on contaminated soils are monitored. Different parameters, such as soil and plant enrichment factors, translocation factor, estimated daily intake, hazard quotient, maximum allowable level of plant consumed, bio-accumulation potential, health risk index and life time cancer risk, are used to estimate Cr health risks to humans as a result of contaminated food consumption (Wang et al., 2011; Shahid et al., 2017).

1.6 The study area of Asopos river basin and framework of analysis

In Greece, the main ophiolite outcrops of the Hellenides are distinguished in four ophiolite belts (Papanikolaou, 2009) (Fig. 1.3). Pindos Mountains are covered with ultramafic rocks, which have been eroded and their fragments have been combined with Tertiary and Quaternary deposits that reside in valleys. Serpentine soils with high geogenic origin Cr contamination were derived from the weathering of these rocks.

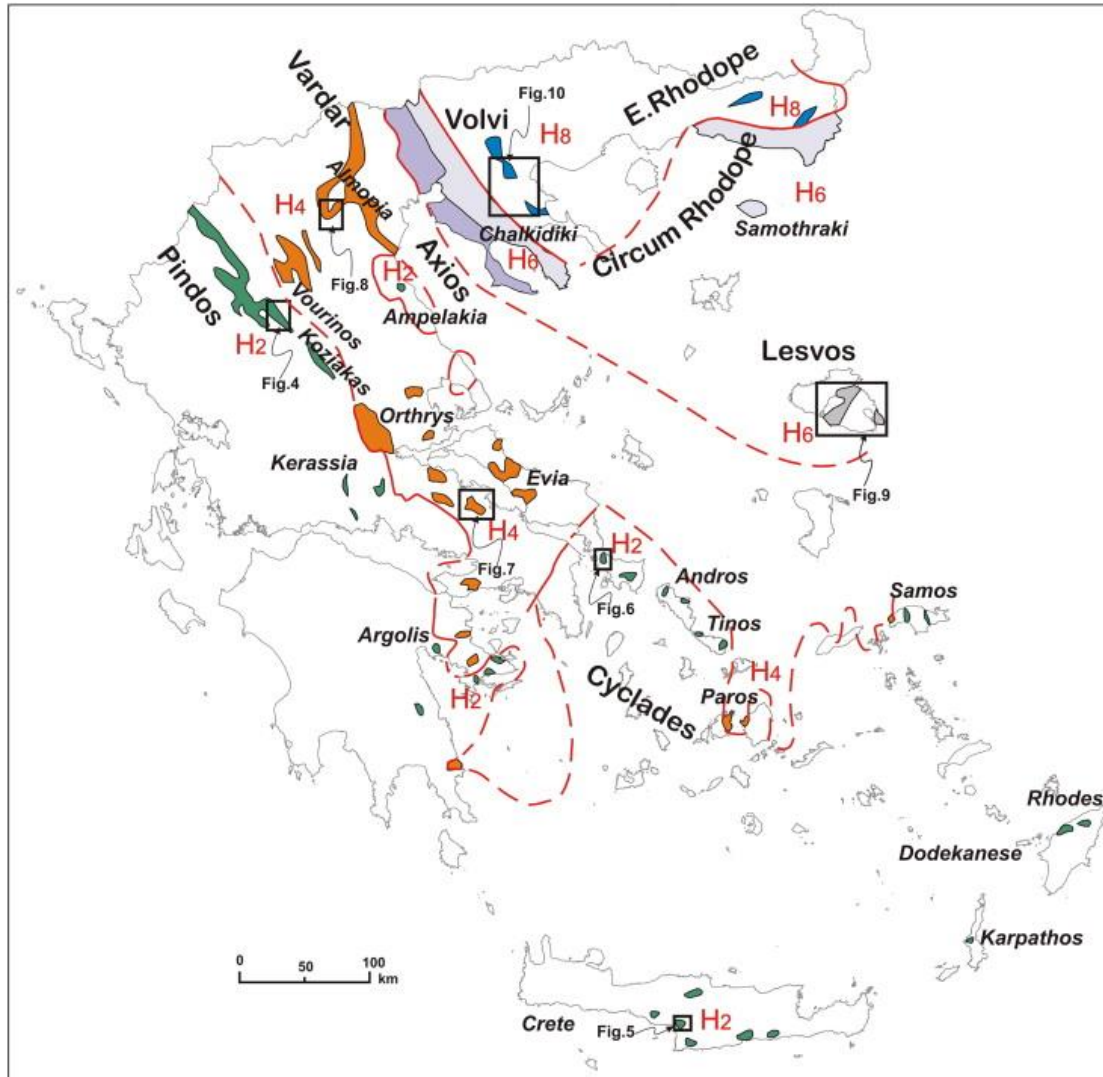


Figure 1.3. The main ophiolite outcrops of the Hellenides are distinguished in four ophiolite belts corresponding to the oceanic terranes of H2, H4, H6 and H8 (Papanikolaou, 2009).

The study area of the present thesis is situated in central Greece and specifically in the basin of Asopos river. Asopos river received waste discharges from metallurgies and industries related to textile, dyes, and food production. The waste release has been ceased at least 5 years ago after the rising of public awareness. Additionally, the western part of Asopos watershed (Thiva valley) is covered with topsoil enriched in Cr and Ni as a result of transport of the weathered parent rock fragments from the higher elevations of the basin (Kelepertzis et al., 2013). Moreover, according to Moraetis et al. (2012), weathered ultramafic rock fragments in the Neogene and alluvial deposits have resulted in producing geogenic origin Cr(VI) in the groundwater at least in the eastern part of the watershed close to Oropos area.

As a result, the problem of contamination of the Asopos river basin is multi-dimensional because of the combined existence of geogenic and anthropogenic origin Cr. Specifically, the geogenic contamination is wide spread throughout the basin, and there is also anthropogenic origin Cr that is restricted around the industrial park of Oinophyta area. It has been observed in some cases drinking water with concentrations of geogenic Cr(VI) above the acceptable criteria, river contamination with concentrations above aquatic quality standards and irrigation with groundwater that contains geogenic Cr(VI) (Moraetis et al., 2012). Moreover there is a public perception of potential adverse effects due to the consumption of agricultural products, a problem that has not been scientifically proven to exist.

Thus, having already strong evidences of geogenic Cr origin, we proposed to conduct a mapping of the Cr geochemical characterization along the Asopos river basin and the mobility of Cr in the soils in order to understand the critical factors influencing the presence of Cr in the watershed. The present study intended to cover the need to develop methodologies and an integrated approach to outline an area with wide spread geogenic origin Cr contamination, and to develop a framework of analysis to understand the origin and mobility of geogenic Cr in soils of Asopos river basin.

Therefore, the objectives of this study were threefold:

- (1) to assess the geochemical characterization and classification of soils and river sediments with respect to the origin of Cr along Asopos river,
- (2) to assess the processes affecting the mobility of Cr(VI) in soils and sediments along Asopos river and to elucidate the mechanisms of Cr release from soils and,
- (3) to develop a methodology to assess the impact of geogenic origin Cr(VI) uptake by agricultural products (specifically carrots), and the risk of human consumption of carrots grown in Asopos River basin in Greece.

Towards achieving these objectives:

Chapter 2 presents a field, laboratory and modelling study that was conducted in order to characterize the soils and river bed sediments and to assess the origin of Cr in Asopos basin.

Chapter 3 presents a laboratory study that was conducted to assess the mobility and to identify the controlling mechanisms of Cr release from soils obtained from Asopos river

basin.

Chapter 4 presents a methodology that was developed to assess the impact of geogenic origin Cr(VI) uptake by carrots, and the risk of human consumption of carrots grown in Asopos river basin.

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CHAPTER 2. CHARACTERIZATION AND MOBILITY OG GEOGENIC CHROMIUM IN SOILS AND RIVER BED SEDIMENTS OF ASOPOS BASIN



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2.1 Abstract

A field and laboratory study was conducted to assess the origin and mobility of Cr(VI) in Asopos basin in Greece. Sampling was designed in such way as to capture the spatial variability of Cr occurring in sediments and soils in different lithological units in the area. Physicochemical and geochemical characterization of surface agricultural soils obtained from river terraces and river bed sediments was conducted in order to determine the natural background of chromium. Lithologies with strong calcareous, siliceous and ultramafic components were identified using principal component analysis. Laboratory mobility studies quantified the rates of Cr sorption and release from soils and their capacity to adsorb Cr. Heavy metal analysis and local geology study support the hypothesis that the main source of Cr is of geogenic origin. Cr distribution in Asopos river bed was influenced from the eroded products derived from extensive areas with ultramafic rocks the last 5 Ma. The mobility studies showed that leaching process was very fast and sorption capacity was significant and capable to retain chromium in case of waste release in the river. Finally the mobility of Cr release is limited due to existing attenuation capacity controlled by ferric oxides coatings on the soil and sediments.

2.2 Introduction

A growing worldwide concern on human health risks of Cr has stimulated research on its fate and transport in groundwater (Oze et al., 2007). In the environment, Cr is found mainly in two oxidation states, the Cr(III) and the Cr(VI). Cr(VI) is mobile and highly toxic for humans, whereas Cr(III) is immobile, has low toxicity and is considered to be an essential trace element in human metabolism. The differences of the two oxidation states of chromium make the assessment of potential human health risks, difficult (James, 1996). The European Commission (Directive 98/83/EC) established 50 ug/L as the maximum permissible limit of total Cr in drinking water, similar to the one established by the World Health Organization. The Italian regulation defined a maximum allowable concentration of 2 mg/L (on a dry basis) for Cr(VI) in soils for private use, and a maximum acceptable concentration of 150 mg/L for total Cr (Fantoni et al., 2002).

The processes of Cr release from soils and adsorption to soils has been the subject of significant body of research and the understanding of the mechanisms affecting Cr fate in aquifers (oxidation, reduction, adsorption and desorption) is of paramount importance (Hellerich and Nikolaidis, 2005). The kinetics of the Cr redox reactions complicate chromium transport because each Cr species presents a different sorptive behavior depending on the prevailing physicochemical conditions (e.g. pH, organic matter content) (Guha et al., 2001). Transport of chromate is mainly controlled by adsorption on the surfaces of iron oxides such as hematite (Johnston and Chrysoschoou, 2014). Generally, assessment of contaminant mobility is a necessary tool in order to establish either risk-based or mobility-based, site-specific cleanup levels for remediation (Nikolaidis and Shen, 2000).

Cr existence in soils and water can be a result of anthropogenic activities i.e. industrial activities and phosphorus fertilizers (Molina et al., 2009), or of natural processes as the weathering of ultramafic rocks (Cooper, 2002, Fantoni et al., 2002, Garnier et al., 2009, Mills et al., 2011, Robles-Camacho and Armienta, 2000. Serpentine soils are the soils derived from ultramafic rocks or serpentinites (Oze et al., 2004). Cr and nickel in soils are primarily derived from the weathering of minerals contained in the ultramafic rocks like chromite, olivines, pyroxenes etc. (Morrison et al., 2009). Although serpentinites and serpentine soils cover about 1% of the earth's exposed surface, they are common along the Circum-Pacific margin and the Mediterranean (Oze et al., 2004). A two-stage mechanism of chromite weathering was proposed by Cooper (2002): a slow hydrolysis of Cr(III) to Cr(OH)₃, followed by a slow oxidation step to Cr(VI) by Mn oxides. During this process, Cr derived from pyroxene, olivine and chromites weathering, is integrated into minerals, i.e. iron oxides or smectite (Garnier et al., 2009). An appreciable amount of Cr is associated with iron oxides, clay minerals (Mills et al., 2011) and spinels as magnetite, chromite and other spinels containing Al, Mg, Fe, Cr (Oze et al., 2004). The difference in the geochemical characterization of the Cr host minerals is the Cr solubility in acids like HNO₃. According to Morrison et al. (2009), Cr in chromite and other Cr-rich spinels are mainly recalcitrant in acid etching, whereas Cr in pyroxenes, olivines, serpentine, chlorite-chromium mixtures or in clays contain a higher proportion of acid-soluble forms.

Asopos River received waste discharges from metallurgies and industries related to textile, dyes, and food production. The waste release has been ceased at least 5 years

ago after the rising of public awareness. The western part of Asopos watershed (Thiva valley) is covered with topsoil enriched in Cr and Ni as a result of transport of the weathered parent rock fragments from the higher elevations of the basin (Kelepertzis et al., 2013). According to Moraetis et al. (2012), weathered ultramafic rock fragments in the Neogene and Alluvial deposits have resulted in producing geogenic origin Cr(VI) in the groundwater at least in the eastern part of the watershed close to Oropos area (dashed orange line Fig. 2.1.). Despite the last two studies there is no complete characterization of the Asopos watershed and specifically of the river bed sediments where probably both Cr of geogenic and anthropogenic origin may exist. Thus, having already strong evidences of geogenic Cr origin we consider that Cr geochemical characterization along the Asopos river sediments would reveal the critical factors influencing the presence of Cr in the watershed and groundwater.

The objectives of this study are twofold: (1) to assess the geochemical characterization and classification of soils and river sediments with respect to the origin of Cr along Asopos river, and (2) to assess the processes affecting the mobility of Cr(VI) in soils and sediments along Asopos river.

2.3 Materials and methods

2.3.1 Site description

Asopos river basin is located in the Region of Sterea Ellada, about 100 km north from Athens and has a surface area of approximately 703 km². Asopos is considered representative of a Mediterranean intermittent river (Botsou et al., 2008). It has a total length of 54 kilometers and runs through the areas of Thebes, Avlona, Tanagra, Schimatari, Oinofyta, Sikamino, and Oropos, until it discharges into Evoikos gulf. Seven municipalities are within the Asopos basin, with a total population of 200.000 inhabitants (Kontou, 2011). During the last three decades, an intense industrialization took place in its drainage basin that nowadays hosts more than 450 industries (industrial zone of Oinofyta area).

The geology of the study area is shown in Fig. 2.1 and it is comprised of the following units: the alpine basement rocks (limestones and dolomites mainly of Triassic and ophiolite rocks), and the post-alpine sediments (Quaternary and Tertiary-Neogene).

The Asopos watershed is covered with post alpine sediments with thickness from 30 to 150 m, while some of them like the Neogene sediments (Late Miocene) reaching a depth of 300 m. Quaternary deposits comprised of a) alluvial fluvial sediments with 30 m thickness mainly situated in the Asopos active riverbed b) Pleistocene fluvial deposits with conglomerates sands and loam with thickness 120-150 m which are situated mainly along the river and c) pliocene deposits of brackish waters with sand, loam, clays and marls which are situated in the western part of the watershed with limited thickness (30 m). The tertiary deposits are comprised of extensive Neogene marine deposits mainly of marl, sand, clay and loam and are situated in the eastern part of the watershed. Within the Neogene deposits lignite lenses and other coarse unconsolidated material exist (Papanikolaou et al., 1988, Stamatis et al., 2011). The alpine nappes comprised of the pelagonia nappe with thick bedded limestones and dolomites which cover mainly the south boundaries of the watershed. Relics of ophiolite nappe lie unconformably on the pelagonia nappe.

At this point we stress the important geological evidence which showed that ophiolite nappes have been extensively eroded and supplied Neogene and Pleistocene basins with ophiolite fragments. The lithologies of those formations have been described by the Greek Geological Survey and they have been identified also by Moraetis et al. (2012) using micro-XRF analysis. Thus, the green color in Fig. 2.1 depicts either relics of ophiolite nappes or sediments with fragments from ophiolites and cherts (sand, cobbles and gravels). Although ultramafic rocks are limited in Asopos basin and small outcrops exist in the south (ophiolites with green colour in Fig. 2.1), there are extended areas with hornstones and serpentized ophiolites in the vicinity of Asopos watershed (west-north area not shown in Fig. 2.1). Finally, field observations at the present study revealed new sites with visible ophiolite fragments in the area close to relics of the ophiolite nappe as shown with the green arrow in Fig. 2.1.

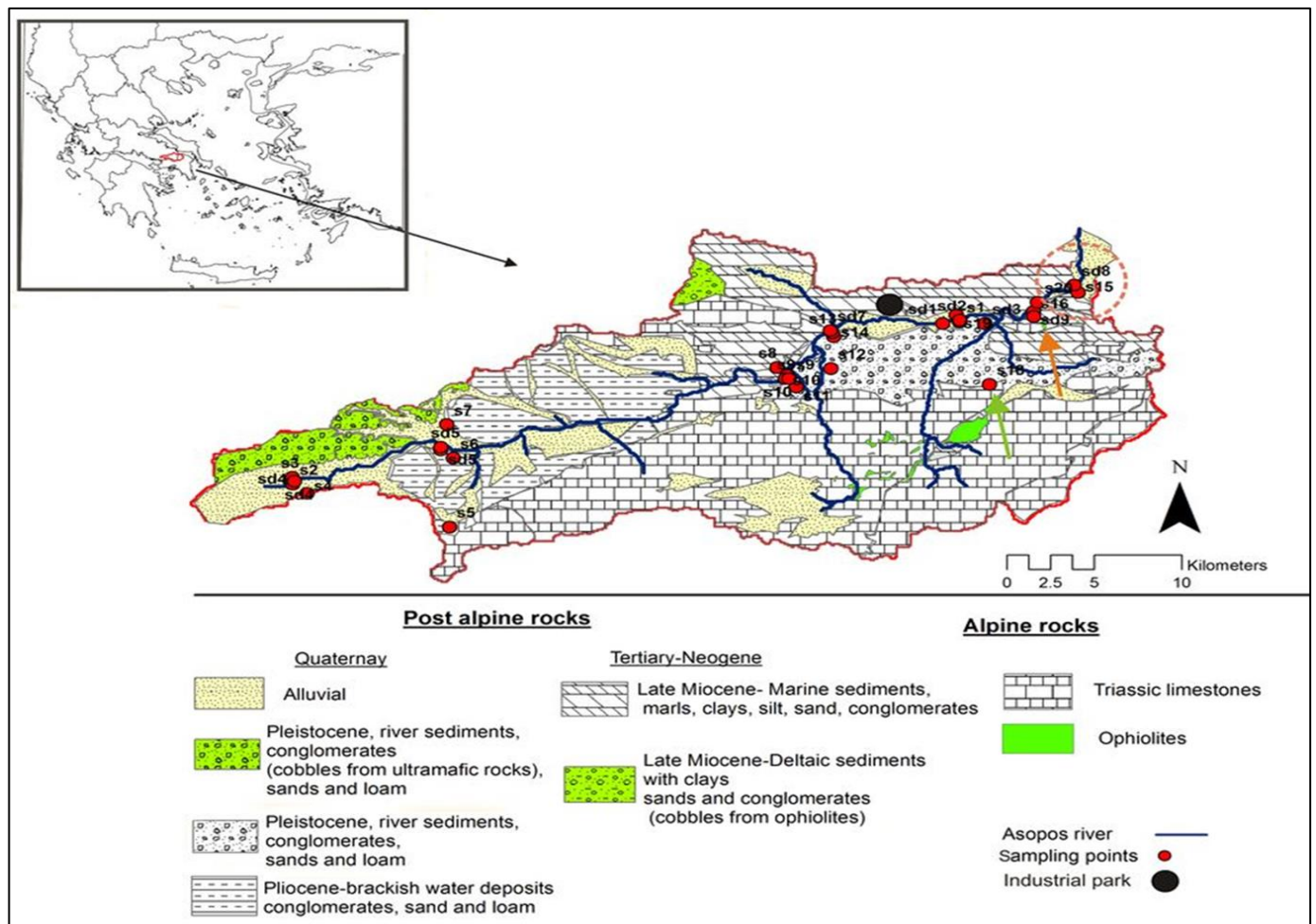


Figure 2.1. Geology map of the study area with the main lithological units. Red circles depict soil and river sediment sampling sites and black circle depicts the industrial park at the Oinophyta area.

2.3.2 Experimental design

The overall experimental design was conducted in two phases and corresponds to the hypothesis of the two objectives of this study respectively. In the first phase, a field monitoring and sampling campaign was designed and soil, surface river bed sediments and core river bed sediment samples were collected. Moreover physicochemical characteristics the soil and river sediment samples were analyzed. Physicochemical characteristics were fed into Principal Components Analysis (PCA) as to categorize the samples according to the geochemical characteristics. The second phase involved mobility studies in the laboratory for process understanding i.e. sorption and release of total and Cr(VI) from the soils. Finally, modeling of Cr adsorption in soil was applied as to elucidate the adsorption mechanism.

A targeted sampling campaign (sediment and soil monitoring survey) was conducted in order to achieve the first objective of this study. The field campaign was carried out in March 2012. The sampling strategy had as objectives on one hand to capture the variability of the lithology of the area and on the other to compare the chemical composition of the river sediments upstream (sd4, sd5, sd7, sd1) and downstream (sd2, sd3, sd9, sd8) from the industrial park (black dot in Fig. 2.1) in order to identify potential contribution of pollution from the industrial park. The surface river bed sediments were taken up to a depth of 20 cm while the core river bed sediments were sample up to a maximum depth of 150 cm using a hand auger. In addition, soil samples (top 15 cm) were taken in cross-sections perpendicular to the river from the first and second terraces to determine the natural background of Cr concentration in each locality.

In general, 8 surface river sediment samples (Sd) and 6 core river sediment samples (Sd core) were collected from the main course of the Asopos River (river bed). 20 soil samples (S) were collected in the first terrace next to the samples from the river bed and in the second terrace. The soils of the first terrace are mostly cultivated alluvial soils, and more brown, indicating recent sedimentation and organic matter deposits. The soils of the second terrace are also cultivated soils mostly Neogene and have less organic matter. Other soil bedrock lithologies included Quaternary sediments (alluvial and Pleistocene sediments) and ultramafic rocks (serpentinite). About 1 kg for each

sample was collected at the top 15cm from different locations and it was homogenized and packed in plastic bags.

2.3.2.1 Physicochemical analysis

Sediment and soil samples were homogenized, air dried and sieved. Part of the sample was kept without drying for alkaline digestion and hexavalent Cr determination. The fraction smaller than 2 mm was used for the experiments. The samples were analyzed for physical and chemical properties including particle size distribution, pH, conductivity, soil moisture, total organic carbon, total organic nitrogen, total major and trace metals. Furthermore, the oxidation and reduction potential capacity was determined. The Bouyoukos hydrometer method was used for determination of the particle size distribution of the surface soil samples (Bouyoucos, 1936). The oxidation and reduction potential capacity was determined using a batch kinetic experiment. During the kinetic experiment pH, oxidation-reduction potential (ORP) and dissolved oxygen were measured in a continuously stirred batch reactor under N₂ blanket conditions. Total organic carbon and total organic nitrogen were analyzed using the multi N/C 2100S Analyzer. Base cations and heavy metals were analyzed according to EPA 3051A method. Specifically, 9 ml HNO₃ were added to 0.2 g soil, followed by microwave digestion at 150-180°C (Multiwave 3000 Digestor). Supernatant solutions were diluted with MilliQ water and analyzed by ICP-MS (Agilent- CX). EPA method 3060A (alkaline digestion) was used for the determination of hexavalent chromium in soils and sediments. Bulk chemical analysis and several trace element analysis were performed on the soil fraction <2mm by X-ray fluorescence using the spectrometer S2 Ranger (Bruker Co).

2.3.2.2 Mobility studies

Two types of mobility experiments were conducted (a batch kinetic leaching experiment and an adsorption experiment) using surface river bed sediment samples. The aim of these experiments was to elucidate the processes that affect the existence and mobility of Cr(VI) in sediments and groundwater. The leaching experiment was used to measure the rate of desorption of Cr from the sediments. The objective of the adsorption experiment was to ascertain the fate of both geogenic and anthropogenic

Cr(VI) in the sediments of the study area. Batch isotherm experiment was used to examine the order of magnitude of soil sorption capacity (using Cr(VI) concentrations higher than current field levels) that could limit the retardation potential of Cr(VI).

The leaching experiment was carried out in duplicates and conducted in plastic bottles using sediment/solution ratio of 1/10 with equilibrium solution of 20 mL NaNO₃ and molarity of 0.01 M. The sorption measuring time spans extended from 1 h to 7 days. Adsorption analyses were carried out in duplicates and solution Cr(VI) concentration ranged from 0.5 mg/L to 500 mg/L. All solutions prepared with MilliQ and aliquots stirred continuously in a vibrating plate. The supernatant solutions were filtered immediately with a nylaflo membrane 0.45 mm filter prior to analysis. Cr speciation in the release experiments was determined using an amberlite IR – 120 resin and measuring the Cr content before and after the resin with an ICP-MS. The adsorption results were modeled using the geochemical model MINEQL+4.6.

2.4 Results and discussion

2.4.1 Physicochemical characterization of soils and sediments

Fig. 2.1 presents the location of the samples in the watershed, contrasted with the geology of the area. The physical and chemical characteristics for the soils and the sediments samples had similar values for pH, electrical conductivity (EC), TOC and TN. The clay content of the surface soil samples was 28-58% measured with the Bouyoukos hydrometer method. The oxidation-reduction potential experiment showed a decrease of ORP with time and after two hours ranged between 60 to 140 mV for the soils and -100 to 80 mV for the river bed sediments. Therefore, the minimum Eh for the soils is 260 mV and for the river bed sediments 100 mV, suggesting the samples are poorly poised and do not have significant reductive potential.

The average heavy metal concentrations were also similar suggesting that the origin of almost all samples analyzed is the same. The total Cr contained in the soil samples ranges between 100 and 2500 mg/kg. Fig. 2.2 presents the vertical distribution of total chromium in the surface river bed sediments and the core river bed sediments taken at different locations along the main axis of the river. Total Cr concentration for the surface river sediments ranges between 631 and 1639 mg/kg. No significant differences

were found, comparing the samples from upstream (sd4, sd5, sd7) with downstream (sd1, sd2, sd3, sd9, sd8) Cr of the industrial park, suggesting that anthropogenic pollution has not affected significantly the downstream river bed sediment Cr concentrations. Core sediments follow a similar trend to surface sediments also suggesting minimum anthropogenic impact.

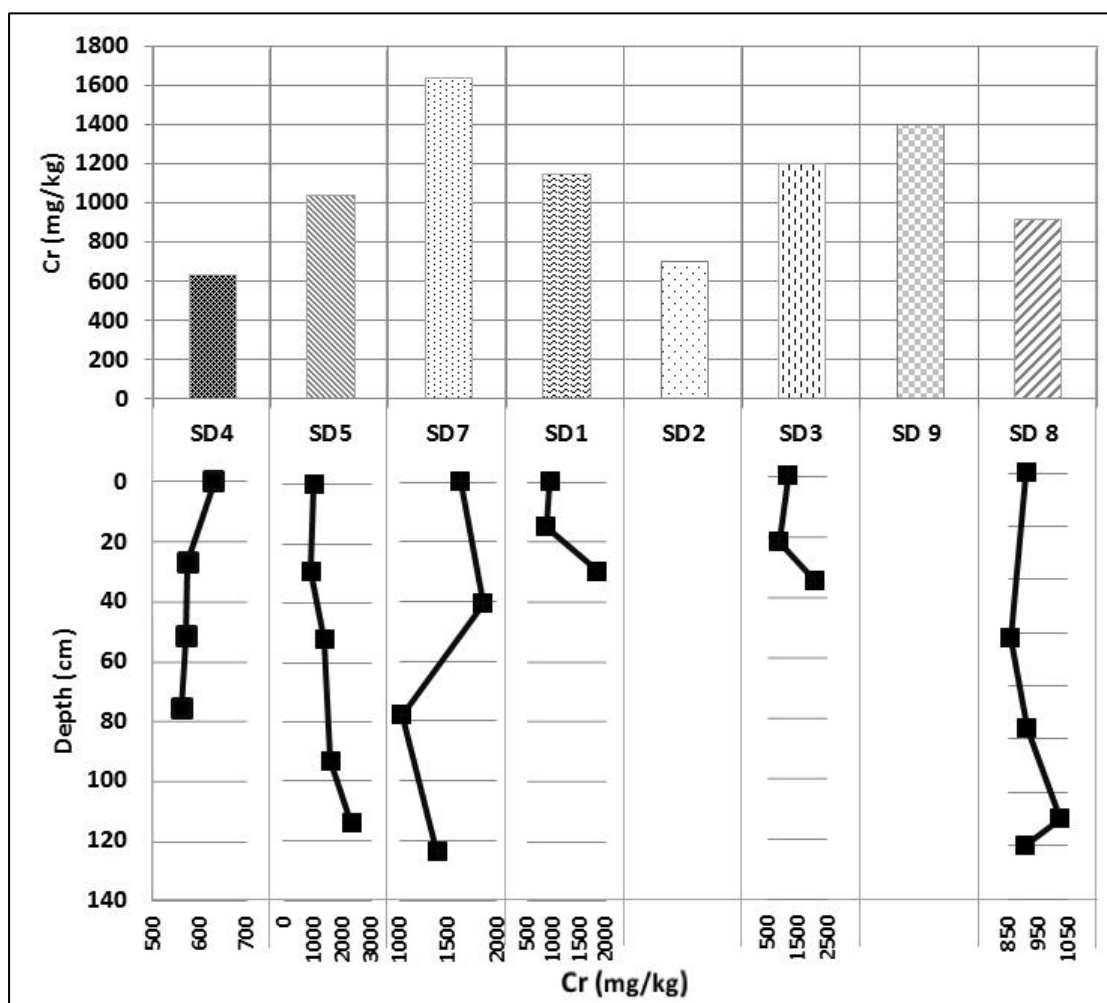


Figure 2.2. Cr distribution of industrial park upstream and downstream surface river bed and core sediments. There are no sd2 and sd9 core samples because of rocky texture sediments.

In order to access the geogenic origin of Cr content of the soils and the river bed sediments, the ratio of Cr to nickel, that is typical of ultramafic rocks, was examined (Fig. 2.3). The samples were analyzed both by XRF and by ICP-MS after HNO_3 digestion. A correlation of the Cr content measured by XRF and HNO_3 digestion had a coefficient of determination of 0.2. The coefficients of determination for Cr-Ni were 0.74 and 0.49, for HNO_3 digestion heavy metal content and XRF bulk analysis heavy

metal content, respectively. High correlation of Cr-Ni suggests geogenic origin of Cr in soils and sediments. The heavy metal content measured with XRF is at least twice as much as the one measured after HNO₃ digestion. The reason for the severe underestimation of acid soluble Cr is the geogenic nature of Cr mainly in chromite grains and other minerals like spinels which are recalcitrant to HNO₃ digestion.

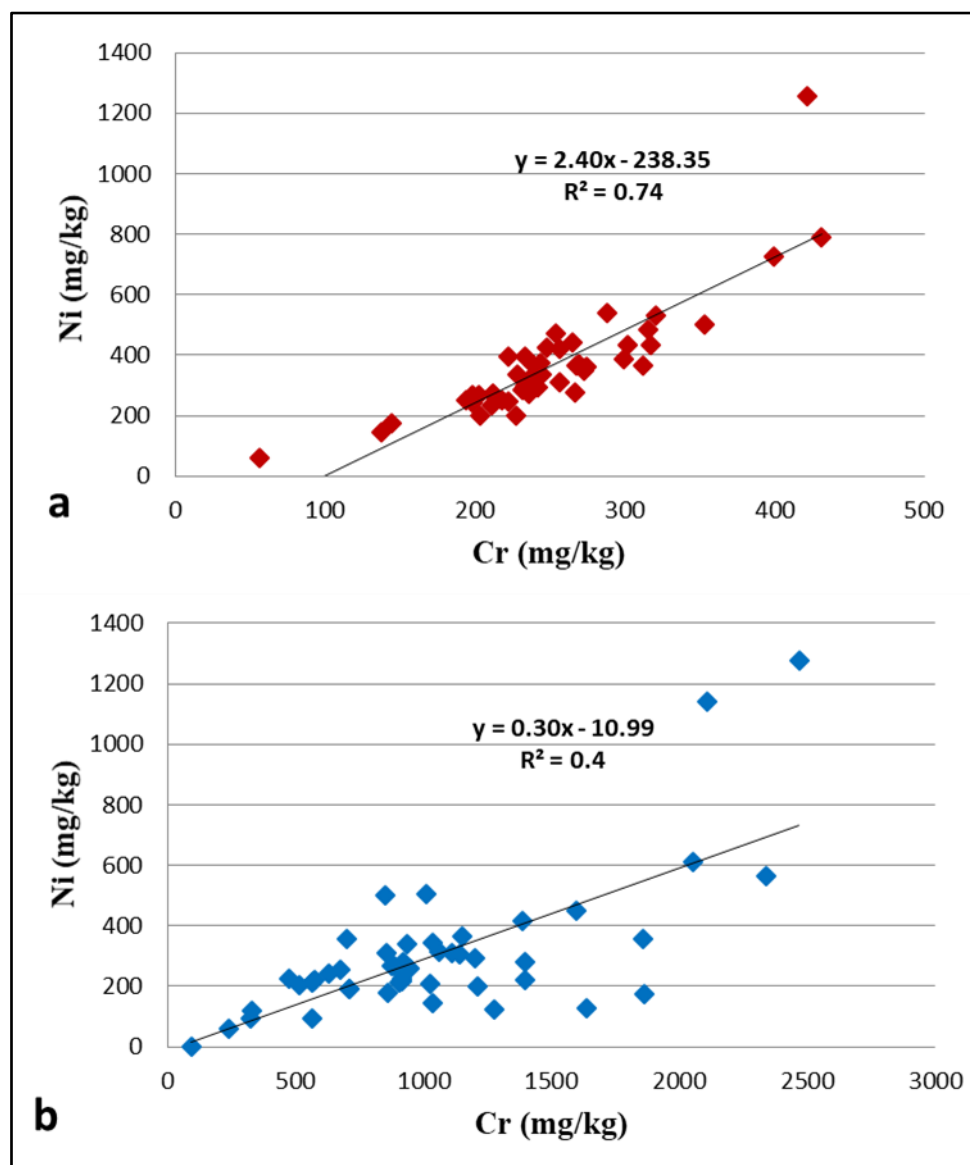


Figure 2.3. Correlation diagram of Ni and Cr content in soils and river bed sediments by a) HNO₃ digestion and b) XRF analysis respectively.

2.4.2 Geochemical analysis and classification of soils and sediments

PCA was conducted using Ni, Mg, total Cr, CaO, Rb, SiO₂, TiO₂, Zr, and Y. The first two principal components (PC1 and PC2) described 55% and 29% of the total variation

whereas the third principal component (PC3) described 7% of the total variation. Specifically, 55% of the variability is related to samples where SiO_2 , TiO_2 , Zr and Y exhibit significant correlation and these are soils with significant siliceous components. 29% is related to samples where Cr, Ni and MgO show correlation. The interpretation of the first two components reveals two sources supplying erosion products; one acidic and the second ultramafic. The samples were clustered in 3 groups (Fig. 2.4). The soils and river bed sediments of Asopos watershed have been developed in sediments with calcareous, siliceous and ultramafic components. The calcareous component was mainly related with the late Miocene marine sediments, while the siliceous component was related with the sediments derived from the western part of the watershed (Pleistocene, Pliocene, and Quaternary). In addition, extensive outcrops of shales, hornstones and serpentized ophiolites outside of the watershed have supplied with eroded products the sediments in the western part of the watershed (sd4, sd5). Two samples have a strong ultramafic component and are located down gradient from the areas close to the exposures of ultramafic rocks (ophiolites and green arrow in Fig. 2.1).

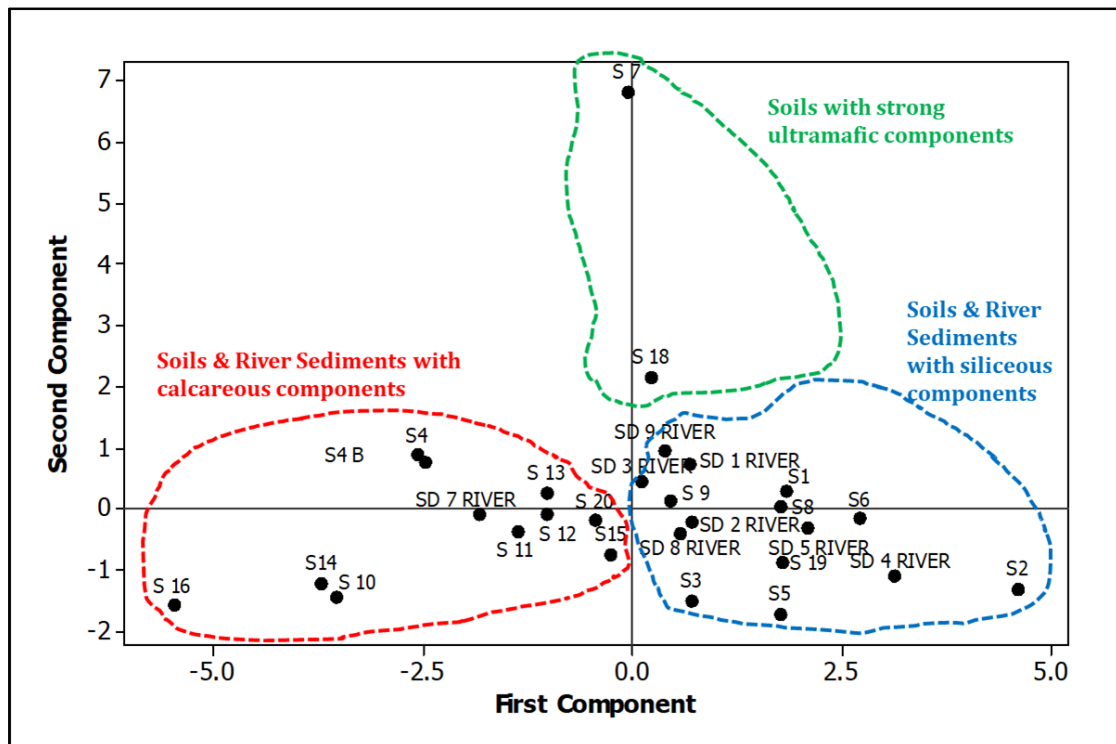


Figure 2.4. Groups resulting from PCA in soils and river bed sediments.

Table 2.1 presents the average chemical characteristics of the 3 clusters. The clustering of the samples is considered meaningful since Ca, Si and Cr exhibited significant differences between them. The concentrations of the oxides and heavy metals were varied depending on the group. Soils with calcareous components had an average concentration of 28% CaO and soils with siliceous components concentration of 47% SiO₂. Soils with ultramafic components exhibited higher Cr (2262 mg/kg) and Ni (943 mg/kg) content. Soils with calcareous and siliceous components presented lower Cr concentrations compare to the soils with the ultramafic components. The average hexavalent Cr content of the samples was about 0.06 mg/kg. As it was expected the values were very low due to the fact that soils derived from serpentine erosion have a great content of Cr(III).

Table 2.1. Average chemical characteristics of the 3 groups of data. Parentheses show standard deviation. The data with different letters (a-c) in the same row indicate significant differences at the $\alpha = 0.05$ level using ANOVA.

Parameter	Soils and river sediments with calcareous components	Soils and river sediments with siliceous components	Soils with ultramafic components
Number of samples	11	15	2
pH	7.9 ^a	7.8 ^a	7.6 ^a
EC (μ S/cm)	581 (459) ^a	763 (631) ^a	823 (632) ^a
Na ₂ O (%)	0.5 (0.96) ^a	0.8 (0.7) ^a	0.7 (0.9) ^a
MgO (%)	3.7 (0.98) ^a	4.5 (1.3) ^a	9.2 (5) ^b
K ₂ O (%)	0.7 (0.2) ^a	1.1 (0.3) ^b	0.7 (0.1) ^a
CaO (%)	28 (6.6) ^a	12.7 (4.3) ^b	16.7 (3) ^b
TiO ₂ (%)	0.2 (0.08) ^a	0.5 (0.1) ^b	0.3 (0.03) ^{ab}
MnO (%)	0.08 (0.04) ^a	0.12 (0.03) ^b	0.11 (0.02) ^{ab}
Fe ₂ O ₃ (%)	5.1 (1.6) ^a	6.3 (0.96) ^b	8.6 (1.9) ^c
Al ₂ O ₃ (%)	3.4 (1.8) ^a	6.8 (2) ^b	2.1 (2.9) ^a
SiO ₂ (%)	29 (9) ^a	47 (3.8) ^b	38 (4.7) ^a
P ₂ O ₅ (%)	0.09 (0.28) ^a	0.18 (0.06) ^a	0.3 (0.28) ^a
LOI (%)	30 (6.8) ^a	19 (2.7) ^b	20 (3.4) ^{ab}
Cr (mg/kg)	775 (466) ^a	900 (316) ^a	2262 (292) ^b
Ni (mg/kg)	230 (166) ^a	252 (86) ^a	943 (472) ^b
Cr(VI) (mg/kg)	0.06 (0.02) ^a	0.06 (0.04) ^a	0.10 (0.07) ^a
CRI	0.4 (0.2) ^a	0.3 (0.15) ^a	0.3 (0.2) ^a
TOC (%)	1.8 (0.8) ^a	1.6 (1) ^a	2.1 (0.5) ^a
TN (g/kg)	1.4 (0.4) ^a	1.4 (0.5) ^a	1.6 (0.6) ^a

Morrison et al. (2009) proposed the Cr reactivity index (CRI) which is the ratio of acid soluble Cr (measured by ICP MS after nitric acid digestion) versus the total Cr by XRF. The results showed that CRI is inversely related to total Cr by XRF both for soils and sediments (Fig. 2.5). The CRI closer to one represents a more soluble Cr sources (Morrison et al., 2009) like natural Cr adsorbents such as iron oxide coatings, clays, and/or soluble Cr bearing minerals like chlorite-chromite minerals, serpentines etc. The soils and sediments in the area of Asopos have a CRI index ranging between 0.1 and 0.6 suggesting that the geogenic component is a mixture of Cr bearing recalcitrant minerals and other soluble minerals. It is worth mentioning the decrease of CRI as the total Cr concentration increases which means that the higher Cr concentration the more recalcitrant minerals are present (e.g chromite). The sample with both high CRI and high Cr shown in Fig. 2.5, represents a soil in close vicinity of the ophiolite nappe where the more soluble chromium sources are present.

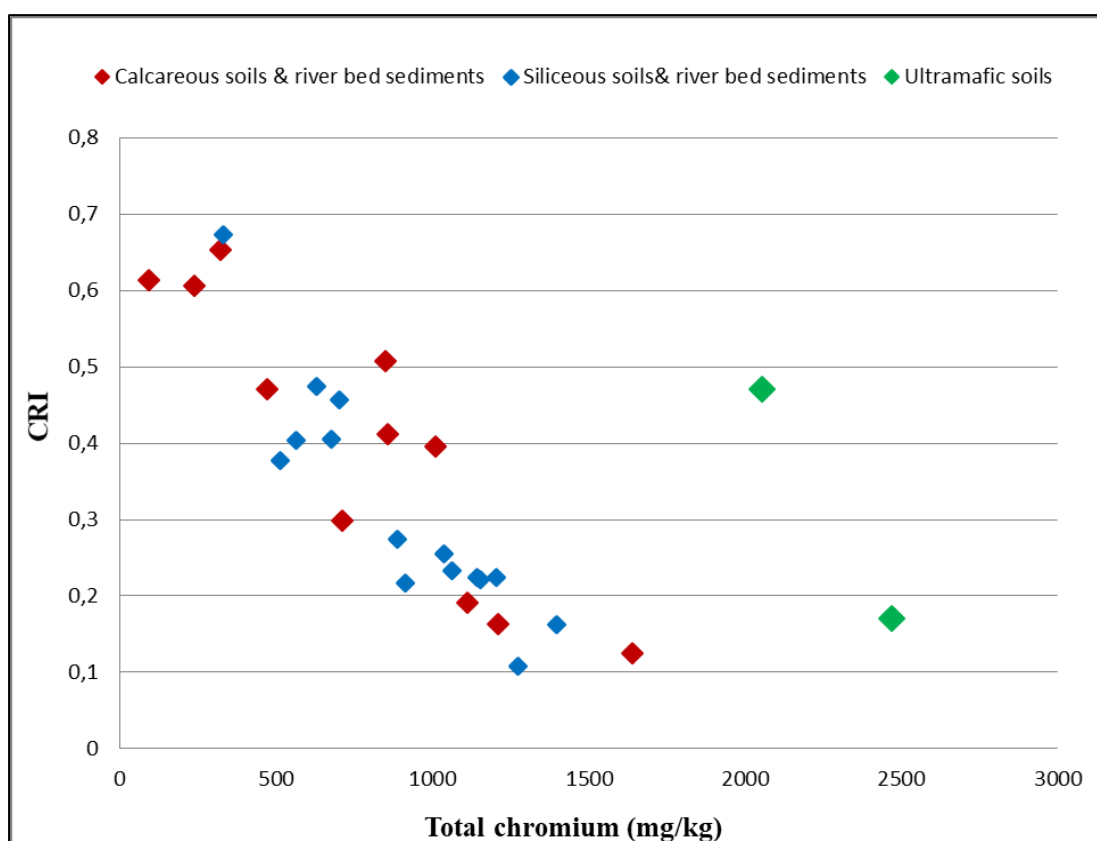


Figure 2.5. CRI and total chromium correlation in calcareous, siliceous and ultramafic soils and river bed sediments.

The Cr/Fe ratio for each of the above groups was used to differentiate non polluted to polluted soil and river sediment samples. According to Shtiza et al. (2005), the ratio Cr/Fe between 130-390 is representative for serpentinite soils, thus, lower than 130 is representative of Pliocene soils (containing lower ultramafic components) and higher than 390 is representative of anthropogenic pollution. Cr/Fe ratio for calcareous soils was 147 ± 86 , for siliceous soils 148 ± 61 , and for soils with ultramafic components 266 ± 23 . (Fig. 2.6).

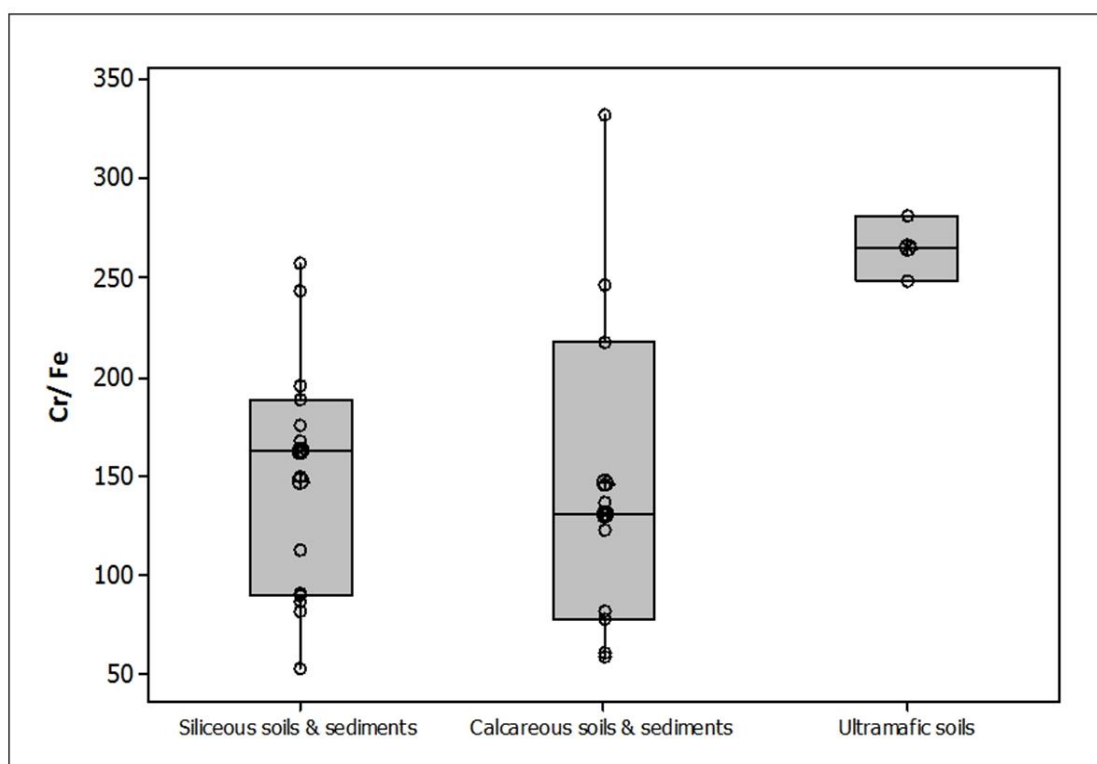


Figure 2.6. Boxplot showing the Cr/Fe ratio for the three groups of soils and river bed sediments. Values of Fe concentration were divided by 10^4 . The top of the box is the third quartile, the bottom of the box is the first quartile, the internal horizontal line indicates the median, and the whiskers extend to the maximum and minimum data value.

Finally all samples had a great content of iron oxide of about 5.1 to 8.6% suggesting that there are possible related with highly reactive surfaces for adsorption processes (Table 2.1).

2.4.3 Mobility studies

Leaching and sorption experiments were conducted using one river sediment sample for the group of soils/sediments with calcareous components (sd7) and one river sediment sample for the group of soils/sediments with siliceous components (sd8). The experiments were not conducted for a river sediment of the third group (soils/sediments with ultramafic components) because soils with clear ultramafic component have limited cover in the surface at Asopos watershed.

Fig. 2.7 presents the leaching experiment results. The results suggest that the kinetics of leaching were very fast. In the first 3-5 hours of the experiment, in a controlled pH of 7.8, the concentration in solution had reached steady state (1.5-2 ug/L). The application of amberlite resin (IR-120) demonstrated that total Cr coincides with hexavalent chromium. The results from the leaching experiment are consistent with the Cr(VI) concentration reported for Asopos groundwater (40-80 ug/L) (Moraetis et al., 2012) with the appropriate scaling up. Assuming an average specific weight of the aquifer sediments 1.2 g/cm^3 , and an average porosity of 30 percent, the solid to solution ratio in the aquifer is 4/1 in contrast to 1/10 used in the leaching test. Scaling up from the laboratory test to the field conditions, the Cr(VI) concentration in the aquifer will be 40 times higher than the one leached from the experiment, yielding hexavalent chromium concentrations in the groundwater of 60-100 ug/L. These values are consistent to field measurements.

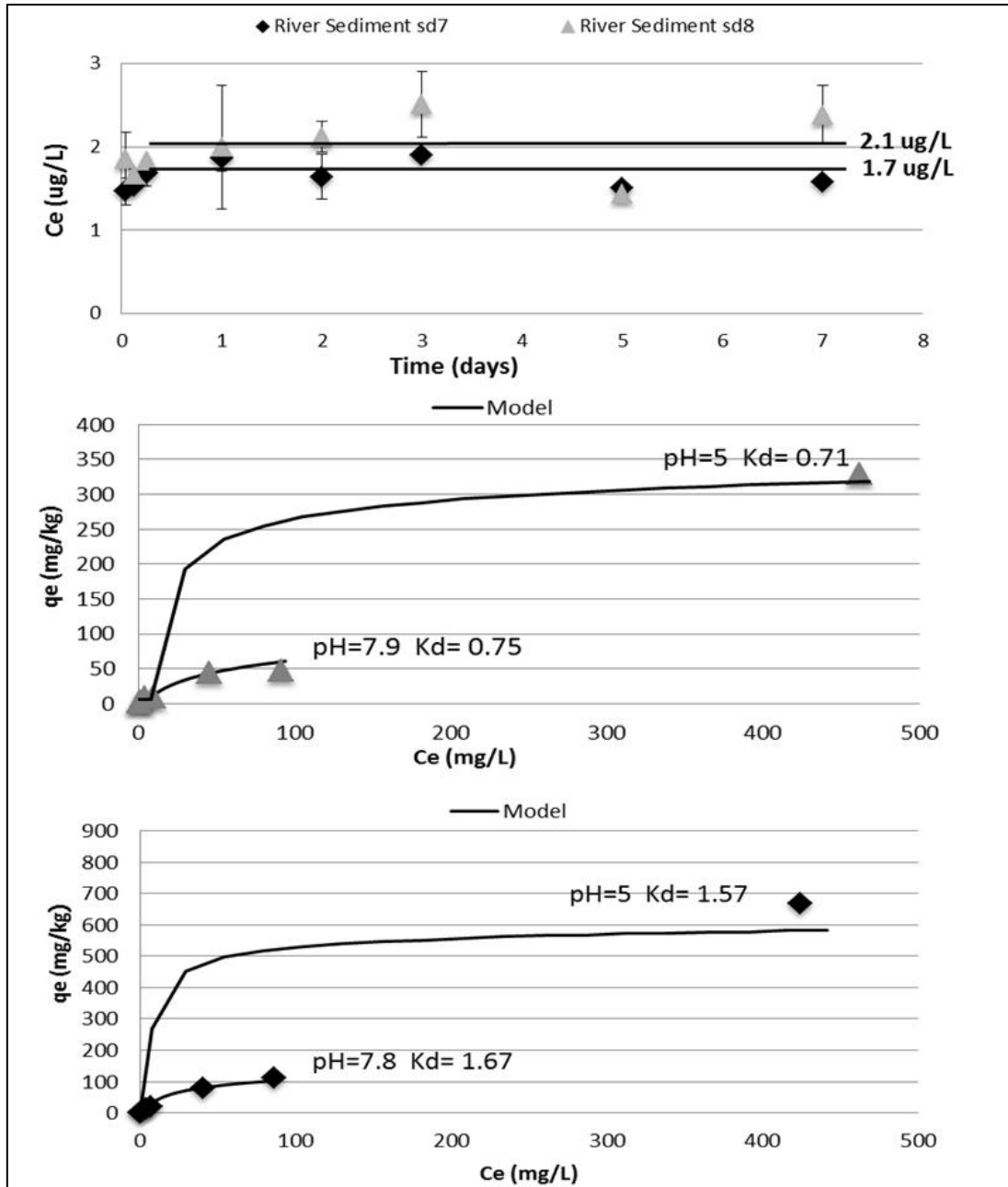


Figure 2.7. Leaching kinetic experiment and adsorption isotherm test using the samples from the surface river bed sediments sd7 (calcareous) and sd8 (siliceous). C_e is the chromium concentration in equilibrium in solution and q_e is Cr(VI) concentration sorbed onto soil.

The sorption results suggest that soils in the basin of Asopos have a significant adsorption capacity (Fig. 2.7). The maximum adsorption capacity at pH 7.8 for the sediment sd8 was 50 mg/kg and the partitioning coefficient 0.75 mL/g and for the sediment sd7 100 mg/kg and 1.67 mL/g respectively. The maximum adsorption capacity at pH 5 for the sediment sd8 was 325 mg/kg and the partitioning coefficient 0.71 mL/g and for the sediment sd7 600 mg/kg and 1.57 mL/g respectively. The retardation

coefficient (i.e. the ratio of the velocity of water to the velocity of Cr) at pH 7.8 was estimated to be 7.6 for sd7 and 4 for sd8, respectively. The retardation coefficient at pH 5 was estimated to be 7.3 for sd7 and 3.85 for sd8, respectively. These results support the hypothesis that the immobilization capacity of both sediments is important suggesting significant Cr retardation in case of wastes release with high Cr concentration and specifically for wastes with high acidity.

The adsorption studies were modeled using the MINEQL+4.6 speciation model (Fig. 2.7). The high adsorption capacity was attributed to Fe oxide coating in the samples that range in content from 5.1 to 8.6%. The assumption that the iron content of the samples play a significant role in the sorption and release of Cr from the sediments was based on the fractionation and XRD analysis of the soils conducted by Moraetis et al. (2012) in the same area. The results of this study showed that the heavy fraction of soils was composed of chromite (in the form of Al chromite and Mg chromite), hematite and goethite.

Regarding the surface complexation modeling, aqueous speciation and surface complexation reactions constants were derived from the literature (Nikolaidis et al., 1999) using the triple layer model. The surface area of the sediment was $2.0 \text{ m}^2 \text{ g soil}^{-1}$ and the inner-layer (1.2 F/m^2) and outer-layer (0.20 F/m^2) capacitance values were obtained from the literature (Nikolaidis et al., 1999). The concentration of surface complexation sites was calibrated and verified with the iron content measurements in order to validate the assumption that all iron in these sediments is iron coatings in the form of goethite and hematite. The calibrated values of the surface complexation sites were 0.1 mol/l . In order to convert these values to an iron content for hematite and goethite, the solid to solution ratio of 1/10 used in the adsorption experiment, the molecular weight of goethite and hematite 88.85 g/mol and 159.98 g/mol respectively, and the iron content of goethite and hematite 63% and 69.94%, were used. The surface complexation site density used in the model was equivalent to an Fe oxide content of 5.6% and 11% for the case of goethite and hematite respectively. These values were within the range of 5.1% and 8.6% measured with XRF. The previous results are consistent with the assumption that iron oxides play a significant role in Cr mitigation in the case of waste release containing Cr.

2.5 Conclusions

The analysis of heavy metals in the area supported the hypothesis of geogenic origin of Cr in soils and sediments. The evidence that supported the geogenic origin of Cr, was the positive and high correlations between Ni and Cr concentrations in soils and sediments and the relatively even distribution of Cr and Ni upstream and downstream from the industrial park. The soils of Asopos watershed exhibited high Cr content regardless the different lithologies which were characterized from calcareous, siliceous and ultramafic components. The Cr/Fe ratio for each of the above clusters confirmed the geogenic origin of chromium. The Cr concentration in equilibrium of 1-2 ug/L resulted from the laboratory experiments was shown to be consistent to field groundwater Cr concentrations. The results show that the adsorption capacity of sediments was significant and the geochemistry of Cr is controlled by ferric oxide surface complexation. Soils and sediments with calcareous components exhibit higher reactivity to Cr(VI) adsorption compared to the siliceous cluster. Further studies that would identify the leaching mechanisms of Cr in different lithologies in the area are necessary to fully elucidate the processes that control the fate and transport of Cr in the subsurface.

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CHAPTER 3. IDENTIFYING THE CONTROLLING MECHANISM OF GEOGENIC ORIGIN CHROMIUM RELEASE IN SOILS



Submitted as:

Lilli, M.A., Nikolaidis, N.P., Karatzas, G.P., Kalogerakis, N. Identifying the controlling mechanism of geogenic origin chromium release in soils.

3.1 Abstract

A laboratory study was conducted to assess the mobility and mechanisms of Cr release from soils obtained from an area of wide spread geogenic contamination. The agricultural soil sample used in this study was taken from the Schimatari area in Asopos River basin in Greece. In order to refine the isolation of minerals contained in the soil, two types of separation analysis were conducted. First, a size fractionation with hydrocyclone and second, a weight fractionation with heavy liquids. The separated fractions were characterized using chemical, mineralogical and surface analysis. The results provided consistent evidence that the heavy fraction of the soil is related directly to the mobile fraction of Cr. At acidic pHs, the clay-sized fraction also plays an additional important role in the mobility of Cr, due to the fact that this fraction has high surface area and Cr reactivity index. In addition, pH-edge leaching studies showed a high correlation between Cr–Ni, Cr–Mn and Cr–Y released from the soil which also suggests that the mobility of Cr is controlled by chromite weathering which is the case observed in Asopos river basin.

3.2 Introduction

Cr is a heavy metal which at high concentrations exhibits toxic behaviour to organisms while at low concentrations is a required micronutrient (Anderson, 1997). Cr exists in both trivalent (Cr(III)) and hexavalent (Cr(VI)) in typical environmental conditions and can change oxidation states due to its electronic configuration and valence chemistry (Prado et al., 2016; Ashraf et al., 2017). These states react geochemically and toxicologically differently with respect to sorption in soil, aquatic bioavailability and uptake by plants (Choppala et al., 2016). The dominant form under reducing conditions is Cr(III), which has low solubility at circum-neutral pH and is readily sorbed onto iron (Fe) oxides. Cr(VI) exists under typical conditions as a soluble oxyanion and is more mobile in soils (Lackovic et al., 1997; Nikolaidis et al., 1999).

Naturally occurring in soils, Cr concentration ranges from 10 to 50 mg/kg, depending on the mineralogy of the parental material (Shanker et al., 2005), while in ultramafic soils, Cr varies within 634 to 125000 mg/kg (Adriano, 1986). Serpentine soils are green to grayish-green “broken rocks”, comprised of thin layers, which can easily break up

and fragment in small particles. Serpentine soils are known for their elevated metal concentrations including Ni, Cr, Mn and Co (Rajapaksha et al., 2012; Vithanage et al., 2014) and these soils provide an opportunity to assess the dynamics of potential metal interactions. Ultramafic rocks such as chromite, olivines and pyroxenes is the primary source of Cr and nickel (Ni) in soils (Morrison et al., 2009). Most of Cr resides as Cr(III) in the source mineral chromite but an appreciable amount is associated also with Fe oxides and clays (Mills et al., 2011). The main Cr containing minerals in serpentines are chromite and magnetite and to lesser extent chlorite, augite, enstatite, Fe hydroxides, and clays (Becquer et al., 2003; Oze et al., 2004). Other processes that retain Cr in soils include co-precipitation with manganese (Mn), aluminum (Al), and Fe, adsorption on oxides, and complexation with organic matter (Hsu et al., 2015).

The fate of metals in soils is determined by their speciation as well as the capacity of the soil to retain them. The cycling of substances in the natural environment is a complex process, thus when measuring the total content of metals is not adequate to determine their mobility and bioavailability (Quevauviller, 2003; Wang et al., 2009).. Speciation studies are important in order to assess the chemical factors that affect the transport as well as the fate of each species in the soil (Jamalia et al., 2009; Rauret, 1998). Soil pH, organic matter and redox potential can influence remarkably the geochemical behavior of Cr by affecting its chemical speciation (Amin and Kassem, 2012; Choppala et al., 2016; Xiao et al., 2015).

Sorption and desorption reactions are relevant to the mobility of Cr (Rai et al., 1989). Cr(VI) transport is mostly controlled by its sorption on Fe oxides (i.e. hematite) (Johnston and Chrysochoou, 2014), amorphous aluminium and organic complexes, and Cr(III) can be adsorbed onto silicaceous components (Bartlett and James, 1988). The maximum Cr adsorption capacity was assessed for the bulk samples and their clay and Fe-free clay fractions of four subtropical soils differing in mineralogy and found that Cr adsorption was the highest in the soil with the highest organic matter content and increased with increasing pH (Bavaresco et al., 2017). Furthermore, Cr, Ni, and vanadium were shown to have lower mobility because they were associated mostly with Fe and Mn oxides and the residual fraction (Jeske and Gworek, 2012).

During the last years, geogenic origin Cr contamination has been found to be wide spread in diverse areas around the world such as in Mexico, California, Zimbabwe and Italy, thus it has stimulated significant scientific interest (Robles-Camacho and

Armienta, 2000; Cooper, 2002; Fantoni et al., 2002; Oze et al., 2004; Garnier et al., 2009; Morrison et al., 2009; Mills et al., 2011; Morrison et al., 2015). In Greece, the main ophiolite outcrops of the Hellenides are distinguished in four ophiolite belts (Papanikolaou, 2009). Pindos mountains are covered with ultramafic rocks, which have been eroded and their fragments have been combined with Tertiary and Quaternary deposits that reside in valleys. Serpentine soils with high geogenic origin Cr contamination were derived from the weathering of these rocks. The study area is situated in central Greece and specifically in the basin of Asopos river where the reported Cr and Ni concentrations in groundwater and agricultural soils and products attributed to the occurrence and weathering of ultramafic rocks (Moraetis et al., 2012; Kelepertzis et al., 2013; Lilli et al., 2017).

The overall objective of this study was to elucidate the mechanisms of Cr release from soils obtained from an area with wide spread geogenic contamination. In order to achieve this, a three step approach was followed:

- firstly a protocol was developed and applied to separate first the bulk soil into secondary fractions using both size and weight fractionation with hydrocyclone and heavy liquids,
- then, the fractions of Cr bound to each of the above fractions of the contaminated soil were characterized using different methods including mineralogical (XRD), chemical (EPA 3051A, EPA 3052, XRF) and surface (XPS) analysis
- finally, a leaching study of each fraction was performed to quantify the variability of Cr release with pH.

3.3 Materials and methods

3.3.1 Site description and sampling

The Basin of Asopos river is located in mainland Greece, north from Athens (Region of Sterea Ellada). The basin has an area of 703 km² and the river is intermittent (Botsou et al., 2008). The structure of the geology is comprised of alpine rocks and the post-alpine sediments. The base geology is Triassic limestones and dolomites and ophiolites which is covered by Quaternary and Tertiary-Neogene deposits. The enrichment of top

soils and sediments in Cr and Ni is the result of weathering of ultramafic rocks that have been transported in fragments from higher elevation and mixed with the Neogene and alluvial deposits both in the eastern (Moraetis et al., 2012) and western part of the basin (Kelepertzis et al., 2013). Top soils and sediments of the area have high Cr content irrespective of the difference in lithology (Lilli et al., 2015). Lilli et al. (2015) have obtained more than 50 soil and sediment samples in order to characterize the geochemical variability in the basin. Principal Component Analysis characterized the soils and sediment into calcareous, siliceous and ultramafic groups. Based on this analysis, the soil sample from the siliceous group was selected to conduct the present study since it was the most widespread soil in the basin.

3.3.2 Experimental design

3.3.2.1 Separation studies

The agricultural soil sample used in this study was taken from the Schimatari area in Asopos river basin. The sample was homogenized, air dried and sieved. The fraction smaller than 2mm was used for the experiments.

In order to refine the isolation of minerals contained in the soil, two types of separation analysis were applied, a size separation using a hydrocyclone (hc) and a weight separation using a heavy liquid (hl) on the sand/silt-sized fraction. The soil sample (about 50 kg) was wet sieved. The fraction smaller than 1mm was used for the separation. Specifically, the bulk soil (1mm) was separated into a sand/silt-sized fraction ($>10\mu\text{m}$) and a clay-sized fraction ($<10\mu\text{m}$) by using a hydrocyclone (hc). Then the sand/silt-sized fraction (hc) was further separated into a heavy fraction and a light fraction by using a liquid (hl) with the specific gravity of 2.96 g/cm^3 (tetrabromoethane). The heavy fraction (hl) was the sink material and the light fraction (hl) the float material in the liquid. Fig. 3.1 depicts a schematic view of the separation procedure.

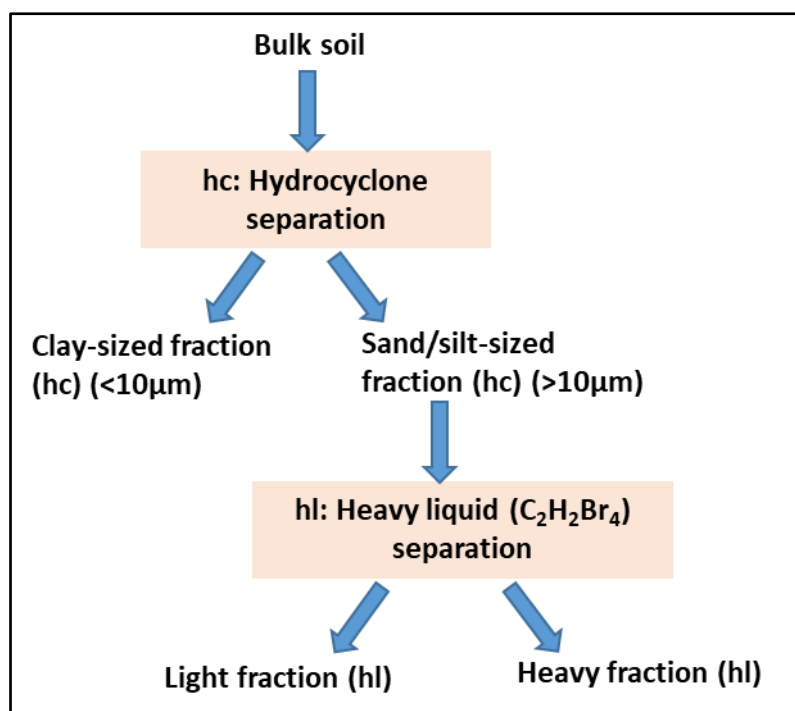


Figure 3.1. Schematic diagram of the separation procedure.

3.3.2.2 Characterization

The mineralogical composition of the bulk soil and of all derived fractions (Fig. 3.1) was determined by X-ray diffraction (XRD) using a Bruker D8 Advance powder diffractometer. A graphite monochromator using CuK α radiation were used at 35 kV and 35 mA to obtain the counts. The instrument was able to differentiate between the light density minerals (quartz, calcite, dolomite) and the heavy (magnetite chromite).

Base cations and heavy metals were determined for the bulk soil and for the derived fractions of the soil sample according to EPA 3051A and EPA 3052 methods. For the EPA 3051A microwave digestion (Multiwave 3000 Digestor), 9 ml HNO₃ were added to 0.2 g soil. In addition, digestion with a combination of acids was used for the complete solubilization of metals using the EPA 3052 microwave digestion method. In this case, 10 ml HNO₃, 2 ml HCl and 1 ml HF were added to 0.1 g soil. MilliQ water was used to dilute the supernatant and the resulting solution was analyzed by ICP-MS (Agilent- CX) for heavy metal and trace elements. Finally, the bulk soil, the sand/silt-sized and the clay-sized fractions (hc) were analyzed for trace elements using an X-ray fluorescence spectrometer S2 Ranger (Bruker Co). This analysis could not be conducted for the heavy and light fractions (hl) due to lack of the required quantities.

The XPS surface analysis were conducted in a UHV chamber ($P < 10^{-9}$ mbar) equipped with a SPECS LHS-10 hemispherical electron analyzer and the measurements were obtained at room temperature using unmonochromatized AlK α radiation. The conditions of the instrument were optimized in order to maximize the signal (constant ΔE mode with pass energy of 97 eV giving a full width at half maximum (FWHM) of 1.7 eV for the Ag 3d $_{5/2}$ peak). The Shirley background was removed from the XPS spectra and allowed for the deconvolution of each spectrum into Gaussian-Lorentzian components using a fitting routine. The samples were pressed into pellets with diameters 1 cm². The XPS analysis was performed for the clay-sized fraction (hc) and for the light and heavy fractions (hl).

3.3.2.3 Chromium and other metals release studies

A pH-edge leaching study ascertained the release patterns of Cr, Ni, Y, and Mn, elements typical of ultramafic rocks, as a function of pH for the different fractions of soil. The batch pH-edge experiment was conducted for the sand/silt-sized (hc) and clay-sized fraction (hc) and for the light (hl) fraction of soil in five different pH values (5, 6, 7, 8 and 9). This experiment was conducted also for an ophiolite rock sample from Avlonas area so as to compare the results. The triplicate experiments were conducted in plastic bottles. The soil/solution ratio was 1/5, the equilibrium solution was comprised of 20 mL NaNO₃ and had an ionic strength of 0.01 N. All solutions were prepared using MilliQ water. All aliquots were placed in a vibrating plate at 200 rpm for 7 days. pH was adjusted three times a day for each bottle. A nylaflo membrane filter (size 0.45 μ m) was used to filter the supernatant solutions prior to analysis and metals were analyzed with ICP-MS (Agilent 7500-CX). The HACH method was used for the determination of Cr(VI).

3.4 Results and discussion

3.4.1 Mineral and chemical characterization of the separated fractions

Two different separation methods were used, a size separation using a hydrocyclone (hc) and a weight separation on the sand/silt-sized fraction (hc) using a heavy liquid (hl) so as a fractionation to be conducted. These separation studies showed that the clay-

sized fraction (<10µm) obtained with the hydrocyclone separation (hc) constituted 55% of the total bulk soil, while the rest (45%) separated by the heavy liquid was divided into the light fraction (hl) 44.4% and the heavy fraction (hl) 0.6% of the total bulk soil, respectively (Table 3.1). Table 3.1 presents also % clay mineral of each fraction, which is the sum of % chlorite, kaolinite, muscovite, illite and montmorillonite, and % iron oxides, which is the sum of % chromite, goethite and hematite derived from XRD analysis (Table 3.2).

Table 3.1. Quantitative separation of the bulk soil. “—” shows that this element is not detected in the related fraction.

	1 st separation	2 nd separation	% of bulk soil (weight)	% clay mineral of fraction	% iron oxides of fraction
Bulk soil	Clay-sized fraction (<10µm) (hc)		55	63	-
	Sand/silt-sized fraction (>10µm) (hc)	Light fraction (hl)	44.4	24.1	-
		Heavy fraction (hl)	0.6	10.9	16.8

XRD analysis suggested that the bulk soil contained mainly quartz (32.2%), calcite (17.5%) and chlorite (27.9%) (Table 3.2). The clay-sized fraction (hc) of soil enclosed light minerals like calcite (22.0%) and chlorite (43.6%) (Table 3.2). The sand/silt-sized fraction (hc) and the light fraction (hl) of soil showed exactly the same composition in all minerals, containing mainly quartz (50.5%), calcite (14.2%) and chlorite (16.3%), derived possibly from the soil parent material (Table 3.2). The XRD analysis of the heavy fraction (hl) identified chromite (6.9%), hematite (6.7%) and goethite (10.1%) as the main minerals (Table 3.1, Table 3.2). Chromite was derived from ultramafic rocks and was detected only in this fraction of soil (heavy fraction). It is also possible that the clay-sized fraction (hc) of the soil contains chromite fragments in a smaller percentage, but they were not detected due to high detection limit (LOD) of chromite by XRD (Morrison et al., 2015). The mineralogical analysis showed also that the total % of clay (chlorite, kaolinite, muscovite, illite, montmorillonite) of the clay-sized (hc),

light (hl) and heavy (hl) fraction of soil is 63%, 24.1% and 10.9%, respectively (Table 3.1, Table 3.2). Albite was also present in low content in all fractions of soil and it is probably related to the erosion products of felsic rocks.

Table 3.2. Mineralogical analysis for the bulk soil and for all derived fractions. “–” shows that these minerals are not detected in the related fraction.

Mineral (%)	Bulk soil	Clay-sized fraction (hc)	Sand/silt-sized fraction (hc)	Heavy fraction (hl)	Light fraction (hl)
Quartz	32.2	8.3	50.5	38.5	50.5
Calcite	17.5	22.0	14.2	10.3	14.2
Chlorite	27.9	43.6	16.3	4.3	16.3
Albite	3.7	1.2	10.1	7.8	10.1
Kaolinite	0.8	4.2	-	0.3	-
Muscovite	12.1	11.9	7.8	6.3	7.8
Dolomite	4.6	5.4	1.2	8.6	1.2
Illite	1.2	3.0	-	-	-
Montmorillonite	-	0.4	-	-	-
Chromite	-	-	-	6.9	-
Goethite	-	-	-	10.1	-
Hematite	-	-	-	6.7	-

Fig. 3.2 presents the total Cr, Fe and Ni concentrations for the fractions of soil using two measuring methods (EPA 3051A, EPA 3052). EPA 3051A presents an underestimation of the content of metals (Fig. 3.2). Specifically, measuring an average Cr content in bulk soil 32 mg/kg using EPA3051A method, the Cr content using EPA3052 method is 271 mg/kg (Fig. 3.2). XRF analysis showed that Cr concentration in bulk soil is 465 mg/kg. Moreover, measuring an average Cr content in sand/silt-sized fraction (hc) 43 mg/kg using EPA3051A method, the Cr content is 217 mg/kg and 856 mg/kg using EPA3052 method and XRF analysis, respectively. Measuring an average Cr content in clay-sized fraction (hc) 116 mg/kg using EPA3051A method, the Cr content is 160 mg/kg and 267 mg/kg using EPA3052 method and XRF analysis, respectively. The HNO₃ digestion underestimates the Cr content due to the recalcitrant behavior of geogenic Cr found in soil minerals (chromite and spinels) (Lilli et al., 2015). Measuring an average Cr content in bulk soil 271 mg/kg, the Cr content in the heavy

fraction (hl) of soil was 939 mg/kg (Fig. 3.2). Higher Cr and Fe concentrations were observed in the heavy fraction (hl) of soil (Fig. 3.2), while higher Ni concentrations in the clay (hc) fraction (Fig. 3.2).

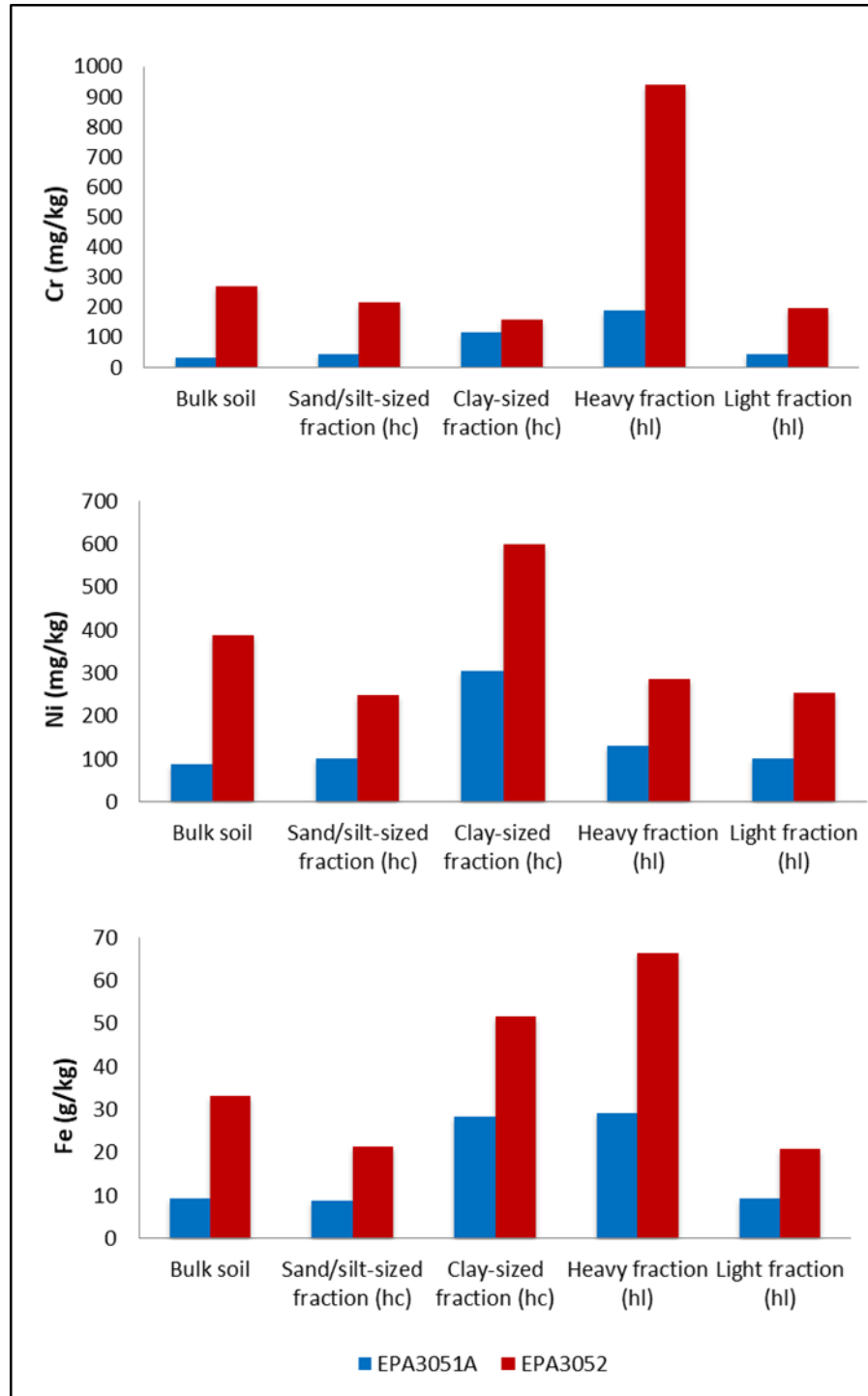


Figure 3.2. Total Cr, Ni and Fe concentration for the different fractions of soil using EPA3051A and EPA3052 measuring methods.

Morrison et al. (2009) characterized the reactivity of Cr (CRI) as the ratio of Cr (acid soluble) to Cr (total). That means that the CRI conditions correspond to acidic conditions and not alkaline. Morrison et al. (2015) also indicate that the grain size contributes to the CRI. The grain size is positively correlated with the Cr (total) concentration and negatively with CRI. Soluble Cr sources are represented by CRI values close to one. CRI was calculated 0.58, 0.60 and 0.25 in bulk soil, in clay-sized fraction (hc) and in sand/silt-sized fraction (hc), respectively. The secondary minerals of clay have a high surface area and are likely to be reactive in soil systems. Higher CRI of the clay-sized fraction (hc) suggests higher reactivity for this fraction at acidic conditions (pH values 5 and 6). The results also suggest the presence of more recalcitrant minerals such as chromite since there is a negative correlation between CRI and total Cr concentration. These results are consistent with CRI estimates ranging between 0.1 and 0.6 in soils and sediments of Asopos (Lilli et al., 2015).

3.4.2 Surface characterization of the separated fractions

The clay-sized (hc), heavy (hl) and light (hl) fractions of soil were used for the XPS analysis to elucidate the speciation of Cr at their surfaces. The wide spectra recorded (Fig. 3.3) from the samples shows the presence of elements: C, O, Fe, Si and Al. The core level peaks that were recorded in detail were: C1s, O1s, Fe 2p, Si2p, Cr2p and Mn2p. In this analysis, only the deconvoluted Fe2p (Fig. 3.3 A) and Cr2p (Fig. 3.3 B) peaks are presented. Si and Al are in the oxide form and C1s peak is the usual contamination form as in every sample exposed in the atmosphere. Errors in our quantitative data are found in the range of ~10% (peak areas) while the accuracy for BEs assignments is ~0.1 eV. Fig. 3A and B shows the detailed spectra of the deconvoluted Fe2p and Cr2p core level peaks, respectively. Fe2p peak is analyzed in one doublet with spin orbit splitting of 13.5 eV and a satellite peak around 722 eV characteristic for Fe oxide. The binding energy of Fe2p_{3/2} is at 714 eV which is assigned to Fe⁴⁺. Fig. 3B shows the Cr2p peaks which is analyzed into two doublets with a spin-orbit splitting 9.1 eV. The binding energy of Cr2p_{3/2} of the two components is at 574.0 eV assigned to Cr⁰ and at 578.1 eV assigned to Cr(VI) (Mikolaichuk et al., 1993). Finally, the spectra of Mn2p region did not show any peak, so the Mn on the surface is below detection limit of XPS technique (0.1% at.). Table 3.3 shows the % atomic ratio of Cr to Fe and the intensity ratio of Cr(VI) to the total intensity which represents the

number of atoms in the exacovalent bonds. The Cr/Fe atomic ratio was found 0.40 - 0.5 and the Cr(VI)/Cr intensity ratio 0.3-0.39 for the 3 fractions of soil. This is an evidence that there is Cr(VI) (about 30-40% of the total Cr) on the surface of the soil that potentially can be released.

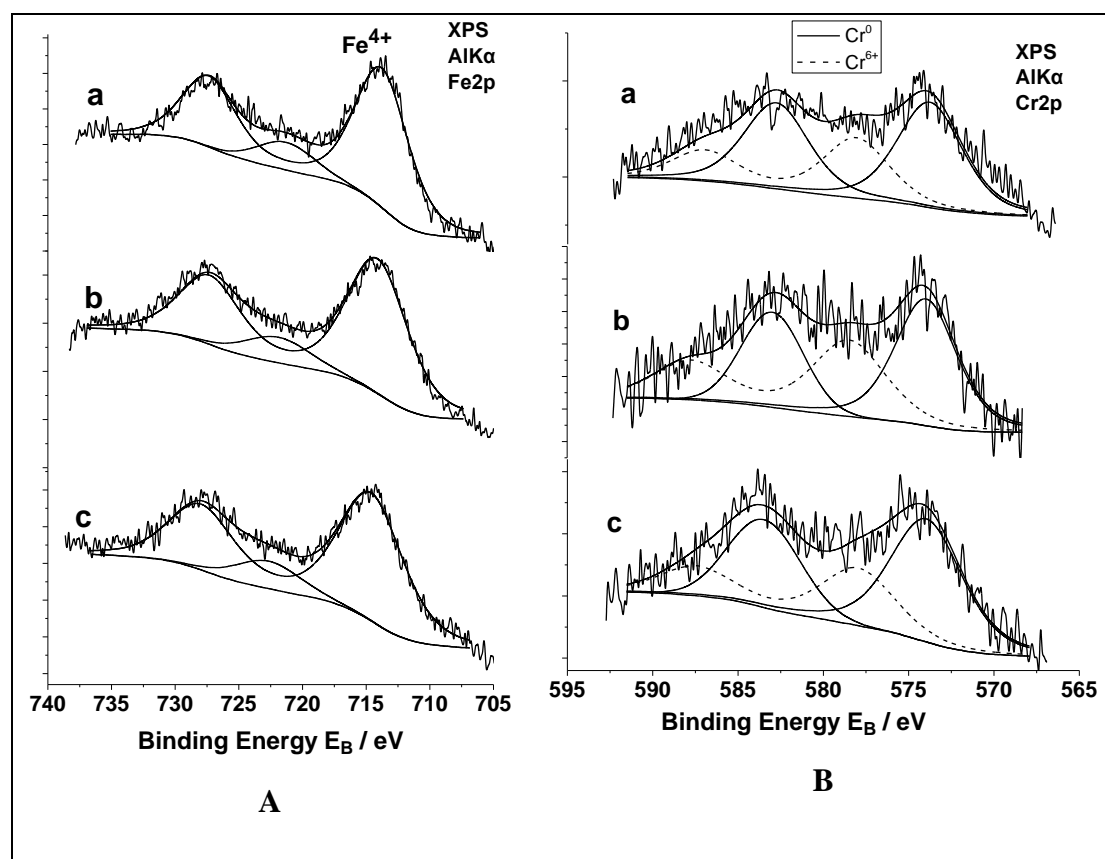


Figure 3.3. XP Deconvoluted Spectra of (A) Fe2p and (B) Cr2p of the clay-sized (hc) (a), heavy (hl) (b) and light (hl) (c) fraction of soil.

Table 3.3. Atomic ratio (%) of Cr/Fe and intensity ratio of Cr(VI) to the total intensity.

	Atomic Ratio (%) Cr/Fe	Intensity ratio: Cr(VI)/Cr
Light fraction (hl)	0.40	0.30
Heavy fraction (hl)	0.40	0.39
Clay-sized fraction (hc)	0.50	0.35

3.4.3 Mobility of chromium and other metals at different pH values

pH edge leaching studies were conducted to further elucidate the controlling mechanism of Cr(VI) release in geogenically contaminated soils. Fig. 3.4 presents the pH edge of Cr, Ni, Mn and Y release experiments for the sand/silt-sized (hc), clay-sized (hc) light (hl) fractions of soil and for the ophiolite rock sample from the area. Both the clay-sized (hc) and light (hl) fractions had similar response to Cr mobility and similar levels of Cr release (Fig. 3.4a). Given that the sand/silt-sized fraction (hc) is divided into the heavy (hl) and light (hl) fractions of soil, Cr release for the heavy fraction (hl) can be determined by the difference between the two curves (sand/silt-sized fraction (hc) and light fraction (hl)) (Fig. 3.4a). In the pH range of 7 to 9, the release of Cr for both the clay-sized (hc) and light (hl) fractions is minimum, and the mobility of Cr is related to the sand/silt-sized fraction (hc), while the Cr concentration released from the soil, increased to more than 200 ug/kg as the pH dropped to 5 (Fig. 3.4a). Total Cr in all fractions is a combined concentration of Cr(VI) and Cr(III), with Cr(VI) occurring mainly at alkaline pHs and Cr(III) at acidic pHs.

According to the above observations, the controlling mechanism of Cr mobility is directly related to the heavy fraction (hl) of soil. In the case that the pH is low (5 and 6), in addition to the heavy fraction (hl), the clay-sized fraction (hc) plays also an important role and controls additionally Cr release, due to its high reactivity. The Cr concentration released from the clay-sized fraction (hc) of soil, was more than 250 ug/kg at pH 5 (Fig. 3.4a). The Cr concentration released from the ophiolite rock sample, presented an increasing trend from approximately 30 ug/kg at pH 9, to 135 ug/kg as the pH dropped to 5 (Fig. 3.4a). The pH edge studies show that the behavior of Cr release in ophiolite sample is associated with the other fractions of soil, indicating that the mobility of Cr is related to ophiolite weathering.

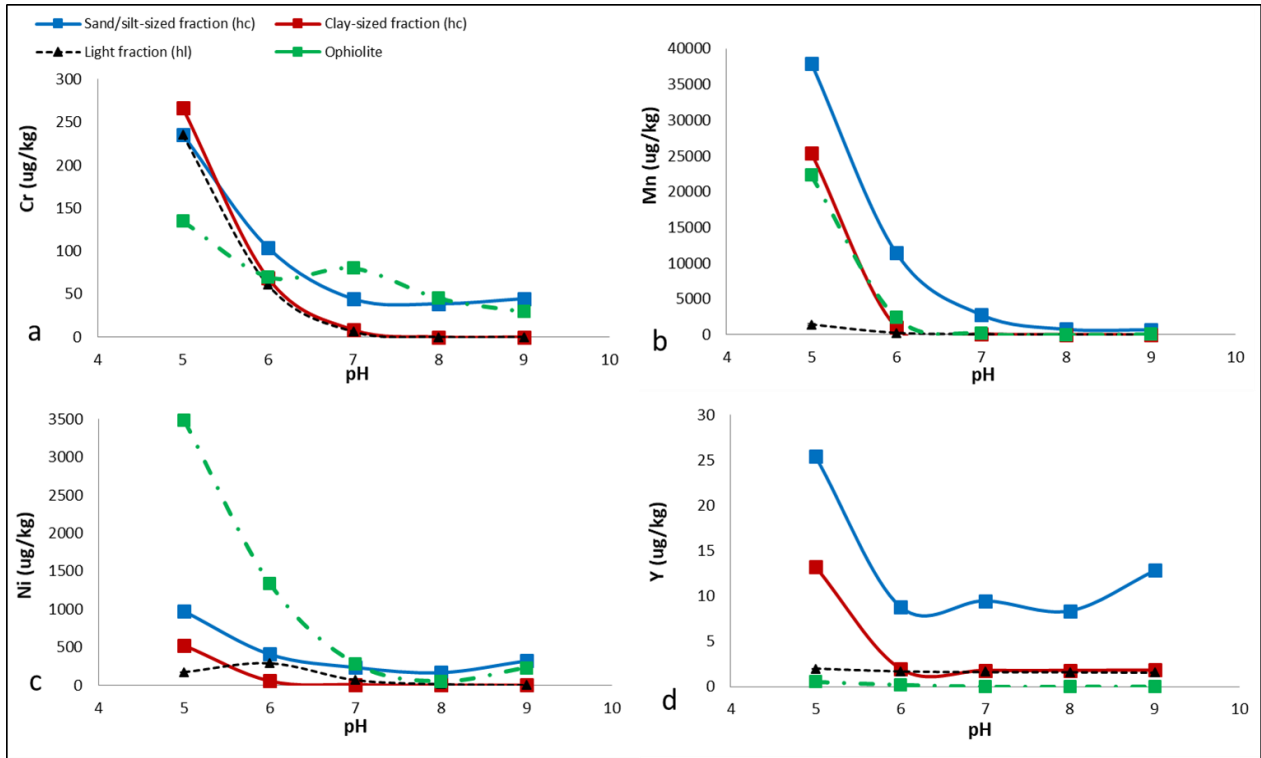


Figure 3.4. Concentration of Cr (a), Mn (b), Ni (c), Y (d) desorbed from the different fractions of soil and from an ophiolite rock sample as a function of pH. Error bars correspond to the standard error of the triplicates.

To further test the evidence that ophiolite weathering is responsible for Cr(VI) levels at pH around 8 in the soils and ground waters of the area, we evaluated the release of three more elements that relate to serpentine composition (Mn, Ni and Y). Both the clay-sized fraction (hc) and ophiolite rock sample had similar response to Mn mobility and similar levels of Mn release (Fig. 3.4b). In the pH range 6-9, the release of Mn for the clay-sized (hc) and light (hl) fractions and for the ophiolite rock sample is minimum, and the mobility of Mn is related to the sand/silt-sized fraction (hc). In pH 5, the Mn concentration increased to more than 20000 ug/kg for the clay-sized fraction (hc) and ophiolite rock sample, to more than 35000 ug/kg for the sand/silt-sized fraction (hc), while the Mn concentration is minimum for the light (hl) fraction (Fig. 3.4b).

In pH range 6-9, the release of Ni for the clay-sized fraction (hc) is minimum, and the Ni content increased to approximately 500 ug/kg in pH 5 (Fig. 3.4c). Low Ni content is presented also for the light (hl) fraction in pH range 7-9, while Ni increased in pH values 5 and 6. The mobility of Ni is related to the sand/silt-sized fraction (hc), while the Ni content desorbed from this fraction ranges between about 160-1000 ug/kg.

Ophiolite rock sample extracts high amounts of Ni (up to 1000 ug/kg) in pH values 5 and 6.

As far as the Y mobility, in pH range 5-9, the release of Y for the light (hl) fraction and ophiolite rock sample is minimum (Fig. 3.4d). In pH range 6-9, the release of Y for the clay-sized fraction (hc) is also minimum and the Y concentration desorbed from this fraction increased to approximately 15 ug/kg as the pH dropped to 5 (Fig. 3.4d). So, the mobility of Y is related to the sand/silt-sized fraction (hc).

The chromite dissolution rates at pH value of 8, which is predominant in field conditions of Asopos basin, and at pH value of 5, which is an extreme condition, was calculated about 0.96 nM/hour and 5.4 nM/hour, respectively. This calculation was attained multiplying the first order rate with the release in the two different pH values. For this calculation, at pH 8 the release of Cr of the sand/silt-sized fraction (hc) was used, as Cr in solution for the other fractions were minor. Moreover, at pH value of 8, the Cr release of the sand/silt-sized fraction (hc) coincides with this of ophiolite sample (Fig. 3.4a). At pH 5 the release of Cr for all fractions was about the same. The chromite dissolution rates between 0.96 and 5.4 nM/hour are consistent to the rates calculated by Oze et al. (2007) and Moraetis et al. (2012).

The results from the release experiment (at pH value of 8, which represents field conditions) are consistent with Cr(VI) groundwater data obtained in the area (40-80 ug/L) (Moraetis et al., 2012) if they are scaled-up to field conditions since the aquifer solid to solution ratio is 4/1 while in the release test is 1/5. The concentration will be 20 times higher in the aquifer than of the release test, assuming a bulk density of 1.2 g/cm³, and a 30% porosity. Taking into account also that the sand/silt-sized fraction (hc) represents 45% of the total bulk soil, Cr(VI) concentration in the groundwater can be up-scaled to be 72 ug/L, a value consistent to field measurements.

The geogenic origin of Cr of the different soil fractions was assessed by examining the relationship of Cr with elements (Ni, Y, Mn) found in ultramafic rocks, at different pH (5, 6, 7, 8, 9) values (Prokisch et al., 2000; Morrison et al., 2009; Bonifacio et al., 2010) (Fig. 3.5). The coefficients of determination for Cr–Ni were 0.96 and 0.97 for the sand/silt-sized (hc) and clay-sized (hc) fraction of soil, respectively. The coefficients of determination for Cr–Mn were 0.99 and 0.94 for the sand/silt-sized (hc) and clay-sized (hc) fraction of soil, respectively. The coefficients of determination for Cr–Y were 0.73 and 0.93 for the sand/silt-sized (hc) and clay-sized (hc) fraction of soil, respectively

(Fig. 3.5). The Cr–Y correlation indicated geogenic Cr in the fractions of soil since both Y and Cr are quite stable and resistant to weathering (Prokisch et al., 2000). The significant correlations of Cr–Ni, Cr–Mn and Cr–Y suggested that the release of hexavalent chromium from the soil is related to the weathering of chromite which was the erosion product of ultramafic rocks of Asopos basin (Moraetis et al., 2012).

3.5 Conclusions

The experimental design of this study used size and weight fractionation and further physical, chemical, mineralogical and surface characterization of the fractions generated, to refine the identification of minerals in order to elucidate the mechanism of Cr release from soils obtained from an area with geogenic contamination. The fractionation studies showed that the clay-sized fraction obtained using hydrocyclone separation constituted 55% of the total bulk soil. The remaining fraction was further separated by heavy liquid. The light fraction (hl) and the heavy fraction (hl) was 44.4% and 0.6% of the total, respectively. The surface studies showed that there is Cr(VI) (about 30–40% of the total Cr) on the surface of the fractions of soil that potentially can be released. As far as the mobility of Cr, it is related directly to the heavy fraction of soil, which contains chromite (6.9%), hematite (6.7%) and goethite (10.1%) as the main minerals, according to mineralogical analysis. In case of acidic pHs (5 and 6), in addition to the heavy fraction, the clay-sized fraction (hc) plays also an important role in the mobility of Cr and controls Cr release, due to its high reactivity. The main conclusion of this study is that chromite weathering is the controlling mechanism of Cr release. The pH edge studies showed that the behavior of Cr release in ophiolite sample is associated with the other fractions of soil, indicating that the mobility of Cr is related to ophiolite weathering. Cr, Ni, Mn, Y enrichment in the fractions of soils of Asopos basin is dominantly the result of erosion, transport and weathering of ultramafic sources of Avlonas area. The significant correlations of Cr–Ni, Cr–Mn and Cr–Y in the fractions of soil also confirm that the soil was mostly generated from the erosion of ultramafic rocks of Asopos basin. The results of the pH edge experiment are consistent with Cr field concentration when up-scaled using typical physical parameters of the soils.

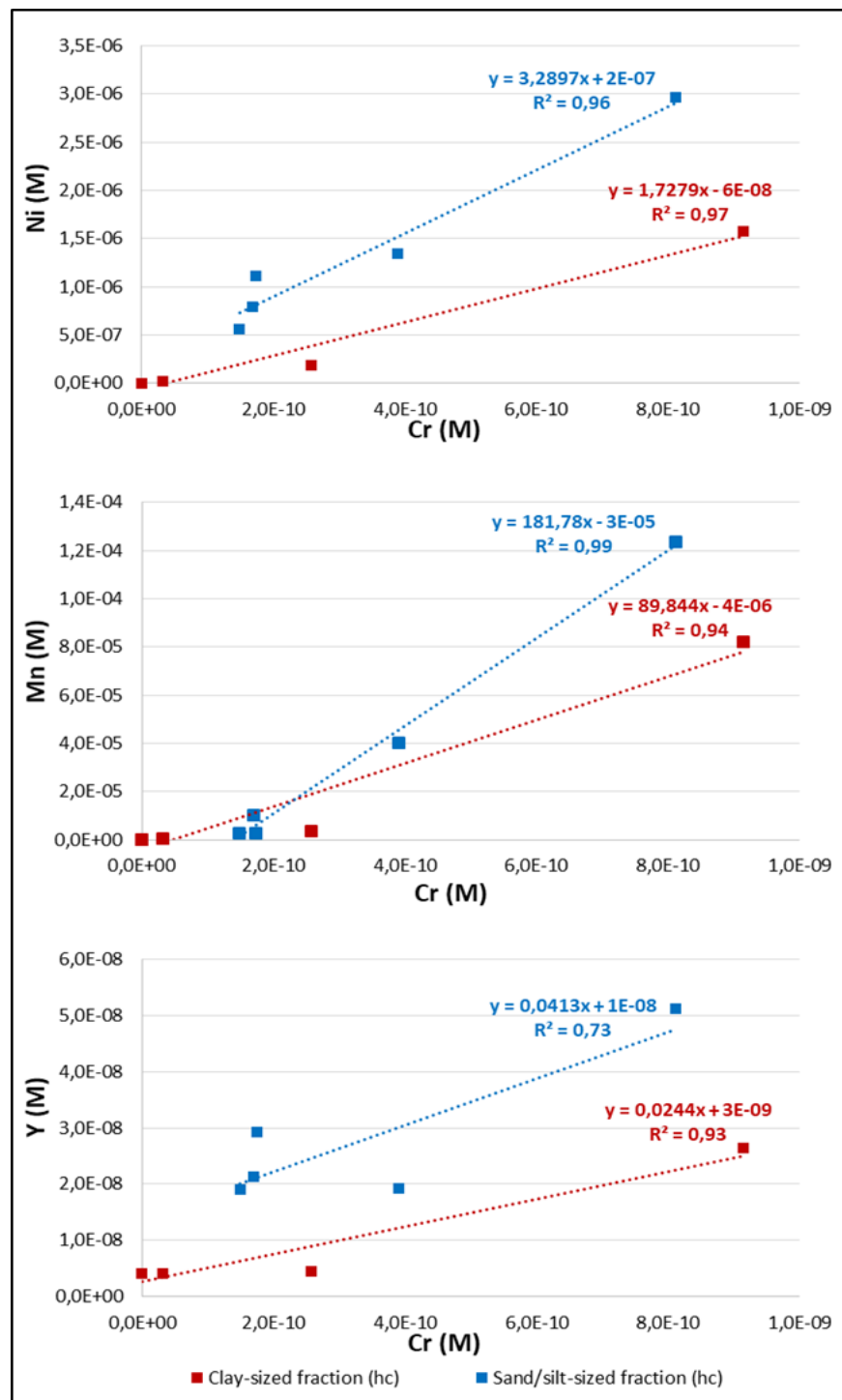


Figure 3.5. Correlation diagram of Cr with Ni, Mn, Y content in sand/silt-sized (hc) and clay-sized (hc) fraction of soil.

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CHAPTER 4. ASSESSING THE IMPACT OF GEOGENIC CHROMIUM UPTAKE BY CARROTS (*DAUCUS CAROTA*) GROWN IN ASOPOS RIVER BASIN



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Lilli, M.A., Syranidou, E., Palliou, A., Nikolaidis, N.P., Karatzas, G.P., Kalogerakis, N., 2017. Assessing the impact of geogenic chromium uptake by carrots (*Daucus carota*) grown in Asopos River Basin, *Environmental Research* 152, 96-101.

4.1 Abstract

A methodology was developed to assess the impact of geogenic origin Cr(VI) uptake by carrots, and the risk of human consumption of carrots grown in Asopos River basin in Greece. A field scale experiment was conducted with carrots cultivated in treatment plots, with and without compost amendment, in order to assess the impact of carbon in the mobility and uptake of Cr by plants. The results suggested that there is a trend for Cr mobilization and uptake in the surface and the leaves of the carrots cultivated in the treatment plot with the higher carbon addition, but not in the core of the carrots. Limited mobility of Cr(VI) in the soil-plant-water system is presented due to the affinity of Cr to be retained in the solid phase and be uptaken by plants. Cr(VI) tolerant bacterial strains were isolated from the carrots. These endophytic bacteria, present in all parts of the plant, were able to reduce Cr(VI) to Cr(III) form to levels below the detection limit. Finally, a site-specific risk assessment analysis suggested no adverse effects to human health due to the consumption of carrots. These findings are of particular importance since they confirm that carrots grown in soils with geogenic origin Cr does not pose any adverse risk for human consumption, but could also have the beneficial effect of the micronutrient Cr(III).

4.2 Introduction

The increasing demand of food safety is a major public concern worldwide and it has stimulated researches regarding the risk associated with food contaminated by pesticides, heavy metals and toxins (D Mello, 2003). Risk assessment of heavy metal contaminated food products is one of the most important aspects of food quality assurance (Sharma et al., 2005), due to its toxicity and bioaccumulation effect (Yusuf et al., 2003).

Cr is one of the heavy metals with growing concern about its toxicity due to diet. Trace amounts of Cr(III) appear to be essential for animal and human glucose and lipid metabolism, while Cr(VI) is commonly regarded as toxic to all forms of life and classified as human carcinogens (Stout et al., 2009, IARC, 1990). Co-exposure to Cr and arsenic in the process of incineration of debris including chromated copper arsenate-treated wood, showed increased carcinogenic risk (Ohgami et al., 2015). Cr

existence in soils and water can be a result of anthropogenic activities i.e. industrial activities and phosphorus fertilizers (Molina et al., 2009), or of natural processes as the weathering of ultramafic rocks (peridotites and pyroxenites) (Garnier et al., 2009, Mills et al., 2011, Robles-Camacho and Armienta, 2000). Geogenic origin Cr(VI) have appeared in many areas around the globe such as California, USA (Oze et al., 2004, Morrison et al., 2009), Zimbabwe (Cooper, 2002) and Italy (Fantoni et al., 2002). In Greece, Asopos River basin has agricultural soils containing geogenic origin Cr (Kelepertzis et al., 2013, Lilli et al., 2015, Moraetis et al., 2012) and other heavy metals that have the potential to pose a threat to human health and the environment.

In plants, particularly crops, Cr at low concentrations (0.05 – 1 mg/L) was found to promote growth and increase yield, but there is no evidence that plays a role in their physiological function (Oliveira, 2012). On the other hand, Sridhar et al. (2007) has shown that Cr can accumulate in plants, reducing their growth, altering mineral nutrition and causing oxidative stress via formation of reactive oxygen species (Raj et al., 2011). Cr(III) had different effects on cress and cucumber shoots: low toxicity was found in cucumber seeds exposed to a concentration of chromium of 300 mg/kg in soils, while biostimulating effects were recorded in cress plants exposed to up to 100 mg/kg of Cr (Baderna et al., 2015). Cr(VI) is harmful to many plants for concentrations ranging from 0.16 mg/L (lettuce) to 75 mg/L (sweet orange) (Barcelo and Poschenrieder, 1997, Pawlisz et al., 1997, Poschenrieder et al., 1987). Dai et al. (2009) have suggested the determination of a soil environmental quality standard for farmland based on the effects of Cr on crop growth and Cr accumulation in the edible part.

Cr(III) uptake is a passive process, whereas Cr(VI) uptake is performed by carriers of essential elements such as sulphate (Hossner et al., 1998, Oliveira, 2012). Cr(VI) absorbed by the root is reduced to Cr(III) and retained mostly by the roots (James and Bartlett, 1984). Studies on uptake by onions (Srivastava et al., 1994), maize and groundnut plants (Mishra et al., 1995) showed that the uptake of Cr was higher when it was supplied by irrigation water. A significant amount of Cr was absorbed by the plant roots which played an important role in modifying the state of Cr to a bioavailable form. The amount of Cr translocated to grain of paddy was less compared to the above three crops (Mishra et al., 1997). Studies on chamomile plants presented similar results regarding Cr accumulation in roots (Kovacik et al., 2014).

The presence of plants in wetland vegetated systems enhances the reduction of Cr(VI) in sediments by modifying the governing biogeochemical cycle (Zazo et al., 2008). Furthermore, bacterial mediated reduction of Cr(VI) to Cr(III) is a widely - known and accepted technology for the remediation of contaminated soils and groundwaters (Hellerich and Nikolaidis, 2005). Similarly, endophytic bacteria (defined as the bacteria that are present in the plant internal tissues without imposing any negative effect) isolated from many plant species have shown to be able to reduce Cr(VI) (Kuklinsky et al., 2004). Experiments conducted using *J. acutus* showed that isolated endophytic bacteria were found to be Cr(VI) tolerant and have a high potential to reduce Cr(VI) to Cr(III) (Dimitroula et al., 2015). The microbial reduction of Cr(VI) to Cr(III), which is a useful process, takes place intra-cellularly (Narayani and Shetty, 2013) and reduces Cr toxicity.

Carrot (*Daucus carota*) is a root crop which is rich in bioactive compounds such as carotenoids and dietary fibers with appreciable levels of other functional components with significant health-promoting properties (Sharma et al., 2012). Carrots have been suggested as biomarkers for toxicity assessment in terrestrial ecosystems (U.S. EPA, 1996). A greenhouse experiment conducted in Chinese soils showed that, the carrot yield significantly decreased in soils with Cr addition, and the Cr content of carrot grown in soils with pH>8.0 exceeded the maximum allowable level (0.5 mg/kg), according to the Chinese General Standard for Contaminants in Foods (Ding et al., 2014).

Given the health effect concerns due to the exposure to Cr(VI), the overall objective of this study was to develop a methodology to assess the impact of geogenic origin Cr(VI) uptake by carrots, and the risk of human consumption of carrots grown in Asopos River basin in Greece.

4.3 Material and methods

4.3.1 Design of analysis

The overall experimental design of this study aimed to determine the bioavailability of Cr uptake by carrots, the fate of Cr(VI) within the plant and a risk assessment of carrot consumption using standardized EPA methodologies. Carrots were selected as one of

the main root product cultivated in the Schimatari area of Asopos River basin and it is hypothesized that they could have the highest uptake and potential accumulation.

In order to assess the risk of human consumption of carrots that are grown in an area with geogenic origin Cr, a three prong approach was followed:

- Cr(VI) uptake by carrots grown under field conditions was determined,
- the fate of Cr(VI) within the carrot was investigated evaluating also the potential of Cr reduction by endophytic bacteria, and
- finally, a site-specific risk assessment of carrot consumption using the results from the first two stages was conducted to define possible diet - induced adverse effects on human health.

4.3.2 Field scale experiment

The field scale experiment was conducted in Schimatari area. Carrots were cultivated in 4×4 meters treatment plots with and without carbon amendment in the form of compost as soil improver. The aims of the preliminary experiment was the quantification of Cr content in the carrots grown in various field conditions depending on the soil organic content. The municipal solid waste compost (MSW-compost) obtained from MSW Management Co. of the Municipality of Chania, was incorporated to the soil. The Cr concentration in MSW-compost was found equal to 26.6 mg/kg (Giannakis et al., 2014). Three conditions were tested: the “control” with no compost amendment, the “dose 1” compost treatment corresponding to 50000 kg of compost per ha and the “dose 2” compost treatment with 100000 kg of compost per ha. Each treatment was conducted in duplicates. An initial soil sampling was done before the application of the compost in June 2014 and 6 soil samples were collected from the field. Specifically, 2 soil samples were collected from each of the three treatments (triplicates). The final soil sampling was conducted at the end of the growing cycle in October 2014 (one growing season tested). Soil samples were taken from the top 0-15 cm, were homogenized, air dried and sieved. The fraction smaller than 2 mm was only used for the experiments.

Carrots were grown for 4 months and the final growth was recorded. 6 carrot samples were collected from each treatment condition. Samples were washed with tap water (in order to remove adhering soil), grinded, homogenized and dried. The leaves, the surface

and the core of carrot (edible part) were separately analyzed. One-way analysis of variance (ANOVA) combined with the post- hoc Tukey test was used in order to assess the statistical differences in Cr uptake among the treatments.

Soil and carrot samples were analyzed for moisture, total organic carbon (TOC), total organic nitrogen (TN), total cations and heavy metals. TOC and TN were analyzed with multi N/C 2100S Reactor. Base cations and heavy metals and specifically total Cr of soils and carrots were analyzed with the procedure described by EPA method 3051A (2007). Specifically, 9 ml HNO₃ were added to 0.2 g soil / carrot, followed by microwave digestion at 150-180°C (Multiwave 3000 Digestor). Supernatant solutions were diluted with MilliQ water and analyzed by inductively coupled plasma mass spectroscopy (ICP-MS) (Agilent 7500-CX).

Groundwater samples were also collected from wells irrigating the carrots. These samples were analyzed for nitrate, ammonia, phosphate, hexavalent and total Cr, and cations (Mn, Fe, Ca, Mg, Na, K). Nutrients (N-NO₃, N-NH₃, P-PO₄) analyzed with a HACH 2800DR spectrophotometer and metals with ICP-MS (Agilent 7500-CX). Amberlite IR – 120 resin was used for the determination of Cr(VI).

4.3.3 Endophytic bacteria experiments

In order to overcome the lack of reliable method for the determination of Cr(VI) in tissues, an endophytic bacteria study was conducted to isolate Cr(VI) tolerant strains and assess their ability to reduce Cr(VI) to Cr(III). This process could be of major importance since the ability of the carrots to reduce accumulated toxic Cr(VI) to Cr(III) leads to minimizing potential human health risk. The objectives of this phase were: first, the isolation and identification of Cr(VI)-resistant cultivable endophytic bacteria, second, genotyping characterization of these bacteria, and finally, the reduction of Cr(VI) assays by the selected endophytic bacteria.

4.3.3.1. Isolation of Cr(VI)-resistant cultivable endophytic bacteria

For the isolation of the endophytic bacteria, the plant samples taken from the “control” treatment, were washed with tap and deionized water in order to remove soil particles. Samples (1 g) of leaves and roots divided into core (edible part) and surface (the first 10 mm) of two carrots were transferred in sterile falcons filled with 10 mL 10 mM

MgSO₄. The surface was sterilized for 20 sec in 70% sterile ethanol solution and in 1% active chloride solution amended with 1 droplet Tween 80 per 100 mL solution for 5 min.

The tissue samples were subsequently rinsed three times in sterile distilled water for 1 min. Aliquots of the third rinsing were plated on 869 medium (10.0 g tryptone, 5.0 g yeast extract, 50 g NaCl, 1.0 g glucose, 0.35 g CaCl₂·2H₂O in 1 l distilled water) and if no growth was observed after 7 days at 30°C, the surface sterilization process was considered successful. The surface sterile samples were macerated with sterile mortar and pestle for 60 sec in 10 mL 10 mM MgSO₄. 10-fold serial dilutions were plated on 1/10 strength 869 agar medium supplemented with 1 mg/L Cr(VI) (K₂Cr₂O₇) for the leaf and root isolates. After incubation for 7 D at 30°C, colonies per gram fresh weight were calculated. All morphologically different colonies were subcultured at least 3 times to ensure purity and were preserved at -80°C in 15% (v/v) glycerol.

4.3.3.2 Genotyping characterization

The total genomic DNA was extracted from purified colonies using the STE method (Sodium Chloride-Tris-EDTA (STE), 10X, pH 8.0) which is suitable for bio molecular lab procedures (10X Solution contains 100 mM Tris-HCl, 10 mM EDTA, and 1M NaCl) and was amplified using the BOX A1R primer (5'-CTACGGCAAGGCGACGCTGACG-3') (Becerra-Castro et al., 2011). PCR reactions were performed in volumes of 50 µL, containing 1X Taq buffer, 1.5 mM MgCl₂, 0.1 mM of each dNTP, 2 µM of BOX A1R primer, 0.5U Taq polymerase and 1 µL extracted DNA. Cycling conditions were: initial denaturation step of 5 min at 95°C, followed by 30 cycles of 1 min at 94°C, 1.5 min at 50°C and 8 min at 65°C with a final elongation step of 8 min at 65°C. The PCR products were separated by electrophoresis in 1.8% agarose gels after running for 3 h at 3.3 Vcm⁻¹ gel, stained by Midori Green and visualized under UV light. The isolates with the same BOX-PCR profile were grouped.

4.3.3.3 Cr(VI) reduction capacity of the cultured isolates

The Cr(VI) resistant isolates were cultured aerobically in 869 medium, 150 rpm at 30°C until they reached the late exponential phase. The grown cells were inoculated into 869 medium amended with 10, 20 or 100 mg/L filter (0.2 µm pore diameter) sterilized

Cr(VI) solution. The flasks were incubated at a shaker (150 rpm) at 30°C. All treatments were performed in triplicates and sterile medium with Cr(VI) ($K_2Cr_2O_7$) was used as a control. Samples were taken at different time intervals, depending on the growth and the initial Cr(VI) concentration. Optical densities were measured at 600 nm (OD 600 nm) in a HACH DR-2800 spectrophotometer. Concerning Cr(VI) and total Cr measurements, samples were centrifuged at 4000 rpm for 15 min and aliquots of the supernatants were analyzed for Cr(VI) by the 1,5-diphenylcarbohydrazide colorimetric method and for total Cr by ICP-MS.

4.3.4 Risk assessment methodology

A risk assessment analysis was conducted, in order to calculate the risk of potential chronic exposure due to carrot consumption, following EPA methodologies.

The risk effects consist of carcinogenic and non-carcinogenic risk assessments for all the metals through various pathways. In this study, only the pathway of ingestion was considered. The average metal concentrations measured in the carrot samples (edible part) from the “control” treatment, were used to calculate the chronic daily intake (CDI) which was then used to characterize the exposure to metals resulting from carrot consumption. The following equation was used to determine the CDI of the metals analyzed in this study:

$$CDI = \frac{C_{carrot} \times IR_{carrot} \times EF \times ED}{BW \times AT}$$

where CDI is the chronic daily intake (mg/kg/day), C is the concentration of each metal found in the edible part of carrot (mg/kg), IR is the average daily intake rate of carrot: 0.0151 kg/D (U.S. EPA, 2011), BW is the body weight: 70 kg of an individual (U.S. EPA, 1991), EF is the exposure frequency: 350 day/a (U.S. EPA, 2011), ED is the exposure duration: 30 a (U.S. EPA, 2011) and AT is the average time for non-carcinogens: 365*30 days (U.S. EPA, 2011).

The non-carcinogenic risk from individual heavy metals can be expressed as the hazard quotient:

$$HQ = CDI/RfD$$

where HQ is the non-cancer hazard quotient and RfD is the chronic reference dose of the toxicant (mg/kg/day). Oral RfDs for Cr(VI), Ni, Mn, Zn and Cu (metals examined in this analysis) are 0.003, 0.011, 0.14, 0.30 and 0.40 respectively (U.S. EPA, 1998, Tvermoes et al., 2014).

The cumulative non-carcinogenic risks were expressed as a hazard index (HI) which is the sum of the HQs from all the metals considered in this analysis (U.S. EPA, 2005). This provides a worst-case scenario assessment of the non-carcinogenic risks that these metals may pose due to carrot consumers.

$$HI = HQ_{Cr} + HQ_{As} + HQ_{Cd} + HQ_{Hg} + HQ_{Pb} + HQ_{Mn} + HQ_{Zn} + HQ_{Cu} + HQ_{Al} + HQ_{Ni}$$

As, Hg, Cd and Al concentrations measured in the edible part of the carrot samples were below detection limit, so these metals were not included in the HQ or HI analysis. Because of the lack of an oral RfD for Pb, this metal was not included in the HQ or HI analysis. HI values >1 shows that there is a chance that non-carcinogenic risk may occur.

Cancer risk represents the probability of an individual lifetime chronic health risk from carcinogens and can be evaluated from:

$$Cancer\ risk = LADD \times SF$$

where LADD is the lifetime average daily dose (mg/kg/day); SF is the slope factor of hazardous substances (for Cr(VI): 0.5 mg/kg/day (U.S. EPA, 1998)).

The following equation was used to calculate LADD:

$$LADD = C_{Cr} \frac{mg}{kg} \times 0.001 \frac{kg}{g} \times \left(\frac{IR_{1-12mths} \times ED_{1-12mths}}{AT} + \frac{IR_{1-2yrs} \times ED_{1-2yrs}}{AT} + \frac{IR_{2-5yrs} \times ED_{2-5yrs}}{AT} + \frac{IR_{5-12yrs} \times ED_{5-12yrs}}{AT} + \frac{IR_{12-19yrs} \times ED_{12-19yrs}}{AT} + \frac{IR_{19-49yrs} \times ED_{19-49yrs}}{AT} + \frac{IR_{49-70yrs} \times ED_{49-70yrs}}{AT} \right)$$

where IR_i is body weight normalized carrot consumption rate for the i th age group (g/kg/day) (U.S. EPA, 2011), Ed_i is exposure duration for the i th age group (years), and AT is a lifetime of 70 years.

The acceptable or tolerable risk for regulatory purposes is within the range of 10^{-6} - 10^{-4} (U.S. EPA, 2001).

4.4 Results and discussion

4.4.1 Cr concentrations in carrots cultivated in compost amended soils

The initial soil sampling, prior to compost amendment, showed that the average Cr concentration in soils was 126 ± 20 mg/kg, suggesting geogenic origin Cr concentration similar to those found in our previous studies (Lilli et al., 2015). Lilli et al. (2015) has obtained more than 50 soil and sediment samples from the greater Asopos area in order to establish the geochemical variability within the basin. High correlation (R^2 of 0.74) of the Cr–Ni chemical relationship was found in soils and sediments of Asopos basin suggesting geogenic origin of Cr. The irrigated water and soil data collected in this study had similar geochemistry with other studies conducted in the area by Moraetis et al. (2012) and Lilli et al (2015) that provided evidence for the geogenic origin of the Cr in the soils. Based on the above analyses, soils taken in this study belong to the calcareous group of soils have soil pH 7.9 and low content of organic carbon and nitrogen (1% and 0.09% respectively). The average total Cr and Cr(VI) concentrations of this group of soils are 775 mg/kg and 0.06 mg/kg, respectively.

The average Cr(VI) found in the groundwater used to irrigate the carrots was 6.11 ± 6.02 μ g/L. The application of amberlite resin (IR-120) in groundwater samples demonstrated that total Cr and Cr(VI) measurements were statistically the same. The groundwater chemistry is characterized by geogenic origin metals at low levels, weathering products of calcaric soils and high agricultural pollution levels as indicated by the nitrate (9.37 mg/L), ammonium (1.47 mg/L) and phosphorous (0.08 mg/L) concentrations.

The sampling conducted in the final stage of the growth of carrots indicated that the average dry weight Cr concentration in the core of the carrots was 0.19 mg/kg, 0.34 mg/kg and 0.23 mg/kg for carrots cultivated without compost amendment (control), with “dose 1” and “dose 2” compost treatment respectively (Fig. 1). The statistical

analysis showed that at the 0.05 significance level there was no statistical difference between the means of Cr concentration in the core of the carrots for the three conditions suggesting that carbon content of the soils does not influence the mobility of geogenic chromium within the core of the carrot (Fig. 4.1). The average dry weight Cr concentration on the surface of the carrots was 0.73 mg/kg, 1.45 mg/kg, and 1.42 mg/kg for carrots cultivated in treatment plots without compost amendment (control), with “dose 1” and “dose 2” compost treatment, respectively (Fig. 4.1). At the 0.05 significance level there was no statistical difference between the means of Cr concentration in the control and the “dose 1” compost treatments, however, there was statistical higher concentrations in the “dose 2” compost treatment compared to the other two (control, “dose 1” compost treatment) (Fig. 4.1). Finally, the average dry weight Cr concentration in the leaves of the carrots was 7.71 mg/kg, 8.23 mg/kg and 15.17 mg/kg for carrots cultivated in treatment plots without compost amendment (control), with “dose 1” and “dose 2” compost treatment, respectively (Fig. 4.1). The statistical results were similar as the surface of the carrots (Fig. 4.1). The results suggested that there is a trend for Cr mobilization and uptake in the surface and the leaves of the carrots cultivated in the treatment plot with the higher carbon addition (“dose 2”), but not in the core of the carrots.

The results obtained from our previous leaching studies showed that the concentration of Cr in soil solution was about 2 µg/L (Lilli et al., 2015). Taking into account that the maximum Cr uptake in carrots was about 15 mg/kg, the partitioning coefficient between plant and water found 7500 mL/g. Furthermore, taking into account that Cr concentration in soil from the field of carrots was about 126 mg/kg, the partitioning coefficient between soil and water found 63000 mL/g. Both of these estimates of partitioning coefficients suggest limited mobility of Cr(VI) in the soil-plant-water system due to the affinity of the Cr to be retained in the solid phase and be uptaken by plants.

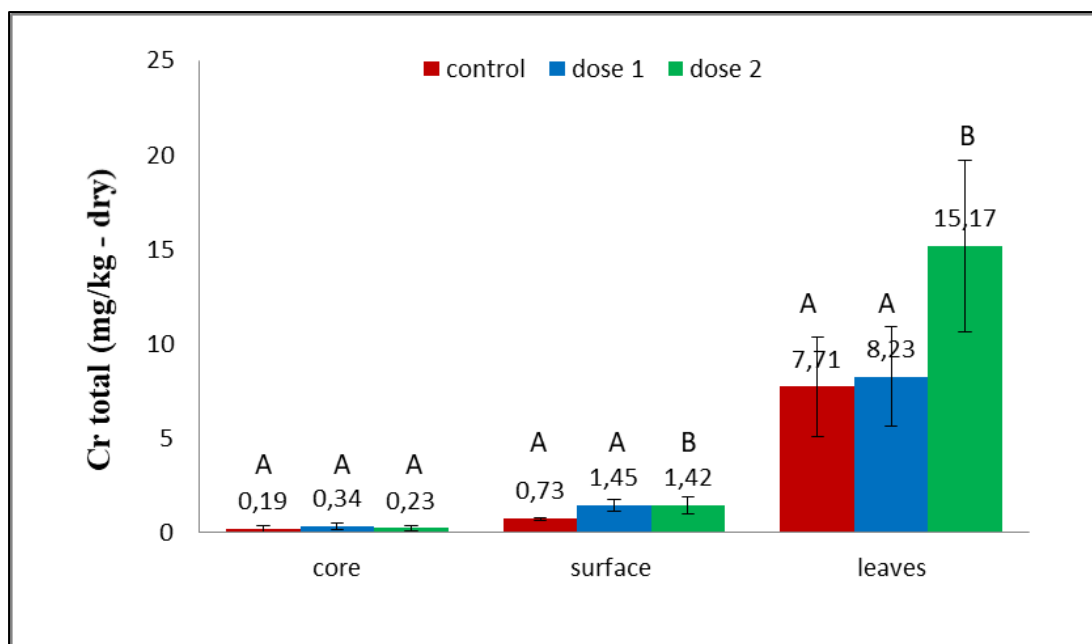


Figure 4.1. Total chromium concentration in the parts of carrots (core, surface, leaves) for the control and compost treatments (“dose 1” and “dose 2”). Error bars correspond to standard deviation of the replicates. The data with different letters (A, B) in the same carrot part indicate significant differences at the $\alpha=0.05$ level using ANOVA. e.g. for the surface of the carrots, “dose 2”compost treatment presents significant differences compared with the other two, while “dose 1” compost treatment and control do not differ significantly with each other.

4.4.2 Endophytic bacteria

4.4.2.1 Isolation and genotyping characterization of Cr(VI) tolerant endophytic bacteria

Since Cr was measured in the surface and the leaves of the carrot the contribution of the endophytic community in plant detoxification was assessed. The cultivation media was supplemented with 1 mg/L Cr(VI) in order to isolate only the resistant strains. A similar number of culturable Cr(VI) tolerant endophytic bacteria was recovered from the root ($7.6 \cdot 10^5$ CFU g^{-1} FW tissue) and the leaves ($9.9 \cdot 10^5$ CFU g^{-1} FW tissue) of the carrot. Concerning the morphologically different colonies, eighty isolates from the root and forty-two isolates from the leaves were found able to grow on medium supplemented with 1 mg/L Cr(VI). According to their BOX-PCR profiles the leaf endophytes were allocated into 31 groups and the root endophytes were allocated into 65 groups.

4.4.2.2 Reduction of Cr(VI) assays by the selected endophytic bacteria

Two leaf (L1, L2), three root (R1, R2, R3) isolates from the core of the carrot and seven root (RS1, RS2, RS3, RS4, RS5, RS6, RS7) isolates from the surface of the carrot were selected for investigation of their potential ability to reduce Cr(VI) through biotransformation to Cr(III).

Fig. 4.2 presents the total Cr concentration, Cr(VI) reduction and growth curves of an indicative root (core) endophytic bacterium (strain R1) at 5 mg/L Cr(VI) initial concentration. The endophytic root strain R1 not only was tolerant to 5 mg/L Cr(VI) concentration but also reduced 5 mg/L of Cr(VI) in 20 h of cultivation (Fig. 4.2). The endophytic strains R2 and R3 had similar trends. More specifically, all isolates for the core of the carrot R1, R2 and R3 removed the 95%, 84% and 87% Cr(VI) respectively, in 20 h of cultivation. It was noticed that the cell growth (OD 600nm) and the Cr(VI) removal started simultaneously for the three strains. At 5 mg/L of Cr(VI) the lag phase of these strains were 4 h, 3 h and 3 h, respectively (Table 4.1). The reaction rate (k) and half-life ($\tau = \ln 2/k$) were 0.18 /h, 0.1 /h, 0.12 /h and 3.85 h, 6.93 h, 5.78 h for the strains R1, R2, R3, respectively, suggesting very fast transformation rates for the Cr(VI) and a very potent detoxification mechanism (Table 4.1).

Table 4.1. Lag phase, reaction rate (k) and half-life (τ) for the root (core and surface) endophytic bacteria and leaf endophytic bacteria

	lag phase (h)	k (/h)	τ (h)
Root (core) endophytic bacterium			
R1	4	0.18	3.85
R2	3	0.1	6.93
R3	3	0.12	5.78
Root (surface) endophytic bacterium			
RS1	4	0.16	4.33
RS2	2	0.17	4.08
RS3	4	0.17	4.08
RS4	2	0.17	4.08
RS5	5	0.21	3.30
RS6	4	0.13	5.33
RS7	4	0.13	5.33
Leaf endophytic bacterium			
L1	3	0.09	7.70
L2	3	0.09	7.70

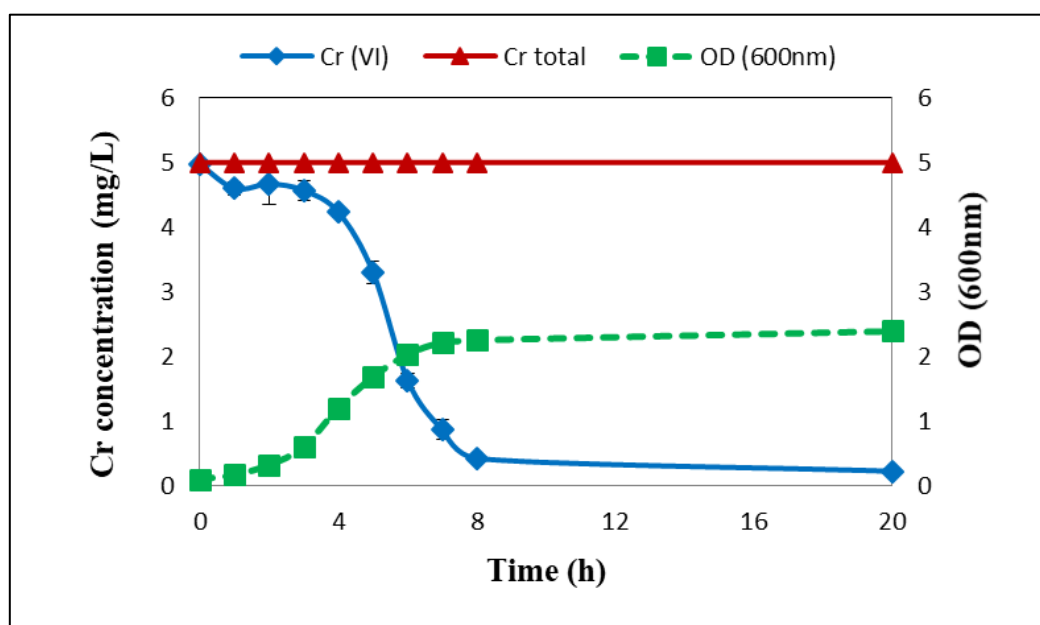


Figure 4.2 . Total chromium concentration, Cr(VI) reduction and growth curves of the root (core) endophytic bacterium, strain R1 at 5 mg/L Cr(VI) initial concentration, pH 7.0 and 30°C. Error bars correspond to standard deviation of the triplicates.

Fig. 4.3 presents the total Cr concentration, the Cr(VI) reduction and growth curves of a selected endophytic bacterium (strain RS1) isolated for the surface of the root at 5 mg/L Cr(VI) initial concentration. The endophytic strain RS1 was tolerant to 5 mg/L

initial Cr(VI) concentration and could completely reduce 5 mg/L Cr(VI) in 20 h of cultivation (Fig. 4.3). The endophytic bacteria RS2-RS7 presented similar trends. The root isolates RS1, RS2, RS3, RS4, RS5, RS6, RS7 removed the 94%, 96%, 94%, 96%, 96%, 89% and 92% Cr(VI) respectively, in 20 h of cultivation. It was noticed that the cell growth and the Cr(VI) removal started simultaneously for the seven strains. At 5 mg/L of Cr(VI) the lag phase of strains RS1, RS2, RS3, RS4, RS5, RS6, RS7 were 4 h, 2 h, 4 h, 2 h, 5 h, 4 h, 4 h, respectively (Table 4.1). The reaction rate (k) and half-life ($\tau = \ln 2/k$) for the strains RS1-RS7 are presented in Table 4.1 and the results show fast reaction rates.

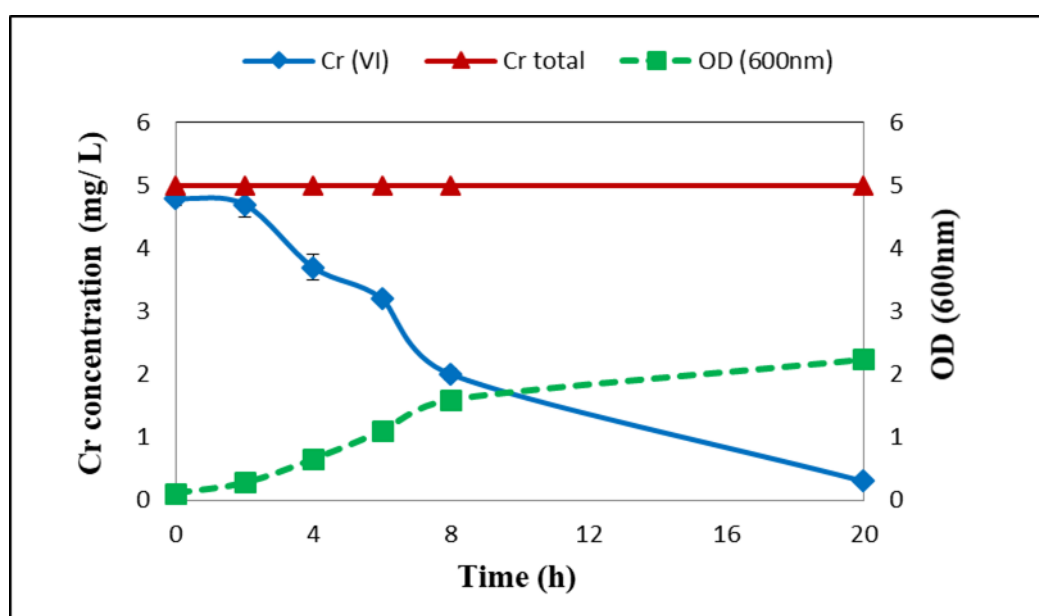


Figure 4.3. Total chromium concentration, Cr(VI) reduction and growth curves of the root (surface) endophytic bacterium, strain RS1 at 5 mg/L Cr(VI) initial concentration, pH 7.0 and 30°C. Error bars correspond to standard deviation of the triplicates.

Fig. 4.4 presents the total Cr concentration, the Cr(VI) reduction and growth curves of an indicative leaf endophytic bacterium (strain L1) at 3 mg/L Cr(VI) initial concentration. The endophytic bacterium strain L1 was tolerant to 3 mg/L initial Cr(VI) concentration and completely reduced 3 mg/L Cr(VI) in 20 h of cultivation (Fig. 4.4). The endophytic bacterium strain L2 had similar trend. The leaf isolates L1 and L2 removed the 85% Cr(VI) in 20 h of cultivation. It was noticed that the cell growth and the Cr(VI) removal started simultaneously for both strains. At 3 mg/L of Cr(VI) the lag phase of strains L1 and L2 were 3 h (Table 4.1). The reaction rate (k) and half-life ($\tau =$

$\ln 2/k$) were 0.09 /h and 7.7 h for both strains L1 and L2 (Table 4.1). The reaction rates of leaf endophytic bacteria are lower than the rates of root endophytic bacteria.

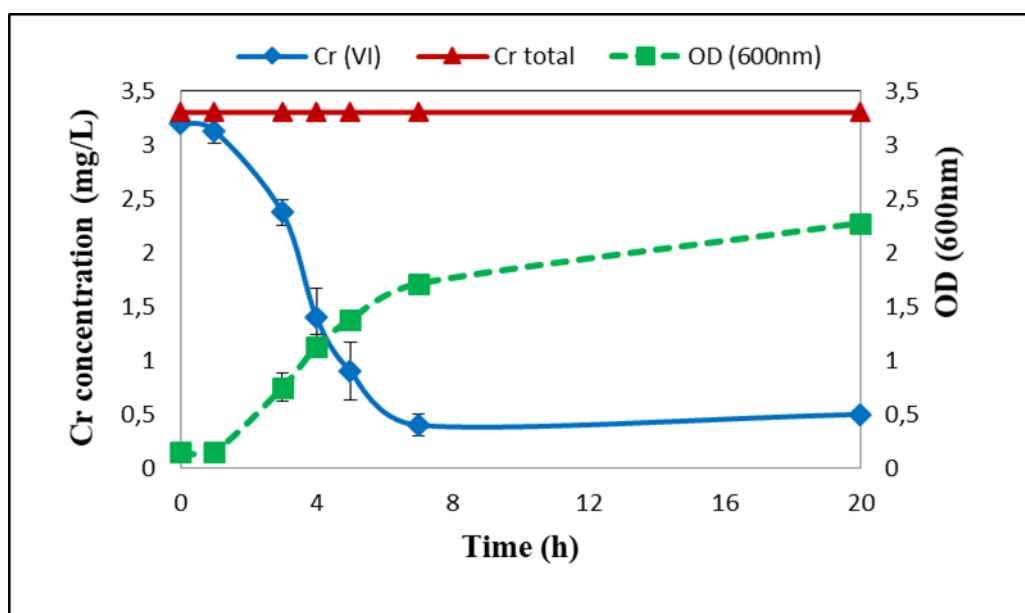


Figure 4.4. Total chromium concentration, Cr(VI) reduction and growth curves of the leaf endophytic bacterium, strain L1 at 3 mg/L Cr(VI) initial concentration, pH 7.0 and 30°C. Error bars correspond to standard deviation of the triplicates.

4.4.3 Risk assessment

The chronic daily intake (CDI) through ingestion of metals was determined following the EPA methodology and was found to be $3.93 \cdot 10^{-6}$, $4.32 \cdot 10^{-5}$, $1.6 \cdot 10^{-4}$, $5.19 \cdot 10^{-5}$ and $7.84 \cdot 10^{-5}$ mg/kg/D for Cr, Ni, Mn, Zn and Cu, respectively. Similarly, the non-carcinogenic risk (HQ) through ingestion of metals was found to be $1.31 \cdot 10^{-3}$, $3.93 \cdot 10^{-3}$, $1.12 \cdot 10^{-3}$, $1.73 \cdot 10^{-4}$ and $1.96 \cdot 10^{-4}$ for Cr, Ni, Mn, Zn and Cu, respectively. To estimate the cumulative non-carcinogenic risk, a hazard index (HI) was calculated from the mean contaminant concentration and was found to be $6.73 \cdot 10^{-3}$. The above value did not exceeded one, suggesting that the population is unlikely to experience obvious adverse effects due to the consumption of carrots.

The conversion of Cr(VI) to Cr(III) is occurring within the carrots by endophytic bacteria and specifically, the root (core) isolated endophytic bacteria removed the 84-95% Cr(VI) in 20 h of cultivation, as described in 4.3.2.2 section. The carcinogenic risks were calculated using this conversion. The lifetime average daily dose (LADD) of Cr was found equal to $4.92 \cdot 10^{-7}$ mg/kg/day and $1.54 \cdot 10^{-7}$ mg/kg/day for the 84% and

95% Cr(VI) reduction, respectively (Table 2). The cancer risk was found equal to $2.4 \cdot 10^{-7}$ and $7.7 \cdot 10^{-8}$ for the 84% and 95% Cr(VI) reduction, respectively (Table 4.2). The cancer risk value of less than 1 in a million additional cancer is well below the acceptable range for regulatory purposes (1 - 100 additional cancers in a million cases). The results of the risk assessment suggested no adverse effects to human health due to the consumption of carrots.

Table 4.2. Risk assessment values for chromium

Cr	
CDI (mg/kg/day)	$3.93 \cdot 10^{-6}$
HQ	$1.31 \cdot 10^{-3}$
LADD (mg/kg/day)	$1.54 \cdot 10^{-7} - 4.92 \cdot 10^{-7}$
Cancer risk	$7.7 \cdot 10^{-8} - 2.4 \cdot 10^{-7}$
Chinese General Standard for Contaminants in Foods (mg/kg)	0.5

4.5 Conclusions

The field scale experimental showed that there is a trend for Cr mobilization and uptake in the surface and the leaves of the carrots cultivated in the treatment plot with the higher carbon addition, but not in the core of the carrots. The results of the endophytic bacteria study reveal that carrots have the ability through their endophytic bacteria community to reduce Cr(VI) to Cr(III). The endophytic bacteria community consists of Cr(VI) tolerant strains present in all plant parts (core, surface, leaves) able to grow on medium with 1 mg/L Cr(VI) and able to reduce different concentrations Cr(VI) to Cr(III). These results lead us to investigate the endophytic bacteria of two leaf and three root (core) isolates which were found to be able to grow on medium with 3 mg/L Cr(VI) and seven root (surface) which were found to be able to grow on medium with 5 mg/L Cr(VI). Furthermore all of these strains were found to be able to decrease initial Cr(VI) concentration (3 mg/L and / or 5 mg/L) below the detection limit in 20 h. Moreover it was noticed that the cell growth and the Cr(VI) removal started simultaneously for all the strains. The estimation of the reaction rate and half-life for the endophytic bacteria strains isolated from the core and surface of the carrots, suggests very fast transformation rates for the Cr(VI). As far as the estimation of the cumulative non-carcinogenic risk, the hazard index was found to be $6.73 \cdot 10^{-3}$, suggesting that the population is unlikely to experience adverse effects due to the consumption of these

carrots. The carcinogenic risk was estimated to be 0.077 - 0.24 in a million. These results suggested no adverse effects to human health due to the consumption of carrots.

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CHAPTER 5. SUMMARY-CONCLUSIONS

In Greece, the main ophiolite outcrops of the Hellenides are distinguished in four ophiolite belts. Pindos Mountains are covered with ultramafic rocks, which have been eroded and their fragments have been combined with Tertiary and Quaternary deposits that reside in valleys. Serpentine soils with high geogenic origin Cr contamination were derived from the weathering of these rocks. The study area is situated in central Greece and specifically in the basin of Asopos river where the reported Cr and Ni concentrations in groundwater and agricultural soils and products attributed to the occurrence and weathering of ultramafic rocks. Moreover, the greater area of the Asopos river, is a unique case of combined anthropogenic and geogenic origin Cr contamination. Although the anthropogenic origin Cr contamination has been extensively studied due to the remediation of industrial contaminated sites around the world, there was a gap in knowledge about geogenic Cr contamination and mobility especially in soils and river bed sediments. Specifically, there was a need to develop methodologies and an integrated approach to outline an area with wide spread geogenic origin Cr contamination, and to develop a framework of analysis to understand the origin and mobility of geogenic Cr in soils of Asopos river basin. The present study intended to cover this need and therefore, the objectives were threefold: (1) to assess the geochemical characterization and classification of soils and river sediments with respect to the origin of Cr along Asopos river, (2) to assess the processes affecting the mobility of Cr(VI) in soils and sediments along Asopos river and to elucidate the mechanisms of Cr release from soils and (3) to develop a methodology to assess the impact of geogenic origin Cr(VI) uptake by agricultural products (specifically carrots), and the risk of human consumption of carrots grown in Asopos River basin in Greece. In order to achieve this, a three prong approach was followed:

In the first phase, a field monitoring and sampling campaign was designed and soil, surface river bed sediments and core river bed sediment samples were collected. Moreover physicochemical characteristics of the soil and river sediment samples were analyzed. Physicochemical characteristics were fed into Principal Components Analysis as to categorize the samples according to the geochemical characteristics. The analysis of heavy metals in the area supported the hypothesis of geogenic origin of Cr in soils and sediments. The evidence that supported the geogenic origin of Cr, was the positive and high correlations between Ni and Cr concentrations in soils and sediments and the relatively even distribution of Cr and Ni upstream and downstream from the

industrial park. The soils of Asopos watershed exhibited high Cr content regardless the different lithologies which were characterized from calcareous, siliceous and ultramafic components (Fig. 5.1). This phase also involved mobility studies in the laboratory for process understanding i.e. sorption and release of total and hexavalent Cr from the soils. Modeling of Cr adsorption in soil was applied as to elucidate the adsorption mechanism. The Cr concentration in equilibrium of 1-2 ug/L resulted from the laboratory experiments was shown to be consistent to field groundwater Cr concentrations. The sorption results suggest that soils in the basin of Asopos have a significant adsorption capacity.

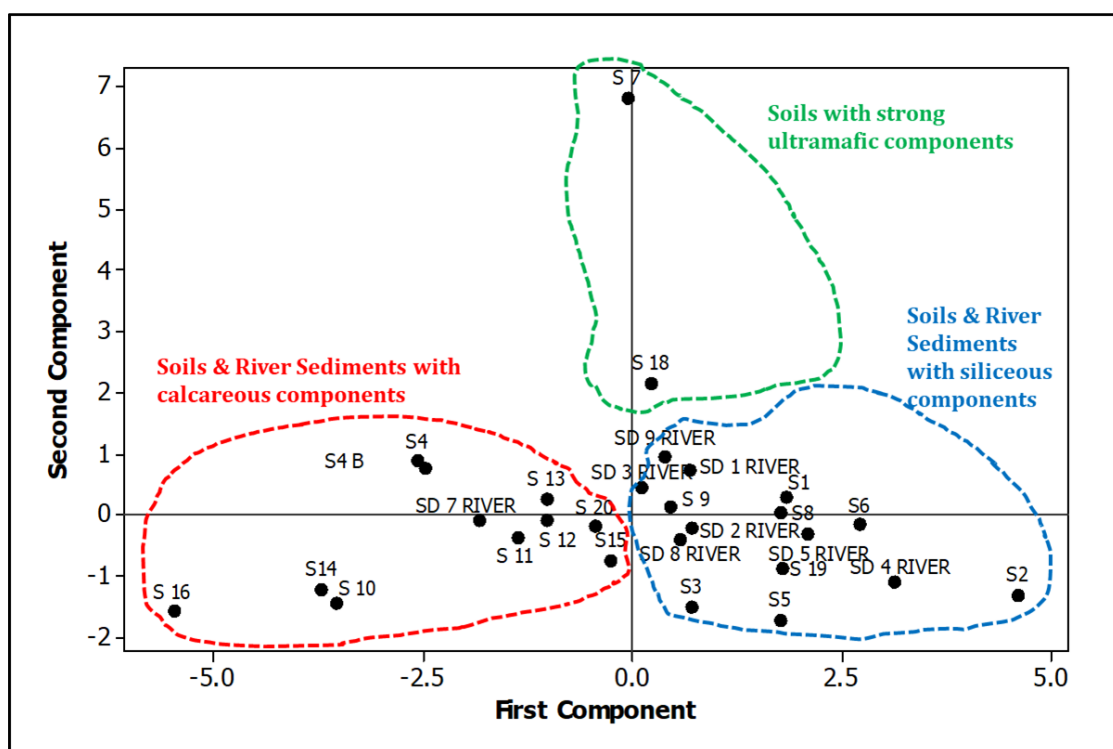


Figure 5.1. Groups resulting from PCA in soils and river bed sediments.

In the second phase a representative agricultural soil sample of Asopos basin used in order to elucidate the geogenic component of Cr contamination by isolating the Cr containing minerals in the soil. The methodology was designed firstly, to separate first the bulk soil into secondary fractions using both size and weight fractionation with hydrocyclone (hc) and heavy liquids (hl) and secondly, to characterize the above fractions of contaminated soil using different methods including mineralogical, chemical and surface analysis. The current phase also involved leaching studies of each

fraction to ascertain the release patterns of Cr as a function of pH and to identify the controlling mechanism of Cr mobility. The fractionation studies showed that the clay-sized fraction obtained using hydrocyclone separation constituted 55% of the total bulk soil. The remaining fraction was further separated by heavy liquid. The light fraction (hl) and the heavy fraction (hl) was 44.4% and 0.6% of the total, respectively. The surface studies showed that there is Cr(VI) (about 30-40% of the total Cr) on the surface of the fractions of soil that potentially can be released. As far as the mobility of Cr, it is related directly to the heavy fraction of soil, which contains chromite (6.9%), hematite (6.7%) and goethite (10.1%) as the main minerals, according to mineralogical analysis. In case of acidic pHs (5 and 6), in addition to the heavy fraction, the clay-sized fraction (hc) plays also an important role in the mobility of Cr and controls Cr release, due to its high reactivity. The main conclusion of this study is that chromite weathering is the controlling mechanism of Cr release. The pH edge studies showed that the behavior of Cr release in ophiolite sample is associated with the other fractions of soil, indicating that the mobility of Cr is related to ophiolite weathering. Cr, Ni, Mn, Y enrichment in the fractions of soils of Asopos basin is dominantly the result of erosion, transport and weathering of ultramafic sources of Avlonas area (Fig. 5.2). The high correlations of Cr–Ni, Cr–Mn and Cr–Y in the fractions of soil also confirm that the soil was mostly generated from the erosion products (transported mechanically as Cr-bearing clasts) of ultramafic rocks of Asopos basin. The results of the pH edge experiment are consistent with Cr field concentration when up-scaled using typical physical parameters of the soils.

The final phase of this study aimed to determine the bioavailability of Cr uptake by carrots, the fate of Cr(VI) within the plant and a risk assessment of carrot consumption using standardized EPA methodologies. Carrots were selected as one of the main root product cultivated in the Schimatari area of Asopos River basin and it is hypothesized that they could have the highest uptake and potential accumulation. In order to assess the risk of human consumption of carrots that are grown in an area with geogenic origin Cr: the Cr(VI) uptake by carrots grown under field conditions was determined, the fate of Cr(VI) within the carrot was investigated evaluating also the potential of Cr reduction by endophytic bacteria, and finally, a site-specific risk assessment of carrot consumption using the results from the first two stages was conducted to define possible diet - induced adverse effects on human health. The field scale experimental in the

Schimatari area showed that there is a trend for Cr mobilization and uptake in the surface and the leaves of the carrots cultivated in the treatment plot with the higher carbon addition, but not in the core of the carrots (Fig. 5.3).

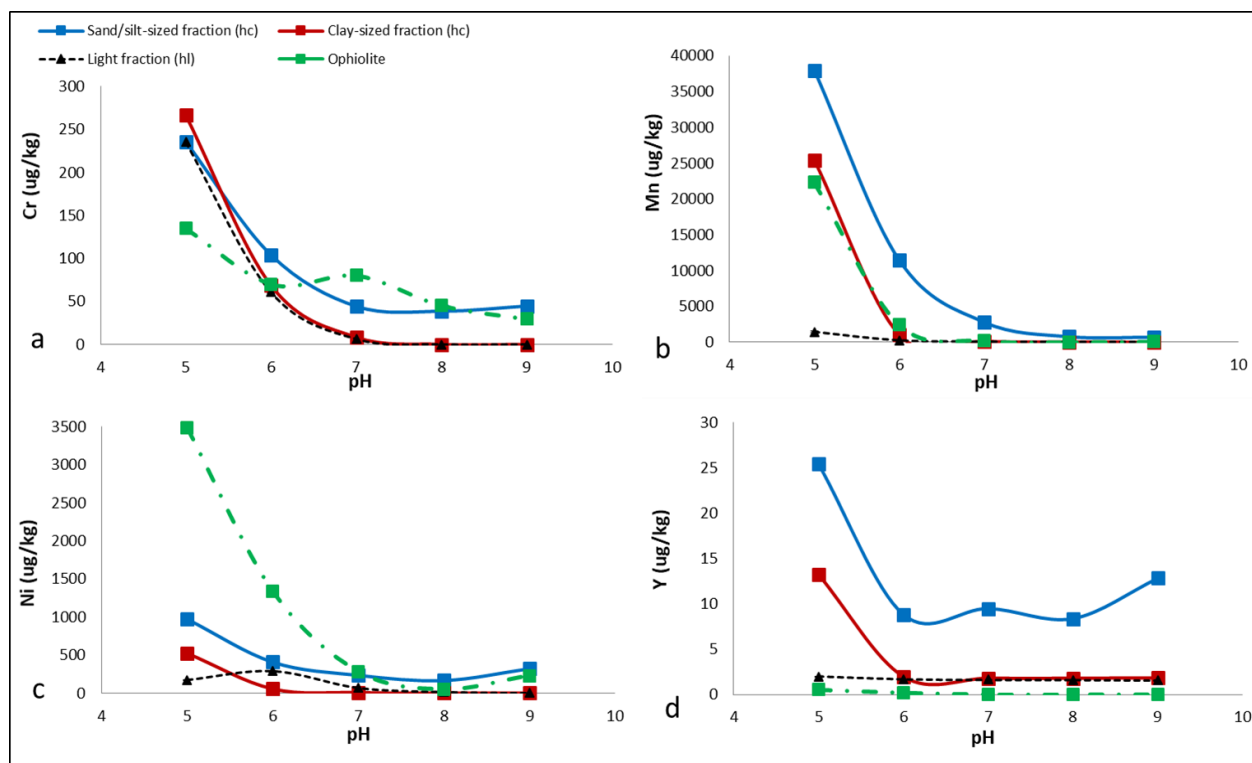


Figure 5.2. Concentration of Cr (a), Mn (b), Ni (c), Y (d) desorbed from the different fractions of soil and from an ophiolite rock sample as a function of pH.

The results of the endophytic bacteria study reveal that carrots have the ability through their endophytic bacteria community to reduce Cr(VI) to Cr(III) (Fig. 5.4). The endophytic bacteria community consists of Cr(VI) tolerant strains present in all plant parts (core, surface, leaves) able to grow on medium with 1 mg/L Cr(VI) and able to reduce different concentrations Cr(VI) to Cr(III). These results lead us to investigate the endophytic bacteria of two leaf and three root (core) isolates which were found to be able to grow on medium with 3 mg/L Cr(VI) and seven root (surface) which were found to be able to grow on medium with 5 mg/L Cr(VI). Furthermore all of these strains were found to be able to decrease initial Cr(VI) concentration (3 mg/L and / or 5 mg/L) below the detection limit in 20 h. Moreover it was noticed that the cell growth and the Cr(VI) removal started simultaneously for all the strains (Fig. 5.4). The estimation of the reaction rate and half-life for the endophytic bacteria strains isolated

from the core and surface of the carrots, suggests very fast transformation rates for the Cr(VI).

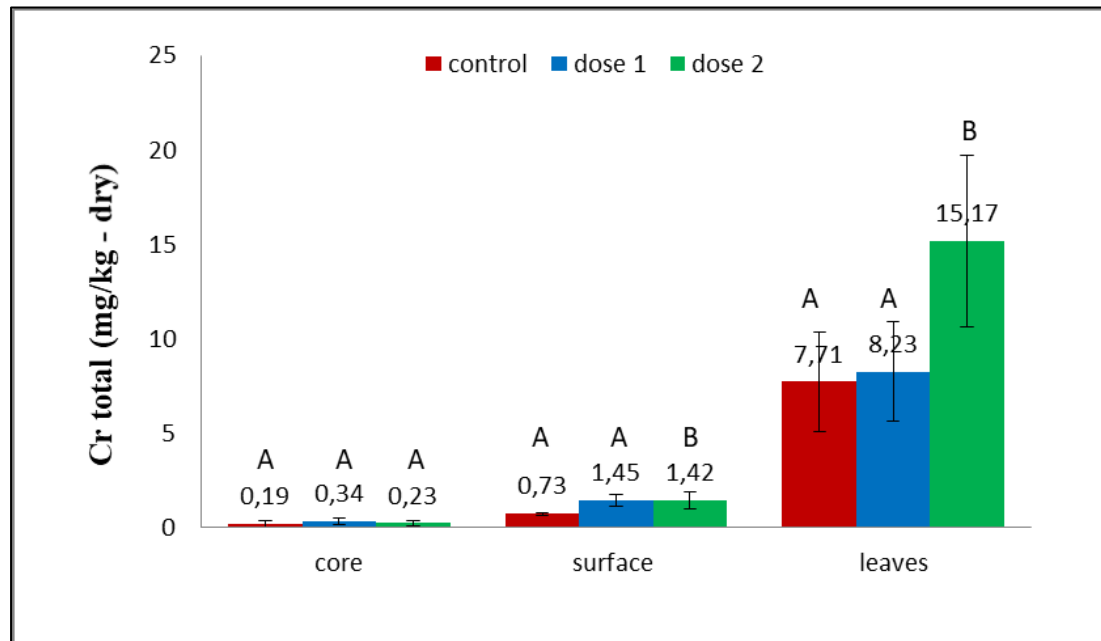


Figure 5.3. Total chromium concentration in the parts of carrots (core, surface, leaves) for the control and compost treatments (“dose 1” and “dose 2”).

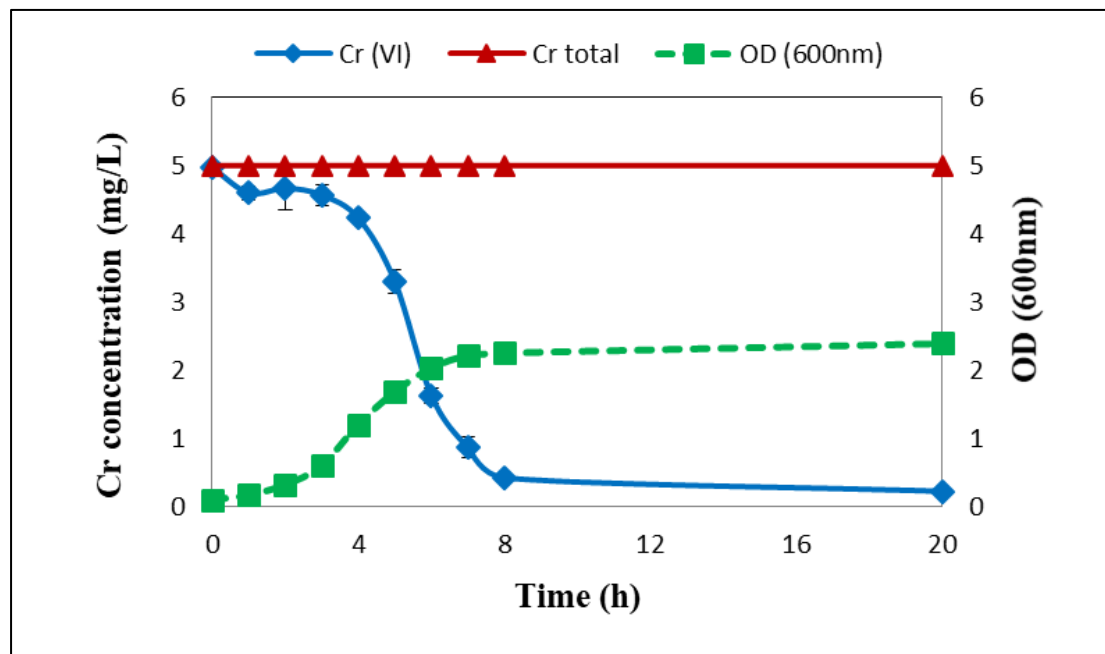


Figure 5.4. Total chromium concentration, Cr(VI) reduction and growth curves of the root (core) endophytic bacterium, strain R1 at 5 mg/L Cr(VI) initial concentration, pH 7.0 and 30°C.

As far as the estimation of the cumulative non-carcinogenic risk, the hazard index was found to be $6.73 \cdot 10^{-3}$, suggesting that the population is unlikely to experience adverse effects due to the consumption of these carrots. The carcinogenic risk was estimated to be 0.077 - 0.24 in a million. These findings are of particular importance since they confirm that carrots grown in soils with geogenic origin Cr does not pose any adverse risk for human consumption, but could also have the beneficial effect of the micronutrient Cr(III).

The overall impact of this thesis was aimed to develop an integrated approach to outline an area with wide spread geogenic origin Cr contamination, and to give answers to questions concerning Cr existence in groundwater, soils and agricultural products. The general findings showed Cr concentrations in groundwater of Asopos basin below the drinking water standards. Increased geogenic Cr(VI) concentrations are only observed in areas directly affected by ophiolite weathering. Chromite weathering is the controlling mechanism of Cr release in soils of Asopos basin. The high adsorption and immobilization capacity of soils and sediments are important suggesting significant Cr retardation in case of wastes release with high Cr concentration and specifically for wastes with high acidity. Finally, there is a case of Cr uptake by agricultural products, however, it is not important due to the direct reduction of Cr(VI) by the endophytic bacteria. The human health risk assessment showed that the population is unlikely to experience adverse effects due to the consumption of these products.

PUBLICATIONS RELATED TO THIS THESIS

PUBLICATIONS IN SCIENTIFIC JOURNALS

Maria A. Lilli, Nikolaos P. Nikolaidis, George P. Karatzas, Nicolas Kalogerakis, 2018. *Identifying the controlling mechanism of geogenic origin chromium release in soils*, Journal of Hazardous Materials (in review).

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Maria A. Lilli, Daniel Moraetis, Nikolaos P. Nikolaidis, George P. Karatzas, Nicolas Kalogerakis, 2015. *Characterization and mobility of geogenic chromium in soils and river bed sediments of Asopos basin*, Journal of Hazardous Materials, 281: 12-19.

CONFERENCE PRESENTATIONS

Maria A. Lilli, Nikolaos P. Nikolaidis, Nicolas Kalogerakis, George Karatzas, *Extent, mobility and impact of geogenic chromium in soils of Asopos basin*. 6th International Conference on Industrial and Hazardous Waste Management (CRETE 2018), 4-7 September 2018, Chania, Crete, Greece (oral presentation).

Maria A. Lilli, Nikolaos P. Nikolaidis, Nicolas Kalogerakis, George Karatzas, *Impact of geogenic Cr(VI) contamination on human health – Risk assessment of carrot consumption*. 5th International Conference on Industrial and Hazardous Waste Management (CRETE 2016), 27-30 September 2016, Chania, Crete, Greece (poster presentation).

Maria A. Lilli, Nikolaos P. Nikolaidis, George Karatzas, Nicolas Kalogerakis, *Chromium Uptake by Carrots in Asopos Basin*. 6th European Bioremediation conference (EBC-VI, 2015), 29 June - 2 July 2015, Chania, Crete, Greece (poster presentation).

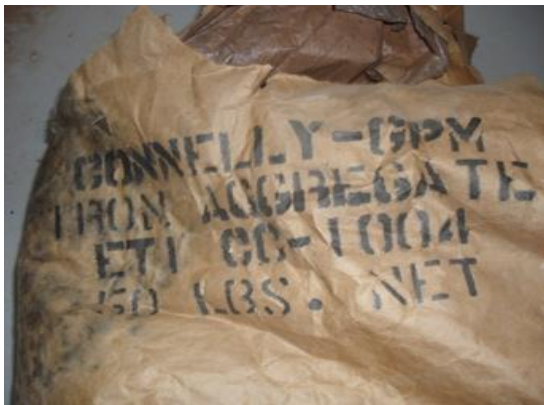
Maria A. Lilli, Daniel Moraetis, Nikolaos P. Nikolaidis, Nicolas Kalogerakis, George Karatzas, *Elucidating the mechanisms of chromium release from geogenic processes*. 4th International Conference on Industrial and Hazardous Waste Management (CRETE 2014), 2-5 September 2014, Chania, Crete, Greece (oral presentation)

Maria A. Lilli, Nikolaos P. Nikolaidis, Nicolas Kalogerakis, George Karatzas, Mike Mueller, *Mitigation Measures for geogenic chromium rich soils*. 11th International Phytotechnologies Conference, 30 September – 3 October 2014, Heraklion, Crete, Greece (oral presentation)

Maria A. Lilli, Daniel Moraetis, Nikolaos P. Nikolaidis, George Karatzas, Nicolas Kalogerakis, *Characterization and mobility of hexavalent chromium in soils and sediments*. 13th International Conference on Environmental Science and Technology (CEST 2013), 5-7 September 2013, Athens, Greece (oral presentation)

Maria A. Lilli, Nikolaos P. Nikolaidis, George Karatzas, Nicolas Kalogerakis, Mike Mueller, *Mitigation measures for hexavalent chromium contaminated groundwater and soil using zero valent iron technologies*. 13th International Conference on Environmental Science and Technology (CEST 2013), 5-7 September 2013, Athens, Greece (oral presentation)

APPENDIX – STABILIZATION OF HEXA VALENT CHROMIUM IN CONTAMINATED SEDIMENT USING ZERO VALENT IRON TECHNOLOGIES



A.1 Materials and methods

A.1.1 Sampling

An additional study was conducted to evaluate ZVI and ZVI-based technologies for Cr stabilization in a representative sediment sample from Asopos river, which has a demonstrated serious geogenic and anthropogenic problem of Cr contamination.

Lilli et al. (2015) has obtained more than 50 soil and sediment samples in order to characterize the geochemical variability with the basin. Soil and sediment samples were taken in eight cross sections along the basin. Soil samples (top 15 cm) were taken from the first and second terraces from these cross-sections that were perpendicular to the river. Surface river bed sediments were taken up to a depth of 20 cm while core river bed sediments were sample up to a maximum depth of 150 cm. Principal Component Analysis characterized the soils and sediment into calcareous, siliceous and ultramafic groups and showed no differentiation between soils and river sediments. Based on this analysis, the sediment sample (sd8) from the siliceous group was selected to conduct the stabilization experiments since it was representative of the area.

A.1.2 Physicochemical analysis

The sediment sample was homogenized, air dried and sieved. Part of the sample was kept without drying for alkaline digestion and Cr(VI) determination. The fraction smaller than 2mm was used for the experiments. The sample was analyzed for physical and chemical properties including pH, conductivity, soil moisture, total organic carbon, total organic nitrogen, total major and trace metals. Total organic carbon and total organic nitrogen were analyzed using the multi N/C 2100S Analyzer. Base cations and heavy metals were analyzed according to EPA 3051A method. Specifically, 9 ml HNO₃ were added to 0.2 g soil, followed by microwave digestion at 150-180°C (Multiwave 3000 Digestor). Supernatant solutions were diluted with MilliQ water and analyzed by ICP-MS (Agilent- CX). EPA method 3060A (alkaline digestion) was used for the determination of Cr(VI). Bulk chemical analysis and several trace element analysis

were performed on the soil fraction <2mm by X-ray fluorescence using the spectrometer S2 Ranger (Bruker Co).

A.1.3 Experimental design

The stabilization studies included batch experiments that were conducted to study the effectiveness of ZVI and ZVI based technologies in reducing the amount of Cr released from sediment.

The stabilization of Cr in the sediment sample was conducted using three different ZVI-based products (Connelly's ZVI, EHC®, DARAMEND®). The Connelly's ZVI ("iron filings") used was obtained from Connelly GPM, Inc., Chicago, IL (stock number CC-1004). Chemical analysis of the product indicated the following composition: iron 89.8%; carbon 2.9%; manganese 0.6%; sulfur 0.1%; phosphorous 0.1%; silicon 1.9%; copper 0.2%. The remaining content was oxygen (Fe and Si oxides) and other impurities. The EHC® and DARAMEND® products were obtained by FMC Environmental Solutions company. The composition of these products was provided by the manufacturer and is the following: for EHC®, iron 18-48% and for DARAMEND®, iron 40-50% and wheat 50-60%.

Eight different ZVI-to-soil ratios were used in batch experiments to determine the efficiency of Cr and other trace elements stabilization. The experiment was carried out in triplicates by adding different mass ratios of ZVI to soil in plastic bottles filled with 20 mL NaNO₃ 0.01 N. The sediment/solution ratio was set to 1/10 (2 g of sediment) and all solutions prepared with MilliQ and stirred continuously in vibrating plate. The supernatant solutions filtered immediately with a nylaflo membrane 0.45 mm filter prior analysis. The speciation of Cr was obtained using an amberlite IR – 120 resin and measuring the Cr content before and after the resin using the ICP-MS. In addition to chromium, other metals like As, Ni, Mg and Fe were measured. The duration of the experiment was 7 days. This reaction time was based on previous kinetic experiments and selected in order to allow the system to reach equilibrium.

A.1.4 Statistical analysis

A statistical analysis was carried out to estimate the effect of the different products and ZVI to soil ratios on the leachability of metals. The leachability results of five metals (Cr, Ni, Mg, As and Fe) and eight different ZVI to soil ratios for the three different ZVI based products, were used in a two-way analysis of variance (ANOVA) combined with the post- hoc Tukey test. The analysis was conducted to assess the statistical differences in metals leachability among the different ZVI to soil ratios and products and it was carried out using the OriginPro 9.1 software.

A.2 Results and discussion

A.2.1 Physicochemical characterization

The physical and chemical characteristics of the sediment sample (sd8) are presented in Table A.1. The other soil and sediment samples taken from Asopos river basin had similar values for pH, electrical conductivity (EC), TOC and TN to this one (sd8) (Lilli et al., 2015). The sediment sample belongs to the siliceous group of soils and sediments (Lilli et al., 2015) with SiO₂ concentration of 44% (Table A.1). The total chromium and nickel contained in the sample was 914 and 224 mg/kg, respectively (Table A.1). Similar Cr and Ni concentrations present the soil and river bed samples of Asopos watershed. The total Cr concentration for the sediments ranged between 631 and 1639 mg/kg (Lilli et al., 2015). High Cr and Ni content suggest geogenic origin of Cr in soils and sediments. The Cr/Fe ratio was calculated to be 163, value that is representative for serpentinite soils (Shtiza et al., 2005). Low hexavalent chromium concentration (0.05 mg/kg) was normal because the soils and sediments derived from serpentine erosion have a great content of trivalent chromium. The selected sediment sample had also a significant content of iron oxides (5.6%) (Table A.1).

Table A.1. Chemical characteristics of river sediment sample

Parameter	Value
pH	7.88
EC ($\mu\text{S}/\text{cm}$)	477
Soil moisture (%)	21.5
TOC (g/kg)	9.9
TN (g/kg)	0.95
Na₂O (%)	1.3
MgO (%)	4.5
K₂O (%)	1.1
CaO (%)	16.6
TiO₂ (%)	0.4
MnO (%)	0.1
Fe₂O₃ (%)	5.6
Al₂O₃ (%)	6.3
SiO₂ (%)	44
P₂O₅ (%)	0.14
LOI (%)	18.5
Cr (ppm)	914
Ni (ppm)	224
Cr(VI) (ppm)	0.05
Cr/Fe	163
CRI	0.22

A.2.2 Trace elements stabilization

The results of the metal stabilization experiment using Connelly's ZVI, EHC® and DARAMEND® are presented in Fig 1a-e. Concentration in solution was measured for 28 elements (Li, B, Mg, Al, Si, K, Ca, Ti, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Se, Rb, Sr, Y, Cd, Sb, Cs, Ba, Hg, Pb, and U), however, the results of five elements that had significant leachability are presented in Fig. A.1a-e. Cr, Ni and As were chosen for further analysis because of their toxic nature, Cr, Ni and Mg are typical of serpentine soils, and Fe is related to iron oxide coating, which contributes to the adsorption capacity of soils and sediments (Lilli et al., 2015; Nikolaidis and Tyrovola, 2006). The experiment was conducted for 8 ZVI to soil ratios (0%, 0.1%, 0.5%, 1%, 1.5%, 2%, 2.5% and 5% of the ZVI products), however only the first 5 ratios are presented in Fig. A.1. The degree of stabilization did not increase for ratios higher than 1.5%.

Cr leachability was reduced exponentially with the increase in ZVI content for all three products (Fig. A.1a). Stabilization studies confirmed that Connelly's ZVI, EHC® and

DARAMEND® can reduce the leachability of Cr(VI) by 78%, 82% and 94% respectively, using only 1% of the product to soil ratio.

Connelly's ZVI also reduced the concentration of the other toxic metals (As and Ni) in solution with the increase in ZVI content (Fig. A.1b-c). Specifically, Connelly's ZVI reduced the leachability of Ni by 57% using 1.5% of the product, while reduced the As concentration in solution to zero using only 0.1% of the product. However Fe and magnesium concentration in solution was high (10 - 19 ppb and 10 - 13 ppm for Fe and Mg respectively) regardless of the ZVI content (Fig. A.1d-e). Connelly's ZVI reduced the concentrations of Cr, As, and Ni while contributed to and increased the concentration of Fe and Mg.

EHC® and DARAMEND® reduced the concentration of As in solution (Fig. A.1b) and increased the concentration of Ni and Mg (Fig. A.1c and e). EHC® and DARAMEND® can efficiently remove As from solution and stabilize it on soils and sediments. Iron concentration in solution was high regardless of the product content (Fig. A.1d). EHC® increased the leachability of Ni and Mg by 66% and 55% respectively, using 1.5% of the product, while reduced the As concentration in solution to zero using only 1% of the product. DARAMEND® increased the leachability of Ni and Mg by 73% and 48% respectively, using 1.5% of the product, while reduced the As concentration in solution to zero using only 1% of the product. Specifically, Cr and As were stabilized while Ni, Mg and Fe were produced by EHC® and DARAMEND®.

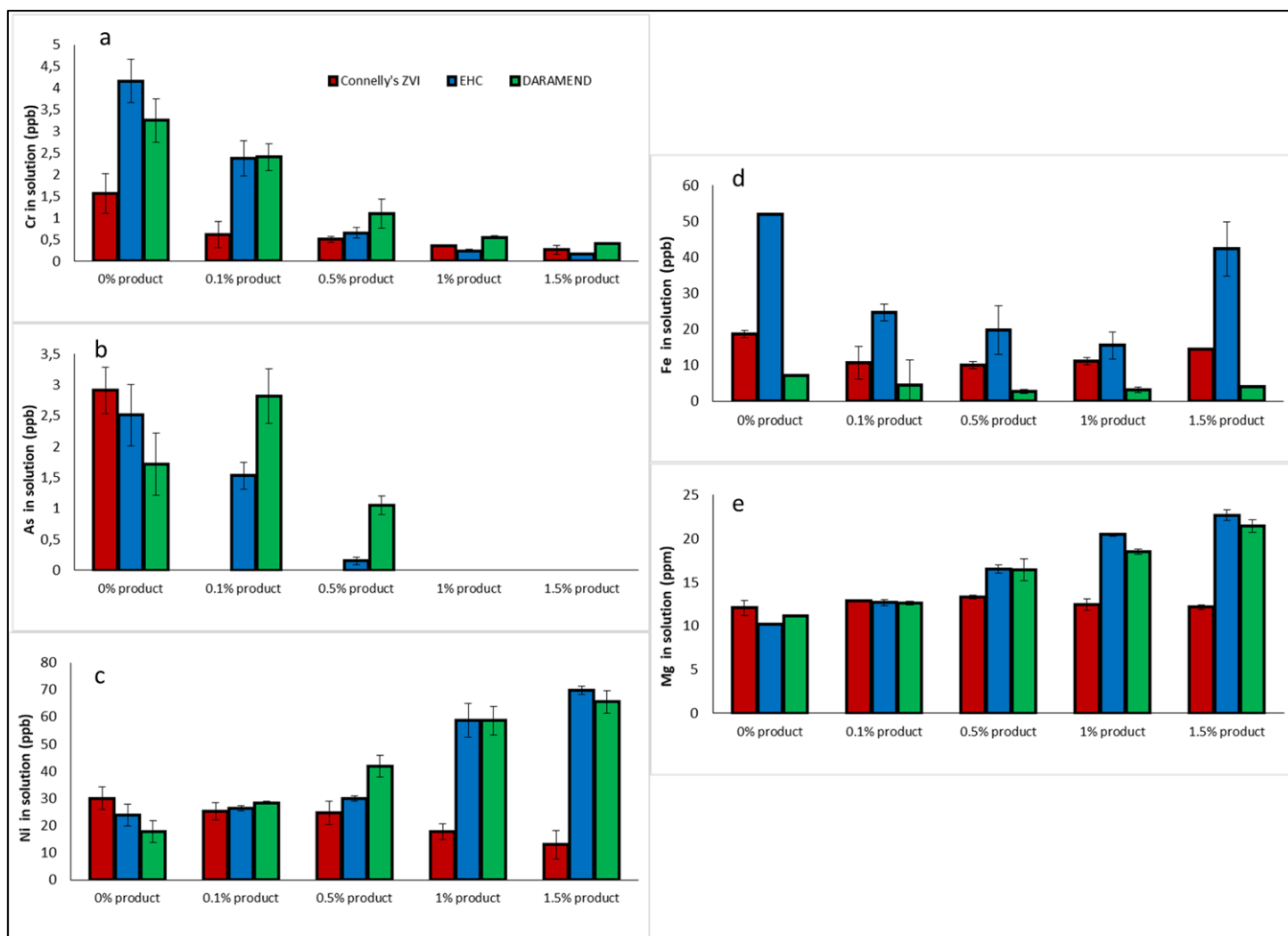


Figure A.1. (a) Cr, (b) As, (c) Ni, (d)Fe and (e) Mg concentrations in solution at different Connelly's ZVI, EHC® and DARAMEND® to soil ratios. Error bars represent the standard deviation of three replicates.

A.2.3 Correlations

Regarding Cr, at the 0.05 level, the population means of ZVI based products (Connelly's ZVI, EHC®, DARAMEND®) and ZVI to soil ratios were significantly different. Specifically, Connelly's ZVI was significantly different from EHC® and DARAMEND®, whereas the FMC's products did not differ significantly. Increasing the ZVI to soil ratio, increased the removal of chromium. Values of leachability of Cr resulting from ZVI ratios between 0.5% and 5% did not differ statistically.

Regarding As, at the 0.05 level, the population means of ZVI based products were not significantly different but for the ZVI to soil ratios were. Increasing the ZVI to soil ratio, increased the removal of As. Values of leachability of arsenic resulted from 0%

and 0.1% ZVI ratios differed significantly in comparison with the values of leachability resulted from the ratios between 0.5% and 5%.

Regarding Ni, at the 0.05 level, the population means of ZVI based products and ZVI to soil ratios were significantly different. Specifically, Connelly's ZVI was significantly different from EHC® and DARAMEND®, whereas FMC's products did not differ significantly. Increasing the Connelly's ZVI to soil ratio, increased the removal of Ni, while increasing the EHC® and DARAMEND® to soil ratio, the removal of Ni decreased. Values of leachability of Ni resulted from the 0% - 2.5% ZVI ratios differed significantly only from the values of leachability resulting from the 5% ZVI to soil ratio.

Regarding Fe, at the 0.05 level, the population means of ZVI to soil ratios were significantly different. Specifically, values of leachability of Fe resulted from the 0.1%, 0.5%, 1% and 1.5% ZVI ratios differed significantly from the values of leachability resulted from the 2.5% ZVI to soil ratio.

Regarding Mg, at the 0.05 level, the population means of ZVI based products and ZVI to soil ratios were significantly different. According to post- hoc Tukey test, Connelly's ZVI was significantly different from EHC® and DARAMEND®, whereas FMC's products did not differ significantly.

The results of this statistical analysis suggested that all three ZVI products can stabilize efficiently the concentrations of Cr, As and Ni in the sediment sample studied, reducing in this way its mobility and bioavailability. The amount of ZVI used to obtain high stabilization results is 1% ZVI to sediment ratio.

A.3 Conclusions

This work is a first step towards reducing Cr mobility from top soils and sediments and therefore its bioavailability and toxicity. ZVI products have shown the potential to reduce Cr mobility in top soils and sediments. Stabilization experiments showed that Connelly's ZVI, EHC® and DARAMEND® can efficiently remove Cr(VI) from solution and stabilize. The results confirmed that Connelly's ZVI, DARAMEND® and EHC® can reduce the leachability of Cr(VI) by 78%, 82% and 94% respectively, using 1% of the product. The kind of the ZVI product and the ratio of ZVI/soil affected the

efficiency of Cr stabilization in sediments. Regarding the reduction of other metals, As and Ni were stabilized while Fe and Mg were produced by Connelly's ZVI. In addition, As was stabilized while Ni, Mg and Fe were produced by EHC® and DARAMEND®. This findings are of particular importance for agricultural soils where a reduction of the bioavailability of Cr(VI) to crops and plants in certain cases could be necessary. More studies are necessary to elucidate the longevity of the products and its biogeochemical behavior under field conditions.

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