Jordan Journal of Biological Sciences (JJBS)

The **Jordan Journal of Biological Sciences (JJBS)** welcomes submissions of articles. The **JJBS** is to publish refereed, well-written original research articles, and studies that describe the latest research and developments in Biological Sciences. The JJBS is to be published quarterly and issued by Higher Scientific Research Committee at the Ministry of Higher Education and Scientific Research and the Deanship of Research and Higher studies at the Hashemite University.

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Contributions in all areas at the interface of biology and other disciplines such as chemistry, physics, and mathematics are welcomed. Interested authors are encouraged to read "Instructions for Authors" while preparing their manuscripts. Send your contributions to:

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INSTRUCTIONS FOR AUTHORS

All submitted manuscripts should contain original research not previously published and not under consideration for publication elsewhere. Papers may come from any country but must be written in English or Arabic with two abstracts, one in each language.

Research Paper: We encourage research paper of a length not exceeding 25 double-spaced pages. It should have a set of keywords (up to 6) and an abstract (under 250 words, unreferenced), followed by Introduction, Materials and Methods, Results, Discussion, Acknowledgments, and References.

Short Research Communication: It presents a concise study, or timely and novel research finding that might be less substantial than a research paper. The manuscript length is limited to 2500 words (excluding references and abstract). It should have a set of keywords and an abstract (under 200 words, unreferenced), containing background of the work, the results and their implications. Results and Discussion Section should be combined followed by Conclusion. Materials and Methods will remain as a separate section. The number of references is limited to 60 and there should be no more than 4 figures and/or tables combined.

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Title page:

The title page of manuscript should contain title, author's names, addresses of university or institution of affiliation, a short title, and the name and address for correspondence including telephone number, fax number, and e-mail address, if available. Authors with different affiliations should be identified by the use of the same superscript on name and affiliation. In addition, a sub- field of submitted papers may be indicated on the top right corner of the title page.

Abstract:

The abstract should provide a clear and succinct statement of the findings and thrusts of the manuscript. The abstract should be intelligible in itself, written in complete sentences. Since JJBS is an interdisciplinary journal, it is important that the abstract be written in a manner which will make it intelligible to biologists in all fields. Authors should avoid non-standard abbreviations, unfamiliar terms and symbols. References cannot be cited in the Abstract.

Authors should submit with their paper two abstracts (English and Arabic), one in the language of the paper and it should be typed at the beginning of the paper before the introduction. As for the other abstract, it should be typed at the end of the paper on a separate sheet. Each abstract should not contain more than 250 words.

Introduction:
This section should describe the objectives of the study and provide sufficient background information to make it clear why the study was undertaken. Lengthy reviews of the past literature are discouraged.

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Periodicals:
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EDITORIAL PREFACE

It is my great pleasure to publish the first issue of the Jordan Journal of Biological Sciences (JJBS). JJBS is a refereed, peer reviewed quarterly international journal issued by the Jordanian Ministry of Higher Education and Scientific Research in cooperation with the Hashemite University. The journal covers a wide range of research and development concerning biological sciences. Through the publication, we hope to establish and provide an international platform for information exchange in different fields of biological sciences.

Jordan Journal of Biological Sciences aims to provide a highly readable and valuable addition to the literature, which will serve as an indispensable reference tool for years to come. The coverage of the journal includes all new findings in all aspects of biological sciences and or any closely related fields. The journal also encourages the submission of critical review articles covering advances in recent research of such fields as well as technical notes.

The Editorial Board is very committed to build the Journal as one of the leading international journals in biological sciences in the next few years. With the support of the Ministry of Higher Education and Scientific Research and Jordanian Universities, it is expected that a valuable resource to be channeled into the Journal to establish its international reputation.

I have received a good response to the first issue of JJBS from biologists in Jordanian universities. I am pleased by this response and proud to report that JJBS is achieving its mission of promoting research and applications in biological sciences. In the first issue, there are six interesting papers dealing with various aspects of biological sciences.

JJBS will bring you top quality research papers from an international body of contributors and a team of distinguished editors from the world's leading institutions engaged in all aspects of biological sciences. Now, the JJBS invites contributions from the entire international research community. The new journal will continue to deliver up to date research to a wide range of biological sciences professionals. The JJBS will assure that rapid turnaround and publication of manuscripts will occur within three to six months after submission.

I would like to thank all members of the editorial board and the international advisory board members for their continued support to JJBS with their highly valuable advice. Additionally, I would like to thank the manuscript reviewers for providing valuable comments and suggestions to the authors that helped greatly in improving the quality of the papers. My sincere appreciation goes to all authors and readers of JJBS for their excellent support and timely contribution to this journal.

I would be delighted if the JJBS could deliver valuable and interesting information to the worldwide community of biological sciences. Your cooperation and contribution would be highly appreciated. More information about the JJBS guidelines for preparing and submitting papers may be obtained from www. jjbs.hu.edu.jo

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Middle Cerebral Artery Occlusion Increases the Sensitivity of Cortical Neurons to Acetylcholine and Impairs Cognitive Function in Rats

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Abstract

The goal of the present study was to relate the degree of cortical cholinergic deafferentation induced by middle cerebral artery (MCA) occlusion to changes in the sensitivity of frontal cortical neurons to iontophoretic administration of acetylcholine (ACh) and to changes in cognitive performance in the Morris water maze in rats. In Wistar rats, MCA occlusion reduced the density of acetylcholinesterase (AChE)-positive fibers in the frontal cortex by 57% (n = 8) and the activity of cholineacetyl-transferase (ChAT) by 59% (n = 5). The MCA occluded rats took significantly longer to locate the submerged platform in the water maze than sham-operated rats, although swim speeds were similar, at a time when neurological deficits were minimal. Basal neuronal firing rates were reduced after MCA occlusion. The percentage of neurons responding to ACh was increased but responses to carbachol and glutamate were unaffected. The increased sensitivity of cortical neurons to ACh correlated positively with the loss of AChE activity and with the impaired performance of the MCA-occluded rats in the water maze. The increased size of the responses to the cholinomimetics is probably due to increased sensitivity of post-synaptic cholinoceptors. These data confirm that MCA occlusion in the rat causes a loss of cortical AChE-positive fibers and behavioral effects which are suggestive of memory disruption. The increased proportion of neurons responding to ACh is likely to result from the loss of AChE activity. Loss of cholinergic neurons may contribute to the cognitive impairment seen in patients with cerebrovascular accidents or stroke.

الملخص

هدفت هذه الدراسية لمعرفة درجة علاقة الموصلات العصبية المخية عن طريق عمل غلق للشريان المخي الأوسط إلى التغيرات التي تحدث في حساسية الأعصاب المخية الأمامية لإفراز الموصلات الكيميائية العصبية والوظائف المعرفية لحيوان مائي (مورز). فئران (وستر) غلق الشريان المخي الأوسط خفض من كثافة الإنزيم الاستيليكولين (الالياف الموجبة) في الفص الأمامي للمخ بحوالي 57% ونسبة 59% الإنزيم الناقل للإستيليكولين في 5 فئران وكان مهارة العوم لم تتغير في المجموعتين. وكان معدل نشاط الألياف القاعدية للمخ انخفض بعد غلق الشريان. وكانت نسبة الألياف التي استجابت للاستيليكولين قد زادت ولكن الاستجابة بالنسبة للكارباكول والجلوتاميد لم تتأثر. وزيادة حساسية الألياف المخية للاستيليكولين أثبت زيادة ضرورية ايجابية لنقص انزيم الاستيليكولين قد زادت ولكن الاستجابة بالنسبة للكارباكول والجلوتاميد لم تتأثر. وزيادة حساسية الألياف المخية للاستيليكولين أثبتت زيادة ضرورية ايجابية لنقص انزيم الاستيليكولين. ونقص الافراز الوظيفي للفئران في الماء ويعزى سبب الزيادة في الاستجابة على الحساسية في العقد المستقبلة للكولين. وعلى هذا أثبتت هذه الدراسية أن غلق الشريان المخي الأوسط للفئران قد تسبب في نقصان انزيم الاستيليكولين وتغير سلوك الفئران يعزى الى اضطرابات الذاكرة. وأن زيادة نسبة الألياف العصبية التي تستجيب الى الاستايل كولين يمكن ان تنتج عن فقدان نشاط انزيم الأستايل كولين. الخلل المعرفي الذي يرى في مرض بعد السكتة الدماغية قد يؤدي على فقدان ألياف كولنيرجية.

Keywords: Ischemia, McAocclusion, Ach, Stroke, Cognitive impairment.

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1. Introduction

Stroke is the third most common cause of death and the most common cause of functional disability in adults (American Heart Association, 2003). In addition to the many physical signs and symptoms, acute stroke and other forms of cerebrovascular diseases are well-recognized causes of cognitive impairment (Nas et al., 2004; Srikanth et al., 2004; Talell et al., 2004; Werring et al., 2004; Zhou et al. 2004). Personality changes had also been reported after stroke (Stone et al., 2006).

Two to 24 h after inducing permanent focal cerebral ischaemia (MCA occlusion), edema and pan-necrosis evolves, affecting neurons and glia, in the frontoparietal cortex and lateral striatum of rats. Neurological and behavioral effects are apparent including forelimb flexion, unilateral circling (Materossi et al., 1982; Bederson et al., 1986; Persson et al., 1989), decreased locomotor performance, and decreased performance in the step-through passive avoidance procedure (Yamamoto et al., 1988; Togashi et al., 1996). After 1 week, there is a loss of AChE-positive fibers from the frontoparietal cortex, probably because of subcortical damage to the cholinergic projection from the nucleus basalis magnocellularis (Kataoka et al., 1991; Togashi et al., 1996).

The causes of post-stroke cognitive impairment are not fully understood (Zhou et al. 2004). The present study attempts to correlate the loss of AChE-positive fibers produced in Wistar rats by combined permanent left MCA and left common carotid artery (CCA) occlusion with two functional measures, sensitivity of cortical neurons to iontophoreticalIy administered ACh and performance of rats in 2 tasks of cognitive ability, the Morris water maze and the step-through passive avoidance procedure.

2. Materials and methods

2.1. Animals and housing

Adult male Wistar rats weighing 280-330 g were housed in groups of 4-6 in PVC cages (350 x 530 mm long x 180 mm high) in an environment maintained at 19-22 °C and a relative humidity of 55% respectively with a 14 h/l0 h light/dark cycle (light on from 06.00 to 20.00 h). Food and water were available *ad libitum.*

2.2. Permanent MCA occlusion surgery

The operative procedures were undertaken using halothane anesthesia (4% in a mixture of 70% N_2O and 30% O_2 for induction reducing to 2% for maintenance) in 13 rats. Each rat was allowed to breathe spontaneously. Cranial and rectal temperatures were maintained between 36.5 and 38 ^oC.

Unilateral left CCA occlusion was followed by subtemporal, subperiosteal craniectomy (with intact zygoma) and exposure of the main trunk of the left MCA under 25-fold magnification of an operating stereomicroscope (Tamura et al., 1981; Shigeno et al., 1985). The MCA was electrocauterised from a point proximal to the lenticulostriate artery to the level of the MCA with the inferior cerebral vein (n = 13). In sham-operated rats, both vessels were exposed but not occluded (n = 5). The ipsilateral CCA occlusion was carried out to reduce variability of the infarct volume.

2.3. Neurological effects of MCA occlusion

The rats were assessed for neurological deficits using the following rating scale: 0, no observable deficit; 1, forelimb flexion; 2, forelimb flexion and decreased resistance to lateral push; 3, forelimb flexion, decreased resistance to lateral push and unilateral circling in three successive trials (Bederson et al., 1986).

2.4. Behavioral effects of MCA occlusion

2.4.1. Locomotor performance

Three weeks after MCA occlusion locomotor performance of the rats was assessed on three consecutive trials at 15 min intervals using a rotarod (12 rpm, 9 cm diameter, 2 mm grooves, 60 s cut-off).

2.4.2. Step-through passive avoidance

The day after evaluating the locomotor performance of the rats on the rotarod, they were tested in a step-through passive avoidance procedure. On the first day, each rat was placed in the illuminated chamber and was allowed free access to the darkened chamber. The latency to enter the darkened chamber was recorded and on entering, the rat was exposed to foot-shock (0.8 mA, 1 s). On the second day, the rat was again placed in the illuminated chamber and the latency for it to enter the darkened chamber was recorded (cut-off 300 s).

2.4.3. Morris water maze

Over the next two weeks the rats were evaluated for their ability to locate a hidden platform in the Morris water maze. Each rat was placed in the maze for two trials a day over 12 days and the latency for each rat to swim to the submerged escape platform was recorded (cut-off 60 s).

On the 13th-15th days, a visible cue was placed on the platform. On the 16th and 17th days, this cue was removed and the latency for each rat to swim to the platform was recorded.

2.5. Electrophysiological effects of MCA occlusion

After completing the behavioral experiments (6 weeks after MCA occlusion), the rats were anaesthetized with urethane (1.5 g/kg i.p.), placed in a stereotaxic frame and the cranium overlying forelimb representation areas (Hall and Lindholm, 1974) in the frontal cortex (motor area 4, Lysakowski et aI., 1989) was removed. The cortex was exposed by a narrow slit in the dura and covered with 4% agar in 0.9% w/v NaCl solution. Rectal temperature was maintained at 37 ± 1°C. Spontaneously active neurons were recorded from the central barrel (containing 2 M NaCl) of a 6-barrelled glass microelectrode (tip diameter 6-8 µm) during vertical penetrations of the frontal cortex (4 µm steps). Records were kept of recording depth

relative to cortical surface. Unit activity was amplified, filtered (500 Hz-l0 KHz) and displayed on a digitizing oscilloscope to facilitate discrimination and quantification (see below).

Drugs were applied iontophoretically through the outer barrels of the micro-electrode. These contained: ACh chloride (0.2 M, pH 4.0, Sigma), carbamylcholine chloride (carbachol, 0.2 M, pH 4.0, BDH), Na L-glutamate (0.2 M, pH 8, Sigma) and atropine sulphate (50 mM, pH 5.0, Sigma). A 5-9 nA backing current was applied to each drug-containing barrel and compensated through a barrel filled with 2 M NaCl.

All drugs were applied with a current of 30 nA for 20 s and current compensation was routinely used. Each drug was applied 3 times (separated by recovery periods of 1 min) to each neuron and the average of the three applications was stored for analysis. The interval between ejections of two different drugs was at least 2 min. The firing rate before drug application was compared with the firing rate during, and for the 20 s period immediately following, drug application. If ACh or carbachol produced significant effects (see Abdulla et al., 1994 for details), atropine was applied for 25 s commencing 5 s before the agonist to confirm the muscarinic nature of the response. Responses which were not blocked by atropine were not included in the analyses.

2.6. Histological verification and AChE staining

At the end of each experiment, the rats were deeply anaesthetized and perfused by transcardiac infusion of 300 ml of 4% paraformaldehyde and 15% saturated picric acid in phosphate buffer (0.1 M, pH 7.4) by means of a peristaltic pump. The brains were removed and kept in 4% paraformaldehyde for 3-4 h, and then repeatedly washed with phosphate buffer at 4 °C to remove colouration due to picric acid. AChE staining was visualized on 30 µm sections with acetylthiocholine iodide as substrate and iso-OMPA (Sigma) as an inhibitor of nonspecific esterase and with 3,3' -diaminobenzidine intensification (Geula and Mesulam, 1989). The total length of AChE-staining axons per unit area was determined using an IBAS 2000 image analyzer. Briefly, the AChE-containing axons and cell bodies were discriminated from background, converted to a binary image and the cell bodies and any artifacts were eliminated interactively. The axon images were thinned to 1 pixel and their total length expressed as a proportion of the field area (Abdulla et al., 1994).

2.7. Choline acetyltransferase activity

Frontal cortical ChAT activity was measured in a separate group of 5 sham-operated and 5 MC A-occluded rats. The rats were killed by cervical dislocation, their brains rapidly removed and tissue (about 30 mg) was dissected from the frontal cortex of each hemisphere and stored at -70 ^{o}C until assay. ChAT activity was calculated from the formation rate of [^{14}C]ACh from [^{14}C]acetylcoenzyme A by a method derived from Fonnum (1975). Briefly, samples were homogenised in NP-40 (1 % phosphate buffer, Sigma) using 8.3 µl/mg tissue wet weight. Ten µl aliquots of tissue homogenate were incubated, in duplicate, at 37 ^{o}C for 5 min with 10 µl incubation medium at pH 7.4 containing NaCl (0.75 M), NaH_2PO_4 (135 mM), choline (20 mM), EDTA (50 mM), acetylcoenzyme A (1 mM), physostigmine sulphate (0.4 mM) and 2 µCi, in 10 µl of [14C]-acetylcoenzyme A (New England Nuclear). Incubation was stopped with 5 ml of cold NaH_2PO_4 (10 mM, pH 7.4). [^{14}C]ACh was extracted with acetonitrile containing tetraphenylboron (20 mgl ml) and counted in a PPO-POPOP toluene scintillant.

2.8. Statistical analysis

Unit responses to drugs were recorded as the average firing rate of 3 drug applications, and consisted of three 20 s blocks (each of eighty 250 ms epochs) obtained immediately before (control), during and for 20 s after drug application as described above. Epochs recorded during drug application were compared to control activity using Wilcoxon tests for each neuron. Zones which differed significantly from control activity were automatically detected and used to calculate response latency and duration.

Independent t-tests were used to compare the averaged firing rates, latencies to onset and durations of drug action and the lengths of AChE-containing axons of the control and ischemic rats. A $\chi 2$ test was used to compare the sensitivity of different drugs between the control group and the ischemic groups. The regressions of the ACh-induced changes in firing rate vs. baseline rate for the sham-operated and MCA-occluded rats were compared using MANOVA.

3. Results

3.1. Neurological effects of MCA occlusion

One day after permanent proximal MCA occlusion, 4/11 animals exhibited unilateral circling (neurological score of 3). The following day 9/11 animals had neurological scores of 2. After 7 days the majority of the animals (8/11) had neurological scores less than 2 and exhibited mainly forelimb flexion (6/11) or no apparent neurological deficit (2/11). After 19 days only two animals had a neurological score of 2.

3.2. Behavioral effects of MCA occlusion

The performance of MCA-occluded rats on the rotarod 3 weeks after occlusion was not significantly different from that of sham-operated rats over three consecutive trials (sham-operated 50 ± 6 s, 38 ± 6 s, 37 ± 6 s, MCA-occluded 42 ± 6 s, 49 ± 6 s, 37 ± 6 s). However, MCA occluded rats performed less well than sham operated rats in the step-through passive avoidance procedure with a mean latency to enter the darkened chamber on the test day of 210.7 ± 34.7 s compared with 300s (cut-off time) for the sham-operated rats (Figure 1). There were no significant differences between the 2 groups on the training session (26.7 ± 4.0 s for the control group and 28.1 ± 4.8 s for the MCA-occluded rats).

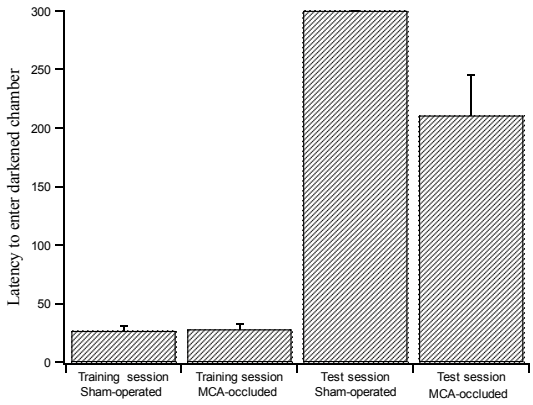

Figure 1. Histogram of mean latencies ± s.e.m. of sham-operated and MCA-occluded rats to enter the darkened chamber in the step-through avoidance procedure.

On the final two training days in the water maze (days 11 and 12), MCA-occluded rats took longer to find the submerged platform than sham-operated rats, 40.0 ± 7.0 s compared with 8.3 ± 1.0 s ($P<0.001$). When the cue was placed on the submerged platform, the MCA-occluded rats located the submerged platform more rapidly and by the third day (day 15), performed as well as sham-operated rats (Figure 2). As can be seen from the figure, the MCA-occluded rats continued to locate the hidden submerged platform with similar latencies to sham-operated rats when the cue was removed (days 16 and 17).

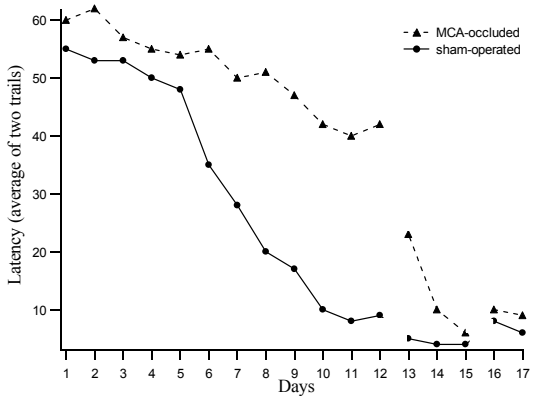

Figure 2. Mean latencies (2 trials/day) of rats to locate the hidden escape platform in the Morris water maze. MCA-occluded rats took longer to locate the platform than sham-operated rats. On days 13-15, when platform position was indicated by a visible cue, latenies of MCA-occluded rats approximated that of sham-operated rats and remained similar when the cue was removed (days 16 and 17).

3.3. Electrophysiological effects of MCA occlusion

Basal neuronal firing rates were reduced in MCA-occluded rats from 3.1 ± 0.2 to 2.6 ± 0.2 imp S^{-1} ($P<0.05$). In sham-operated rats, 52.3% of neurons showed a muscarinic response to ACh compared with 80.3% in MCA-occluded rats (Figure 3). In contrast, the proportions of neurons responding to carbachol and glutamate were unaffected by ischemia (Figure 4). Regression analysis showed that the direction and magnitude of the response to ACh were closely correlated with the firing rate immediately before administration. The regression line obtained in MCA-occluded rats was significantly steeper than that obtained in sham-operated control rats ($P< 0.0001$, MANOVA). MCA occlusion also significantly increased the slope of the regression line for responses to carbachol.

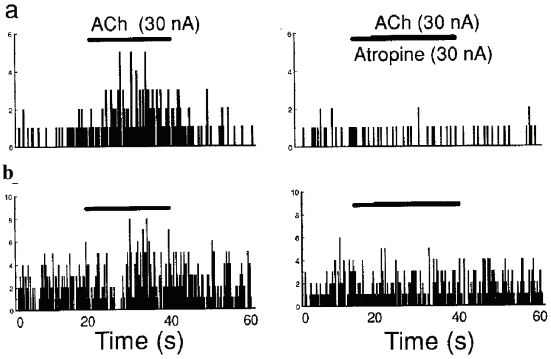

Figure 3. Spike frequency/time histograms showing the firing rates of two different frontal cortical neurons from (a) MCA occluded rat and (b) sham operated rat responding to iontophoretic application of ACh (30 nA for 20 s. The effects of ACh in both neurons were completely antagonized by iontophoretic application of atropine (30 nA for 25 s) commencing 5 s before ACh and continuing throughout the period of ACh application. Note that the base line firing rat is less in the neuron from the MCA occluded rate while the response to ACh was from such rat than the sham operated rat.

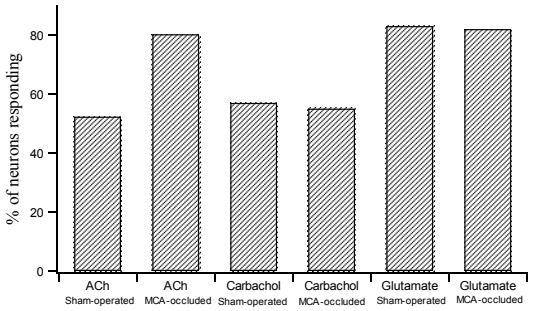

Figure 4. Histograms showing the influence of MCA occlusion on the percentages of cortical neurons responding to iontophoretic administration of Ach, carbachol and glutamate.

3.4. Histological/biochemical effects of MCA occlusion

The infarct boundaries were clearly delineated after MCA occlusion and there was a greater neuronal cell loss in the striatum than in the frontal cortex. There was a generalized loss of AChE staining from ipsilateral cortical and subcortical brain regions (Figure 5). The density of AChE-positive fibers in the frontal cortex was reduced by 57%, from 145.7 ± 6.6 mm mm^{-2} to 62.9 ± 8.2 mm mm^{-2} and this was associated with a 59% reduction in cortical ChAT activity, from 168.4 ± 14.1 to 69.2 ± 5.1 pmol ACh min^{-1} mg $tissue^{-1}$.

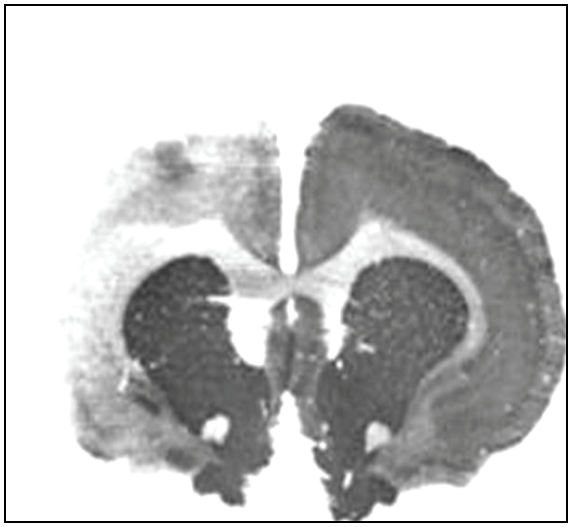

Figure 5. A 30 µm coronal brain section from an MCA-occluded rat showing loss of AChE staining in the lesioned hemisphere.

3.5. Relation between behavioral, iontophoretic and morphological studies

The relations between the performances of MCA - occluded rats in the water maze; the loss of cortical AChE-positive fibers and the sensitivity of cortical neurons to ACh were investigated by correlation analyses. Latency for the MCA-occluded animals to reach the platform in the water maze correlated positively with the loss of AChE ($r = 0.85$) and with the sensitization to ACh ($r = 0.90$). The increased sensitivity of cortical neurons to ACh also correlated positively among the MCA-occluded animals with the loss of AChE-positive fibers ($r = 0.81$).

4. Discussion

Middle cerebral artery occlusion produces ischemic damage which, in rats, typically affects areas of the frontoparietal cortex and lateral striatum. Susceptibility of the frontoparietal cortex varies among stains being less damaged in Wistar rats compared to the substantial damage which occurs in Sprague-Dawley and Fischer rats (Duverger and MacKenzie, 1988). For this reason, Wistar rats were used in the present study.

Middle cerebral artery occlusion increased the latency for rats to locate the submerged platform in the water maze. This could be explained by an impaired spatial ability during the acquisition phase of learning because the performance of the MCA-occluded rats equaled that of the sham-operated rats when a cue was placed on the submerged platform and because the improvement was maintained when the cue was removed. This indicates that once learnt the ability to retain information (i.e. how to locate the escape platform) was not impaired. In contrast, rats with excitotoxic lesions of the nucleus basalis had difficulty locating the platform when the cue was removed (Abdulla et al., 1994; 1997b). The ability of MCA-occluded rats to localize tactile stimuli delivered to the side of the body contralateral to the lesion (Grabowski et al., 1988) is impaired but this could not explain the behavioral deficits obtained in the present study because, when the cue was in place, the MCA-occluded rats located the platform with similar latencies to the sham-operated rats. An injection of microspheres into the internal carotid artery of rats produces a more severe focal cerebral ischemia, in terms of neuronal cell loss, than that observed in this study but the selective deficit in acquisition of spatial information rather than in retention or retrieval, is similar (Lyden et al., 1992). The deficits observed in ischemic rats are consistent with findings obtained in the step-through passive avoidance procedure (this study; Yamamoto et al., 1988; Tamura et al., 1989; Wahl et al., 1992; Togashi et al., 1996). As well as data obtained from many clinical studies performed on human after stroke (Nas et al., 2004; Srikanth et al., 2004; Talell et al., 2004; Werring et al., 2004; Zhou et al., 2004; Park et al., 2005; 2007). However, the results differ from those described in CFWl mice in which MCA occlusion did not produce a decrement in water maze performance (Stollenwerk et al., 1992).

Iontophoretic application of ACh and carbachol to frontal cortical neurons of sham-operated rats produced muscarinic responses (atropine-sensitive) in 52% and 55%, respectively, of neurons tested. These percentages are similar to those described previously in control rats (Abdulla et al., 1994; 1997a). MCA occlusion increased the percentage of neurons responding to ACh, without affecting the neuronal population responding to carbachol and glutamate. Since carbachol is not susceptible to hydrolysis by acetylcholinesterase the increase in the number of neurons responding to ACh was therefore probably due to decreased AChE activity. This is supported by the loss of AChE positive fibers from cortical regions. However, the sizes of the individual responses to both ACh and to carbachol were increased after MCA occlusion suggesting an increased sensitivity of post-synaptic muscarinic receptors.

A more selective cortical cholinergic deafferentation produced by an excitotoxic lesion of the nucleus basalis (Abdulla et al., 1994; 1997a) also produced similar changes in unit responses to iontophorised ACh and carbachol.

In conclusion Loss of AChE-positive fibers may be responsible for the cognitive impairment seen after stroke. Additionally, strategies designed to increase cortical cholinergic functions (grafting or pharmacological) may have therapeutic value in alleviating stroke-induced cognitive impairment.

Acknowledgment:

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Modeling the biodegradation efficiency and growth of *Pseudomonas alcaligenes* utilizing 2,4-dichlorophenol as a carbon source Pre- and Post-exposure to UV radiation

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Abstract

A bacterial strain capable of utilizing 2,4-dichlorophenol as the sole carbon source was isolated by using enrichment and isolation techniques. The strain was identified as *Pseudomonas alcaligenes*. Ultraviolet radiation was used to enhance the biodegradation efficiency of the isolated bacterium. The growth pre- and post-irradiation of the isolated bacterium was modeled using nonlinear equations. Furthermore, the biodegradation efficiency of 2,4-dichlorophenol at temperatures of 25, 30, 35 and 40 ºC and pH values of 6.5, 7 and 8 was modeled using Gompertz type equations. The models indicated that ultraviolet irradiation enhanced the growth capabilities of the *Pseudomonas alcaligenes* and that the highest biodegradation efficiency reached 79% at 35 ºC and pH 7.

الملخص

تم عزل سلالة بكتيرية قادرة على استخدام 2 و4 ثنائي الفينول كمصدر وحيد للكربون والتعرف عليها بأنها Pseudomonas alcaligenes و تم استخدام الأشعة فوق البنفسجية لتحسين كفاءة التحطيم البيولوجي لهذه البكتيريا حيث تم نمذجة نمو هذه البكتيريا قبل وبعد تعرضها للأشعة فوق البنفسجية باستخدام معادلات غير خطية. بالإضافة إلى ذلك تم نمذجة التحطيم البيولوجي لثنائي الفينول عند درجات حرارة 25 و 30 و 35 و 40 درجة مئوية ورقم هيدروجيني 6.5 و 7 و 8 باستخدام معادلات Gompertz وقد أشار النموذج الرياضي إلى أن أعلى كفاءة تحطيم بيولوجي هي عند درجة حرارة 35 درجة مئوية ورقم هيدروجيني 7 حيث بلغت 79.%

Keywords: Nonlinear modeling; Biodegradation; Pseudomonas alcaligenes Gompertz model; Ultraviolet radiation;

1. Introduction

Biodegradation and bioremediation have become the most rapidly developing fields of environmental restoration (Dua *et al*., 2002). It is estimated that 25% of the Earth's biomass is composed of compounds that have a benzene ring as the main structural constituents (Gibson and Harowood, 2002). Chlorophenols are toxic for a wide range of organisms. They are used in pressure treatment in the wood preservation industry, herbicides and fungicides, and are also found in pulp bleaching effluents and industrial wastewater (Bae *et al.*, 2002). Bielicka et al. (2002) have isolated a number of enzymes that catalyze the biodegradation reactions from various microorganisms.

A large number of bacterial and fungal species have the capability to degrade chlorophenolic compounds (Jong and Field, 1997; Reddy and Gold, 2000; Schlosser et al., 2000; Bollag et al., 2003; Steinle *et al*., 1998).

Pseudomonades have been identified to be of importance in bioremediation as a result of their high capacity for biodegradation. *Pseudomonas* species have been used by Premalatha and Rajakumar (1994), Kiyohara et al. (1992) and Farrell and Quilty (2002) for the biodegradation of a variety of chlorophenolic compounds. On the other hand, the efficiency of biodegradation of all bacterial or fungal species is questionable. For example, Koh *et al*. (1997) have reported a 69% dehalogenation of 2,4-dichlorophenol using *Alcaligenes eutrophus*, while Wang *et al*. (2000) reported that the removal of 2,4-dichlorophenol using *Bacillus insolitus* at high concentrations was less than 50% and Tomasi *et al*. (1995) have reported various success rates for the biodegradation of dichlorophenols using *Pseudomonas cepacia*.

Ultraviolet radiation (UV) has been extensively used in the water disinfection and treatment industry. A number of studies have examined the effects, either negative or positive, due to the exposure of different organisms to UV radiation and the potential repair mechanisms of these organisms (Lysetska *et al.*, 2002; Zimmer and Slawson,

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2002; Cooke and Williamson, 2006; Alonso-Saez *et al*., 2006).

The mutagenic capability of UV radiation makes it a useful tool for the genetic modification of microorganisms. Since a study of the capabilities of a bacterial species to grow and utilize an organic compound is a prerequisite for its use in the biodegradation or bioremediation of this compound, the aims of this study are to mathematically model the difference in the growth of *Pseudomonas alcaligenes* utilizing 2,4-dichlorophenol as the sole carbon source pre- and post-exposure to UV radiation and to model the efficiency of biodegradation at different temperatures and pH values.

2. Materials and Methods

2.1. Isolation and Identification of *Pseudomonas spp.*

The bacterial species were isolated from wastewater samples taken from two pharmaceutical companies that their wastewater have been analyzed to contain phenolic compounds and centrifuged at 3000 rpm for 30 minutes to obtain sludge. Two hundred milligram of the sludge was added to pre-sterilized flasks containing 20 ml of chlorophenol enrichment culture media supplemented with 100mg/L of 2,4-dichlorophenol (Zhang and Weigel, 1990; Kiyohara *et al*., 1992) and allowed to grow in a shaking water bath for four days at 30 ºC. Flasks containing 50 mL of nutrient broth (HiMedia Laboratories Limited, Mumbai, India) were inoculated with 0.5 mL of the first set suspension and grown for four days under the same conditions. Nutrient agar plates supplemented with 100 mg/L of 2,4-dichlorophenol were inoculated with 0.3 ml from the flasks and incubated for four days at 37 ºC. Biochemical characterization of the bacterial colonies was carried out according to Cowan and Steels manual for identification of medical bacteria (Barrow, 1993). Confirmation of the identity of the bacterial species was carried by Jordan University Hospital, using a "REMEL system and API 20 NE kit".

2.2. Examination of the Efficiency of Biodegradation of 2,4-dichlorophenol isolates prior to UV irradiation

Colonies that appeared on the plates were streaked onto nutrient agar supplemented with cetrimide (1g/L) and inoculated for three days at 37 ºC. Pink colonies were then transferred to nutrient agar plates supplemented with 120, 140, 160, 180, 200, 220, 240, 260, 280 and 300 mg/L of 2,4-dichlorophenol in order to examine the ability of *Pseudomonas alcaligenes* in using 2,4-dichlorophenol as the only carbon source. Enumeration of the bacterial colonies, at different 2,4-dichlorophenol concentrations, was carried out by using a colony counter to count the bacterial colonies.

2.3. Pseudomonas alcaligenes Exposure to Ultraviolet radiation

The isolated colonies were streaked onto nutrient agar plates in duplicated and irradiated by UV radiation (365 nm, 11 W/m2) for 24, 48, 72 and 96 hours. Colonies from UV irradiated plates were transferred to nutrient agar plates supplemented with 2,4-dichlorophenol concentrations of 240, 260, 280, 300, 320, 340, 360, 380 and 400 mg/L in order to examine the effect of UV radiation on the capability of *Pseudomonas alcaligenes* in utilizing 2,4-dichlorophenol for its growth.

2.4. Biodegradation of 2,4-dichlorophenol

The UV irradiated *Pseudomonas alcaligenes* was cultivated in a benchtop bioreactor (Laboratory benchtop fermenter unit model 300, and EMC unit model 351, Hamburg, Germany) at temperatures of 25, 30, 35 and 40 ºC and pH values of 6.5, 7 and 8. The initial concentration of 2,4-dichlorophenol was 340 mg/L and aeration was kept constant at 0.6 L/min and continuous agitation of 40 rpm. Samples were taken daily and the concentration of the 2,4-dichlorophenol was determined using a UV-spectrophotometer (UV-visible spectrophotometer, 100 Bio:Cary Varian, USA) .

2.5. Modeling the Growth of *Pseudomonas alcaligenes*

A nonlinear regression model is used to determine the relationship between the concentration of 2,4-dichlorophenol and the number of colonies of *Pseudomonas alcaligenes* that can grow on this concentration. This relationship is modeled as:

$$DCP = A / e^{(k*CFU)}$$

Where,

DCP = the concentration of 2,4-dichlorophenol (mg/L)

A = A theoretical value of the maximum the concentration of 2,4-dichlorophenol that Pseudomonas alcaligenes can use as the carbon source

k = regression model parameter

CFU = number of colonies (log 10 of CFU/mL)

Levenberg-Marquardt, a nonlinear least squares method, is used to calculate the A and K parameters. This method is a modification of the Gauss-Newton algorithm which in using the least-squares loss function, the second order partial derivative do not have to be computed in order to find the least-square estimates; instead, the algorithm in each iteration solves a set of linear equations to compute the gradient.

2.6. Modeling the biodegradation of 2,4-dichlorophenol

Two nonlinear models are used to model the biodegradation of 2,4-dichlorophenol. The first, models the nonlinear relationship between each temperature used in the biodegradation (25,30,35,40 ºC) and time of biodegradation. Therefore, four temperature models are formulated. The second, models the nonlinear relationship between each pH (6.5, 7, 8) and time of biodegradation, thus three models are formulated. These nonlinear relationships are modeled as Gompertz equation. The general formulae for these models are:

$$BDCPT = A*(e^{-e(-k*T)}) \quad \text{and,} \quad BDCPH = A*(e^{-e(-k*T)})$$

where,

BDCPH = the percentage of 2,4-dichlorophenol biodegraded at a specific pH value

BDCPT = the percentage of 2,4-dichlorophenol biodegraded at a specific temperature

T = time of biodegradation

A = a theoretical value of the maximum concentration of 2,4-dichlorophenol that can be biodegraded

k = model parameter

Quasi-Newton method is used to calculate the model parameters (A and k). This method uses an algorithm that approximates the second-order derivatives of the loss function to guide the search for the best parameter estimates, given the respective loss function.

3. Results

The model for the biodegradation of 2,4-dichloropheno indicates that there are differences between the UV irradiated *Pseudomonas alcaligenes* and the non-irradiated species. A comparison of the calculated A values by the model indicates that the UV irradiated *Pseudomonas alcaligenes* can utilize, as a carbon source, a much higher concentration of 2,4-dichlorophenol than the non-irradiated *Pseudomonas alcaligenes* as shown in table (1).

Table 1: Parameters of the Pseudomonas spp. growth models

Model Parameters	*Pseudomonas spp.*	UV irradiated *Pseudomonas spp.*
A	412.67	1228.85
K	0.0967	0.169
r	0.8783	0.9504

r = correlation coefficient of the model

This is further supported by the value of the k parameter, which is higher in the UV irradiated *Pseudomonas alcaligenes* than the non-irradiated. The model can determine the concentration of 2,4-dichlorophenol that can be utilized as a carbon source by inputting the number of bacteria in Log $_{10}$ of CFU/mL and the model parameters A and k (refer to table 1). The relationship between the number of *Pseudomonas alcaligenes* and the concentration of 2,4-dichlorophenol is indicated in figure (1) for the non-irradiated and UV irradiated species, respectively.

The biodegradation models show that the temperature that resulted in the highest biodegradation percentage is 35 ºC and the pH value that resulted in the highest biodegradation percentage is pH 7. This is indicated by the A values shown in table (2). The models can accurately determine the biodegradation percentage for each temperature and each pH value. For example, the model for the temperature of 35 ºC is:

*DCPT = 79 * e(-e-0.0166*35)*

The equation for pH 7 is:

*DCPH = 68 * e (-e-0.0192*7)*

The analysis of variance (ANOVA) test showed that there are statistically significant differences between the mean values of the temperatures (25, 30, 35 and 40 ºC) tested in the biodegradation (p=0.0108) and the pH values (6.5, 7 and 8) tested in the biodegradation (p=0.0059). The mean values for both the temperature and pH biodegradation results are shown in figures (2) and (3).

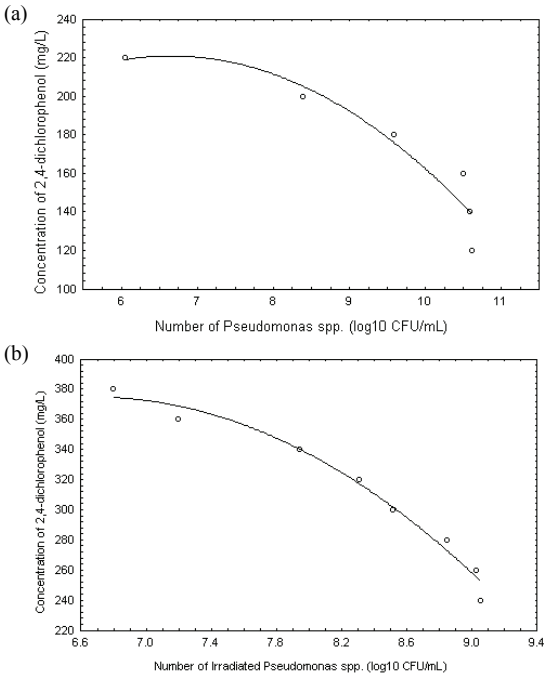

Figure 1: Comparison of the maximum concentration of 2,4-dichlorophenol that can be used as a carbon source by the non-irradiated (a) and irradiated (b) *Pseudomonas spp.*

Table 2: Biodegradation model parameters for the temperature and pH values

		Parameters of the Model		
		A	k	r
Temperature ºC	25	68	0.0192	0.8793
	30	76	0.0183	0.8818
	35	79	0.0166	0.8642
	40	64	0.0107	0.7473
PH values	6.5	59	0.0153	0.8581
	7	68	0.0192	0.8793
	8	48	0.0116	0.8116

r = correlation coefficient of the model

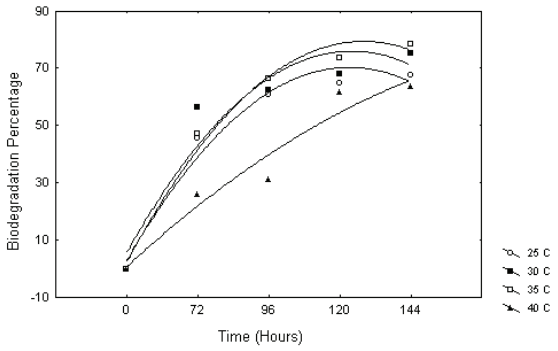

Figure 2: Biodegradation percentages of 2,4-dichlorophenol by *Pseudomonas spp.* at 25, 30, 35 and 40 ºC

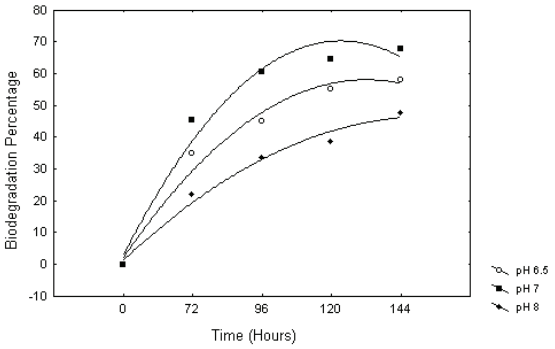

Figure 3: Biodegradation percentages of 2,4-dichlorophenol by *Pseudomonas spp.* at pH values of 6.5, 7 and 8

4. Discussion

There are a number of studies that explain the genetic bases of biodegradation. For example, *Flavobacterium* ATCC biodegrades pentachlorophenol by a catabolic pathway encoded by many genes one of which is pcpA that encodes information for a 30-kDa polypeptide pcpA, found in the periplasm of the bacterium (Chanama and Crawford, 1997). Although several studies have been carried out on the biodegradation capabilities of a number of *Pseudomonas spp.* and still others have determined the biodegradation pathway of 2,4-dichlorophenol (Perkins *et al*., 1990; Bhat *et al*., 1994; Xun, 1996; McFall *et al*., 1997; Tarao and Seto, 2000), the scarcity of studies that used UV radiation to enhance the biodegradation efficiency of this bacterium makes it difficult to compare their results with our results. Even though the aim of this study was not to find out the genetic modification that occurred to the *Pseudomonas alcaligenes* after the UV irradiation, it is clear that a large improvement in the biodegradation efficiency occurred due to this UV irradiation. The model even predicts about three times increase in the theoretical maximum concentration of 2,4-dichlorophenol that can be biodegraded (table 1) by the UV irradiated *Pseudomonas alcaligenes* compared to the non-irradiated species. Further studies in this area are needed to elucidate the genetic alterations and the new biodegradation pathway that occurred due to UV irradiation.

Temperature and pH values obviously affect the biodegradation efficiency. It has been reported that the amount of 2,4-dichlorophenol absorption to microbial cells increased with decreased pH values (Gillian *et al*., 1999). Therefore, at pH 4 there was a great deal more absorption than at pH 8. This could explain our results, which show that the best biodegradation occurred at pH 7 and not 8 (table 2). Furthermore, an increase in hydrogen ion concentration at lower pH than 7 influences and limits the growth rate of microorganisms (Armenante *et al*., 1993). Thus, it is reasonable to expect biodegradation to be the highest at pH 7. Temperature affects biological reaction rates and biological growth rates. Hence, it is expected that biodegradation efficiency to increase with the increase in temperature, which is indicated by our results (table 2). The models of the influence of the temperature indicated that 35 ºC had the highest maximum biodegradation percentage. On the other hand, a higher temperature than the optimal temperature for the microorganisms could have a fast drop in the biological reaction rates and thus, decrease the biodegradation efficiency.

The use of mathematical model to determine the growth of microorganisms has been carried out successfully. For example, Ng and Schaffner (1997) formulated mathematical models to test the effects of pH, temperature and sodium chloride on the growth of *Bacillus stearothermophilus*. They used quadratic polynomial models for determining the germination, outgrowth and the growth rate of the bacterium. They concluded that the models provided an estimate of bacterial growth in response to combinations of the variables studied within the specified ranges. Our results also indicate that the use of the growth and biodegradation models successfully predicted *Pseudomonas alcaligenes* growth and the variation in growth after UV exposure and the biodegradation efficiency at different temperature and pH values. It is worth mentioning that the highest biodegradation percentage obtained in our study was 79% at temperature 35 ºC and pH 7 is higher than the reported percentage of 50% by Tomasi *et al*. (1995) and the 69% reported by Koh *et al*. (1997).

In conclusion, modeling the biodegradation efficiency of 2,4-dichlorophenol by *Pseudomonas alcaligenes* has shown that the biodegradation efficiency was enhanced by UV irradiation for 144 hours and that the highest biodegradation efficiency occurred at temperature of 35 ºC and pH 7. Further studies are needed to explain the genetic alterations that occurred due to UV irradiation and the alterations in the catabolic pathways responsible for the 2,4-dichlorophenol biodegradation.

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Heavy Metals in Eleven Common Species of Fish from the Gulf of Aqaba, Red Sea

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Abstract

The muscles, livers, gills, gonads, and stomachs of eleven common fish species collected at three sites in the northern Gulf of Aqaba, were analyzed for the heavy metals Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb, and Zn. Large differences in trace metal concentrations (in µg g^{-1} dry weight) were observed between different tissues or organ parts within each fish. In muscle tissue Cd ranged from 0.5 to 2.0 µg g^{-1} dry weight, while higher concentrations were found in liver, gill, and stomach tissues (up to 7.0 µg g^{-1}). Cobalt concentrations were lowest in muscles (1.7-7.1) and livers (1.9-6.8) and highest in gills (4.3-15.0) and stomachs (1.8-11.0). Similarly, Cr concentrations were highest in gills (4.3-44.2) and stomachs (1.8-22.0), and lowest in livers (1.9-11.5). Copper was also low in muscles (0.5-2.0), but highest in liver tissue (6.7-40.6). Iron levels were highest in liver (30-1031) and lower in other organs (3.9-391.0). Muscle tissues contained lower concentrations of Mn (1.0-3.3) than other organs, particularly the gills (3.8-19.0), which contained also higher concentrations of Ni and Pb (4.5-19.2 and 8.7-35.0, respectively) compared to the livers (1.0-11.4 and 1.9-6.3) and muscles (1.0-5.0 and 2.5-8.3). Gonads contained the highest concentration of Zn (77.5-271.7), while muscles contained the lowest (1.9-35.0).

الملخص

تم تحليل مستوى العناصر الثقيلة (Zn, Pb, Ni, Mn, Fe, Cu, Cr, Co, Cd) في عضلات و كبد و خياشيم و مناسل و معد إحدى عشر نوعاً من الأسماك السائدة في خليج العقبة . لوحظ وجود فروقات كبيرة في تراكيز هذه المعادن في الأنسجة المختلفة لكل نوع من السمك . تراوح تركيز Cd (0.5 – 2.0) ugg^{-1} dry wt. في العضلات بينما وصل إلى 7.0 ugg^{-1} dry wt في الأنسجة الإخرى . أما Co فقد كان تركيزه أقل ما يمكن في العضلات (1.7-7.1) و الكبد (1.9-6.8) و أعلى ما يمكن في الخياشيم (15.0 – 4.3) و المعد (11.0 – 1.0) . و كان تركيز Cr أعلى ما يمكن في الخياشيم (44.2- 4.3) و المعد (22.0 – 1.8) و أقل تركيز كان في الكبد (11.5- 1.9) . كذلك كان النحاس منخفضاً في العضلات (0.5-2.0) و مرتفعاً في الكبد (6.7-40.6) ، كما كان الحديد مرتفعاً في الكبد (30-1031) . أما تراكيز Mn فكانت منخفضة في العضلات (1.0-3.3) بينما بلغت في الخياشيم (3.8-19.0) ، و اشتملت كذلك الخياشيم تراكيز عالية من Ni (4.5-19.2) و Pb (35.0 – 8.7) بينما كانت التراكيز في الكبد (11.4 – 1.0 و 6.3-1.9) و العضلات (8.3-2.5 و 5.0 – 1.0) . كانت تراكيز Zn أعلى ما يمكن في المناسل (271.7 – 77.5) ، بينما كانت في العضلات (35.0 – 1.9) .

Keywords: Heavy metals; Gulf of Aqaba; Red Sea; Jordan;

1. Introduction

There are relatively few studies available on the content of many of the important toxic and essential trace elements in marine organisms of the Gulf of Aqaba. This is in spite of the rapid coastal development at the northern most tip of the Gulf at Aqaba town during the last three decades. During the past three decades, there has been a noticeable increase in the interest to measure these elements in marine sediments (Abu-Hilal, 1987; Abu–Hilal and Badran, 1990; Abu-Hilal, 1993), crustaceans (Abu-Hilal *et al.,* 1988), fish species (Wahbeh, 1985), mussels (Ababneh, 2004; Al-Batainh, 2004), seagrasses (Wahbeh, 1984; Abu–Kharma, 2006), algae (Wahbeh and *et al.,* 1985; Abu-Kharma, 2006), zooplankton (Bani Fawwaz, 2005) and corals (Al–Shloul, 2006; Al-Tarabeen, 2006). The work by Abu-Hilal (1987) described the high degree to which eight toxic heavy metals have contaminated the sediments of the Gulf in the submarine discharge zone of the previous Aqaba town municipal sewage outfall and the phosphate loading berth. Wahbeh and Mahasneh (1987) attributed the considerable variations in the elements

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among the tissues to diet although they did not consider the feeding habits of the species. Moreover, it is important to determine the spatial variability of these trace metals using fish from localities with various degrees of impact. This paper examines the degree to which common coastal fishes collected from polluted and unpolluted sites at the northern portion of the Gulf of Aqaba have accumulated the metals in their organs and edible tissues and compares these results to previously published data in order to evaluate the degree of anthropogenic trace metal contamination on the local fish population.

2. Materials and Methods

Fish were collected from three localities along the northern portion of the Gulf of Aqaba. The first locality was south of and adjacent to a phosphate polluted area near the port of Aqaba characterized by urban anthropogenic impact. The second site was a relatively unpolluted area in front of the Marine Science Station and the third was in front of the industrial complex zone adjacent to the Jordan-Saudi border representing industrial impact. In total, fish specimens of 11 different species were examined. These include the sergeant major fish *Abudefduf saxatilis*, the exquisite butterfly fish *Chaetodon austricus*, the striped butterfly fish C. fasciatus, the black tip grouper *Epinephelus fasciatus*, the cornet fish *Fistularia petimba*, the sea chub fish Kyphosus sp., the mullet fish Mugil sp., the goat fish *Mulloidichthys auriflama*, the goat fish *Parupenus cyclostomus*, the sparid fish *Polysteganus coeruleopunctatus*, and the wrass fish *Thalassorna sp*. Sampling, pretreatment, preparation of subsamples and analysis were made according to FAO Technical Paper No. 212 (1983). After capture the samples were weighed, measured, cleaned with deionized-distilled water, stored in pre-cleaned plastic bags, and kept frozen at -18 ^{0}C until further analysis. Frozen fish were partially thawed and dissected on cleaned plastic sheets using scalpels with steel blades and plastic forceps. Flesh, liver, gill, gonads and stomach were taken out and dried in a precleaned glass container at 103 ± 2 ^{0}C to a constant weight. A suitable volume of a mixture of hydrogen peroxide/nitric acid solution 1:1 v/v was used for the wet acid digestion of a preweighed tissue or organ. A Perkins Elmer 3030 atomic absorption spectrophotometer with digital read-out, deuterium lamp background corrector, and automatic zero to compensate the blank, was used for the determination of the metals. Settings were those recommended by the manufacturer. A standard curve was run with each analysis. A blank treated exactly as for the sample was also run with each batch of samples. Blanks were always of negligible values. The effect of interferences attributable to the matrix and the validity of the results were checked with the standard addition method. Recoveries were between 98 and 103% .The precision was confirmed by carrying out ten replicate analyses for three different samples. The coefficient of variation was less than 5% for all elements. Detection limits were considered as equal to 2 × standard deviation of the blank.

3. Results and Discussion

Concentrations of the heavy metals Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb, and Zn in the muscles and selected organs of the eleven fish species collected from the northern portion of the Gulf of Aqaba are presented in Tables 1 and 2. Levels of each metal are discussed below with reference to data available for fish species from the Red Sea and other areas of the world. Concentrations of metals are presented in μg g^{-1} dry weight unless otherwise mentioned.

Table 1: concentrations (μg g^{-1}g^{-1} dry weight) of heavy metals in the muscles of eleven species of common fish collected from the Gulf of Aqaba at the Phosphate Loading Berth (PLB), Marine Science Station (MSS), and Industrial Complex (IC).

Species	Site	Cd	Co	Cr	Cu	Fe	Mn	Ni	Pb	Zn
Abudefduf saxatilis	PLB	0.6	3.2	10.3	1.9	20.5	1.3	3.9	5.6	10.9
	MSS	0.5	3.0	5.0	2.0	5.5	1.5	3.5	6.5	35.0
Chaetodon austricus	IC	0.6	1.7	2.9	1.7	6.4	0.6	1.2	4.1	12.2
	MSS	0.5	2.5	1.0	1.0	4.5	2.0	1.5	1.5	10.0
	PLB	1.7	3.3	5.0	1.7	8.3	3.3	5.0	8.3	16.7
C. fasciatus	PLP	0.5	2.0	5.0	1.0	6.5	1.0	4.0	4.0	2.5
Epinephelus fasciatus	MSS	1.0	3.8	4.0	1.0	6.5	1.5	1.0	5.0	11.0
	MSS	0.9	7.1	4.6	0.9	8.3	1.9	4.6	5.6	7.4
	PLB	1.0	3.5	9.0	1.0	3.0	1.5	1.5	3.8	9.0
Fistularia petimba	PLB	2.0	2.5	2.0	1.0	13.0	1.5	2.5	3.5	7.5
Kyphosus sp.	PLB	0.5	1.5	2.3	0.8	6.0	0.5	1.0	4.0	28.3
Mugil sp.	PLB	0.5	2.5	1.5	1.0	14.0	1.5	2.0	3.0	9.5
Mulloidichthys auriflama	MSS	0.5	3.0	2.5	1.5	8.5	2.0	3.0	6.5	11.0
	PLB	0.8	5.0	3.5	1.0	2.5	1.0	2.5	2.5	17.0
Parupenus cyclostomus	MSS	0.5	2.5	1.5	1.0	6.8	1.5	2.0	2.5	18.0
Polysteganus coeruleopnctatus	MSS	0.5	2.0	1.5	0.5	6.0	1.0	1.5	4.0	8.0
Thalassoma sp	MSS	0.5	3.5	2.8	0.5	4.5	1.0	2.5	4.0	23.0
	PLB	ND*	ND	ND	ND	3.9	1.9	1.9	5.8	1.9

* ND: Not determined.

Table 2: Concentrations (μg g^{-1} dry weight) of heavy metals in selected organs in eleven species of fish from the Gulf of Aqaba collected at the Phosphate Loading Berth (PLB), Marine Science Station (MSS), and Industrial Complex (IC).

Organ	Species	Site	Cd	Co	Cr	Cu	Fe	Mn	Ni	Pb	Zn
Liver	*Abudefduf saxatilis*	MSS	2.3	6.8	4.5	13.6	1027	6.8	11.4	ND	52.3
	Chaetodon austricus	IC	10.4	ND	6.3	14.6	256	4.2	2.1	4.2	122.9
	C. fasciatus	PLB	ND	1.8	8.9	7.1	411	5.4	ND	ND	101.8
	Epinephelus fasciatus	MSS	0.6	1.9	3.8	40.0	1031	3.1	1.9	6.3	64.4
		MSS	ND	ND	3.1	40.6	494	6.3	ND	ND	93.8
		PLB	2.0	3.0	11.5	19.0	30	3.0	4.0	3.5	54.0
	Fistularia petimba	PLB	0.5	2.0	4.0	6.0	470	3.0	2.5	4.0	58.0
	Kyphosus sp.	PLB	2.0	2.5	2.5	42.5	569	3.0	1.0	6.0	137.5
	Mulloidichthys aurirlama	PLB	0.8	4.2	4.6	23.5	386	3.8	1.5	3.8	74.2
	Parupenus cylostomus	MSS	0.5	2.0	ND	9.0	510	4.0	2.3	4.0	57.0
	Polysteganus coeruleopunctatus	MSS	1.9	2.0	1.9	6.7	208	4.8	2.9	1.9	63.5
	Thalassoma sp.	MSS	1.2	3.6	3.6	11.9	474	2.4	1.0	6.0	45.2
Gonad	*A. saxatilis*	MSS	0.5	3.0	3.0	4.0	16	2.5	3.5	5.0	205.0
	E. tasciatus	PLB	ND	5.0	10.0	6.7	105	3.3	5.0	6.7	271.7
	F. petimba	PLB	0.6	3.2	5.1	5.1	17	1.3	2.6	5.1	123.1
	M. auriflama	MSS	0.5	3.5	2.5	6.0	41	2.0	5.0	5.0	77.5
Gill	*A. saxatilis*	MSS	2.0	12.0	13.5	4.5	67	9.5	10.0	19.0	85.0
		PLB	2.9	13.5	44.2	7.7	ND	17.3	19.2	11.5	15.8
	C. austricus	IC	2.3	5.87	5.6	4.6	125	8.0	8.0	14.8	53.4
		MSS	2.2	4.3	4.3	3.3	62	8.7	6.5	16.3	45.7
	C. fasciatus	PLB	1.9	9.6	26.9	3.8	52	4.8	4.8	8.7	31.7
	E. fasciatus	MSS	2.0	13.5	15.0	5.0	68	5.0	7.5	15.0	71.5
		MSS	2.0	14.5	4.5	3.5	47	4.0	7.5	21.0	65.0
		PLB	2.5	15.0	34.0	4.0	40	6.0	8.5	19.0	52.0
	F. petimba	PLB	1.6	7.8	4.2	3.1	47	7.3	4.7	10.4	43.2
	Kyphosus sp.	PLB	1.5	7.5	9.0	3.5	91	5.0	4.5	12.5	43.5
	Mugil sp.	PLB	3.1	9.4	7.3	4.2	199	7.3	9.4	20.8	32.3
	M. auriflama	MSS	2.0	13.0	12.5	5.5	97	10.0	10.0	35.0	260.0
		PLB	2.3	12.1	9.1	4.6	67	3.8	9.0	18.9	74.2
	P. cyclostomus	MSS	2.5	17.0	ND	4.0	40	4.5	6.8	24.0	69.0
	P.coeruleo-punctatus	MSS	1.5	10.0	5.5	3.5	84	19.0	6.0	20.0	33.0
	Thalassoma sp.	MSS	2.2	11.8	11.0	3.7	121	5.9	6.6	14.7	80.2
Stomach	*A. saxatilis*	MSS	0.6	1.8	1.8	3.0	63	3.0	1.8	3.0	29.8
		PLB	3.1	7.8	12.5	10.9	27	10.9	12.5	10.9	31.3
	C. austricus	IC	2.8	ND	2.8	8.3	153	ND	2.8	ND	52.8
		MSS	4.2	8.3	8.3	8.3	279	12.5	16.7	4.2	66.7
	C. rasciatus	PLB	1.6	4.7	20.3	6.3	120	4.7	6.3	1.6	37.5
	E. fasciatus	MSS	0.5	3.0	3.0	8.5	38	2.5	2.5	4.0	97.5
		MSS	0.6	1.1	1.1	8.9	84	1.7	1.7	4.4	63.3
		PLB	1.3	3.8	8.3	8.3	57	2.6	2.6	3.9	53.5
	F. petimba	PLB	0.8	3.0	5.0	5.5	56	2.0	3.0	5.5	48.0
	Kyphosus sp.	PLB	1.0	4.5	22.0	9.5	63	4.5	2.8	15.0	36.5
	Mugil sp.	PLP	2.5	11.0	17.5	8.5	53	7.5	10.5	22.5	75.5
	M. auriflama	MSS	0.5	2.5	2.5	5.0	180	3.0	4.0	9.0	144.5
		PLB	1.1	7.1	6.5	7.1	391	14.7	5.0	6.5	71.2
	P. cyclostomus	MSS	0.2	9.5	ND	4.5	51	6.0	4.9	12.5	84.5
	P.coeruleo punctatus	MSS	1.0	1.5	1.5	10.0	73	7.5	1.5	5.0	56.5
	Thalassoma sp.	MSS	7.0	ND	20.5	8.5	64	9.0	23.0	10.0	41.5

3.1. Cadmium:

Cadmium is accumulated primarily in major organ tissues of fish rather than in muscles (Moore and Ramamurthy, 1984). This was the case in the present study as Cd in muscles ranged from 0.5 to 1.7 with a mean of 0.77 µg g^{-1}, whereas higher means were found in stomachs (1.8), livers (2.2), and gills (2.2). In the gonads of the eleven fish species sampled, Cd was similar to tissues and ranged from 0.5-0.6. These values are within the ranges reported for 21 species from the Red Sea (Hanna, 1989). However, higher means of Cd (1.3-8.6) in muscles of other fish species from the Gulf of Aqaba were reported (Wahbeh, 1985; Wahbeh and Mahasneh, 1987). These same authors reported high Cd levels in livers of these fishes (2.0-15.6). In contrast, Cd levels in muscles of fish from the Great Barrier Reef were consistently lower than 0.1 (Denton and Burdon Jones, 1986), while in livers of these fishes Cd concentrations varied from less than 0.1 to 209 µg g^{-1}. By comparison, most of the fish from the Mediterranean Sea examined contained Cd concentrations of less than 0.4 in their muscles, with the highest values reported from *Mullus barbatus* and *Sardinella aurita* (0.6-0.7) (Roth and Hornung, 1977). In general, it can be stated that the concentrations of Cd found in the present study are still considered as those of uncontaminated fish (< 1.5) reported by Moore and Ramamurthy (1984), and within the range of 0.2-3.5 µg g^{-1} reported for the Red Sea (Hanna, 1989) and the Mediterranean Sea, but are relatively high compared to those reported from Australia.

3.2. Cobalt:

There was a wide variation in mean concentration of Co in various organs of fish species examined. The highest mean was in gills (11.0) and stomach (5.0). Lower means were found in muscles (3.0), livers (3.3), and gonads (3.7). In addition, Co concentration varied among species. It ranged from 1.5 in muscles of *Kyphosus sp*. to 7.1 in E. *fasciatus*. In gills, Co concentration ranged from 4.3 in *C. austricus* to 17.0 in *P. cyclostomus*. In livers, it ranged from 1.9 in *E. fasciatus* to 6.8 in *A. saxatilis*. The concentration was low in stomach of *E. fasciatus* (1.1) and high in *Mugil sp*. (11.0), *P. cyclostoznus* (9.5), and *C. austricus* (8.3). In gonads, it ranged from 3.0 in *A. saxatilis* to 5.0 in *E. fasciatus*. Wahbeh and Mahasneh (1987) reported lower means of Co concentration in fish species from the Gulf of Aqaba, which ranged from 2.3 to 6.1 in gills, 0.3 to 6.1 in gonads, and 0.2 to 4.3 in muscles. Our results suggest that the comparisons could only be made within a species using similar organs because of the large intra species and intra organ variability. Regardless, the data is suggestive of increased Co contamination in the Gulf.

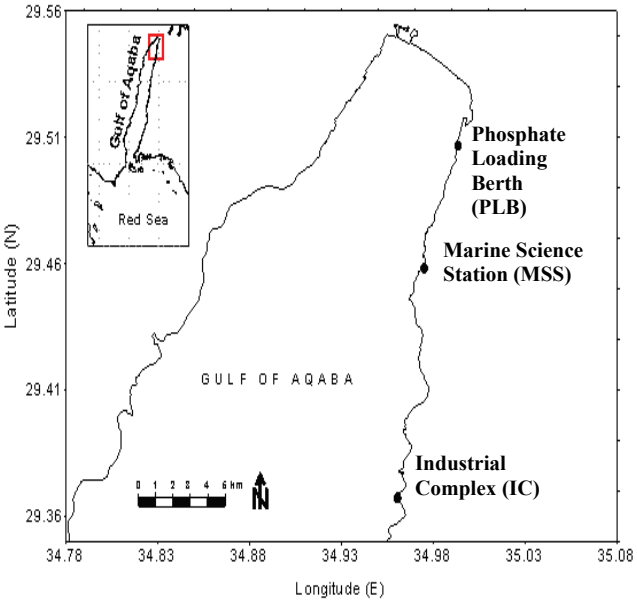

Figure 1: Locations of the sampling sites

3.3. Chromiurn:

Chromium does not normally accumulate in fish and hence low concentrations of Cr were reported even from the industrialized parts of the world (Moore and Ramamoorthy, 1984). The mean concentration of Cr found in the fish muscle samples from the Mediterranean coastal waters was 2.1. Even lower means were also reported from the Gulf of Aqaba for the muscle of *P. barberinus* (0.4) and *Scarus variegatus* (0.6) (Wahbeh and Mahasneh, 1987). However, in the present study higher concentrations were found in muscles of the examined fish (3.8), while it ranged from 4.9 in livers to 13.8 in gills. The values of Cr in muscles and livers are still in the range of values reported by Hanna (1989) for many Red Sea fishes. While this may suggest no large changes in Cr between 1989 and the present, it may indicate long term contamination of the Gulf waters with Cr as the values measured are high compared to other locations in the Red Sea region.

3.4. Copper:

Relatively high mean concentrations of Cu were found in livers (19.1) and stomachs (7.6) of the fish examined, compared to those found in muscles (1.1), gills (4.3), and gonads (5.5). There was also a wide variation in Cu content in livers among species as it ranged from 6.0 for *F. petimba* to 42.5 for *Kyphosus sp*. In muscles, there was a narrower range of concentration (0.5- 2.0). The same trend of difference between muscles and livers was reported by Powell et al. (1981) who found low Cu level in muscles of eight species of marine fish from Bougainville Island, Papua New Guinea (< 1.0 µg g^{-1} wet weight), while higher means were found in the livers (7.2- 15.2) of these fishes. Similar mean values were found in muscles of fish from coastal waters around England (0.67-1.80 µg g^{-1} wet

weight) (Portmann, 1972). In muscle tissue of 50 species of fish from the Great Barrier Reef Cu ranged from 0.47 to 2.4 (Denton and Burdon-Jones, 1986). This range is similar to that obtained in the present study. However, the livers of these fishes contained almost 5 times Cu (up to 226) higher than those obtained in the present study and than those found in the livers of 21 species from the Red Sea (3.49-61.00 µg g^{-1}) (Hanna, 1989). The data indicate that the fish in the Gulf compare to other fish world wide and are not more contaminated with Cu than that at other marine environments.

3.5. Iron:

There was a wide variation in mean concentrations of Fe among organs, particularly between livers (30-1031; mean 530) and muscles (2.5-20.5; mean 7.5). Similar variations were also found in stomachs (26.6-391.3; mean 109.3), gills (40.0-199.0; mean 80.5), and gonads (16.0-105.0; mean 44.8). Comparatively high levels of Fe were also reported for livers (413.3-1333.3), gills (80.0-320.4), gonads (88.9-160.0), and muscles (35.6-71.2) of fish species from the Gulf of Aqaba previously analyzed (Wahbeh and Mahasneh, 1987). On the other hand, Cross et al. (1973) reported lower mean levels of Fe in the muscles of the blue fish, *Pomatomus saltatrix* (4.5-5.0 µg g^{-1} wet weight). While we can not report increased Fe contamination in recent years in the Gulf like for Cr, values are high and suggest long term contamination.

3.6. Manganese:

Although this element is of low toxicity, it has a considerable biological significance and seems to accumulate in certain fish species (Eustace, 1974; Uthe and Bligh, 1971). The lowest mean concentration of Mn was 1.5 (range 0.5-3.3) in the muscles of the fish examined, while it was 2.3 (1.3-3.3) in gonads, 4.4 (2.4-6.8) in livers, 6.1 (2.0-14.7) in stomachs, and 7.9 (3.3-17.3) in gills. Cross et al. (1973) reported lower Mn concentration (0.20-0.28 µg g^{-1} wet weight) in the muscle of the blue fish *P. saltatrix*. Eustace (1974) found that 39 species of marine fish from Derwent Estuary, Tasmania, contained up to 0.6- 4.4 µg g^{-1} wet weight Mn when homogenized whole. By comparison, Wahbeh and Mahasneh (1987) reported higher mean concentrations (5.6-26.8) in various organs of fish they examined from the same study area within the Gulf of Aqaba. Our data are generally within the range of fish from other water bodies and do not indicate any particular contamination issue.

3.7. Nickel:

Similar to manganese the lowest mean concentration of Ni was 2.5 (range 1.0-4.6) in the muscles of fish examined, while it ranged from 8.1 (4.5-19.2) in gills to 6.4 (1.5-16.7) in stomachs, 4.0 (2.6-5.0) in gonads, and 3.9 (1.0—11.4) in livers. Other studies have shown that Ni concentrations in fish muscles are generally lower than those in other tissues (Moore and Ramamoorthy, 1984). Wright (1976) reported more or less the same range of concentrations in muscles (0.5-7.2), livers (1.7-10.8), and gills (3.3-4.5) of several fish species off the NE coast of England. In contrast, Hanna (1989) reported higher values of Ni in the muscles (0.2-7.2), livers (1.58-42.90), and gonads (3.2-1.3.9) of 21 fish species from the Red Sea. Our data show more pristine conditions than those in the Red Sea and similar to values in the Atlantic, thus not necessarily indicative of a Ni pollution problem.

3.8. Lead:

The mean concentration of Pb was lowest in the muscles (4.5) and livers (4.4) of the fish examined, while the highest was in the gills (17.6) of these fishes. These results are consistent with what has been reported by Moore and Ramamoorthy (1984) that there is often little accumulation of Pb in the muscles of marine and freshwater fish species. Low concentrations of Pb in the muscles of marine fish were reported from coastal areas of England and Wales (< 1.0 µg g^{-1} wet weight) (Portmann, 1972), West Malaysia (< 0.5 mg kg' wet weight) (Baji et al., 1979), Gulf of Mexico (0.05-0.73 µg g^{-1} wet weight) (Taylor and Bright, 1973), and Gulf of Aqaba (0.8-2.6 µg g^{-1}) (Wahbeh and Mahasneh, 1987). In fishes of the Red Sea Pb ranged between 0.01-0.66 µg g^{-1} in the muscles, 0.1-2.4 in the livers, and <0.05-0.14 in the gonads (Hanna, 1989). Our data indicate that the fish in the Gulf contain higher levels of Pb compared to other fish at other marine environments world wide.

3.9. Zinc:

There was great variation in Zn concentrations among species. The mean concentration in muscles was 13.3 and ranged from 1.9 in *Thalassorna sp*. to 35.0 in *A. saxatils*. The highest concentration was in the livers of these fishes (45.2-137.0; mean 75.2). Powell et al. (1981) recorded lower mean concentration of 3.0-4.5 µg g^{-1} wet weight) in muscles of fish from Bougainville Island. However, they reported higher means from the livers of these fishes (30.0-44.9). Similar range of concentration (4.3-41.8) was found in the muscles of fish species from the Great Barrier Reef (Denton and Burdon-Jones, 1986). However, they reported relatively high concentrations in the livers of these fishes. By comparison, Hanna (1989) found much higher and wider concentrations of Zn in the muscles (8.4-195.0 µg g^{-1}), livers (43-620), and gonads (72-259) of fishes from the Red Sea. Our data show that Zn levels in the fish of the study area are within the levels reported from the Red Sea and other regions world wide.

4. Conclusions

- Cadmium is accumulated primarily in major organ tissues of fish rather than in muscles. In the present study Cd in muscles ranged from 0.5 to 1.7 with a mean of 0.77 µg g^{-1}, compared to higher means in stomachs (1.8), livers (2.2), and gills (2.2).
- However, the concentrations of Cd found in the present study are still considered as those of uncontaminated fish (< 1.5 µg g^{-1}) reported by Moore and Ramamurthy (1984), and within the range of 0.2-3.5 µg g^{-1} reported for the Red Sea (Hanna, 1989) and the Mediterranean

Sea, but are relatively high compared to those reported from Australia.

- The data is suggestive of increased Co contamination in the Gulf. Cobalt concentration varied among species. Highest means were in gills (11.0) and stomachs (5.0) while lower means were found in muscles (3.0 μg g^{-1}).
- High concentrations of Cr were found in gills (13.8 μg g^{-1}) of the examined fish while the values in muscles (3.8), and livers (4.9) are still in the range of values reported by Hanna (1989) for many Red Sea fishes. However, while the results may suggest no large changes in Cr between 1989 and the present, it may indicate long term contamination of the Gulf waters with Cr as the values measured are high compared to other locations in the Red Sea region.
- Relatively low mean concentrations of Cu were found in muscles (1.1 μg g^{-1}) of the fish examined, compared to those found in gills (4.3) gonads (5.5) livers (19.1) and stomachs (7.6).
- The data indicate that the fish in the Gulf are compared to other fish world wide and are not more contaminated with Cu than those at other marine environments. The range of Cu in muscle tissue in the present study is similar to that obtained in muscle tissues (0.47 to 2.4 μg g^{-1}) of 50 species of fish from the Great Barrier Reef (Denton and Burdon-Jones, 1986).
- There was a wide variation in mean concentrations of Fe among organs, with higher concentrations in liver (30-1031; mean 530 μg g^{-1}) and lower concentrations in muscles (2.5-20.5; mean 7.5 μg g^{-1}).

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Phenotypic and Genotypic Characterization of Three Novel Halophilic *Bacillus* Strains from Jordanian Hot Springs

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Abstract

Three strains of facultatively anaerobic thermotolerant and moderately halophilic bacteria were isolated from two Jordanian hot spring locations. The novel *Bacillus* strains were short rods, Gram-positive, motile and spore formers. The strains were capable of anaerobic (but not aerobic) growth at 63°C, moderately halophilic, able to grow at NaCl concentration range of 12 - 15 % and at temperatures range of 37 -63°C. Moreover, the strains were resistant to aztreonam, bacitracin and oxacillin. The range of the guanine-plus-cytosine content of the strain's DNAs was 43 – 54 mol%. The three strains HAR7100^T, HAR720^T and HAR200^T, differ phenotypically from each other in terms of antibiotic resistance, compositions of polar lipids, presence and absence of several enzyme activities such as amylase, protease alkaline phosphatase, G6PDH and 6PGDH. Moreover, the novel *Bacillus* strains were shown to differ genotypically by random amplified polymorphic DNA fingerprinting and 16S rDNA amplification patterns. Phenotypic and genotypic variations may support establishment of new strains of the same genus *Paenibacillus*.

الملخص

ثلاث عترات من البكتيريا اللاهوائية المحبة للحرارة و للملوحة المتوسطة الموسومة ب HAR200^T HAR720^T HAR100^T تم عزلها من موقعين للينابيع الساخنة في الاردن . و تتصف هذه العترات بانها تنمو في وسط ملحي 12-15% من كلوريد الصوديوم ولها القدره على النمو لاهوائيا (وليس هوائيا) على درجات حراره 37-63 مئويه ؛ وهي عصيات قصيرة متحركة ومتجرثمة وتفاعلها ايجابي مع صبغة غرام. وجدت هذه الدراسه ان محتوى قواعد (الجوانين + السيتوسين) في الحامض النووي لهذه العترات يتراوح ما بين 43-54 % كما ان تواجد او غياب انزيمات الاميليز والبروتييز والفوسفاتيز القلويه وانزيمي جلوكوز 6 فوسفات و 6 فوسفو جلوكونيت النازعين للهيدروجين ومحتواها من الدهنيات القطبيه ومقومتها لبعض الادويه والبصمه الوراثيه لمحتوي دي.ان. اي. العشوائي وتحليل بصمة 16 sDNA تثبت ان هذه العترات الثلاثه تختلف اختلافا جذريا بالصفات الجينية والنوعية مما يدعم كونها عترات جديدة من الجنس *Paenibacillus* .

Keywords: Paenibacillus Hashemite; Paenibacillus rimawi; Paenibacillus zara; moderate halophilic; hot springs.;

1. Introduction

Organisms isolated from hot springs have received considerable interest in recent years. The thermotolerant microorganisms are members of the genus *Paenibacillus.* Bacteria of the genus *Paenibacillus* are facultative anaerobic, endospore-forming, Gram-positive rods. Representatives of this genus are widely distributed in soil, water, air, the plant rhizosphere, food, and diseased insect larvae (Daane et. al., 2002). The genus currently contains over 30 species and is phenotypically related to other genera belonging to the family Bacillaceae (Chung et. al., 2000).

Few publications are devoted to the study of thermotolerant halophilic *Paenibacillus* species isolated from the hot spring due to their ubiquity and capability to survive under adverse conditions (McDonald, 2001).

This study describes the isolation and characterization of a moderate halophile and thermotolerant bacteria. We have concluded based on detailed phenotypic, chemotypic, and genotypic investigations. Followed by numerical analysis that the three novel isolates represent a new *Paenibacillus* species, for which the names *P. hashemite sp.* nov. (HAR710^T), *P. rimawi sp*. nov. (HAR720^T) and *P. zara sp.* nov. (HAR200^T) are proposed.

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2. Materials and Methods

2.1. Isolation of bacteria

Samples of hot springs water were collected from hot springs located in Ma'in and Zara, Jordan. Water samples were transported without temperature control and analysed within 24 hr. Untreated, one hundred microliter samples of water, were spread directly onto Thermus agar plates (Williams and Da Costa, 1992) which were sealed in plastic bags and incubated at 53°C for 24-48 hr. Colonies were purified by serial transfers. All isolates were preserved in Thermus media containing 15% glycerol at –70°C. Three isolates designated HAR710^T, HAR720^T, and HAR200^T were chosen for in depth characterization.

2.2. Phenotypic characterization

Strains isolated in the present study were characterized by conventional microbiological methods, using API20E, morphology of vegetative cells and sporangia, shape and position of spores. In addition, the following characteristics were studied: utilization of citrate; hydrolysis of casein, starch and gelatine; catalase; oxidase; urease; motility; haemolytic type; aerobic growth at 37°C and 63°C; anaerobic growth at 37°C and 63°C; oxidation/fermentation; acid production from glucose, lactose, mannitol, inositol, sorbitol, rhabinose, saccharose, melebiose, amylase and arabinose; growth at (4°C, 10°C, 25°C, 30°C, 37°C, 43°C, 53°C, 63°C and 73°C); and NaCl requirement (0, 0.5, 1, 3, 7, 10, 12, 15 and 18%). Growth at different pH (2-12) was detected on the medium that contained (wt/vol): 0.2% Bacto-peptone, 0.2% casein hydrolysate, 0.2% yeast extract, 0.1% glucose, 0.02% KH_2PO_4, 0.005% $MgSO_4.7H_2O$, 1.5% Bacto-agar, 50% (vol/vol) of natural hot-spring water and 50% distilled water. The pH was adjusted with 10 M NaOH.

Cluster analysis was performed using SPSS 12.0.1 software for windows. An unweighted pair group average method was used for cluster analysis, and using a percentage disagreement method to drew a dendrogram.

2.3. DNA base composition and fatty acid analysis

DNA was isolated from the cells grown overnight in nutrient agar. The G + C contents of the DNA were determined by the method of (Marmur, and Doty, 1962). Fatty acid composition was essentially studied as described by (Reischl, 1998).

2.4. Antibiotic resistance

Resistance to antibiotic was determined by using the routine diffusion plate technique. Cultures were grown overnight on the nutrient medium at 37°C and were used to prepare suspensions with optical density of 0.5 McFarland Standard (1.5 x 10^8 cells per ml). A 0.1 ml portion of suspension was plated onto agar and disks containing antibiotics which were plated onto surface of the medium. After overnight incubation at 37°C, the diameters of zones of growth inhibition were measured. The following antibiotics were used (mcg/disk): azlocillin (75 mcg), amoxicillin (25 mcg), aztreonam (30 mcg), cefadroxil (30 mcg), bacitracin (10 U), carbenicillin (100 mcg), cefactor (30 mcg), ciprofloxacin (5 mcg), cefoxitin (30 mcg), imipenem (10 mcg), cephalothin (30 mcg), cefamandole (30 mcg), norfloxacin (10 mcg), piperacillin (100 mcg), streptomycin (10 mcg), risemycin (30 mcg), tobramycin (10 mcg) and oxacillin (1 mcg).

2.5. Polar lipid composition

The isolates were cultivated to late log phase, harvested by centrifugation at 8000 rpm for 20 min, then washed in distilled water and reharvested. Organisms were lyophilised and stored anhydrously as a fine powder until required.

Polar lipids were analysed by two-dimensional thin layer chromatography (TLC) using Silica Gel prepared plates (Merk HPTLC RP-18F 259). The chromatograms were developed using chloroform: methanol: water (65:25:4) as the first solvent system and chloroform: methanol: acetic acid: water (80:12:18:5) as the second solvent system. A variety of reagents was used to detect and partially identify the lipids. Ethanolic molybdophosphoric acid was used as a non-specific destructive detection reagent. Sprayed plates were charred in a forced draught at 180-190°C for 15 min. Lipids containing free amino groups were identified by using 0.2% w/v of ninhydrin solution in water saturated butanol. Sprayed chromatograms were heated at 100-105°C for 10 min. Lipids counting free amino groups were identified as a red-violet spots. Alpha naphthol reagent 10.5 ml of 15% (wt/v) alpha naphthol in 95% ethanol solution to 6.5 ml concentrated sulphuric acid, 405 ml of 95% ethanol and 4 ml water were added. The lightly sprayed chromatogram that was heated for 10 min at 120°C was used for detection of sugars that appeared as purple-blue spots.

2.6. Enzymatic activity

The following condition was used to analyse enzyme activity at 53°C, pH 6.5 and different NaCl concentrations (0.5, 1, 5, 7 and 10%).

Protease enzyme activity was detected by measuring the diameter of the clear zone produced in peptone yeast extract agar plates in the presence of 2% skim milk (casein) as substrate (Al Baker et al, 2000). A positive result was indicated by a clear zone around a streak inoculation after flooding with 10% (wt/v) $HgCl_2$.

Amylase enzyme activity was detected by measuring the diameter of the clear zone produced in peptone yeast extract agar plates in the presence of 1% starch as substrate (Al Baker et al, 2000). A positive result was indicated by a clear zone around a streak inoculation after flooding with 1% (wt/v) Lugol's iodine solution.

Alkaline phosphatase activity was detected by measuring the yellow diameter zone produced in peptone yeast extract agar plates in the presence of 5 mM p-nitrophenyl phosphate as substrate in 50 mM glycine/NaOH buffer, pH 9, containing 10 mm $CaCl_2$, 1 mM $MgCl_2$ and 1 mM $ZnCl_2$.

Glucose-6-phosphate dehydrogenase (G6PDH) and 6-phosphogluconate dehydrogenase (6PGDH) activity were measured at 43°C using the method of (Hohorsts, 1965)

with slight modification, which depends on the reduction of $NADP^+$ to NADPH by G6PDH and 6PGDH. The activity measurement was made by monitoring the increase in absorbance at 340 nm, and the calculation of enzyme unit per litre (U/L) was done by assuming a molar extinction coefficient of 6270 U/L/mole for NADPH as described by (Wei-Ying and Tang, 1999).

2.7. Random amplified polymorphic DNA fingerprinting (RAPD) of the new isolates

PCR was performed in a volume of 50 µl containing 1 µl bacterial genomic DNA solution, 5 µl of 10X PCR reaction buffer, 200 µM of each nucleotide, 2.5 mM of $MgCl_2$, 1 µM of primer RAP-1 (5'-CAGCGACAAG-3'), 1 U of Taq polymerase and endonuclease free water up to 50 µl. All PCR materials were obtained from Promega (USA). The temperature profile was as follows: 5 cycles consisting of 94°C for 1 min, 31°C for 45 sec and 72°C for 2 min, and 25 cycles consisting of 92°C for 1 min, 40°C for 45 sec and 72°C for 45 sec; the final cycle was followed by an additional 7 min at 72°C. After amplification, 20 µl of the PCR product was electrophoresed in 1.5% agarose gel in TAE buffer and photographed by Digi-Doc camera (Vilber Lourmat).

2.8. 16S rDNA-based phylogenetic analysis

A DNA 16S-23S spacer region polymorphism analysis was performed in a volume of 25 µl containing: 1 µl of bacterial genomic DNA solution obtained as described by (Mora et al.; 1998), 2.5 µl of 10 x PCR reaction buffer, 200 µM of each dNTP, 2.5 mM of $MgCl_2$. 0.3 µM of each primer and 1.3 U of Taq polymerase. Primer set C (forward primer 5-GTCGTAACAAGGTAGCCGTA-3' and reverse primer 5'-CAAGGCATCCACCGT-3') was used to amplify the IIS region between the 16S and the 23S rDNA genes. The temperature profile was the following: 5 cycles consisting of 94°C for 1 min, 55°C for 1 min and 72°C for 2 min, and 30 cycles consisting of 92°C for 45 sec, 60°C for 45 sec, 72°C for 2 min and the final cycle was followed by an additional 7 min at 72°C. After amplification, 8 µl of product was electrophoresed in agarose gel.

3. Results

Differentiation between novel isolates and other related *Paenibacillus* species:

3.1. Phenotypic characteristics.

Morphological, biochemical, physiological and genetic composition tests of the novel isolates and other selected *Paenibacilli* are shown in Table-1. Cells of the novel isolates were Gram-positive, motile, spore forming rods of approximately 1.0-3.0 x 0.5-1.5 µm size. The new isolates produced spherical endospores in swollen sporangia in the central or subterminal region of the cell (Figure-1). Novel isolates were facultatively anaerobic, able to grow at 18% NaCl, catalase and oxidase positive and grew well on sheep blood and chocolate agar but not on MacConkey and xylose lysine desoxycholate agar. On sheep blood agar, the beta hemolytic colonies were grayish white with a convex elevation and regular margins.

Growth was found to be optimal at 43°C, 52°C and 50°C, with a range from 37°C to 63°C. In contrast, the optimal growth temperature for the majority of *Paenibacillus* species were 28 to 30°C (Shida et. al.; 1997) and 42°C (Bosshard et. al.; 2002). Contrary to almost all other *Paenibacillus* species, novel isolates were negative for Voges-Proskauer test, lactose, xylose, sucrose, melebiose and growth in 0.001% lysozymes. They were able to grow at 63°C and at pH 4 with optimum NaCl concentration of 15% (unpublished data). The new isolates had low G+C DNA content compared with other known *Paenibacillus* species (Table-1).

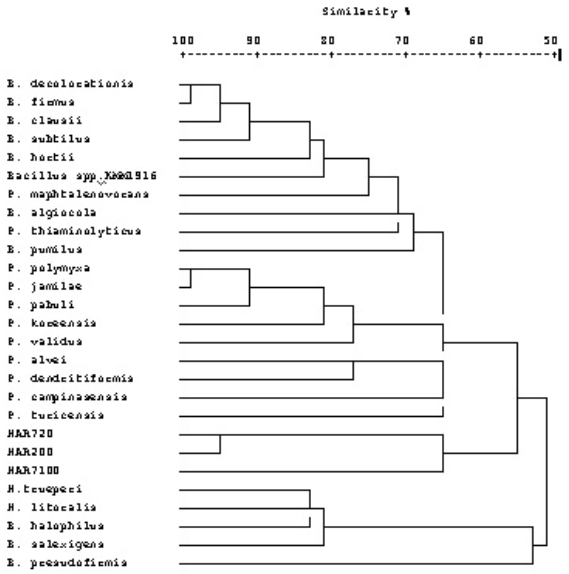

Figure-1. Dendrogram of relationship based upon similarity of average linkage clustring based on data obtained from this study; data obtained from Bosshard et al. (2002); data obtained from Bosshard et al. (2002), Daane et al. (2002), Shida et al. (1997a, b); data obtained from Bosshard et al. (2002) and Arguilera et al. (2001); data obtained from Enright et al. (2003) and Elo et al. (2001); data obtained from Chung et al. (2000); data obtained from (Velázquez et al. 2004).

3.2. Chemotaxonomy.

Cellular fatty acids analysis is shown in Table-2. The predominant fatty acids in whole-cell methanolysate of novel species were anteiso-15:00 (59.7, 38.6 and 29.1%, respectively), 16:00 (20.5, 15.2 and 8.9%, respectively), 17:00 (15.4, 20.1 and 9.0%, respectively) and iso-16:00 (20.1, 14.5 and 15.1%, respectively).

Table-1. Phenotypic characteristics of novel isolates and other related *Paenibacillus* species; (1) *P. hashemite*; (2) *P. rimawi*; (3) *P. zara*; (4) *P. turicensis*; (5) *P. alvei*; (6) *P. pabuli*; (7) *P. validus*; (8) *P. jamilae*; (9) *P. polymyxa*; (10) *P. macerans*; (11) *P. koreensis*; (12) *P. favisporus*; C= Central; St= Subterminal; T= Terminal; Sph= Spherical; Elp= Ellepsiodal; LTk= Long thick rods; SR= Short rods; β= Beta hemolysis; γ= Gamma hemolysis (nonhemolytic); WFRF= White, filamenteous, raised and filamanteous; WCUU= White, circular, umbonate and undulate; WFRU= White, filamentous; raised and undulant; GCRE= Green, circular, raised and entire; GCUU= Green, circular, umbonate and undulant; WCRU= White, circular, raised and undulant; GCUE= Green, circular, umbonate and erose; WCCT= White, circular, convex and translucent; LAS= layer at surface only. NR = not reported; $ Data obtained from this study; # Data obtained from (Bosshard et al.; 2002); & Data obtained from (Daane et al 2002; Shida et al.; 1997, and Bosshard et al.; 2002); * Data obtained from (Bosshard et al.;2002 and Aguilera et al,2001); ^ Data obtained from Enright et al.; 2003 and Elo et al.; 2001); Data obtained from (Chung et al.; 2000); ~ Data obtained from (Velázquez, et al.; 2004)

Characteristics	1[$]	2[$]	3[$]	4[#]	5[&]	6[&]	7[&]	8[*]	9[&]	10[^]	11[!]	12[~]
Microscopic examination:												
Spore position	C/St	C	C/St	T/St	C/T/St	NR	NR	NR	C/St	T/St	T	St
Spore morphology	Sph	Sph	Sph	Elp	Sph	NR	NR	NR	Sph	Sph	Elp	Sph
Cell morphology	LTk	SR	SR	SR	LTk	NR	NR	NR	LTk	LTk	SR	SR
Culture examination:												
Colony morphology	WFRF	WCUU	WFRU	GCRE	GCUU	NR	NR	NR	WCRU	GCUE	WCUU	WCCT
Broth growth	LAS	LAS	LAS	LAS	LAS	NR	NR	NR	LAS	LAS	LAS	LAS
Type of hemolysis	β	β	β	γ	γ	NR	NR	NR	β	β	NR	NR
Temperature growth (ºC):												
Minimum	28	37	37	15	15	10	15	30	28	NR	10	28
Optimum	43	52	50	42	28	30	30	30	30	NR	40	37
Maximum	63	63	63	48	43	48	53	40	42	NR	50	43
pH growth:												
Minimum	4	4	4	5.5	6.5	5.6	NR	5	4	4	NR	NR
Optimum	6.5	8	9	7	7	7.5	NR	7	6.5	6.5	NR	5
Maximum	7	11	11	9.5	8.5	8	NR	12	8	7.5	NR	7
NaCl growth:												
Minimum	12	12	12	3	4	3	NR	NR	NR	NR	NR	NR
Optimum	15	15	15	5	4	3	NR	NR	NR	NR	NR	NR
Maximum	18	18	18	5	5	4	NR	NR	3	NR	NR	5
Utilization of citrate	-	-	-	-	-	-	-	-	-	NR	-	-
Hydrolysis of:												
Casein	+	-	-	-	-	-	-	-	+	-	+	-
Starch	+	+	+	+	+	+	+	+	+	+	+	+
Gelatin	+	-	-	-	+	-	-	+	+	-/+	NR	+
VP test	-	-	-	+	+	-	-	+	+	-	NR	+
Nitrate reduction	-	+	+	-	-	-/+	-/+	+	+	+	-	+
N2 gas	-	+	+	NR	NR	NR	NR	NR	NR	NR	+	NR
Indole	+	+	-	-	+	-	-	NR	-	NR	-	-
Catalase	+	+	+	-	+	+	+	+	+	NR	+	+
Oxidase	+	+	+	-	+	-	-	-	-	NR	+	+
Urease	-	-	-	-	+	-	+	NR	-	NR	-	+
TDA	-	-	+	-	-	-	-	NR	-	NR	+	-
ODC	-	+	+	-	-	-	-	NR	-	NR	-	NR
LDC	+	-	-	-	-	-	-	NR	-	NR	NR	-
ONPG	+	+	-	+	+	+	-	-	+	+	+	+
LV	+	-	-	NR	NR	NR	NR	NR	NR	NR	NR	NR
Growth in 0.001% lysozyme	-	-	-	+	+	-	NR	+	+	NR	+	NR
Anaerobic growth	+	+	+	+	+	-/+	-	+	+	+	+	+
% G+C	50	54	43	NR	46	49	54	NR	44	52	54	53
Carbohydrates, acid from:												
Glucose	+	+	+	+	-	+	+	+	+	NR	+	+
Lactose	-	-	-	+	-	+	-	+	+	+	+	+
Arabinose	-	-	-	-	-	+	-	-	+	+	+	-
Mannitol	-	-	+	-	-	+	+	+	+	+	+	+
Xylose	-	-	-	+	-	+	+	+	+	+	-/+	+
Sucrose	-	-	-	+	+	+	+	+	+	+	+	+
Amylase	-	-	-	-	-	-	-	-	-	-	-	+
Melebiose	-	-	-	-	+	+	+	+	+	+	NR	+
Inositol	-	-	-	-	-	-	+	-	-	-/+	+	-
Sorbitol	-	-	-	-	-	-	-	-	-	-	+	-

Table-2. Fatty acid composition (% of total) of novel isolates and several other species of the genus *Paenibacillus* (Data were taken from (Daane et al 2002; Bosshard et al.; 2002 and Enright et al.; 2003).

Paenibacillus species	Saturated acids							
	Straight-chain			Iso-branched		Antesio-branched		
	$C_{14:00}$	$C_{15:00}$	$C_{16:00}$	$C_{15:00}$	$C_{16:00}$	$C_{15:00}$	$C_{16:00}$	$C_{17:00}$
P. hashemite	6.2	1.5	15.6	12.1	20.1	59.7	20.5	15.4
P. rimawi	4.1	0.5	8.9	10.6	14.5	38.6	15.2	20.1
P. zara	3.5	2.1	1.5	6.1	15.1	29.1	8.9	9.0
P. turicensis	8.9	ND	25.4	5.8	4.1	41.1	ND	ND
P. macerans	3.7	0.5	17.9	2.6	17.1	34.5	ND	16.1
P. korensis	ND	ND	ND	ND	20.6	51.1	28.3	ND
Other *Paenibacillus* species	0.2-3.7	ND-3.5	1.5-24.7	0.2-27.1	0.2-7.1	34.5-81	ND	1-30.2

ND, not detected.

3.3. Numerical analysis.

The numerical study of the selected species and the reference species using Jaccard coefficient (Sj), and clusters of species were obtained by average linkage (UPGMA) analysis. Clustered by the unweighted average linkage methods, gave the dendrogram shown in Figure 1. Phenotypic and chemotaxonomic characters resulted in dendrogram divided into three areas at similarity level 50%.

Area I divided into two clusters at similarity level 53%, cluster I contains one species *B. presudofirmis* in class I while class II contains four species *B. salexigens*, *B. halophilus, H. litoralis* and *H. trueperi* showing 81-83% intrasimilarity level.

Area II contains twelve species and divided into two subareas. Subarea I contains three species divided into two clusters at similarity level 65.0%. It contains one species *P. hashemite* sp. nov., while cluster II contains two species *P. rimawi* sp. nov. and *P. zara* sp. nov. at similarity level 96%. On the other hand, Subarea II contains nine species clustered into three clusters. Cluster I contains two species *P. turicensis* and *P. campinasensis* at intrasimilarity level 65%. Cluster II contains two species *P. dendritiformis* and *P. alvei* at intrasimilarity level 76%. Cluster III is divided into four subclusters at similarity level 91%. Subcluster I contains species *P. validus* at overall similarity level 76%; subcluster II contains species *P. koreensis* at overall similarity level 81%; subcluster III contains species *P. pabuli* at overall similarity level 91% and subcluster IV contains two species *P. jamilae* and *P. polymyxa* at intrasimilarity level 99%.

Area III is divided into nine clusters, 1-9 contains species *B. pumilus* (69%), *P.* thiaminolyticus (71%), *B. algiocola* (71%), *P. maphtalenovorans* (75%), *Bacillus spp.* KMM1916 (81%), *B. hortii* (83%), *B. subtilus* (91%), *B. clausii* (96%), *B. firmus* and *B. decolorationis* (99%) similarity level, respectively.

3.3.1. Differentiation of the three novel isolates

The phenotypic variability between the three novel species, were further discriminated using antibiotics resistance, polar lipid composition, enzymatic activity, RAPD , 16s rDNA and advanced PCR with specific markers for *Bacillus* species.

3.3.2. Resistance to antibiotics by the novel isolates.

The resistance of the new isolates to several antibiotics is shown in Table-3. They were resistant to aztreonam, bacitracin and oxacillin. Whereas, *P. zara* sp. nov. was resistant to streptomycin and *P. hashemite* sp. nov. was resistant to cefadroxil. On the other hand, both *P. zara* sp. nov. and *P. rimawi* sp. nov. were resistant to tobramycin.

Table-3. Resistance to antibiotics by the novel isolates.

Antibiotics	*P. hashemite*	*P. rimawi*	*P. zara*
Azlocillin (75 mcg)	N	N	N
Amoxicillin (25 mcg)	N	N	N
Aztreonam (30 mcg)	P	P	P
Cefadroxil (30 mcg)	P	N	N
Bacitracin (10 U)	P	P	P
Carbenicillin (100 mcg)	N	N	N
Cefactor (30 mcg)	N	N	N
Ciprofloxacin (5 mcg)	N	N	N
Cefoxitin (30 mcg)	N	N	N
Imipenem (10 mcg)	N	N	N
Cephalothin (30 mcg)	N	N	P
Cefamandole (30 mcg)	N	N	P
Norfloxacin (10 mcg)	N	N	N
Piperacillin (100 mcg)	N	P	N
Streptomycin (10 mcg)	N	N	P
Risemycin (30 mcg)	N	N	N
Tobramycin (10 mcg)	N	P	P
Oxacillin (1 mcg)	P	P	P

N: Negative P: Positive

3.3.3. Polar-lipid composition of the novel isolates.

The polar lipid compositions of the novel isolates are shown in Table-4. All tested organisms had phosphatidylethanolamine (PE), phosphatidylglycerol (PG), phosphatidylcholine (PC) and phosphatidylinositol (PI); on the other hand, phosphatidylserine (PS) and phosphoglycolipid (PGL1, PGL2 and PGL3) were absent.

Simple lipid (SL) was present in *P. hashemite* sp. nov. and *P. rimawi* sp. nov. but was absent from *P. zara* sp. nov.. Moreover, biphosphatidylglycerol (DBG) and sulfoquinovosyldiacylglycerol (SQDG) were absent in *P. hashemite* sp. nov.. On the other hand, digalactosyldiacylglycerol (DGDG) was present in *P. rimawi* sp. Nov. only.

3.3.4. Enzymatic activity.

The enzymatic activities of the novel isolates are shown in Table-5. All enzymes were tested at 53°C. All tested isolates showed activity in the presence of high concentration of NaCl up to 7%. The exception was for alkaline phosphatase activity, from *P. rimawi* sp. nov. that showed activity only in the presence of 7% NaCl; whereas, *P. zara* sp. nov. had activity in NaCl concentrations of up to 5%, but no activity observed for *P. hashemite* sp. nov.

Table-4. Polar lipid composition of the novel isolates.

Polar lipid composition	*P. hashemite*	*P. rimawi*	*P. zara*
Simple lipid	P	P	A
Glycolipid	P	A	P
Monogalactosyldiacylglycerol	P	A	P
Phosphatidylethanolamine	P	P	P
Biphosphatidylglycerol	A	P	P
Phosphatidylglycerol	P	P	P
Phosphatidylcholine	P	P	P
Digalactosyldiacylglycerol	A	P	A
Sulfoquinovosyldiacylglycerol	A	P	P
Phosphatidylinositol	P	P	P
Phosphatidylserine	A	A	A
Phosphoglycolipid III	A	A	A
Phosphoglycolipid II	A	A	A
Phosphoglycolipid I	A	A	A

A: Absent P: Present

3.3.5. Genotypic characterization.

Polymorphism of these organisms is highlighted by both RAPD test and 16S rDNA. Genetic variability studies by RAPD test are presented in (Figure-2a); while the 16S rDNA studies are shown in (Figure-2b). Genetic variability among these novel strains was clearly demonstrated.

4. Discussion

In this paper we describe the characterization of a group of moderate halophilic spore forming bacteria, including strains HAR7100^T, HAR720^T and HAR200^T, isolated from two Jordanian hot springs located in different geographical areas. The novel isolates appear to resemble species within the genus *Peanibacillus* based on their phenotypic, chemosystematic standard and phylogenetic properties.

The chemotaxonomic data, i.e. G+C content of DNA (range from 43 to 50) and the antesio-15:00 fatty acid as the major cellular fatty acid (range from 59.7 to 29.1%) also falls within the ranges exhibited by *Paenibacillus* species (Shida et al., 1997; Yoon et al., 1998; Chung et al., 2000). A comparison of the phenotypic characteristics of strains HAR7100^T, HAR720^T and HAR200^T with those of phylogenetically related *Paenibacillus* species such as: *P. turicensis, P. alvei, P. pobuli, P. validus, P. jamilae, P. polymyxa, P. macerans, P. koreensis* and *P. favisporus*, revealed that these novel strains were very different. The novel strains were negative for VP test, able to grow at 63^{o}C at pH 4 with optimum growth at NaCl concentration of 15%. Lactose, xylose sucrose and melebiose were not fermented by the isolates. The isolates were also able to grow anaerobically but not aerobically at 63^{o}C.

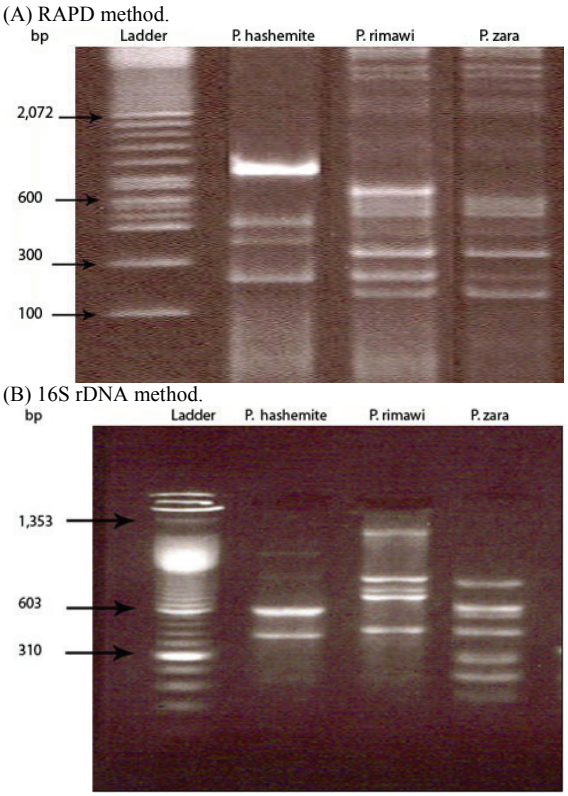

Figure -2. Comparison between patterns of the novel isolates by (A) RAPD and (B) 16S rDNA methods using PCR technique.

The three novel *Paenibacillus* strains have slight different phenotypic characteristics among each other in terms of growth conditions, resistance to antibiotics, and composition of polar lipid, enzymatic activity and fatty acid composition as demonstrated by this study.

DNA finger printings analysis, showed distinctive amplification patterns that attain a useful clustering at the genus level. RAPD and 16S rDNA amplification patterns explain polymorphism present among the three novel strains that belong to the same genus. The DNA analysis provides a more stable determination for the identification of the new isolates (Wisotzkey, et al.; 1992, Aguilera et al.; 2001).

Further DNA genetic tests such as DNA sequencing, 16s rRNA and DNA-homology are needed for precise characterization of these new different isolates.

Table-5. Some enzymatic activity at different NaCl concentrations, 53^{o}C and pH 6.5 of the novel isolates.

Bacillus	***P. hashemite***					***P. rimawi***					***P. zara***				
NaCl (%)	0.5	1	5	7	10	0.5	1	5	7	10	0.5	1	5	7	10
Amylase	P	P	P	N	N	P	P	P	N	N	P	P	P	N	N
Protease	P	P	P	P	N	P	P	P	N	N	P	P	P	N	N
Alkaline phosphatase	N	N	N	N	N	N	N	N	P	N	P	P	P	N	N
G6PDH	P	P	P	P	P	P	P	P	P	N	P	P	P	P	N
6PGDH	P	P	P	P	P	P	P	P	P	N	P	P	P	P	N

G6PDH= Glucose-6-phosphate dehydrogenase; 6PGDH= 6 Phosphogluconate dehydrogenase; P= Positive; N= Negative.

Aknowlegment

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Chemical Stress Response of Wild Oat to 1, 2, 7, 8-Diepoxyoctane Treatment

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Abstract

This work aimed for positive genetic or physiological alteration in the quantitative traits of oat species specifically and other cereals in general in a way that may reflect human benefits in terms of increasing crop yield for both human and livestocks consumption. The results indicated that the treatment of both diploid oat species *(Avena clauda* and *A. eriantha*) with 100 μl of the chemical mutagen 1,2,7,8-Diepoxyoctane (DEO) as compared with the 200 µl and the untreated ones (control) had resulted in an increased final plant height (82-104 cm as compared with 70-9 cm for the treated with 200 µl and 12-48 cm for the untreated plants of *A.clauda* and *A. eriantha* respectively). Also, the former treatment had lead to an increase in ; leaf number (18-16 respectively as compared to 15-2 in the 200 µl treatment and 4-14 in the untreated). Furthermore, the 100 μl treatment had induced the germination of fertile node tillers (2-3) and apical tillers (6-9) which had increased the spikelets number between 16-26 within the node and apical tillers for *A. clauda* and between 4-5 for *A. eriantha* compared to 1-6 in the other treatments which is related to the complete absence of node tillers and to the presence of just single apical panicle in the other treatments. The presence of several fertile apical and node tillers in the diploid species as a result of treatment with 100 μl – DEO had reflected 4-5 folds increase in plant productivity in terms of increasing leaf number and seed density. These results may suggest that such tillers had arised as a result of physiological and/or chemical stress responses to DEO treatment rather than to genetic effects, Since the F2 progeny which is resulted from DEO-untreated F1 had shown no tillers as compared to the re-treated ones. These findings may suggest that the re-application of the 100 μl DEO treatment on cultivated oats as well as other cereals may be beneficial in terms of increasing productivity and this requires further studies on such plants.

الملخص

تهدف هذه الدراسه إلى إحداث تغيير أو تعديل وراثي أو فسيولوجي ايجابي في الصفات الكميه لمحصول الشوفان والمحاصيل ألحقليه عامة بطريقة تعكس منفعة بشريه ممثله بزيادة المحصول للاستهلاك البشري وتغذية الحيوانات الداجنة. تظهر التحاليل الإحصائية للنتائج بان معالجة أنواع الشوفان التي تحوي أربعة عشر كرموسوما (*Avena clauda* and *A. eriantha*) بمقدار 100 µl من المطفر الكيميائي DEO -Diepoxyoctane 1,2,7,8 أدت إلى زيادة كبيره في كل من طول النبات النهائي, عدد الأوراق (زياده بمقدار 3.5 ضعف), زياده في طول الشمراخ الزهري (بمقدار 2 - 15 ضعف) وزيادة في طول موسم النمو. مقارنة مع العينات المعالجة بمقدار 200 µl من نفس المركب.DEO حيث عكست المعالجه الثانيه 200 µl تأثيرات سلبيه على الصفات المذكوره أعلاه في جميع الأنواع الخاضعه للدراسة. إضافة إلى ذلك فأن المعالجة الأولى 100 µl للأنواع ذات الأربعة عشر كروموسوم أدت إلى ظهور تفرعات خصبه في كل من منطقه القمة النامية للنبات وعند العقد على طول الساق. نستطيع أن نستنتج بأن ظهور صفات كمية مرغوبه هو ناجم عن استجابة للتغيرات الفسيولوجية في النبات أو استجابة لتأثير المركب الكيميائي المستخدم DEO وليس لتأثيرات وراثيه , حيث أن أفراد الجيل الثاني F2 غير المعالجه بالمركب الكيميائي أظهرت نموا طبيعيا في حين أن المعالج منها أظهر زيادة إنتاجيه عن طريق زيادة إنتاجية الصفات الكمية من حيث زيادة طول النبات وعدد الأوراق والبذور وكذلك ظهور التفرعات الخصبه من القمم الناميه وعند العقد لبعض النباتات . وهذا يعد صفة ايجابيه مرغوبه في النبات كونها تؤدي إلى مضاعفة الانتاجيه على الرغم من أن مثل هذه الصفات لم تكن مورثه من جيل إلى آخر. تفيد هذه النتائج بان اعادة تطبيق هذه المعالجه 100 µl من المركب DEO على النباتات الاقتصاديه التي تزرع في البيوت البلاستيكيه أو الزجاجيه للاستهلاك البشري قد تؤدي الى زياده كبيره في كمية المحصول وهذا يتطلب اعادة الدراسه على مثل هذه النباتات مستقبلا.

Keywords: Avena species; 1,2,7,8-diepoxyoctane treatment; Apical and node Tillers;

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1. Introduction

Wild Oats (*Avena L.*) Species grow as weeds in Jordan and mostly found as contaminants in fields of cultivated crops, but as a crop in world production, it ranks fifth among all cereals and is distinctive among cereals due to their relatively high protein content. Seven species of Oats occur at three ploidy levels, diploids (5 species), tetraploids (1 species) and hexaploids (1 species) (Ladizinsky and Zohary, 1971; Kanan, 1987). Concerning the three oat species which were included in this particular work, The diploid species (*A. clauda Dur* and *A. eriantha Dur*) were found to be very restricted in their microenvironments when compared with the other species from different ploidy levels including the wild hexaploid species *Avena sterilis* which is characterized by having a wide range of distribution world wide and throughout the geographical regions of Jordan (Sampson, 1954; Ladizinsky and Zohary, 1971; Rajhathy and Thomas, 1974; Price and Kahler, 1983; Kanan, 1987; Jaradat, 1991; Kanan and Jaradat, 1996). The geographical differentiation between the species of the three ploidy levels may be related to specific genetic variability that provides each particular species with specific features to enhance its adaptation to the microenvironment in which it survives (Imam and Allard, 1965). The aim of this study is to evaluate the potential genotoxic or cytotoxic effects of the chemical mutagen 1,2,7,8-Diepoxyoctane (DEO) treatment by means of in-vitro short-term mutagenicity tests on growth and survival of wild oat plants collected from Jordan. Also, to specify its significant effects on various (19) quantitative traits as compared with the control (DEO-untreated) or the natural populations, since no previous work had indicated the effect of DEO or other related compounds on oats or other cereals up to our knowledge. Several previous works had reported the genotoxic activity of several mutagens including DEO on the filamentous fungi including *Aspergillus nidullans* and *A. niger* were the mutagenic activity was tested by selecting chlorate and /or bromate resistant mutant strains that lie in genes responsible for nitrate assimilation (Kanan, 1996; Appleyard, *et al*., 1998). The obtained results had confirmed that DEO was serving as a strong mutagen (Kanan, 1996; Appleyard, *et al*., 1998; Kanan, *et al*., 2002; Kanan, 2002; Al-Najar, 2005 and Kinghorn *et al.*, 2005). In order to discriminate between genotoxic and cytotoxic mechanisms of DNA fragmentation, time-dependant dose response relationships for the induction of DNA double-strand breaks (DSB) was assessed by pulsed-field gel electrophoresis (PFGE) using cultured human lung epithelial cells treated with diepoxides where, the viability was evaluated by cytotoxicity tests (Cedervall, *et al*., 1995; Vamvakas, *et al*., 1997; Vock, *et al*., 1998; and Vock *et al*., 1999). Results obtained from such tests had confirmed that the chemical agent DEO and other diepoxides have induced DSB by a genotoxic mode with concentrations that did not affect cell survival (Blocher, *et al*., 1989; philips and Morgan, 1993; and Vock *et al*., 1999) . The diepoxybutane induced cross-links were of the unreparable types while, considerable repairs were observed for the DEO induced cross-links. However, DSB induction by formaldehyde and glutaraldehyde were found to be the consequence of extragenomic damage and viability loss (Saladino, *et al*., 1985 and Vock *et al*., 1999). Furthermore, the chemical mutagen 1,2,7,8-diepoxyoctane (DEO) whose deletogenic activity was also demonstrated in ad-3 system of the fungus *Neurospora crassa* and then in different fungal and bacterial species, has also been tested in *Samonella typhimurium* tester strain *(hisG428*) (de Serres, *et al*., 1995 and Picada, *et al*., 1999; Martinez, *et al*., 2000). The obtained results had confirmed that DEO is a cross-linking deletogenic agent and considered as a direct acting mutagen (Picada, *et al*., 1999). The sensitivity responses of two groups of *pso* mutants of *Saccharomyces cerevisiae* (The first; is involved in repairing damaged DNA or in RNA processing whereas the second group is related to metabolic steps but not responsible for DNA repair) towards three mutagens including DEO indicated that all mutants of the DNA repair group were sensitive to DEO while the other group of mutants had revealed wild-type or near wild-type resistance to all tested mutagens (Henriques, *et al*., 1997 and Pungartnik, *et al*., 2002). The relative DNA intra-strand cross-linking capabilities of several diepoxides (including DEO) with respect to chain length, molecular flexibility, carcinogenic potential, and DNA sequences targeted had been examined with a fragment of the 5S RNA gene of *Xenopus borealis*. Sites and efficiencies of intrastrand cross-linking were probed through denaturing polyacrylamide gel electrophoresis and quantitative phosphorimagery (Millard and Wilkes, 2001). Results obtained from such conducted mutagenicity and carcinogenicity tests using Mono-and Diepoxides had confirmed that the diepoxides (1,2,5,6-diepoxihexane and 1,2,7,8-diepoxyoctane) share the 5'-GNC target sequence and DEO also targeted 5'—GNNC sites where the efficiency of cross-linking this sequence may reflect carcinogenicity (Yunes, *et. al*., 1996 and Millard and Wilkes, 2001). The carcinogenicity of epoxide compounds has been attributed to covalent binding to DNA. Whereas, monoepoxides form only mono-adducts, diepoxides can form both mono-adducts and intra-strand cross-links (ICL) and show higher carcinogenicity and mutagenicity than their monoepoxide analogues (Yunes, *et al*., 1996 and Millard and Wilkes, 2001) where it is believed that ICLs are more significant cytotoxic lesions (Yunes, *et al*., 1996, Picada, *et al*., 1999, Vock, *et al*., 1999).

2. Materials and Methods

2.1. Collection of wild oat samples

The wild oat samples were collected from natural populations within the two restricted environmental sites for the included diploid species within the mountainous region of Jordan. The species *A. clauda Dur.* was found to be restricted to Mehna-Ajlun area (Lat: 32 22; Long: 35 46, Alt: l029) whereas, *A. eriantha Dur.* was found to be restricted to AL-ramaimeen-Al-Salt area (Lat: 32 07; Long 35 47; Alt: 671). In contrast, *A. sterilis* has been shown to be widely distributed throughout the mountainous region and Jordan valley. Fifty samples of mature (i.e. fruiting stage) whole plants and seeds from each species were

collected from the target regions. 19 quantitative traits were recorded for each plant and these are: plant height; date of seedling emergence; date of leaf emergance; leaf number; leaf length; leaf width; internode distance; date of panicle emergance; mature panicle length; number of flowering whorles; number of spikletes; distance between whorles; glume length; glume width; floret size; awn length; ground tillers. The collected seeds were subjected to a lobratory work where each treatment was repeated just once (i.e.Two independent experiments).

2.2. Germination of 1,2,7,8-diepoxoctane (DEO)-untreated seeds

Germination of seeds was induced by dehulling and piercing their coats with a 5 ml syringe needle (2-3 holes) followed by placing the seeds on three discs of filter paper (9 cm in diameter) in 9 cm petridishes. The seeds were considered germinated when the radical and the plumule become visible. Germinated seeds were transplanted at a depth of 3-4 cm in 12 x 12 cm plastic pots containing 3:1 claylaom: peatmoss. 20 pots with 4 plants/each Pot were used for each species. Pots were placed in lightened shelves (18.81KLx) inside the laboratory (10 x l0 m) under 16 hours light period and the following physical conditions were scored at least once /day throughout the growing season: light intensity, air temperature and %RH. Where the scored ranges of temperature and %RH were as follows: June 25.2C°-28.8C° and 26.1 – 80.3 %RH; July : 27.6C° – 30.3C° and 87.9 - 95.2 %RH; August, 26.4C° - 33.2C° and 77.3 – 95.4 % RH.

2.2.1. Germination of 1,2,7,8-diepoxoctane treated seeds

Germination of seeds was induced as described above except that seeds were soaked for 6 hours in tap water then treated with DEO. Where, a wide range (i.e. 5, 10, 20, 40, 80, 100, 200, 300, 400, and 500 μl per 25 ml water) of DEO concentrations for various incubation periods (i.e. 3, 5, 10, 15 and 30 min) was used. After treatment, seeds were washed three times every 5 min with sterile tap water to remove any traces of the mutagen. Seeds were placed on three wet discs of filter paper in 9 cm petridishes to germinate then transplanted in pots as mentioned above. However, 33%-57% of survivals was generated within 10 days with seeds treated for only 3 minutes with 100 µl and 200 µl DEO. Whereas, the other combinations of treatments were showing a range of 0.0%-83% over different incubation periods ranging from 3 to 30 minutes. Therefore, the decision was to treat seeds with 100 µl and 200 µl DEO where only one third to one half of seeds have survived.

2.2.2. Generation of F2-progeny from the studied species

In order to determine that the obtained results (i.e apical and node tillers) had arised as a result of genetical events and will be transmitted to the succeeding generations or due physiological or chemical stress responses as a result of DEO treatment. Fourty DEO–untreated fertile seeds of F1 generation from each of the three studied species were grown to maturity as mentioned above, another fourty seeds from each species were treated with 100 µl DEO (i.e. The treatment that had yielded apical and node tillers) until maturity.

2.3. Measurements of the studied traits

Every single plant was checked daily for scoring the date of emergence for every single leaf or panicle. Plant height was measured every 3 days starting from the date of implantation in soil until each plant has reached maturity (i.e. forming mature panicle) then the internode distance was measured using scaled ruller. Whereas, the remaining traits were scored at maturity.

2.4. Statistical analysis

From the experimental observation there were apparent difference between the treatments on the studied traits. Therefore, to test the statistical significance of these differences, SPSS programme (version 10) was used to carry out one way ANOVA for F1 progeny in order to determine the effect of different treatments on the studied variables while, two way ANOVA was used to study the effect of the applied treatment, the plant species and the interaction between them on the studied variables generated from F1 progeny this is followed by Post Hoc comparisons (Sheffe test). The results of the statstical analysis were used to study the significant differences of applied treatments on the studied quantitative traits within and between studied species. The results of different treatments on plant height, leaf number and percentage of survivals for tested plants from the three studied species were expressed as means ± SE and analyzed statistically by student's t-test using SPSS programe. *P* values of less than 0.05 was considered as the lowest limit of significance.

3. Results

All of the analyzed results presented here were obtained from the F1 (DEO-treated and untreated) generation. The F2 plants generated from the treated with 200 µl DEO or the untreated F1 were just like the natural plant population and no single plant had shown apical or node tillering capacity. Whereas, six *A. clauda* F2 plants (Re-treated with 100 µl DEO) out of fourty that were generated from the 100 µl DEO treated F1 had restored the tillering capacity.

3.1. Statistical analysis

Results presented in table (1) indicated that there is a highly significant effect of treatments on all of the studied quantitative traits (16 traits) from the three studied species except for the awn length in *A. sterilis,* the distance between whorles in *A. eriantha* and the date of emergence in *A. clauda*.

3.1.1. Multiple comparison by sheffe method – one way ANOVA

***A.clauda*/ Plant height**: Results presented in table (2) indicated that the applied treatments had significantly affect the plant height of *A. clauda*. Where, the highest plant height was obtained after 100 μl DEO treatment (104.0 cm) followed by the 200 μl DEO treatment (70.3571 cm) then the untreated (control) plants where the

plant height had reached only 12.6375 cm. However, plants from the natural population had shown a final height of 45.4231 cm.

Leaf number:- No significant difference in leaf number was noticed between plants from natural population and the DEO-untreated (control) ones, where the leaf number has reached 3.7375 and 5.3077 respectively. Also, no significant difference in leaf number was noticed between plants treated with 200 µl DEO or 100 µl where the leaf number has reached 15.5536 and 16.000 respectively. However, both DEO-treatments have shown 3 folds increase in leaf number as compared with the control (Table 2).

***A. eriantha*/Plant height:** The obtained results indicated that the 100 µl DEO treatment had significantly affected the plant height where it had shown a 9-folds increase in plant height (82.1642 cm) as compared to the ones treated with 200 µl DEO (9.2237 cm). In addition, the former treatment had shown a double-fold increase as compared with the control (48.2167 cm). However, no significant difference in plant height was noticed between natural population plants (50.688 cm) and the untreated - control (48.217 cm) (Table 2).

Leaf number: The applied treatments had significantly affect the leaf number of *A. eriantha* plants. Where, the highest number was obtained after 100 µl DEO treatment (17.97) and the least (1.338) was obtained after 200 µl DEO as compared to 14.62 leaves obtained by the untreated (control) plants (Table 2).

Panicle length: The applied treatments had significantly affect the panicle length of *A. eriantha* plants. Where, the longest panicle was obtained after 100 µl DEO treatment (14.81 cm) and the least (0.844 cm) was obtained after 200 µl DEO as compared to 7.82 cm long obtained by the untreated (control) plants. These findings indicated that the 100 µl DEO treatment had shown 15-folds increase as compared with the plants treated with 200 µl DEO. and just 2 folds increase when compared to the untreated ones (Table 2).

Number of whorles: The 100 µl DEO treatment had significantly affected the number of whorles where, it had shown 20-folds (6.48) increase in whorles number as compared with the 200 µl DEO treatment (0.33) and 1.5 folds compared to the control (Table 2).

Number of spikelets: The plants treated with 100 µl DEO have shown an approximate of 2-folds increase in number of spikelets per plant (7.045) compared to the untreated plants (4.1). However, the former traetment had shown an approximate of 25 folds increase as compared to 200 µl DEO treatment (0.3250). These findings were based on a single apical panicle but in fact the 100 µl DEO treatment has lead to the generation of several plants showing uncommonly occurring fertile tillers at the apical meristems and at each node and these will be discussed later (Table 2).

***A sterilis*/ Plant height**:- Results obtained indicated that both DEO treatments (i.e. 100 µl and 200 µl) had negatively influenced the plant hright where, such treatments had lead to the generation of plants with approximately half-length (i.e. in the range of 41.4-44.5 cm respectively) as compared with the untreated ones (73.143). However, no significant difference in plant height was noticed between untreated plants and the ones from the natural population. Furthermore, both DEO-treatments as compared with the control had negatively influenced (shown the least means) all of the remaining *A.sterilis* traits presented in table 2. However, no significant difference between both treatments was noticed (Table 2).

3.1.2. Two-way analysis of variance

Results presented in table (3) indicated that the applied treatment, the plant species and the interaction between them have shown a significant effect on all variables presented except for the internode distance and the distance between the flowering whorles. However, no interaction between the treatment and the species was noticed for these two variables.

3.1.3. Multiple comparison by Sheffe method for means of five quantitative traits that mostly reflect plant productivity (Two-Way ANOVA)

Final plant height. Results presented in table (4) indicated that the 100 µl DEO was the most significantly effective treatment in terms of generating the highest plant height in *A. clauda* plants whereas, the untreated *A. clauda* and the treated *A. eriantha* with 200 µl DEO had shown the least. However, depending on the level of significance presented in table 4 for the effect of applied treatments on species and their interactions, such treatments can be organized as groups in a descending order starting from the most effective treatment (group 1) to the least as presented in table 5. where, all combinations of the treatments within the same group are not significantly effective on the studied trait.

Leaf number. Both DEO treatments applied to *A. clauda* plants and the 100 µl DEO treatement applied to *A.eriantha* plants have shown the highest number of leaves. Whereas, the untreated *A. clauda* and the *A. eriantha* plants treated with 200 µl DEO have shown the least leaf number. However, the untreated *A. sterilis* plants had shown an approximate of double leaf number as compared with both DEO treatments. The desending order of groups as mentioned above is presented in table 5.

Leaf length. The untreated plants of *A. sterilis* have shown the longest leaves whereas, all treatments applied to *A. clauda* plants, natural population of *A. eriantha* and the *A. sterilis* plants treated with 200 µl DEO have shown the shortest leaves where, the reduction was in the range of 2.5-3.0 folds as compared to the former treatments. However,the 100 µl DEO treatments applied to both *A. sterilis* and *A. eriantha* also the untreated *A. eriantha* plants have shown a medium range in terms of leaf length as compared with other treatments. The desending order of groups as mentioned above is presented in table 5.

Panicle length. The 100 µl DEO treatment applied to *A. clauda* plants is not included in the comparison here since such treatment has shown uncommonly occuring several (6-9) apical tillers/plant and a single node tiller/node, and this will be discussed separately. However, the results presented in table 4 showed that both untreated (control) and natural population plants of *A. sterilis* have shown the tallest panicles followed by the 100 µl DEO treatment applied to *A. eriantha*. Whereas, both DEO treatments that were applied to *A. sterilis*, the 200 µl DEO treatment applied to *A. eriantha* and the untreated

plants of *A. clauda* have shown the shortest panicles where these were about $1/8^{th}$-$1/10^{th}$ of *A. sterilis* natural population plants or the untreated ones respectively. The desending order of groups as mentioned above is presented in table 5.

Number of spikelets. The results of the 100 µl DEO treatment applied to *A. clauda* plants is not included in the comparison here since such treatment has shown as mentioned above apical and node tillers each with its own spikelets and this will be discussed separately. However, the presented results in table 4 indicated that both DEO treatments that were applied to *A. sterilis* plants as well as the 200 µl DEO-treatment applied to *A. eriantha* plants and the untreated *A. clauda* plants had shown the least number of spikeletes as compared to other treatments. The desending order of groups as mentioned above is presented in table 5.

3.1.4. The generation of uncommon apical and node tillers with fertile panicles after 100 µl of 1,2,7,8-Diepoxioctane (DEO) treatment

Approximately one tenth of treated *A. eriantha* plants and one half of treated *A. clauda* plants had generated fertile apical and node tillers (Table 6) where instead of having one common panicle (Figure 3) at the apex, several (6-9) apical tillers each with leaves and fertile panicles (Fertile seeds) were generated at the apical meristem (Figure 1). In addition, each single node along the culm had shown one tiller with leaves and fertile panicle (Figure 2). Surprisingly, such uncommonly occuring beneficial traits had reflected several folds increase in total number of leaves per plant also, increased the number of seeds produced by a single plant which leads finally to an increased crop yield. However, *A. eriantha* tiller's type had shown a double leaf length as compared to *A. clauda* tillers leaves. On the contrary, *A. clauda* tiller's panicles have shown a range of 4-5 folds increase in the number of spikelets as compared to *A. eriantha* ones. In addition, these results had indicated that there is no significant difference in leaf length between node tillers (Figure 2) and apical tillers (Figure 1) within the same species. In contrast, the leaf length in both types of *A.eriantha* tillers have shown double length as compared to *A. clauda* leaves.

3.2. General- growth habit

3.2.1. Plant height

A. clauda The growth rate is shown in Figure 4. The results showed that the growth rate for untreated *A. clauda* plants was very slow where the plants have gained very short culms (≈ 10 cm) in the second week then the growth rate has persisted relativley constant until the end of the short growing season (≈ 9 weeks). However, the 100 µl DEO treatment has positively influenced the growth rate in terms of final plant height and the length of growing season where, the plant height has increased almost linearly up to 13, weeks after emergence and reached a final height of approximately, 90 cm. Also, the 200 µl DEO treatment had increased the growth rate of *A.clauda* plants almost linearly up to 15 weeks after emergence where, the plant height has reached ≈ 75 cm then the rate has stayed relatively constant until week 20. Furthermore, there was a high significant difference in plant height between the untreated plants and the treated with either 100 µl DEO ($p<0.05$) or 200 µl DEO ($p<0.05$).

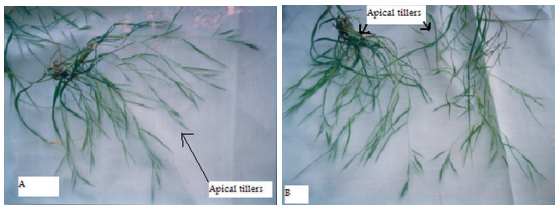

Figure 1. Fertile tillers generated at the apical meristems of plants from diploid oat species (*Avena clauda* (photo A) and *A. eriantha* (photo B)) treated with 100 µl 1,2,7,8-Diepoxyoctane (DEO), Photos were taken 60 days post treatment.

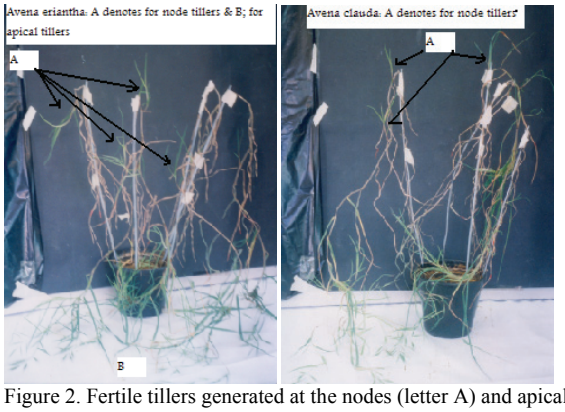

Figure 2. Fertile tillers generated at the nodes (letter A) and apical meristems (letter B) of plants from diploid oat species (*Avena clauda* and *A. eriantha*) treated with 100 µl 1,2,7,8-Diepoxyoctane (DEO). Photos were taken 60 days post treatment.

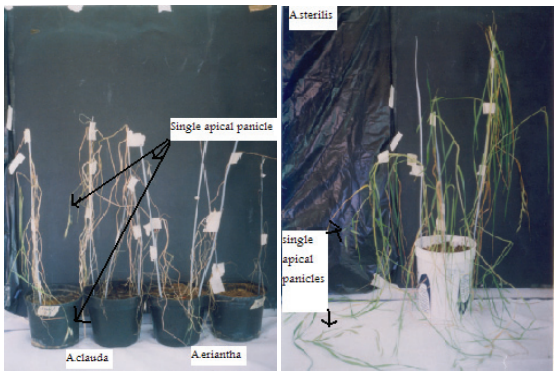

Figure 3. Representatives of 1,2,7,8-Diepoxyoctane (DEO)-untreated plants (control) from the three studied oat species (*Avena clauda*; *A. eriantha* and *A. sterilis*) indicating the formation of only the usual single apical panicle. Photos were taken 60 days post treatment.

A. eriantha The growth rate of untreated *A. eriantha* (Figure 5) plants has been accelarated rapidly during the first 6 weeks where the plant height has reached ≈ 35 cm then slight increase in height with increase in time has occurred until week 14 where, the plant has reached ≈ 50 cm in height. After that a steady state has occurred until week 23 . However, the 100 µl DEO treatment has positively influenced the growth rate in terms of final plant height and the length of growing season where, the plant height has increased almost linearly up to 21 weeks after

emergence and reached a final height of approximately 83 cm.

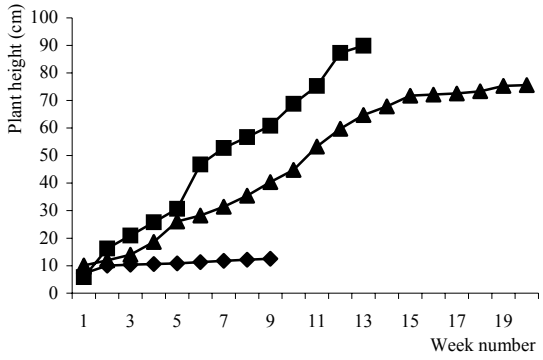

Figure 4. Time–course of growth expressed as means ± SE for *A. clauda* plants measured as plant height of untreated plants (control), treated with 100 µl of the chemical mutagen 1,2,7,8-Diepoxyoctane (DEO) and with 200 µl DEO. ♦-untreated *A. clauda*; ■- treated with 100 µl DEO and ▲- treated with 200 µl DEO. The t-test results indicated that there is a high significant difference in plant height between the untreated plants and the treated with either 100 µl DEO (P<0.05) or 200 µl DEO (P<0.05). The significance was for all weeks of the study. As a result of reaching maturity (end of growing season) earlier than the treated plants there were no scored data for untreated *A. clauda* plants after week number 9. Also there was no increase in final plant height after week 13 for the plants treated with 100 µl DEO.

In contrast *A. eriantha* plants were extremly negatively influenced by the 200 µl DEO treatment where, the plant has shown a very stunted growth in which the height was ranging from ≈7 cm (week 1) to 9 cm (week 4; the end of the growing season). Moreover, there is no significant difference between the untreated plants and the treated with 100 µl DEO on plant height (P=0.186) while there is significant difference in plant height between the untreated and the treated with 200 µl DEO (P <0.05).

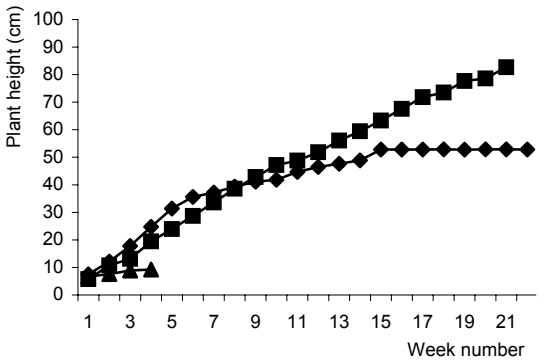

Figure 5. Time–course of growth expressed as means ± SE (for *A. eriantha* plants measured as plant height of untreated plants (control), treated with 100 µl of the chemical mutagen 1,2,7,8-Diepoxyoctane (DEO) and with 200 µl DEO. ♦-untreated *A. eriantha*; ■- treated with 100 µl DEO and ▲- treated with 200 µl DEO. the t-test results indicated that there is no significant difference between the untreated plants and the treated with 100 µl DEO on plant height (P=0.186) while there is significant difference in plant height between the untreated and the treated with 200 µl DEO (P <0.05). The significance was for all weeks of the study. There were no scored data for *A. eriantha* plants treated with 200 µl DEO after week number 4 due to the end of the growing season.

Table 1. One – way analysis of variance for 16 quantitative traits of plants from hexaploid (*Avena sterilis*) and diploid (*A clauda* and *A eriantha)* oat species showing the effect of treatments on the presented quantitative characters. Results indicated that there is a high significant effect of the applied treatments (natural population; treated with 100 µl 1,2,7,8-Diepoxoctane (DEO); treated with 200 µl 1,2,7,8-Diepoxoctane (DEO) and DEO-untreated (control) plants on the studied traits. *NC: indicates not scored.

Character	*A. sterilis (2n = 42)*		*A. eriantha (2n = 14)*		*A. clauda (2n = 14)*	
	Observd F-value	Observed sig. level $\alpha = 0.05$	Observd F-value	Observed sig. level $\alpha = 0.05$	Observd F-value	Observed sig. level $\alpha = 0.05$
Date of emergence (days)	4.448	0.006	7.153	0.000	0.307	0.821
Plant height (cm)	48.955	0.000	202.638	0.000	162.665	0.000
Leaf number	10.594	0.000	389.968	0.000	180.521	0.000
Date of panicle emergence (days)	70.812	0.000	287.808	0.000	73.851	0.000
Panicle length (cm)	200.399	0.000	145.655	0.000	42.114	0.000
Whorle number	190.075	0.000	301.277	0.000	279.349	0.000
Spikelet number	236.872	0.000	251.816	0.000	300.883	0.000
Leaf length (cm)	30.163	0.000	60.407	0.000	24.84	0.000
Leaf width (cm)	161.694	0.000	23.518	0.000	4.95	0.003
Internode distance (cm)	0.000*	1.000	17.088	0.000	* NC	* NC
Distance between whorles (cm)	55.536	0.000	1.454	0.231	* NC	* NC
Ground tiller number	159.561	0.000	3.159	0.027	* NC	* NC
Upper glume length (cm)	4.675	0.004	97.719	0.000	2.719	0.051
Lower glume length (cm)	20.714	0.000	197.514	0.000	* NC	* NC
Floret size (cm)	*NC	1.000	8.582	0.000	9.896	0.000
Awn length (cm)	1.152	0.335	102.183	0.000	15.689	0.000

A. sterilis The growth rate of untreated *A. sterilis* plants (Figure 6) was accelarated rapidly during the growing season ($\approx$13 weeks) where, the plant has reached $\approx$75 cm in height. The 100 μl DEO treatment has positively influenced the growth rate in terms of final plant height and the length of growing season where, the plant height has increased almost linearly up to 14 weeks after emergence, where the final plant height has reached approximately, and 58 cm. The treated plants with 200 μl DEO have shown a rapid increase in height until week 5 where the mean height was $\approx$40 cm, then, the rate has stayed nearly constant until the end of the growing season (week 12) where, the plant height has reached $\approx$50 cm. There is a high siginificant difference in plant height between the untreated and the treated with 100 μl DEO (P=0.009) while there is no significant difference in plant height between the untreated plants and the treated with 200 μl DEO (P=0.074).

Table 2. Multiple comparison by Sheffe method for the means of various quantitative traits of plants from hexaploid (*Avena. sterilis*) and diploid (*A. clauda* and *A. eriantha)* oat species (One -Way ANOVA). Results indicated that the 100 μl DEO – treatment had lead to a significant increase in the quantitative traits that reflect plant productivity (plant height; leaf number; number of seeds) in both diploid species whereas, such treatment had lead to a significant reduction in the above mentioned traits in *A. sterilis*. *Treat (i) and (j) denote for treatment type where: (1): denotes for plants from natural population, (2): plants treated with 200 µl DEO, (3): plants treated with 100 µl DEO and (4): untreated plants with DEO (control).

Variable	Treat type (i)*	Treat type (j)*	Species								
			A.clauda			*A.eriantha*			*A.sterilis*		
			Mean difference (i-j)	Std.Er	Sig. level α= 0.05	Mean difference (i-j)	Std.Er	Sig. level α= 0.05	Mean difference (i-j)	Std.Er	Sig. level α= 0.05
Plant height	1	2	24.9341	3.7942	0	41.4638	3.2872	0	39.1854	1.0769	0
		3	58.5769	5.1839	0	31.4767	3.4047	0	42.316	4.2835	0
		4	32.7856	3.5095	0	2.4708	3.4866	0.918	10.5759	4.5623	0.152
	2	3	33.6429	5.1322	0	72.9404	2.9817	0	3.1306	3.9423	0.889
		4	57.7196	3.4327	0	38.9929	3.0749	0	28.6095	4.2437	0
	3	4	91.3625	4.9254	0	33.9475	3.2002	0	31.7401	4.4425	0
Leaf number	1	2	10.2459	0.6545	0	3.9542	0.5981	0			
		3	10.6923	0.8943	0	12.6785	0.6195	0			
		4	1.5702	0.6054	0.085	9.325	0.6344	0			
	2	3	0.4464	0.8853	0.968	16.6326	0.5425	0			
		4	11.8161	0.5922	0	13.2792	0.5595	0			
	3	4	12.2625	0.8497	0	3.3535	0.5823	0			
Panicle length	1	2				9.4688	0.7543	0	17.1806	0.9433	0
		3				4.4935	0.7812	0	19.625	0.9911	0
		4				2.4958	0.8	0.023	3.125	1.0556	0.036
	2	3				13.9622	0.6842	0	2.4444	0.9121	0.071
		4				6.9729	0.7056	0	14.0556	0.9819	0
	3	4				6.9893	0.7343	0	16.5	1.0279	0
Whorle number	1	2				6.3208	0.262	0	4.0028	0.252	0
		3				0.1682	0.2714	0.943	4.625	0.2648	0
		4				1.7958	0.2779	0	0.2679	0.2821	0.825
	2	3				6.1526	0.2377	0	0.6222	0.2437	0.094
		4				4.525	0.2451	0	4.2706	0.2624	0
	3	4				1.6276	0.2551	0	4.8929	0.2746	0
Spikelets number	1	2				12.7167	0.4765	0	14.3813	0.6062	0
		3				5.9969	0.4935	0	14.7813	0.6369	0
		4				8.9417	0.5054	0	9.5313	0.67884	0
	2	3				6.7198	0.4322	0	0.4	0.5862	0.926
		4				3.775	0.4457	0	4.85	0.631	0
	3	4				2.9448	0.4639	0	5.25	0.6606	0
Ground tillers number	1	2							9.8625	0.4833	0
		3							8.9514	0.5077	0
		4							7.7768	0.5408	0
	2	3							0.9111	0.4673	0.288
		4							2.087	0.503	0.001
	3	4							1.1746	0.5266	0.179

Table (3). Two-way analysis of variance for the treatment type * oat species. Results indicated that the studied quantitative traits were significantly affected by the applied treatment type (natural plant population; treated with 100 µl 1,2,7,8-Diepoxoctane (DEO); treated with 200 µl 1,2,7,8-Diepoxoctane (DEO) and DEO-untreated plants), the plant species (hexaploid (*Avena. sterilis*) and diploid (*A. clauda* and *A. eriantha*)) and the interaction between them. *: Significant (P ≤ 0.05).

Character	Treatment		Species		Treatment*Species	
	F-value	Sig.level α = 0.05	F-value	Sig.level α = 0.05	F-value	Sig.level α = 0.05
date of emergence (days)	3.668*	0.026	160.305*	0.000	5.672*	0.000
plant height (cm)	87.016*	0.000	27.723*	0.000	143.860*	0.000
internode distance (cm)	26.899*	0.000	70.025*	0.000		
leaf number	137.665*	0.000	62.236*	0.000	256.198*	0.000
leaf length (cm)	70.459*	0.000	26.630*	0.000	44.549*	0.000
leaf width (cm)	160.474*	0.000	24.556*	0.000	76.720*	0.000
date of panicle emergence (days)	26.089*	0.000	80.596*	0.000	344.794*	0.000
panicle length (cm)	112.993*	0.000	35.364*	0.000	173.043*	0.000
number of whorles	257.691*	0.000	107.567*	0.000	247.809*	0.000
distance between whorles (cm)	110.680*	0.000	188.478*	0.000		
number of spikletes	635.856*	0.000	9.455*	0.000	62.368*	0.000
glume length (cm)	14.099*	0.000	391.644*	0.000	12.389*	0.000
floret size (cm)	12.933*	0.000	186.598*	0.000	40.222*	0.000
awn length (cm)	81.157*	0.000	1115.594*	0.000	3.370*	0.036
ground tiller number	187.637*	0.000	9.638*	0.000	16.803*	0.000

*: Significant (P ≤ 0.05)

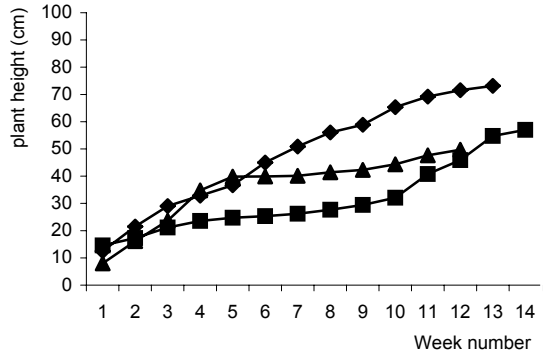

Figure 6. Time–course of growth expressed as means ± SE for *A. sterilis* plants measured as plant height of untreated plants (control), treated with 100 µl of the chemical mutagen 1,2,7,8-Diepoxyoctane (DEO) and with 200 µl DEO. ♦-untreated *A. sterilis*; ■- treated with 100 µl DEO and ▲- treated with 200 µl DEO. The t-test results indicated that there is a high significant difference in plant height between the untreated and the treated with 100 µl DEO (P=0.009) while there is no significant difference in plant height between the untreated plants and the treated with 200 µl DEO (P=0.074). The significance was for all weeks of the study. There were no scored data for *A. sterilis* plants treated with 200 µl DEO after week number 12 due to the end of their growing season.

3.2.2. Leaf number

A. clauda The growth rate (leaf number vs . time in days) of untreated plants is shown in Figure 7. The obtained results indicated that the leaf number has almost increased linearly in all treatments where the leaf number has reached 8 leaves during 77 days, 23 leaves, over 130 days, and 18 leaves, over 98 days in the three applied treatments i.e. the untreated (control), the 100 µl DEO treatment and the 200 µl DEO treatment respectively. There is no significant difference in plant height between the untreated plants and both the treated with 100 µl DEO (P=0.176) or the treated with 200 µl DEO (P= 0.108).

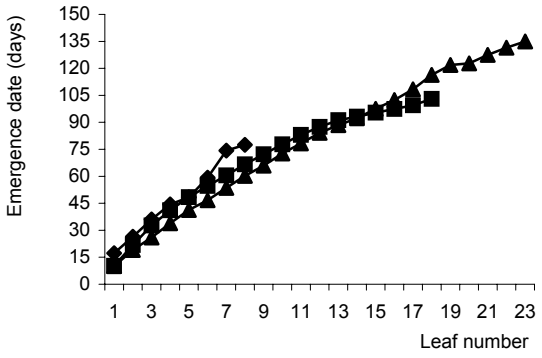

Figure 7. Time–course of growth expressed as means ± SE for *A. clauda* plants measured as as leaf number of untreated plants (control), treated with 100 µl of the chemical mutagen 1,2,7,8-Diepoxyoctane (DEO) and with 200 µl DEO. ♦-untreated *A. clauda*; ▲- treated with 100 µl DEO and ■- treated with 200 µl DEO. The results of the t-test indicated that there is no significant difference in leaf number between the untreated plants and both the treated with 100 µl DEO (P=0.176) or the treated with 200 µl DEO (P = 0.108). The significance was for all weeks of the study. As a result of reaching maturity (end of growing season) earlier than the treated plants there was no emergence of more than 8 leaves for untreated *A. sterilis* plants as compared to 18 leaves and 23 leaves for the treated with 200 µl DEO and 100 µl DEO respectively.

A. eriantha The obtained results of untreated plants (Figure 8) indicated that the leaf number has almost increased linearly in all treatments where the leaf number has reached 21 leaves during 120 days, 24 leaves, over 140 days, and 4 leaves over 50 days in the three applied treatments i.e. the untreated (control), the 100 µl DEO

treatment and the 200 µl DEO treatment respectively. There is a high significant difference in plant height between the untreated plants and the treated with 100 µl DEO (P<0.05). Whereas, no siginificant difference was noticed in plant height between the untreated and the treated with 200 µl DEO (P = 0.0.392).

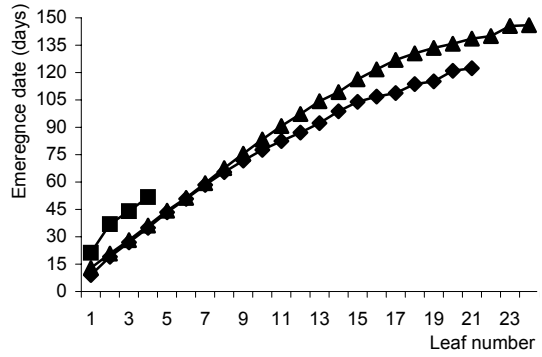

Figure 8. Time–course of growth expressed as means ± SE for *A. eriantha* plants measured as as leaf number of untreated plants (control), treated with 100 µl of the chemical mutagen 1,2,7,8-Diepoxyoctane (DEO) and with 200 µl DEO. ♦-untreated *A. eriantha*; ▲- treated with 100 µl DEO and ■- treated with 200 µl DEO. The results of the t-test indicated that there is no significant difference in leaf number between the untreated plants and the treated with 100 µl DEO (P<0.05). Whereas, high significant difference was noticed between the untreated and the treated with 200 µl DEO (P = 0.0.392). The significance was for all weeks of the study. As a result of reaching the plants that were treated with 200 µl DEO maturity (end of growing season) earlier than other tested plants there was no emergence of more than 4 leaves for *A. sterilis* plants treated with 200 µl DEO as compared to 21 leaves and 24 leaves for the untreated and the treated with 100 µl DEO respectively.

A. sterilis The obtained results of untreated plants (Figure 9) indicated that the leaf number has almost increased linearly in all treatments where the leaf number has reached 12 leaves during 93 days, 12 leaves over 115 days and 9 leaves over 75 days, in the three applied treatments i.e. the untreated (control), the 100 µl DEO treatment and the 200 µl DEO treatment respectively. There is significant difference in plant height between the untreated plants and the treated with 100 µl DEO (P = 0.035). while there is no siginificant difference in plant height between the untreated and the treated with 200 µl DEO (P = 0.410).

3.2.3. Survival of plants up to maturity

A. clauda The untreated plants have shown the highest mortality rate and the shortest growing season where the percentage of survivals has decreased from 100% to ≈ 50% within 12 weeks the end of the growing season. Whereas, the percentage of survivals for the plants that were treated with 100 µl DEO has been kept constant (100%) during the first 10 weeks of the growing season (Figure 10) and then showed a slight decline (≈ 97% to ≈ 90%) starting from week 11 to the end of the season (week 21). However, the percentage of survivals in the treated plants with 200 µl DEO was nearly constant throughout the growing season (16 weeks) where, the percentage of survivals was in the range of 100% to 96%. There is a high significant difference in the percentage of survivals between the untreated plants and either the treated with 100 µl DEO or the treated with 200 µl DEO (P < 0.05).

A. eriantha The untreated plants have also shown the highest mortality rate and the shortest growing season where the percentage of survivals has decreased from 100% to ≈ 50% within 20 week (Figure 11). The treated with 100 µl DEO have shown a gradual decline in percentage of survivals that range form 97% (week 2) to 75% (week 13) then the percentage has kept nearly constant until the end of the growing season (24 weeks). The 200 µl DEO treatment had shown negative influence on both the length of the growing season (9 weeks) and the percentage of survivals for *A. eriantha* plants where it has been droped down from 95% (week 3) to ≈13% (week 9). There is a high significant difference in the percentage of survivals between the untreated plants and either the treated with 100 µl DEO or the treated with 200 µl DEO (P < 0.05).

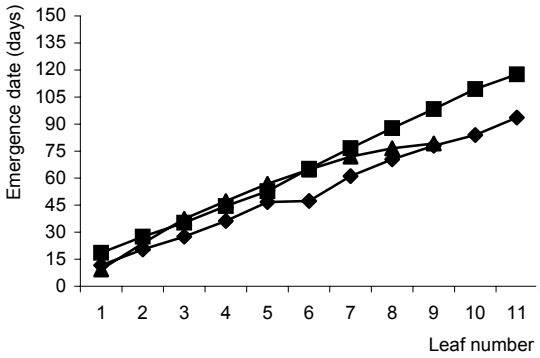

Figure 9. Time–course of growth expressed as means ± SE for *A. sterilis* plants measured as as leaf number of untreated plants (control), treated with 100 µl of the chemical mutagen 1,2,7,8-Diepoxyoctane (DEO) and with 200 µl DEO. ♦-untreated *A. sterilis*; ■- treated with 100 µl DEO and ▲- treated with 200 µl DEO. The results of the t-test indicated that there is significant difference in leaf number between the untreated plants and the treated with 100 µl DEO (P = 0.035). while there is no significant difference in leaf number between the untreated and the treated with 200 µl DEO (P = 0.410). The significance was for all weeks of the study. As a result of reaching the plants that were treated with 200 µl DEO maturity (end of growing season) earlier than other tested plants there was no emergence of more than 9 leaves for *A. sterilis* plants treated with 200 µl DEO as compared to 12 leaves for the untreated and the treated with 100 µl DEO respectively.

A. sterilis The percentage of survivals for control plants (Figure 12) indicated that such percentage was nearly fixed (98%-100%) from the date of emergence until the end of week 15. However, the percentage survivals has decreased from ≈ 98% to ≈40% during the last four weeks (16-19) of the growing season. In contrast, the plants which were treated with 100 µl DEO treatment were the most negatively influenced ones where the percentage of survivals was kept nearly constant (≈98%) during the first 9 weeks then a sharp decline has occured where the percentage was ≈ 45% (week11). The plants which were treated with 200 µl DEO have shown a constant percentage (100%) of survivals during the first 6 weeks and then declined during the last 9 weeks where, the percentage of survivals has decreased to approximately 50% at the end of the season (16 weeks). There is no significant difference in the percentage of survivals between the untreated plants and either the treated with

100 µl DEO (p = 0.722) or the treated with 200 µl DEO ($P = 0.086$).

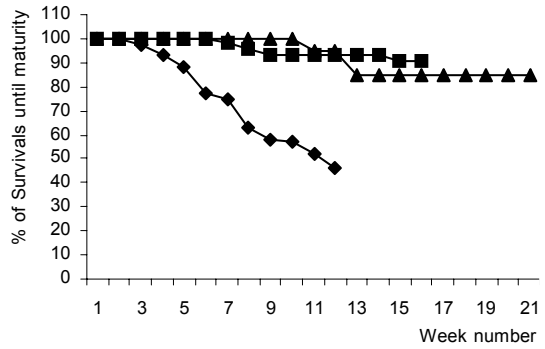

Figure 10. The pattern of change in the percentage of survivals from the date of emergence until reaching maturity for untreated (control), treated with 100 µl DEO and with 200 µl DEO plants of *A. clauda*. ♦-untreated *A. clauda*; ■- treated with 100 µl DEO and ▲- treated with 200 µl DEO expressed as means ± SE. The results of the t-test indicated that there is a high significant difference in the percentage of survivals between the untreated plants and either the treated with 100 µl DEO or the treated with 200 µl DEO ($P < 0.05$). As a result of having the highest mortality rate and the shortest growing season only 45% of the untreated plants have survived until week number 12 as compared to over 90% for the treated with 200 µl DEO (16 weeks) and the treated with 100 µl DEO (21 weeks).

4. Discussion and conclusions

The obtained results from the analysis of variance had indicated that the applied 100 µl DEO treatment to plants from both diploid species (*A. clauda* and *A. eriantha*) had positively influenced the growth rate of such plants.

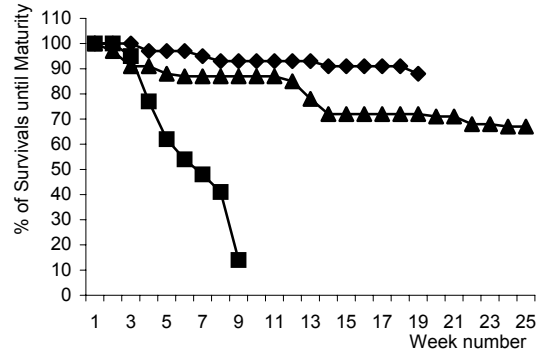

Figure 11. The pattern of change in the percentage of survivals from the date of emergence until reaching maturity for untreated (control), treated with 100 µl DEO and with 200 µl DEO plants of *A. eriantha*. ♦-untreated *A. eriantha*; ▲- treated with 100 µl DEO and ■- treated with 200 µl DEO expressed as means ± SE. The results of the t-test indicated that there is a high significant difference in the percentage of survivals between the untreated plants and either the treated with 100 µl DEO or the treated with 200 µl DEO ($P < 0.05$). As a result of having the highest mortality rate and the shortest growing season only 13% of the treated plants with 200 µl DEO have survived until week number 9 as compared to over 90% for the untreated (20 weeks) and the treated with 100 µl DEO (75% until 24 weeks).

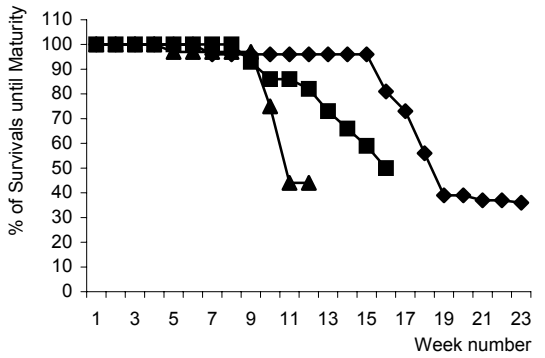

Figure 12. The pattern of change in the percentage of survivals from the date of emergence until reaching maturity for untreated (control), treated with 100 µl DEO and with 200 µl DEO plants of *A. sterilis*. ♦-untreated *A. sterilis*; ■- treated with 100 µl DEO and ▲- treated with 200 µl DEO expressed as means ± SE. The results of the t-test indicated that there is no significant difference in the percentage of survivals between the untreated plants and either the treated with 100 µl DEO ($P = 0.722$) or the treated with 200 µl DEO ($P = 0.086$). As a result of having the highest mortality rate and the shortest growing season only 40% of the treated plants with 200 µl DEO have survived until week number 11 as compared to over 40% for the untreated (23 weeks) and the treated with 100 µl DEO (50% until 16 weeks).

Such influence includes much extended (up to 24 weeks) growing season; several folds delay in panicle emergence; highest final plant height (2-folds increase); highest number of leaves (3.5 folds increase); longest panicles (2-5 folds as compared to untreated and 15-folds as compared to 200 µl treatment); highest number of flowering whorles reduced awn length to one half and the highest seed density. These findings may suggest that such plants are more resistant to intra-specific interferance between seedlings where by this would show a longer developmental period between the date of emergance and flowering or reaching maturity. In addition, such treated plants were subjected to approximately higher day-night temperatures, photoperiods and higher soil fertility than their natural environments. As a result of this more leaves will be produced in shorter periods between every two succession leaves. Also, much taller plants with an increases internode's distance, much longer, wider and healthy leaves and this suggestion would agree with the findings of (Bonaparte, 1975 and Sharma, *et al*., 1977). Where the gene action and its related secondary effects are highly influenced by the internal environmental factors in the external environment in which the plant was surviving. According to this wide range of modified phenotypic expressions will be generated and this may suggest that why several variable vegetative and floral characters could appear as a result of modified or altered gene(s) compared to normally expressed ones in the natural or the untreated plants. Furthermore, approximately one tenth of *A. eriantha* and one half of *A. clauda* plants that were treated with 100 µl DEO had shown several (6-9) fertile apical tillers/plant instead of one at the apical meristem and a single tiller at each stem node where each has its own small leaves and fertile seeds. Such induced traits would reflect human benefits in terms of increasing crop yield for human and farm animals consumption.

Table 4. Multiple comparison by Sheffe method (Two-way ANOVA) for means of five quantitative traits (Plant height; Leaf number; Leaf length; number of spikelets and panicle length) that generally reflect plant productivity. Plants of *A. clauda* and *A .eriantha* treated with 100 µl DEO had shown the highest means for traits reflecting productivity whereas, the same treatment had negatively influenced the same traits in *A. sterilis*. *Treat (i) and (j) denote for treatment type where: (1): denotes for *A. clauda* plants treated with 200 µl DEO, (2): *A. clauda* plants treated with 100 µl DEO, (3): untreated *A. clauda* plants, (4): *A. eriantha* plants treated with 200 µl DEO, (5): *A. eriantha* plants treated with 100 µl DEO, (6): untreated *A. eriantha* plants, (7): *A. sterilis* plants treated with 200 µl DEO, (8): *A. sterilis* plants treated with 100 µl DEO and (9): untreated *A. sterilis* plants.

Variable																									
Plant height					Leaf number					Leaf length					Spikelets number					Panicle length				
Treat type (i)*	Treat type (j)*	Mean difference (i-j)	Std.Er	Sig. level α= 0.05	Treat type (i)*	Treat type (j)*	Mean difference (i-j)	Std.Er	Sig. level α= 0.05	Treat type (i)*	Treat type (j)*	Mean difference (i-j)	Std.Er	Sig. level α= 0.05	Treat type (i)*	Treat type (j)*	Mean difference (i-j)	Std.Er	Sig. level α= 0.05	Treat type (i)*	Treat type (j)*	Mean difference (i-j)	Std.Er	Sig. level α= 0.05
1	2	33.6429	4.8254	0	1	2	0.4464	0.8351	**1**	1	2	1.2443	2.3997	**1**	1	3	3.1339	0.4747	0	1	3	6.9518	0.7048	0
	3	57.7196	3.2275	0		3	11.8161	0.5586	0		3	16.2055	1.6679	0		4	3.7464	0.4747	0		4	8.183	0.7048	0
	4	61.1334	3.2275	0		4	14.2161	0.5586	0		4	8.294	1.7117	0.003		5	2.9733	0.4933	0		5	5.7792	0.7324	0
	5	11.807	3.3539	**0.337**		5	2.4166	0.5804	**0.07**		5	2.0615	1.8442	**0.996**		6	2.86E-02	0.5063	**1**		6	1.2101	0.7516	**0.989**
	6	22.1405	3.4419	0		6	0.9369	0.5957	**0.991**		6	13.3829	1.9679	0		7	3.6714	0.5455	0		7	6.5823	0.8099	0
	7	25.8238	3.7085	0		7	8.9091	0.6418	0		7	29.7321	2.1322	0		8	4.0714	0.5821	0		8	9.0268	0.8642	0
	8	28.9544	3.9572	0		8	9.4147	0.6848	0	7	8	15.4444	2.0599	0		9	1.1786	0.6306	**0.967**		9	7.4732	0.9363	0
	9	2.7857	4.2875	**1**		9	5.9464	0.742	0		9	31.7937	2.2174	0	4	5	6.7198	0.4512	0	4	5	13.9622	0.6699	0
4	5	72.9404	3.0677	0	4	5	16.6326	0.5309	0	2	5	17.4498	2.3473	0		6	3.775	0.4653	0		6	6.9729	0.6909	0
	6	38.9929	3.1636	0		6	13.2792	0.5475	0		6	9.5383	2.3786	0.044		7	7.50E-02	0.5077	**1**		7	1.6007	0.7538	**0.921**
	7	35.3096	3.4517	0		7	5.3069	0.5974	0		7	0.8172	2.4757	**1**		8	0.325	0.5468	**1**		8	0.8438	0.8119	**1**
	8	32.179	3.7176	0		8	4.8014	0.6434	0		8	14.6272	2.5691	0		9	4.925	0.5983	0		9	15.6562	0.8883	0
	9	63.9191	4.0674	0		9	8.2696	0.7039	0		9	30.9764	2.697	0	7	8	0.4	0.6093	**1**	7	8	2.4444	0.9046	**0.696**
7	8	3.1306	4.1421	**1**	7	8	0.5056	0.7168	**1**	5	6	7.9114	1.6374	0.004		9	4.85	0.6558	0		9	14.0556	0.9737	0
	9	28.6095	4.4587	0		9	2.9627	0.7716	**0.145**		7	18.267	1.7755	0	5	6	2.9448	0.4843	0	5	6	6.9893	0.719	0
2	3	91.3625	4.631	0	2	3	12.2625	0.8015	0		8	2.8226	1.9037	**0.974**		7	6.6448	0.5252	0		7	12.3615	0.7797	0
	4	94.7763	4.631	0		4	14.6625	0.8015	0		9	13.5267	2.073	0		8	7.0448	0.5631	0		9	1.694	0.9103	**0.968**
	5	21.8358	4.72	0.032		5	1.9701	0.8169	**0.829**	8	9	16.3492	2.3212	0		9	1.7948	0.6131	**0.574**	8	9	16.5	1.0193	0
	6	55.7833	4.7829	0		6	1.3833	0.8277	**0.986**	6	7	10.3556	1.8166	0	8	9	5.25	0.6866	0	3	4	1.2313	0.6396	**0.959**
	7	59.4667	4.9782	0		7	9.3556	0.8615	0		8	5.0889	1.9421	**0.552**	3	4	0.6125	0.4308	**0.996**		5	12.731	0.6699	0
	8	62.5972	5.1661	0		8	9.8611	0.8941	0		9	21.4381	2.1084	0		5	6.1073	0.4512	0		6	5.7417	0.6909	0
	9	30.8571	5.4233	0.001		9	6.3929	0.9386	0							6	3.1625	0.4653	0		7	0.3694	0.7538	**1**
5	6	33.9475	3.2925	0	5	6	3.3535	0.5698	0							7	0.5375	0.5077	**1**		8	2.075	0.8119	**0.768**
	7	40.7614	3.8279	0		7	11.8313	0.6625	0							8	0.9375	0.5468	**0.983**		9	14.425	0.8883	0
	8	9.0213	4.1685	**0.945**		8	8.363	0.7214	0							9	4.3125	0.5983	0	6	7	5.3722	0.7977	0
8	9	31.7401	4.6676	0	8	9	3.4683	0.8078	**0.051**						6	7	3.7	0.5373	0		8	7.8167	0.8528	0
3	4	3.4138	2.9289	**1**	3	4	2.4	0.5069	0.014							8	4.1	0.5744	0		9	8.6833	0.9258	0
	5	69.5267	3.0677	0		5	14.2326	0.5309	0							9	1.15	0.6236	**0.97**					
	6	35.5792	3.1636	0		6	10.8792	0.5475	0															
	7	31.8958	3.4517	0		7	2.9069	0.5974	0.009															
	8	28.7653	3.7176	0		8	2.4014	0.6434	**0.18**															
	9	60.5054	4.0674	0		9	5.8696	0.7039	0															
6	7	3.6833	3.653	**1**	6	7	7.9722	0.6322	0															
8	8	6.8139	3.9052	**0.99**		8	8.4778	0.6759	0															
	9	24.9262	4.2396	0		9	5.0095	0.7337	0															

Table 5. Multiple comparison by Sheffe method (Two-way ANOVA) for means of five quantitative traits. Treatments were arranged as groups in a descending order starting from the most effective treatment (group 1) to the least, depending on the level of significant effect at ά=0.05 presented in table 4 for the effect of applied treatments on species and their interactions. where, combinations of treatments within the same group reflect non- significant effect on the studied trait. The figure in brackets denotes for the treatment type where: 1, 5, and 9 denote for plants from natural populations of *A. clauda*, *A. eriantha* and *A. sterilis* respectively. 4, 8, and 12 denote for DEO-untreated (control) plants from the above mentioned species respectively. 3, 7, and 11 denote for for 100 µl DEO treratment of plants from the above mentioned species respectively. 2, 6, and 10 denote for 200 µl DEO treratment of plants from the above mentioned species respectively.

character	Group 1	Group 2	Group 3	Group 4	Group 5
Plant height (cm)	(3)104.0000	(2)70.3571 (12)73.1429 (7)82.1642 (9)83.7188	(11)41.4028 (10)44.5333 (1)45.4231 (8)48.2167 (5)50.6875	(6)9.2238 (4)12.6375	
Leaf number	(2)15.5536 (3)16.0000 (7)17.9701	(8)14.6167 (2)15.5536 (3)16.0000	(12)9.6071	(4)3.7375 (5)5.2917 (1)5.3077 (11)6.1389 (10)6.6444	(6)1.3375 (4)3.7375
Leaf length (cm)	(12)50.5714	(8)29.1333 (11)34.2222 (7)37.0448	(1)14.3462 (5)16.0417 (10)18.7778 (3)19.5950 (2)20.8393		
Panicle length (cm)	(12)16.5000 (9)19.6250	*(7)14.8060* *(12)16.5000*	(8)7.8167 (1)7.9423 (2)9.0268 (5)10.3125	(11)0.000 (6)0.8438 (4)2.0750 (10)2.4444	
No. of spikelets	(9)14.7812 (1)16.3846	(5)13.0417 (9)14.7812	(12)5.2500 (7)7.0448	(2)4.0714 (8)4.1000 (12)5.2500	(11)0.000 (6)0.3250 (10)0.400 (4)0.9375

Table 6. Mean ± standard deviation (SD) for quantitative traits measured on fertile tillers generated at the apical meristems and each node along the stem of plants from both diploid species (*A. clauda* and *A. eriantha*) after treatment with 100 µl of 1, 2,7, 8-diepoxyoctane (DEO). Approximately one half of the tested *A. clauda* and one tenth of *A. eriantha* plants had generated several (6-9) apical tiller/plant and a single node tiller per node after DEO treatment. Nc* : denotes for not scored.

Quantitative trait	*A. eriantha*			*A. clauda*		
	Ground tillers	Node tillers	Apical tillers	Ground tillers	Node tillers	Apical tillers
Number of tillers	5.00±4.05	3.06±1.51	5.73±3.64	Nc	3.020±1.67	8.93± 4.11
Length of tillers (cm)	33.22±11.81	34.79±8.39	31.84±12.22	Nc	22.40±8.96	27.03±7.88
Number of leaves/tiller	Nc*	5.70±1.06	5.70±1.06	Nc	8.30±2.367	7.04±1.53
Leaf length (cm)	11.14±6.21	10.28±7.71	10.44±4.33	Nc	4.78±3.91	4.57±2.11
Leaf width (cm)	0.16±0.05	0.17±0.05	0.44±0.14	Nc	0.229±0.05	0.24±0.05
Panicle length (cm)	9.28±3.70	9.59±4.15	10.76±4.36	Nc	9.50±7.54	9.71±3.83
Number of whorles	4.88±1.76	3.75±1.29	5.28±2.06	Nc	5.78±2.22	5.00±1.95
Number of spikelets	5.26±2.31	4.25±2.51	5.56±2.40	Nc	16.22±9.26	26.27±15.38
Internode distance (cm)	7.37±2.33	Nc	7.64±3.16	Nc	Nc	Nc
distance between whorles (cm)	1.21±0.26	Nc	1.39±0.41	Nc	Nc	Nc

However, the obtained results from the F2 progeny had indicated that such traits are not inherited but can be restored after the re-application of the treatment. The possible explanation for such effects that came as a result of DEO toxicity may be traced to alterations that cause low DEO sensitivity in some plants indicating that there is either a very low DNA damage (in number or in lesions) in silent and non-transcriptional regions of the genome (Verhage *et al*.; 1994) or that the still existing residual mismatch, excision, and the post replication repair mechanisms i.e. the error-free mechanisms (Reed *et al*., 1998) are sufficient enough to remove most of DEO induced DNA lesions. Such resistance might be due to genes encoding proteins with known or apparent functions in either the SOS-repair (i.e. Tolerance of the damage that allows the DNA polymerase to replicate past a lesion making replication faster but promote error-prone replication, where it introduces mutations while doing so) or error-free DNA repair. This effect demonstrates the importance of error-free and error-prone repair in handling the DNA lesions induced by the action of DEO mutagen. However, the generation of such apical and node tillers may indicate that the lack of ergosterol does not lead to a larger number of DNA lesions but such lesions might induce physiological changes or chemical stress responses that alter the metabolism or the production of balanced amounts of plant growth regulators such as cytokinines, auxins and gibberellines-GA3 (Devlin and Witham, 1983). where, each hormone has a multiplicity of effects depending on its concentration, site of action, developmental stage of the plant, and the balanced amounts with other hormones rather than on the absolute amount of each individual hormone. Moreover, the induced tillering capacity may arise as a result of altered rapid mitotic cell division, cell proliferation and cell elongation in the meristematic tissues. Where it seems to

be that the 1,2,7,8-Diepoxyoctane treatment has alters the hormonal balance between auxins and cytokinines because these are antagonistic hormones in controlling the apical dominance and the ability of the terminal buds to suppress the development of axillary buds. Auxins from apical bud inhibits the growth of axillary buds this leads to elongation of shoots main axis over lateral branches. Auxins in some way derepress a repressed gene, thereby releasing DNA template for RNA synthesis. The new RNA-presumably mRNA would then induce the formation of one or more new enzymes thus increasing wall plasticity and extension (Cohen and Bandurski, 1982). In addition, cytokinines which are produced in meristematic regions and areas of continued growth potential to induce cell division and enlargment are transported upwards from roots counter auxins and stimulating the growth of axillary buds, this explains why axillary buds near the shoot tips are likely to grow. Because a cytokinin intercalated into DNA causes template modifications that are important to the mechanisms of transcription and translocation and hence important to numerous physiological and morphogenetic processes (Burrows, 1975). Moreover, all gibberellines are able to stimulate cell division as well as elongation in the subapical meristem. Growth retardants retard growth by blocking the biosynthesis of gibberellines and by this inhibiting subapical cell division and induce lateral expansion of the apex (Jacobsen, 1977). In contrast, both DEO-treatments that were applied to *A. sterilis* plants and the 200 µl DEO treatment that was applied to all species have generated adverse antagonistic effects on almost all the studied traits. The altered responses in negatively influenced plants had shown reduced developmental processes that may result from reduced rate of cell division in meristematic tissues, such reduction will lead to reduced cell proliferation and elongation and these in turn will lead to dwarf plants with stunted growth, short growing season, low percentage of survivals, short internodes, reduced leaf number, reduced leaf length and width and mostly be lethal. One of the possible causes for such sensitivity may be traced to altered mutagen metabolism induced by an electron flow impairment (pungartmik *et al*., 1999). However, the extreme sensitivity of DNA repair—proficient altered plants suggest that DEO might be metabolized and this would generate highly genotoxic plants (Schimdt *et al*., 1999). Therefore, we might speculate that DEO metabolism in altered plants could lead to altered derivatives with significantly higher DNA-damaging potential. However, the genes that encode proteins apparently not involved in a DNA repair mechanisms but most probably confer sensitivity to certain mutagens as a result of loss from protection to reactive oxygen species (Schimdt *et al*., 1999). Furthermore, from a genetical point of view since DEO is considered as a DNA cross-linking genotoxic agent so it is suggested that all altered plants in the repair-deficient group were highly sensitive to DEO. More specifically, the altered plants are sensitive to intra-strand cross-links (ICL) producing mutagens including DEO and such suggestion agreed with that of Ruhland *et al*; 1981b. In addition, ICL repair in transcribed region of the genome might be more severily inhibited or lead to lethal RNA processing in the other repair-deficient plants and hence enhances their sensitivity over other plants. In summary, it can be suggsted that the DNA repair genes are rather important to elucidate genotoxicity in terms of survival, repair, mutagenesis (Henriques *et al*., 1997 and Pungartnik *et al*., 2002) of photo-induced DNA damage, thereby, controlling events that when not properly coordinated might eventually lead to cell death.

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Levels of Heavy Metal Cd, Cu and Zn in Three Fish Species Collected from the Northern Jordan Valley, Jordan

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Abstract

Cd, Cu and Zn concentrations were determined in muscle, bone, skin, scales and gills of three fish species (*Oreochromis aureus, Cyprinus carpio* and *Clarias lazera*) collected from northern Jordan Valley (Wadi El-Arab) during March, 2006. Heavy metal concentrations varied significantly depending on the type of the tissue and fish species. Generally, *Oreochromis aureus* showed the lowest levels of both Cd, and Cu metals in all tissues except gills. The other two fish species, *Cyprinus carpio* and *Clarias lazera,* showed less difference in their heavy metal levels but it was a significant difference ($P< 0.05$). Cd and Cu recorded their lowest levels in muscle and their highest levels in gills. *Cyprinus carpio* fish species showed high values of Cu metal in all organs, except muscles. *Oreochromis aureus* accumulated the highest level of Zn in all organs particularly in their skin (432.11± 152.14 mg/ kg dry wt.). Other studied fish species showed a high values of Zn in their skin, but not as high as that in *Oreochromis aureus*. The other two fish species showed no significant difference in Zn level between all of their different organs. It was concluded that the level of heavy metals (Cd, Cu and Zn) in muscles of the three fish species were within acceptable limits by FAO standards, except for the Zn concentration in muscles of *Oreochromis aureus (*70.76± 31.21 mg/ kg dry wt.) which might be due to the increase of agricultural influx and some other anthropogenic activity in that area.

الملخص

تم قياس تركيز ثلاثة عناصر ثقيلة (الكادميوم والنحاس والزنك) في عضلات وعظام وجلد وقشور وخياشيم ثلاثة أنواع من الأسماك جمعت من سد وادي العرب في شمال وادي الأردن وهي على الترتيب البلطي والشبوط وسمك القط والتي جمعت خلال شهر مارس 2006. أوضحت الدراسة أن تراكيز المعادن الثقيلة الثلاث كانت تتغير بتغير نوع النسيج ونوع السمك. ووجد أن سمك البلطي سجل اقل مستوى لكل من الكادميوم والنحاس في جميع أنسجتها ما عدا الخياشيم . أما النوعين الآخرين من الأسماك (الشبوط والقط) فقد كانت تراكيز هذين المعدنين متقاربة أكثر منها مع البلطي ومع ذلك فقد كان الفارق الإحصائي بينهما جوهريا" (أقل من 0.05) . ووجد أيضا أن مستوى هذين المعدنين (الكادميوم والنحاس) كان الأقل في العضلات والأعلى في الخياشيم. وقد لوحظ أن سمك الشبوط قد سجل قيما عاليه من النحاس في جميع أنسجته ما عدا العضلات. لقد كان على العكس من ذلك بالنسبة لتركيز معدن الخارصين (Zn) فقد سجلت اسماك البلطي أعلى مستوى للخارصين في كل أنسجتها خاصة الجلد (432,11± 152,14 ملجم/ كغم من الوزن الجاف) ولوحظ أيضا أن جميع الأسماك تحت الدراسة سجلت مستويات عالية من الخارصين في جلدها أيضا ولكنها لم تصل إلى القدر الذي هو عليه في سمك البلطي إضافة إلى أن النوعين الآخرين (الشبوط والقط) لم يكن هناك فارق جوهري بينهما في مستوى الخارصين في جميع أنسجتهما. وعليه فقد أثبتت الدراسة إن تراكيز جميع العناصر الثقيلة موضوع الدراسة في عضلات الأسماك كان في حدود التراكيز المسموح بها عالميا وذلك حسب منظمة التغذية والزراعة ولكن المستوى العالي للخارصين في عضلات سمك البلطي (70,76 ملجم / كغم) وكذلك في الأنسجة الأخرى لأسماك تلك المنطقة يجب أخذه بعين الاعتبار وذلك لما له من تـأثير سلبي على صحة الأشخاص المستهلكين لها وصحة الأسماك نفسها حيث أن زيادة تركيز الخارصين وغيره من العناصر الثقيلة ربما يعود إلى رمي المخلفات الزراعية وغيرها من أنشطة الإنسان في تلك المنطقة.

Keywords: Heavy metals; Jordan Valley; Jordan; FAO; *Oreochromis; Cyprinus; Clarius*;

1. Introduction

The contamination of freshwaters with a wide range of pollutants has become a matter of great concern over the last few decades. Heavy metals are natural trace components of the aquatic environment, but their levels have increased due to domestic, industrial, mining and agricultural activities (Lelan et al., 1978; Mance, 1987; Kalay and Canli, 2000). Discharge of heavy metals into river or any aquatic environment can change both aquatic species diversity and ecosystems, due to their toxicity and accumulative behavior (Heath, 1987; Allen, 1995). Aquatic organisms such as fish and shell fish accumulate metals to concentrations many times higher than present in water or sediment (Olaifa et al, 2004, Gumgum et al., 1994). They can take up metals concentrated at different levels in their different body organs (Khaled, 2004). Certain environmental conditions such as salinity, pH, water hardness can play an important factor in heavy metals accumulation in the living organisms up to toxic

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concentrations and cause ecological damage (Guven et al., 1999). Thus, heavy metals acquired through the food chain as a result of pollution are potential chemical hazards, threatening consumers. At low levels, some heavy metals such as copper, cobalt, zinc, iron and manganese are essential for enzymatic activity and many biological processes. Other metals, such as cadmium, mercury, and lead have no known essential role in living organisms, and are toxic at even low concentrations. The essential metals also become toxic at high concentrations (Bryan, 1976). Studies carried out on fish have shown that heavy metals may have toxic effects, altering physiological activities and biochemical parameters both in tissue and in blood of fish (Larsson et al, 1985; Nemesok and Huphes, 1988; Abel et al, 1986). The consequence of heavy metal pollution can be hazardous to man through his food. Therefore, it is important to monitor heavy metal in aquatic environments (water, sediment and biota).

In most parts of Jordan, water resources are scarce and insufficient to meet the growing demands of a rapidly increasing population. As a consequence, the water resources situation is now precarious and of great concern to the Government. All water bodies are looked upon as a source of exploitation for urban, agricultural and industrial uses. Many water bodies are affected by increasing salinity, pollution and eutrophication due to intensive agricultural practices.

A 380-kilometer-long rift valley runs from Yarmouk River in the north to Aqaba in the south. The northern part, from Yarmouk River to the Dead Sea, is commonly known as the Jordan Valley. The valley is properly known as (Al Ghawr). Northern Jordan Valley includes: Wetlands of the Yarmouk River basin (including Birket Al Rais pool), Wadi El- Arab and Wadi Ziglab (Budeiri, 1994).

This study was undertaken to investigate the levels of heavy metal in three commercially important fish species (Bolti, *Oreochromis aureus;* common carp*, Cyprinus carpio;* and cat fish, *Clarias lazera*) collected from Wadi El-Arab, Northern Jordan Valley.

2. Materials and Methods

2.1. Study Area

Wadi El- Arab is situated at 3235'N, 3540'E; in the northern highlands and north Jordan Valley, 10-25 km west-northwest of Irbid. Wadi El- Arab and its tributary wadis rise in the hills west of Irbid city and drain west into the Jordan Valley, entering the Jordan River about 10 km south of Lake Tiberias. A dam was constructed on the main wadi in 1987, with a total capacity of 20 MCM, to collect flood water and base flows for use in irrigation in the Jordan Valley area. Since its completion, the dam has filled with water originating from within its catchment area only in the very wet year of 1991/92. In other years, water has been pumped from the King Abdallah Canal during floods to increase the stored amount of water in the dam for use during the dry season. The dam is used for irrigation, and has substantial potential for fish production (Ahmad, 1989). The catchment area is under agriculture. Irbid city is expanding westwards into the catchment, and this may put increasing pressure on the quality of the water collected in the dam **(**Budeiri, 1994). Moreover, due to heavy agricultural, domestic activities and urbanization in the region, the wetlands of this area may receive large quantities of untreated agricultural and domestic sewage. Meanwhile, they have an economical importance for fishery. Thus, contamination in the region is an important issue regarding the health of the aquatic animals and in turn, health of the human.

2.2. Sampling

Three fish species (7-9 individuals of each species) namely, Bolti(*Oreochromis aureus* measuring 26± 1 cm and weighing 358± 14.42 g) *,* common carp (*Cyprinus carpio* measuring 20.79± 1.13 cm and weighing 202.58±12.81 g), and cat fish (*Clarias lazera* measuring 40.58± 1.29 cm and weighing 467.26± 33.81 g) were bought from northern Jordan valley (Wadi El-Arab) during March, 2006 from the local fishermen of the area. Fish were brought to the laboratory and dissected with clean stainless steel instruments on the same day. The tissues from 7-9 fish individuals of the same species were pooled to make 5 subsamples. Muscle, bone, skin, scales and gills organs were dried and put in an oven at 150̊C until reaching a constant weight. Tissues were homogenized and grinded to a powder. Two grams of each dry tissue were weighed out, transferred into polyethylene tubes, 10 ml of freshly prepared nitric acid-perchloric acid (10:4) were added to the sample, and left overnight at room temperature. Then the samples were digested, digestion tubes were put in a water bath set to boiling water temperature; 100̊ C and the contents boiled for about 2 hours until all the tissues were dissolved. The digests were allowed to cool, filtered, transferred to 25ml volumetric flasks and made up to mark with 1% nitric acid (FAO, 1983). The digests were kept in plastic bottles and later, the heavy metal concentrations were determined using an atomic absorption flame emission spectrophotometer (Shimadzu, AA- 6200, Japan), and given as mg/ kg dry weight. The actual concentration of each metal was calculated using the formula:

Actual concentration of metal in sample = *ppmR* × *dilution factor*

Where

ppmR= AAS Reading of digest

Dilution Factor = Volume of digest used / Weight of sample digested

Working calibration standards of cadmium, copper and zinc were prepared by serial dilution of concentrated stock solutions (Merck, Germany) of 1000 mg/ l. These and blank solutions were also analyzed in the same way as for the digested samples.

2.3. Determination of Recovery

The digestion method and the atomic absorption spectrophotometry (AAS) analysis were validated by preparation of a multi-element standard solution (MESS) containing 1000 mg/ l of each metal. Two grams of randomly selected muscle sample powder were spiked with three different concentrations (Table 1) of heavy

metals, each run in triplicate. This was followed by the digestion of the spiked samples and determination of metal concentration using AAS. Blank or unspiked samples were carried through the whole procedure described above. The amount of spiked metal recovered after the digestion of the spiked samples was used to calculate percentage recovery.

Table 1: Recovery (%) of Cd, Cu and Zn in muscle fish samples.

Heavy metal	Spiked concentration (mg/ kg)	Recovery concentration (mg/ kg)	Recovery (%)
Cd	0.025	0.0230	92
	0.050	0.0488	97
	0.10	0.0987	98
Cu	0.25	0.250	99
	0.50	0.489	97
	1.00	0.999	99
Zn	0.25	0.240	96
	0.50	0.480	96
	1.00	1.01	101

2.4. Statistical analysis

Statistical Analysis of data was carried out using SPSS statistical package programs. A one- way analysis of variance (ANOVA) was performed, followed by Scheffe post hoc comparisons for the source of statistically significant difference. Differences in mean values were accepted as being statistically significant if $P< 0.05$.

3. Results

Table1 shows the following % recovery: on average Cd, 95% ; Cu, 98% and Zn 97%.This shows that the digestion method used and the AAS analysis are reliable. Table 2 shows mean metal concentrations in the tissues of fishes and their standard deviations for the fishes *Oreochromis aureus, Cyprinus carpio* and *Clarias lazera*. Comparisons of the data for the three fish species, related with the heavy metal levels of organs, are also given in this table. Figure 1 shows the sampling area.

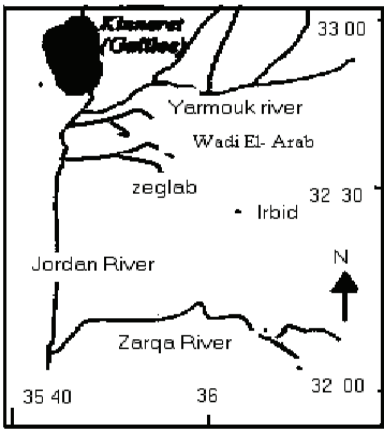

Figure1: Sampling location map of Wadi El-Arab in northern Jordan valley.

Cadmium: Concentration of Cd varied with no significant difference among all tissues of *Oreochromis aureus* except the gills that show the highest concentration of Cd (0.24± 0.12 mg/ kg dry wt.). Its distribution in both *Cyprinus carpio* and *Clarias lazera* was similar, it was in the increasing order of muscle≤ skin< bone< scales< gills with a significant differences among all organs with each other except muscle and skin which showed no significant difference in each of the two fish species. Gills also showed the highest values of Cd (about 0.7 mg/ kg dry wt.) in both fish species. There was no significant difference between *Cyprinus carpio* and *Clarias lazera* in Cd concentration values of their comparable tissues. While there was a significant difference between both of them and *Oreochromis aureus* in almost all comparable tissues. Skin of *Oreochromis aureus* and *Clarias lazera* showed no significant difference in their Cd values. Cd level in *cyprinus carpio* scales (0.66± 0.15 mg/ kg dry wt.) was significantly higher than that in *Oreochromis aureus* (0.07± 0.07 mg/ kg dry wt.). It should be noted that *Clarias lazera* has no scales. Muscles showed the lowest values of Cd in all fish species, particularly in *Oreochromis aureus*.

Copper: In *Oreochromis aureus,* the distribution pattern of copper was in the increasing order of scales≤ bone≤ muscle≤ skin< gills. Cu concentration was low in the first three tissues with no significant difference between each other, also there was no significant difference between muscle and skin, but there was a significant difference ($P< 0.05$) between skin and both scales and bone. The gills recorded the highest Cu concentration (9.32± 1.75 mg/ kg dry wt.) with a significant difference with all other tissues.

In *Cyprinus carpio* and *Clarias lazera* the distribution pattern of Cu metal was different from *Oreochromis aureus*. Its order was as follows: muscle< bone< skin< gills< scales. *Cyprinus carpio* fish recorded the highest values of Cu metal in almost all of its tissues; (Bone, skin, gills and scales with 7.99±3.04, 8.30±1.23, 9.92±2.38 and 9.93±1.74 mg/ kg, respectively) and with no significant difference between each other but with a significant difference in the comparable tissues of the other fish species; *Oreochromis aureus* and *Clarias lazera*, while muscles recorded the lowest mean concentration of copper, and with a significant difference ($P< 0.05$) when compared to the other tissues of *Cyprinus carpio*. Although Cu distribution pattern in *Clarias lazera* followed the same order as in *Cyprinus carpio* but it showed different values; bone and muscle had the lowest Cu concentrations (2.79± 0.60 and 3.04± 0.64 mg/ kg dry wt. respectively). Skin recorded a moderate concentration of Cu (6.26± 1.17) while gills recorded the highest level (9.12± 1.99 mg/ kg). There was significant differences among different tissues of *Clarias lazera* in their Cu concentratios except the difference between muscle and bone was not significant ($P> 0.05$). In general, Cu concentration was low in muscles of all fish species, and also it was low in bones and scales except those of *Cyprinus carpio* which showed a significant high Cu level. Gills in the three fish species had similar values of Cu metal (about 9 mg / kg dry wt).

Zinc: Table 2 shows that all tissues of *Oreochromis aureus* fish tend to accumulate Zn in large amounts particularly in the skin (432.11± 152.14) which was recorded the highest concentration of Zn with a significant difference ($P< 0.05$) compared with all other tissues of *Oreochromis aureus* and of other fish species. The gills

recorded the lowest concentration of Zn (46.22± 22.24). Muscles recorded high concentration of Zn (70.76± 31.21) and it was higher than the acceptable values for human consumption designated by FAO (FAO, 1983). In the other fish species (*Cyprinus* and *Clarias*) the highest concentration of Zn recorded was in their skin, and with a significant difference (P< 0.05) compared with the other tissues. The lowest Zn value was recorded in bone and gills of *Cyprinus carpio* (25.06± 4.09 and 27.85±3.93, respectively) and in muscles of *Clarias lazera* (30.13± 3.04 mg/ kg). Zn concentration in muscles and scales of *Cyprinus carpio* showed no significant difference but there was a significant difference (P< 0.05) between scales and bone. No significant difference (P>0.05) was found between the levels of Zn in bone and gills of *Clarias lazera* but there was a significant difference (P< 0.05) among them and the other tissues such as muscles and skin.

In comparison of Zn level in the three fish species, it was found that *Oreochromis aureus* showed the highest Zn level with a significant difference (P< 0.05) among all comparable tissues, except the gills of *Oreochromis aureus* and *Clarias lazera* which showed no significant difference. While *Cyprinus carpio* and *Clarias lazera* showed no significant difference (P> 0.05) in the Zn level in all of their comparable tissues.

4. Discussion

In the present study the concentration of cadmium was lowest in *Oreochromis aureus* fish and highest in *Clarias lazera* fish in all organs. There was a significant difference (P< 0.05) between the concentrations of Cd in all organs of the three fish species except the skin of *Oreochromis aureus* and *Clarias lazera.* Also, gills of *Cyprinus carpio* and *Clarias lazera* were not significantly different in their Cd concentrations. Gills seemed to be the organ which accumulates the highest value of Cd. The concentration of Cd in scales and bone recorded comparable values with that in gills of *Cyprinus carpio* and *Clarias lazera* which indicated that the uptake of Cd could occur through gills and other hard tissues; scales and bones. This is in agreement with the literature (El-Nemr, 2003; Khaled, 2004; Van Aardt and Endmann, 2004 and Mwashot, 2003) which reported that Cd is stored in the body in various tissues, but the main site of accumulation in aquatic organisms is in the kidney and liver, beside other tissues, notably the gills, bone and exoskeleton. It was also reported that Cd level in gills recorded higher than or comparable value with that in the liver of *Cyprinus carpio* and some other fish species (Canli et al., 1998; Khaled, 2004). There was a significant difference (P<0.05) between the concentrations of Cd in muscles of all collected fish species. Muscles showed the lowest levels of Cd particularly in muscles of *Oreochromis aureus;* 0.02± 0.02 mg/ kg dry wt. The highest value of Cd in muscle organ was in *Clarias lazera*; 0.24± 0.05 mg/ kg dry wt. This value is still in a permissible values of Cd; 0.5 mg/ kg that was proposed by the Food and Agricultural Organizations (FAO, 1983) to be safe for human consumption. Cadmium is rarely found in natural water (Hem, 1989). It is considered to be toxic if its concentration exceeds 0.01 mg/ L both in drinking and irrigation water (Taha, 2004). Cadmium with some other heavy metals lead and mercury are of no biological function in human system and they are potentially toxic even at trace concentrations (Robert, 1991). The effects of acute cadmium are high blood pressure, kidney damage, destruction of testicular tissue as well as destruction of red blood cells (Gupta and Mathur, 1983).

Table 2 shows that gills exhibit the highest level of Cu in addition to Cd metal while the muscle recorded the lowest level of Cu. *Oreochromis aureus* showed low level of Cu in their muscles, bones, scales and skin. Gills accumulate high level of Cu in all fish species (about 9 mg/ kg dry wt.).

Table 2. Concentrations of Cd, Cu, and Zn in mg/ kg dry wt (mean ± standard deviation) in different organs of three fish species collected from Wadi El- Arab, Northern Jordan Valley during March, 2006.

Organ	Fish species	N	Cd	Cu	Zn
Muscle	*Oreochromis aureus*	7	0.02± 0.02 [a]*	2.90± 0.34 [a]	70.76± 31.21 [a]
	Cyprinus carpio	9	0.14± 0.07 [b]	2.48± 1.00 [ab]	30.31± 4.16 [b]
	Clarias lazera	9	0.24± 0.05 [c]	3.04± 0.64 [ab]	30.13± 3.04 [b]
Bone	*Oreochromis aureus*	7	0.11± 0.08 [a]	2.33± 0.96 [a]	169.23± 84.66 [a]
	Cyprinus carpio	9	0.41± 0.06 [b]	7.99± 3.04 [b]	25.06± 4.09 [b]
	Clarias lazera	9	0.53± 0.06 [c]	2.79± 0.60 [a]	39.96± 2.90 [b]
Skin	*Oreochromis aureus*	7	0.12± 0.07 [a]	4.98± 1.43 [a]	432.11± 152.14 [a]
	Cyprinus carpio	9	0.27± 0.08 [b]	8.30± 1.23 [b]	46.88± 8.41 [b]
	Clarias lazera	9	0.15± 0.07 [a]	6.26± 1.17 [a]	65.89± 12.04 [b]
Scales	*Oreochromis aureus*	7	0.07± 0.07 [a]	2.18± 0.97 [a]	152.66± 157.75 [a]
	Cyprinus carpio	9	0.66± 0.15 [b]	9.93± 1.74 [b]	35.96± 6.45 [b]
	Clarias lazera	9	No Scales	No scales	No Scales
Gills	*Oreochromis aureus*	7	0.24± 0.12 [a]	9.32± 1.75 [a]	46.22± 22.24 [a]
	Cyprinus carpio	9	0.70± 0.16 [b]	9.92± 2.38 [ab]	27.85± 3.93 [b]
	Clarias lazera	9	0.77± 0.17 [b]	9.12±1.99 [ab]	40.12± 6.80 [ab]

* Data shown with different letters are statistically significant at the P < 0.05 level .
N: number of fish individuals of each fish species.

Cyprinus carpio accumulated high concentrations of Cu in all of its tissues except muscles, while *Clarias lazera* had no scales and they tend to accumulate Cu only in their gills and skin (9.12± 1.99 and 6.26± 1.17 mg/ kg dry wt. respectively), while bone and muscle recorded low levels (2.79± 0.60 and 3.04± 0.64 mg/ kg dry wt. respectively). Most copper minerals are relatively insoluble and hence little copper is found in natural water. Copper is available in surface water and ground water due to the extensive use of pesticides sprays containing copper compounds for agricultural purposes. It is an essential element in human metabolism but can cause anemia, disorders of bone and connective tissues and liver damage at excessive levels**.** The toxicity of copper depends upon the hardness and pH of the water, and therefore, it is more toxic in soft water and in water with low alkalinity (Taha, 2004). This study shows that the most uptake of Zn metal was through the skin tissue not gills and which showed the lowest level of Zn if compared to the other organs of *Oreochromis aureus.* Generally all the studied organs showed high level of Zn in bone, scales and even the muscles particularly in *Oreochromis aureus.* The other two fish species *Cyprinus carpio* and *Clarias lazera* also showed high level of Zn in their skin but lower level than in *Oreochromis aureus* skin. *Cyprinus carpio* showed the lowest values of Zn in their organs. Zinc concentration in muscles was the highest in *Oreochromis aureus* (70.76± 31.21 mg/ kg dry wt). This value is higher than the acceptable value for Zn in edible fish (30 mg/ kg dry wt) (FAO, 1983). *Cyprinus carpio* and *Clarias lazera* fish showed similar values of Zn in their muscles (30 mg/ kg dry wt). Zinc is one of the essential elements as copper, and cobalt for both animals and humans. A deficiency of zinc is marked by retarded growth, loss of taste and hypogonadism, leading to decreased fertility. Zinc toxicity is rare, but at concentrations in water up to 40 mg/ l, may induce toxicity, characterized by symptoms of irritability, muscular stiffness and pain, loss of appetite, and nausea (NAS-NRC, 1974). Zinc appears to have a protective effect against the toxicities of both cadmium (Calabrese et al., 1985) and lead (Sanstead, 1976).

This difference in the pattern of heavy metals distribution in the three fish species might be a result of their difference in many factors such as; feeding habits, habitats, ecological needs, metabolism, biology and physiology (Arellano et al., 1999). Generally, heavy metal uptake occurs mainly from water, food and sediment (Canli et al., 1998). However, the efficiency of metal uptake from contaminated water and food may differ in relation to ecological needs, metabolism, and the contamination gradient of water, food and sediment, as well as other environmental factors such as salinity, temperature and interacting agents (Heath, 1987 and Pagenkopt, 1983). *Cyprinus carpio* is a benthic, burrowing species and their heavy metal concentrations especially Cu, can indicate the bioavailability of them from sediments (Luoma, 1983). On the other hand, *Clarias lazera* usually feeds near the bottom in natural waters but they take some food from the surface, besides the nature of diet of their youngs differ from that of adults. While *Oreochromis aureus* is a benthopelagic, feeds on plants and zoobenthos which might accumulate heavy metals from their food. This is agreed with the literature which reported that some plant and animal taxa such as crustacea and mollusca have high potential for accumulation of metals and other pollutants even from much diluted solutions without obvious noxious effects (Ali and Fishar, 2005). Although fish muscle is the most important part to be used for human consumption, fish skin and liver may also be consumed to some extent. Target organs such as liver, kidney, gonads and gills, have a tendency to accumulate heavy metals in high values, as shown in many species of fish in different areas (Kargin, 1996; Yilmaz, 2003; Yilmaz, 2005 and Abdel-Moniem, et al., 1994). It is generally accepted that muscle is not an organ in which metals accumulate (Legorburu et al., 1988). Similar results were reported from a number of fish species showing that muscle is not an active tissue in accumulating heavy metals (Karadede and Unlo, 2000). This is agreed with the present study. Skin is not much studied in previous works, although it is a consumed part of the fish. Yielmaz (2003) indicated that concentrations of heavy metals were higher in all of the skin samples than in muscles. This is agreed with the present study. The reason for high metal concentrations in the skin could be due to the metal complexion with the mucus that is impossible to be removed completely from the tissue before the analysis. The present results indicated that the lowest concentrations of the heavy metals were usually recorded in muscle rather than the skin, while the higher values were recorded in the gills and sometimes in bones and scales. This study indicated that gills accumulate more Cd and Cu metals than skins and muscles, but this is reversed in case of Zn metal. The levels of heavy metal in fish vary in various species and different aquatic environments (Canli and Atli, 2003). The presence of trace metals, particularly Zinc in Wadi El-Arab might due to the agricultural influx and sewage via surrounding cultivated lands

Consequently, it can be concluded that the levels of heavy metals in muscle are at acceptable levels for all of the studied samples in this region. Only the zinc level in muscle of *Oreochromis aureus* was higher than the acceptable values for human consumption designated by the FAO, 1983. Absorbed zinc and other heavy metals can be distributed quickly to the other tissues and organs (e.g. bone, gills, kidneys, muscle) rather than accumulating in the liver (Yilmaz, 2005) and in the skin as in the present study. Accumulation of heavy metals in fish viscera and other organs may be considered as an important warning signal for fish health and human consumption. The present study shows that precaution measures need to be taken in order to prevent future heavy metal pollution.

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التقديم

إنه لمن دواعي سروري أن أكتب هذه المقدمة بمناسبة صدور العدد الأول من المجلد الأول من المجلة الأردنية للعلوم الحياتية (Jordan Journal of Biological Sciences)، وهي مجلة علمية عالمية محكمة و مفهرسة، تصدر أربع مرات في العام عن اللجنة العليا للبحث العلمي في وزارة التعليم العالي والبحث العلمي في المملكة الأردنية الهاشمية. ويشرف على إعداد و نشر هذه المجلة عمادة البحث العلمي والدراسات العليا في الجامعة الهاشمية.

تحرص هيئة التحرير للمجلة الأردنية للعلوم الحياتية أن تكون مرجعاً مهماً للدارسين والباحثين، يستنيرون بما ستتضمنه من بحوث أكاديمية أصيلة ودراسات علمية عميقة في شتى موضوعات العلوم الحياتية. وتسعى هيئة التحرير ومن خلال دعم وزارة التعليم العالي والبحث العلمي والجامعات الأردنية كافة المتواصل أن تصل هذه المجلة إلى مرتبة متقدمة ضمن المجلات العلمية العالمية المتخصصة في هذا المجال.

إننا نتمنى أن تشكل هذه المجلة قاعدة أساسية ترفد عملية النشر والبحث العملي في قطاعات العلوم الحياتية المتعددة، وتضع آخر ما توصلت إليه الأبحاث العلمية في متناول أيدي الباحثين والمتخصصين. وتنشر المجلة مقالات البحوث العلمية التي تتسم بالجدة والأصالة، إضافةً إلى الملاحظات الفنية و التقنية ومقالات المراجعة لموضوعات حيوية و تهم الدارسين و الباحثين و في مختلف حقول العلوم الحياتية. ويجري تحكيم مقالات البحوث والملاحظات التقنية من قبل محكمّين من ذوي الاختصاص والخبرة. يشتمل العدد الأول على ستة مقالات أبحاث أصيلة تعالج جوانب متعددة في العلوم الحياتية.

أتقدم بالشكر العميق لأعضاء هيئة التحرير وأعضاء الهيئة الاستشارية الدولية للمجلة الذين قدموا النصح والمشورة لإصدار هذا العدد من المجلة. كما أتقدم بالشكر والعرفان للزملاء الذين قاموا بتحكيم الأبحاث على الوقت و الجهد الذي بذلوه في مراجعة وتحكيم هذه الأبحاث. وكذلك أتقدم بالشكر لكافة الزملاء للذين تقدموا بأبحاثهم للمجلة، سواء ما تم قبوله منها أو من لم يقبل، حيث أن أبحاثهم هي العنصر الأساسي في إصدار هذه المجلة.

وختاما، و بالنيابة عن هيئة التحرير و بالأصالة عن نفسي، أرجو أن أكون قادراً على تقديم كل ما هو مفيد لكافة الباحثين والعاملين في قطاعات العلوم الحياتية المختلفة من خلال هذه المجلة، آملاً من القراء الكرام عدم التردد في المساعدة وإرسال مساهماتهم واقتراحاتهم واستفساراتهم للارتقاء بهذه الدورية العلمية المتخصصة لما فيه مصلحة الجميع . والله ولي التوفيق.

أ.د. نعيم صبحي إسماعيل
رئيس هيئة التحرير
الجامعة الهاشمية

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