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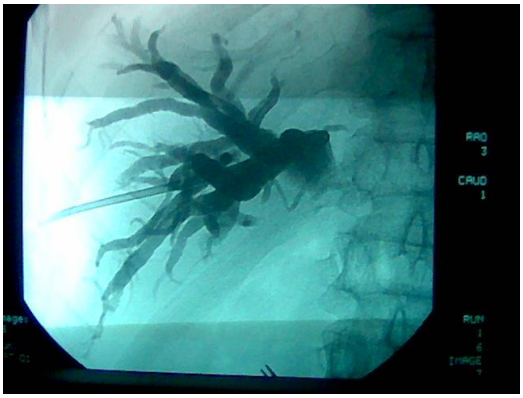
Volume 7 (6); November 25, 2017

Research Paper



Rational Surgical Tactics in Proximal Bile Ducts Tumors.

Nazirov FG, Akbarov MM, Omonov OA.
J. Life Sci. Biomed., 7(6): 76-81, 2017;
pii:S225199391700012-7

Abstract
This study aimed to evaluate the experience of surgical management of 156 patients with tumors of proximal parts of bile ducts. Palliative surgical interventions were performed in 125 patients. Recanalization of the tumor with external drainage of bile ducts was performed in the 61 cases. Palliative resection of billiary ducts and biliodigestive anastomosis formation was performed in 17 patients. Transcutaneous transhepatic cholangiostomy (TTCS) as a final method of treatment was performed in 33 case, and in the 14 cases we have carried out endoprosthesis placement in the stricture caused by tumor through TTCS. The radical and conditional-radical surgical interventions were performed in the 31 patients. The 19 patients have undergone the cholecystectomy, resection of hepaticocholedoch with the tumor and placement of hepatojejunoanastomosis, and in 12 cases were performed resection of hepaticocholedoch with liver resection, placement of hepaticcojejunoanastomosis. Postoperative mortality constituted 10.2%. We studied long-term results of surgical treatment of TPBD. The recurrences of disease were noted at follow-up period from 5 months to 5 years. After operation having relatively-radical character, recurrence occurred in the period from 5 months to 12 months after radical operations – from one year to 5 years. Survival was calculated with method of multiplicand evaluations of Kaplan-Meier. Survival after radical surgeries was achieved 1 years in 94% of patients, 2 years – 82%, 3 years – 45%, 4 years – 15% and 5 years – 12%. At I and II types of tumor by Bismuth-Corlette at the I-II stages of the cancer of proximal bile ducts it would be rationally to perform radical surgical treatment with preliminary decompression of the intrahepatic bile ducts.

Keywords: Klatskin tumor, Mechanical jaundice, Transcutaneous transhepatic endobiliary intervention, Radical surgical interventions

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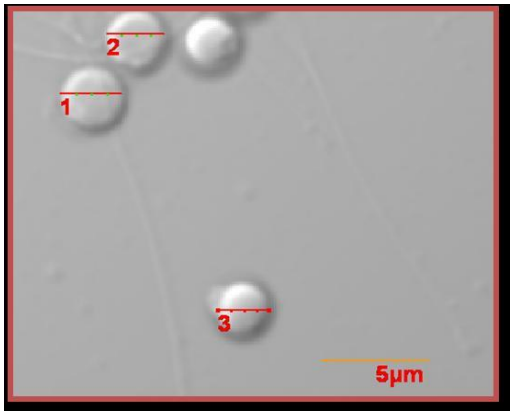
Research Paper



The Effect of Different Electric Field Strength (V/Cm) on Post-Electroporated Quality of Koi Fish Sperms.

Soeprijanto A, Faqih AR, Anitasari S.
J. Life Sci. Biomed., 7(6): 82-89, 2017;
pii:S225199391700013-7

Abstract
This research was to understand the effect of different electric field strength on sperm quality of Koi fish and to identify the best electric field. Various electric field strengths were treated at range of 10, 20, 30, 40 and 50 V/Cm in form of square wave using electroporator Gen Pulser of BIO RAD. The length of shock was 0.5 ms with shock frequency of 4 times. The result showed The highest and lowest parameter tests of treatment Koi fish could be explained as following. (1) The highest motility was 77 % and found at electric field strength 10 V/Cm, and whereas the lowest motility was 58 % obtained from electric field strength 50 V/Cm. (2) The highest viability of Koi fish sperms was 80 % obtained from treatment with electric field strength 10 V/Cm, while the lowest viability was 59 % found at electric field strength 50 V/Cm (3). The highest fertility was 70 % acquired from electric field strength 10 V/Cm whereas the lowest fertility was 51% derived from electric field strength 50 V/Cm. It was suggested from this research that to obtain better motility and viability rates, electric field strength 10 V/Cm should be considered.

Keywords: Electric Field, Sperm Quality, Square Wave, Motility, Viability, Fertility

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Research Paper

Bio Monitoring of Heavy Metals (Cu, Zn, Pb, Cd and Ni) in the West Coast of Peninsular Malaysia Using Giant Mudskipper *Periophthalmodon Schlosseri* (Pallas 1770).

Buhari TR, Ismail A.
J. Life Sci. Biomed., 7(6): 90-109, 2017;
pii:S225199391700014-7

Abstract
The giant mudskipper *Periophthalmodon schlosseri* (Pallas 1770) is the largest notable species of Malaysian mudskippers and invariable the best known example of resident intertidal fish. This paper present an assessment of heavy metals concentration; Cu, Zn, Pb, Cd and Ni in scales, muscle, bone, gills, operculum, intestine, liver and cartilage of *Periophthalmodon schlosseri* (*P. schlosseri*) with aim of proposing it as a biomonitoring agent in the coastal mudflats. The giant mudskippers; *P. schlosseri* were collected from six sampling sites in the west coast of Peninsular Malaysia and analyzed for heavy metals concentrations; Cu, Zn, Pb, Cd and Ni in scales, muscle, bone, gills, operculum, intestine, liver and cartilage by using an air-acetylene flame atomic absorption spectrophotometer, Perkin Elmer Analyst 800. The levels of heavy metals concentrations in the tissues of *P. schlosseri* varied significantly ($P > 0.05$). The studied metals showed different accumulation target in the fish; Cu was observed highest in liver while Zn, Pb, Cd and Ni exhibited their highest concentrations in cartilage. The pattern of metal accumulation was in the order: Zn > Pb > Ni > Cu > Cd, with a similar pattern observed for metal selectivity index (MSI %) value. There was no unique ranking with regards to tissue selectivity index (TSI %) among the tissues, except liver which showed high copper selectivity at all the six sampling sites. However, the result of biota-sediment accumulation factor (BSAF) for Cu and Zn were highest in liver and intestine respectively while the BSAF for Pb, Cd and Ni were highest in cartilage. Results of the present study indicate bioaccumulation of the studied metals in the tissues of giant mudskipper *P. schlosseri* which might suggest it use as a potential bio monitoring agent for heavy metals in the west coast of Peninsular Malaysia.

Keywords: Bio monitoring, Heavy metals, *Periophthalmodon schlosseri,* West coast Peninsular Malaysia.

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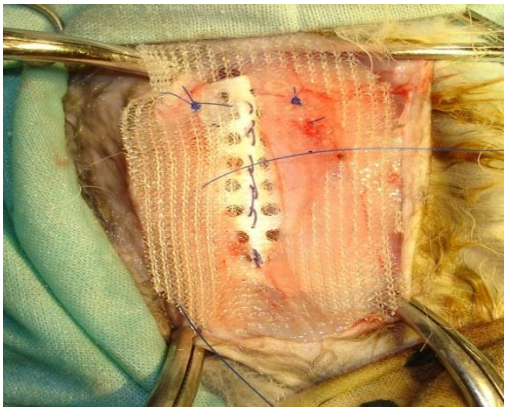
Research Paper



Two-Component Mesh Repair of Medium-Sized Ventral Hernias.

Ametov LZ, Baibekov IM, Khan GV, SHayusupov AR, Egamov BYu.
J. Life Sci. Biomed., 7(6): 110-116, 2017;
pii:S225199391700015-7

Abstract
In order to reduce the frequency of adhesions which are accompanying hernial repair procedure, various types of combined prostheses are offered. At the same time many researchers are still searching for a more profitable combination of synthetic materials. Experimental studies were performed on 8 rabbits. Animal weight was varying from 2.2 to 3 kg. General endotracheal anesthesia was performed. We developed a two-component prosthesis model consisting of two layers, firs layer (lower) was a polytetrafluoroethylene (PTFE) and was placed in the abdominal cavity and the second (upper) was a prolene layer and located above the muscular aponeurosis of the anterior abdominal wall. The absence of adhesions of the visceral peritoneum after establishing PTFE prosthesis was determined by a macroscopic method. On the 10th day, the formation of a thin capsule covering the lower prosthesis was visually observed. Morphological studies have shown that the use of a two-component prosthesis does not affect the course of the wound regeneration process and the wound healing time, the upper polypropylene film is integrated with the surrounding tissues and neovascularization is observed already by the 14th day.

Keywords: Ventral hernia, Synthetic PTFE prosthesis, Two-component mesh for ventral hernia repair, Intraperitoneal prosthesis, Intra-abdominal prosthesis adhesions.

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Rational Surgical Tactics in Proximal Bile Ducts Tumors

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ABSTRACT

This study aimed to evaluate the experience of surgical management of 156 patients with tumors of proximal parts of bile ducts. Palliative surgical interventions were performed in 125 patients. Recanalization of the tumor with external drainage of bile ducts was performed in the 61 cases. Palliative resection of billiary ducts and biliodigestive anastomosis formation was performed in 17 patients. Transcutaneous transhepatic cholangiostomy (TTCS) as a final method of treatment was performed in 33 case, and in the 14 cases we have carried out endoprosthesis placement in the stricture caused by tumor through TTCS. The radical and conditional-radical surgical interventions were performed in the 31 patients. The 19 patients have undergone the cholecystectomy, resection of hepaticocholedoch with the tumor and placement of hepatojejunoanastomosis, and in 12 cases were performed resection of hepaticocholedoch with liver resection, placement of hepaticcojejunoanastomosis. Postoperative mortality constituted 10.2%. We studied long-term results of surgical treatment of TPBD. The recurrences of disease were noted at follow-up period from 5 months to 5 years. After operation having relatively-radical character, recurrence occurred in the period from 5 months to 12 months after radical operations – from one year to 5 years. Survival was calculated with method of multiplicand evaluations of Kaplan-Meier. Survival after radical surgeries was achieved 1 years in 94% of patients, 2 years – 82%, 3 years – 45%, 4 years – 15% and 5 years – 12%. At I and II types of tumor by Bismuth-Corlette at the I-II stages of the cancer of proximal bile ducts it would be rationally to perform radical surgical treatment with preliminary decompression of the intrahepatic bile ducts.

Original Article
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Keywords

Klatskin tumor, Mechanical jaundice, Transcutaneous transhepatic endobiliary intervention, Radical surgical interventions

INTRODUCTION

The tumors of proximal bile ducts (TPBD) accounted for 10-26% of all malignant lesions of the bile ducts. The frequency rate varied from 2 to 8 per 100000 of population [1-3]. The clinical manifestations of the tumors with localizations in the portal fissure were described in the literature for the first time in 1957. In 1965 Klatskin G. published a lot of observations in patients with cancer of the common hepatic duct [4, 5, 7].

At present time the majority of specialists classified the tumor of Klatskin as independent disease related to the tumors originated from epithelium of the biliary ducts in the interval between the place of confluence into the common hepatic duct of the gallbladder and segmental hepatic ducts of the secondary order [9]. There are met

other names, such as portal cholangiocarcinoma, hilus tumor, portal fissure cancer. The survival duration depends directly on the efficacy of the surgical treatment [5].

The tumors of proximal bile ducts are slowly growing neoplasms having distant metastases at the early stages of disease. Local invasion of the tumor in the liver parenchyma or elements of its hilus as well as perineural invasion are found at the early stages of the tumor development, that be conditioned by the difficulties of the radical operative intervention [2, 5, 6].

The diagnosis of tumor is based on the study of the level of tumor markers (CA-19-9), findings of the ultrasound investigation (USI), direct methods of the biliary ducts contrast (retrograde cholangiopancreatography – RPCG, transcutaneous transhepatic cholangiography – TTCG), and computed tomography (CT). In rare cases the angiographic investigation and thin needle biopsy have to be performed [8].

This study aimed to improvement of the results of complex treatment of the patients with tumors of the proximal biliary ducts with choice of rational surgical technique.

MATERIAL AND METHODS

The Republican Specialized Center of Surgery named after Academician V.Vakhidov have experience of the complex treatment of 156 patients with tumors of extrahepatic biliary ducts of proximal localization during the period from 1997 to 2016. The patients were at the age of 18 to 87 years.

For classification of the tumors of proximal biliary ducts we used classification by system TNM for cancer of the extrahepatic biliary ducts and classification proposed by Bismuth-Corlette (1975) for tumor prevalence [9].

Classification for distribution of Bismuth-Corlette (1975):

Type I – tumor of the common hepatic duct without infiltration of its bifurcation;
Type II – tumorous obstruction of confluence of the lobular ducts;
Type III_a – predominant invasion of confluence and right hepatic duct;
Type III_b – predominant invasion of confluence and left hepatic duct;
Type IV – tumorous lesion of the both hepatic ducts.

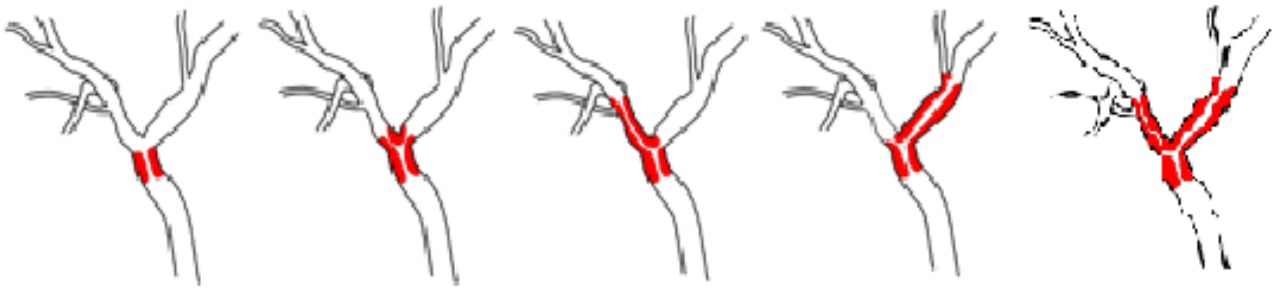

Figure1. Distribution of TPBD by classification of Bismuth-Corlette

According to this classification tumor of type I was found in 18 patients (11.5%), type II – in 26(16.6%), type III_a – in 17(10.8%), type III_b – in 31(19.8%) and type IV was diagnosed in 64 patients (41.0%).

The leading expression of disease is painless obstructive jaundice which was observed in 100% of patients. The level of bilirubinemia fluctuated from 35.6 to 770 μmol/l. The most part of these patients (56.4%) had jaundice of severe degree (higher than 300 μmol/l). In the clinical symptomatics there was also noted pruritus (77%), signs of cholangitis (56.4%) and weight loss (20%).

Diagnosis of proximal tumors of extrahepatic biliary ducts included special instrumental methods of investigation. Instrumental methods: ultrasound investigation was performed in all patients, computed tomography – 44, endoscopic retrograde cholangiography – 97, transcutaneous transhepatic cholangiography – 72 and double direct contrast of the biliary ducts – 16 patients. Ultrasound dopplerography of the hepatic vessels was used in 19 patients. Additionally there was performed radioisotope hepatoscintigraphy in 9 patients.

Ethical approval

The review board and ethics committee of Republican Specialized Center of Surgery named after Academician V. Vakhidov approved the study protocol and informed consent were taken from all the participants.

RESULTS AND DISCUSSION

Analysis of the results showed, that determination of the level of block of bile deviation is one of the key moment in the determination of the further tactics of treatment. The ultrasound investigation is the main oriented noninvasive method (USI). Echographic features of TPBD are presented by dilatation of the intrahepatic ducts, absence of visualization under stricture of the sites of the system of ducts, and presence of the collapsed gallbladder. It is important, that the Klatskin tumor is difficult for identification and its borders are frequently determined according to the secondary signs to which the changes of biliary ducts above the tumorous obstruction are attributed.

The computed tomography (CT) was used for identification of nonspecific signs, such as atrophy of one from liver lobes and hypertrophy of the contralateral that may indirectly indicate about tumor invasion into the branches of the portal vein. The sensitivity of CT achieved 88% of cases, tumorous cause of the obstruction was determined in visualization of the tumorous masses, as a rule, in intravenous bolus contrast and accounted for 55% of cases, the evaluation of respectability appeared to be correct in 42% of cases. Computed tomography gave more information while performing it before drainage of the hepatic ducts, because decompression of the biliary ducts before CT-investigation reduced sensitivity of the method at measurement of the level of the block of biliary ducts. During performance of CT before drainage of biliary ducts the proximal level of obturation was determined in 98.1% of investigation.

Today the nucleic magnetic resonance in the regime of cholangiography appeared to be the most optimal and effective method of diagnosis in TPBD, because it allows to determine clear localization and distribution of the tumor, as well as to visualize in details structure of the hepatic vessels and their link with tumor and blliary ducts. Sensitivity of MRI achieved 93.2% [10].

After the complex of noninvasive diagnostic investigation in jaundice of moderate and severe degree the first step was decompression of the biliary tree for what there were used the modern mini-invasive technologies. The preference was given to TTCG which allows the mostly adequate drainage of the biliary tree and receiving of cholangiogram of high informativity. Sensitivity of TTCG achieved 95.2%, accuracy of the res ectability evaluation – 93%. TTCG remains to be the "golden standard" for determination of the character of bile duct lesions in TPBD. Undoubtful advantage of TTCG is direct link with performance of transcutaneous transhepatic cholangiostomy (TTCS), which is only real way of preventive decompression of biliary tract in the patients with proximal tumorous block before possible radical surgery (Figure 2).

Figure 2. TTCG and TTCS in the patients with TPBD

Endoscopic retrograde pancreatocholangiography (ERPCG) s an alternative of TTCG, however the performance of ERPCG at the proximal blocks appeared to be problematic at absence of patency in zone of tumorous obstruction, and for evaluation of the state of biliary ducts above the place of obstruction this method is not informative [8,11]. At the associated use of RPCG and TTCG it may be determined length and accurate localization of the process.

In order to avoid development of the acute suppurative cholangitis and with purpose to regulate cholestasis the direct contrast methods of investigation were ended by external or external-internal drainage of the biliary ducts.

In bilirubinemia more than 200μmol/l the preoperative decompression of biliary ducts have to be done which may be both uni- and bilateral. The plan operative interventions were carried out 2-3 weeks after moment of decompression. In cases of TPBD non-resectability or in presence of contraindications the preference was given to mini-invasive methods of decompression of the intrahepatic biliary ducts.

Palliative operative interventions were performed in 125 (80,1%) patients with TPBD and in these cases tumor recanalization with external drainage of biliary ducts on the transhepatic drains was made in 61 cases, palliative resection of billiary ducts and biliodigestive anastomosis formation was performed in 17 patients. TTCS (external or externo-internal) – in 33 cases, endoprosthesis of the tumorous stricture through TTCS – in 14 cases. Thirteen patients underwent endoprosthesis of extrahepatic biliary ducts through TTCS (Figure 3).

The character of performed palliative operative interventions was presented in table 1. Radical and relatively-radical surgical interventions in TPBD were performed in 31 (20.3%) patients. Of them in 19(61.2%) patients was type I, in 8(25.8%) – II type and in 4 (12.9%) patients type III of tumor was revealed.

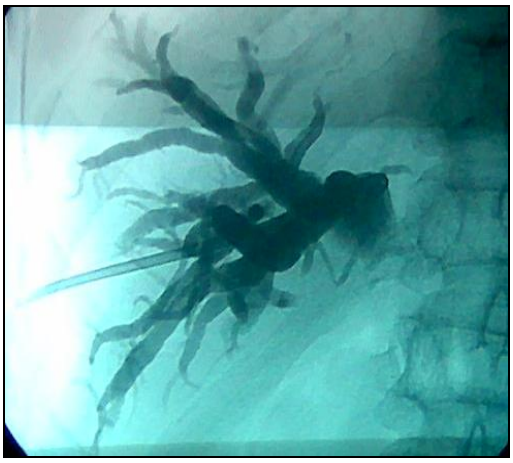

Figure 3. Endoprosthesis of the hepaticocholedoch

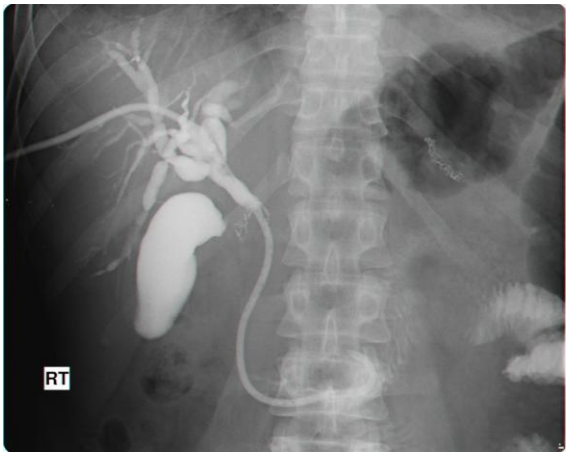

Figure 4. External-internal drainage of the biliary ducts.

The performed radical operative interventions included resection of hepaticocholidoch together with tumor and application of hepaticojejunostomy in 19(61.2%) patients, resection of hepaticocholedoch with tumor, liver resection, application of hepaticojejunoanastomosis in 12(38.7%) patients.

The term of relatively radical operation presents performance of resection surgeries at IVA and IVB stages of disease when the radical procedure is impossible due to stage of disease or due to impossibility of complete tumor resection because of its anatomic localization and growth character, for example IV type (by Bismuth) for lesion of the common hepatic duct bifurcation when tumorous invasion extends highly to segmental ducts. Radicalism in such situation may be achieved only by performing liver transplantation.

Analysis of complications after all operative interventions revealed that hepatic insufficiency was observed in 17,9% of cases, insufficiency of bile digestive anastomosis (BDA) – in 4,48%, gastro-intestinal hemorrhages from acute ulcers connected with operative stress as well as marked shifts in the system of hemocoagulation (reduction of prothrombine time, prothrombine index, increase in fibrinolitic activity, thrombocytopenia) – in 4,48%, intra-abdominal hemorrhages – in 5,76% and cholangitis – in 28,8%. Intra-abdominal hemorrhages and cholangitis occurred more frequently after TTCS and endoprosthesis. In the structure of lethality the association of peritonitis with recurrent gastrointestinal hemorrhages and purulent abscess formating cholangitis with development of biliary sepsis and hepatic-renal insufficiency were of the most importance. After all radical, relatively-radical and palliative interventions 23 patients (14,7%) dead (Table 3).

We studied long-term results of surgical treatment of TPBD. The recurrences of disease were noted at follow-up period from 5 months to 5 years. After operation having relatively-radical character, recurrence occurred in the period from 5 months to 12 months after radical operations – from one year to 5 years. Survival was calculated with method of multiplicand evaluations of Kaplan-Meier. Survival after radical surgeries was achieved 1 years in 94% of patients, 2 years – 82%, 3 years – 45%, 4 years – 15% and 5 years – 12%. Survival after palliative external drainage accounted for 3.2 ±1.8 months, after internal drainage – 7.0±3.4 months.

We believe that resection of the hepatic ducts in TPBD has radical character only at II stage of tumors with localization of type I by Bismuth-Corlett and only in some cases for type II. For other localizations and stages of cancer of TRBD this intervention has relatively-radical or palliative character.

Table 1. The characteristic of palliative operative interventions

Types of interventions	Quantity of patients	%
Tumor recanalization with external drainage of the bile ducts	61	48,8
BDA	17	13,6
TTCS	33	26,4
Endoprosthesis	14	11,2
Total	125	100

BDA= bile digestive anastomosis; TTCS = transcutaneous transhepatic cholangiostomy

Table 2. Character of the radical operative interventions

Types of interventions	Quantity of patients	%
Resection of hepaticocholedoch together with tumor and application of hepatojejunostomy	19	61.2
Resection of hepaticocholedoch together with tumor, liver resection and application of hepaticojejunoanastomosis	12	38.7
Total	31	100

Table 3. Frequency rate of complications after operative intervention (abs/%).

Variant of surgery	Quantity	Acute hepatic insufficiency	Insufficiency of BDA	Gastrointestinal hemorrhage	Intra-abdomen hemorrhage	Cholangitis	Lethality
Radical	31	4 (12.9)	5 (16.1)	1 (3.2)	2 (6.45)	4 (12.9)	4 (12,9)
Recanalization and external drainage	61	12 (19,6)	-	3 (4,9)	5 (8,1)	23 (37,7)	10 (16,3)
Palliative resection of hepatic ducts with formation of anastomosis	17	4 (23,5)	2 (11,7)	3 (17,6)	-	1 (5,8)	2 (11,7)
Transcutaneous transhepatic cholangiostomy	33	7 (21,2)	-	-	2 (6,1)	9 (27,2)	5 (15,1)
Endoprosthesis	14	1 (7,14)	-	-	-	8 (57,1)	2 (14,2)
Total	156	28 (17,9)	7 (4,48)	7 (4,48)	9 (5,76)	45 (28,8)	23 (14,7)

BDA= bile digestive anastomosis

Diagnosis of these tumors is difficult, since in the first period the disease is asymptomatic. Affecting the proximal bile ducts, the tumor leads to their obturation. The greatest diagnostic value is computed tomography, ultrasound, angiography of the hepatic vessels, retrograde pancreaticocholangiography, percutaneous transhepatic cholangiography [12].

Biochemical investigations reveal disorders characteristic of mechanical jaundice and cholestasis. The level of bilirubin and the activity of alkaline phosphatase can be very high. Almost 100% of tumors of the proximal bile ducts show severe changes in the blood coagulation system. The level of tumor markers is increased: carcinoembryonic antigen (CEA) (44-46%), carbohydrate antigen CA19-9 (47-70%), alpha-fetoprotein (19%) [13]. Currently, one of the most informative methods is magnetic resonance imaging (MRI), which allows a clear visualization of tumor, ductal system of the liver and vascular structures.

Ultrasound diagnosis (ultrasound) is one of the most informative methods. Already in the conditions of the clinic, a qualified doctor can determine the syndrome of biliary hypertension with the presence of a block at the level of the gates of the liver [14]. Ultrasound reveals the dilatation of the intrahepatic bile ducts of one or both lobes of the liver and the level of the block without the expansion of the common bile duct below the level of obstruction and without an increase in the gallbladder.

The most clinically important diagnostic method for proximal bille duct cancer identification is the direct contrast of the biliary ducts. Percutaneous transhepatic cholangiography is highly informative in determining the proximal level of biliary tract obstruction and the prevalence of the tumor along the bile ducts. The sensitivity of transhepatic cholangiostomy reaches 95.2%, specificity, according to different data - 85-88%, correctness in the evaluation of resequence - 58%. From the literature review one can observe that there is still no generally accepted diagnostic algorithm for perepherial bile cancer. However, most authors believe that ultrasound, CT or MRI, transhepatic cholangiostomy are of greatest importance for the choice of treatment tactics; Cholangioscopy, angiography, biopsy, laparoscopy are used according to strict indications

CONCLUSION

The optimal complex of differential-diagnostic methods of investigation together with general methods of examination should include the following techniques in the special order: USI of the organs of abdominal cavity, duplex scanning of the portal fissure vessels, CT(SCT) of the abdominal cavity organs, MRI with regimen of cholangiography, ERPCG and TTCG.

At I and II types of tumor by Bismuth-Corlette at the I-II stages of the cancer of proximal bile ducts it would be rationally to perform radical surgical treatment with preliminary decompression of the intrahepatic bile ducts at marked mechanical jaundice that allows reduction of the operation risk and quantity of complications in the patients with severe stage of jaundice.

At diagnosis of clearly nonresectable TPBD and presence of contraindications for surgery the decompression of bile ducts with use of TTCS and, if possible, with use of external-internal drainage or endoprosthesis appeared to be method of choice.

Acknowledgements

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Authors' Contributions

All authors contributed equally to this work.

Competing interests

The authors declare that they have no competing interests.

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The Effect of Different Electric Field Strength (V/Cm) on Post-Electroporated Quality of Koi Fish Sperms

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ABSTRACT

This research was to understand the effect of different electric field strength on sperm quality of Koi fish and to identify the best electric field. Various electric field strengths were treated at range of 10, 20, 30, 40 and 50 V/Cm in form of square wave using electroporator Gen Pulser of BIO RAD. The length of shock was 0.5 ms with shock frequency of 4 times. The result showed The highest and lowest parameter tests of treatment Koi fish could be explained as following. (1) The highest motility was 77 % and found at electric field strength 10 V/Cm, and whereas the lowest motility was 58 % obtained from electric field strength 50 V/Cm. (2) The highest viability of Koi fish sperms was 80 % obtained from treatment with electric field strength 10 V/Cm, while the lowest viability was 59 % found at electric field strength 50 V/Cm (3). The highest fertility was 70 % acquired from electric field strength 10 V/Cm whereas the lowest fertility was 51% derived from electric field strength 50 V/Cm. It was suggested from this research that to obtain better motility and viability rates, electric field strength 10 V/Cm should be considered.

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Electric Field, Sperm Quality, Square Wave, Motility, Viability, Fertility

INTRODUCTION

Fish sperm was potential to be developed into a vector in transgenic process. Besides being a relatively new process, genetic transfer process was also relatively natural [1]. Transgenic technology in animal was an attractive technology to develop [2]. In modern biotechnology, the characters of certain living creatures could be changed by moving genes from a species to other species. Transgenic technology had been suggested since 1980 by Gordon and colleagues. In recent development, establishing transgenic fish by transferring "DNA construct" was facilitated through several methods [3], such as Micro-Injection, Retroviral Injection (intentionally injected with virus), Sperm-Mediated Gene Transfer (sperm as gene carrier), Particle Bombardment (gun particle or biolistic), and Electroporation [4]. Of these methods, electroporation method was the most frequent method used for gene transfer because it was more efficient, faster, simpler, and relatively natural. Gene transfer on fish using electroporation method with fish sperm as gene transfer medium, however, was rarely found in Indonesia [1].

Electroporation was a mechanical method used to embed certain molecule into host cell through cell membrane. In this procedure, electric shock was used to open temporarily phospholipids layers which allowed alien molecule, such as DNA, to enter into the cell and be the part of the cell [5].

Transfer of DNA molecules into cells, a transfer of gene from cells that contain foreign DNA through electric shock. Two different types of electric shocks that are Square wave and Exponential wave have been used in transferring the gene into the fish sperm. The voltage of the Square wave is more directed to the required amplitude

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which is to maintain the length of the shock time, then returned to zero. While the Exponential wave, the voltage is intended for a desired amplitude, then gives exponential or continuous damage. Amplitude is the furthest distance from the equilibrium point, but in this wave the amplitude depends on the applied voltage [6].

The entry of DNA molecule into the cell was facilitated by electric shock against the DNA-carrying cell. Two wave forms were used, respectively square wave and exponential wave. Both were useful in transferring gene into fish embryo and fish sperm [7]. Square wave method could produce great voltage which kept more efficient the internalization of DNA due to the relatively wide opening of the pores. Other benefit was very short wave (*pulse length*) which could prevent cell damage and help the cell to recover faster because the involved heat was relatively low. *Square Wave* could also conduct higher electric field strength at short wave, and therefore, it produced low heat which made possible that certain molecule was transferred without killing cell or embryo [8].

Sperm is spermatozoa cells that are in seminal solution and are produced by hydration testes, one part of the fish reproduction apparatus [9]. Spermatozoa are solid and very distinctive cells, do not grow or divide and have no physiological role to the animals that produce them, just to fertilize eggs of the same kind [10]. As a gene transfer medium, sperm is potentially developed in transgenic fish because of its relatively natural and more efficient procedure [11]. Sperm have advantages as gene transfer media, because in the process of transfer of genetic material, sperm as a natural vector. Sperm cells have been used as gene transfer vectors in Carp, Catfish and Tilapia [6].

Koi fish belonged to a family of Cyprinadae, and represented ornamental fish favored by Indonesians or foreigners. There was a future possibility that genetic engineering might be done to produce certain color and pattern, and it was realized through transgenic process. This paper would be a review against the optimization of different electric field strength with Koi fish sperm as transgenic vector in electroporation method.

The objective of research was to understand the effect of different electric field strength on sperm quality of Koi fish and to identify the best electric field

MATERIAL AND METHODS

Materials in this research were (1) Male prime of Koi Fish that was dissected for obtaining sperm to examine its motility and viability; (2) Female prime of Koi Fish that was stripped and taken its egg to be used in fertilization process; (3) ovaprim that was injected to fish prime to accelerate gonad maturity; (4) Fructose solution as the diluter before and after electroporation; (5) HCl 0.2 M to clean cuvette; (6) Ethanol 70% used to soak cuvette after cleaning with HCl; (7) Ice rock used to cool cuvette before cuvette was applied in sperm electroporation; and (8) Eosin-negrosin dye to color the sperm to facilitate the observation of its viability.

Several instruments were used, such as no (1) rearing batch of Koi fish primes; (2) spuit as sperm container during stripping; (3) micro pipette to move sperm cell, fructose, and aquadest; (4) digital weight METER PE 22 to weight Koi fish primes; (5) bowl to contain eggs from stripping; (6) ruler to measure total length of Koi fish; (7) fiber batch as the container of Koi fish primes after injected with ovaprim; (8) a set of electroporator (BIO-Rad) to give electric shock at Koi fish sperm; (9) cuvette as sperm container during electroporation; (10) Microscope DIC to observe motility of Koi fish sperm; (11) Ependorf as sperm container after electroporation; (12) Petri dish as the container of fertilized eggs; (13) Digital camera to take picture of the motility and viability of sperms and the development of ovum; (14) Set of incubator tools to incubate the eggs that had been fertilized by sperms.

Research was conducted at the Laboratory of Fish Biology and Reproduction (Breeding Laboratory), Faculty of Fishery and Marine Science, and also at the Central Laboratory of Natural Science (LSIH), University of Brawijaya, Malang, East Java. Research begun from May 2011 to finished.

Research design

Various electric field strengths were treated at range of 10, 20, 30, 40 and 50 V/Cm in form of square wave using electroporator Gen Pulser of BIO RAD. The length of shock was 0.5 ms with shock frequency of 4 times (1).

(a) Research procedure

Before exposing Koi fish sperm to electric field strengths, electroporation instruments must be prepared and programmed based on research design. Cuvette must be also ready, and cuvette size should be 0.2 cm. The sample was prepared through following steps: (1) 25 µl sperms were poured into cuvette using micro pipette and the cuvette was sealed tight; (2) The lid of "Shock Pod" was opened; (3) Cuvette with sample was set into "shock pod"; (4) "Shock Pod" was closed; (5) PULSE button was pressed; (6) Data were displayed on screen and it must be recorded;

(7) Physiological solution of 275 µl was added into 25 µl post-electroporated sperms in cuvette; (8) One drop was taken for motility observation and one other drop was obtained for viability observation, and sperms were colored with dye; and (9) Result of observation was recorded and compared with the quality of control sperm.

(b) Test parameters

1) Spermatozoa motility; 2) Spermatozoa viability; 3) Spermatozoa fertility

Fertility, or also called as fertilization, was a process when ovum core fused into cytoplasm to produce zygote. Basically, fertilization was the unification or fusion of male and female gamete cells to produce a cell [12]. The fertilized and unfertilized eggs might be enormous, and therefore, fertility percentage was counted (%).

Ethical approval

The review board and ethics committee of Fisheres and Marine Science Faculty approved the study protocol.

RESULTS AND DISCUSSION

Data of motility, viability and fertility

Data of observation on percentage rate of test parameters show that the higher electric field strength (V/Cm) could produce lower percentage rate of test parameters of Koi fish sperms. Table 1 showed that percentage rate of sperm quality of control Koi fish was still higher than that of treatment Koi fish.

The highest and lowest parameter tests of treatment Koi fish could be explained as following:

(1) The highest motility was 77% and found at electric field strength 10 V/Cm, and whereas the lowest motility was 58% obtained from electric field strength 50 V/Cm (Figure 1).

(2) The highest viability of Koi fish sperms was 80 % obtained from treatment with electric field strength 10 V/Cm, while the lowest viability was 59 % found at electric field strength 50 V/Cm (Figure 3).

(3) The highest fertility was 70% acquired from electric field strength 10 V/Cm whereas the lowest fertility was 51% derived from electric field strength 50 V/Cm.

Table 1. Percentage Rate of Test Parameters

Electric field strength (V/Cm)	Sperm quality of Koi fish		
	Motility (%)	Viability (%)	Fertility (%)
Control	82	85	72
10	77	80	70
20	72	75	65
30	67	73	60
40	62	65	55
50	58	59	51

a. Motility of Koi fish sperms

Figure 1 indicated that higher electric field strength would produce lower motility of Koi fish sperms. Spermatozoa plasma membrane was damaged with higher electric field strength, and it disturbed sperm metabolism. The consequence was that sperms lost their motility and even died. Higher electric field strength reduced the percentage of the living sperms. The disturbance against sperm's membrane or film permeability could reduce metabolism activity and damage sperm cells. According to Dewi et al. [13], sperm motility was reduced with higher electric field strength. Tsai [14], had said that sperm motility depended on the level of electric field strength (V/Cm) during electroporation.

According to Kalkianto [15], motility average rate of Koi fish sperms was 31.67% after treating sperms with electric shock at electric field strength 40 V/Cm and with shock length 0.5 ms using square wave method. The reduction of motility rate was due to the adaptation of sperms to electric field strength, and thus, it disturbed sperm's membrane or film permeability which led to metabolism activity decrease, cell damage and motility reduction.

As noted by Faqih [1], electric field strength 40 V/Cm brought relatively small negative impact effect on catfish, indigo fish and gold fish. The application of electric field strength 80 and 120 V/Cm had caused all sperms of gold fish and indigo fish to death. It could be said that sperms of gold fish and indigo fish could not survive normally in the medium exposed to electric field strength above 40 V/Cm, but catfish sperms were successfully survived until electric field strength 520 V/Cm although motility and viability of the sperms were relatively low.

Statistic measurement on the motility of Koi fish sperms indicated that F-table 5% < F-count < F-table 1 %, meaning that Ho was accepted. It also meant that treatment with electric field strength (V/Cm) in different forms of square wave was not influential obviously to the motility of Koi fish sperms.

The motility of Koi fish sperms was a measure of sperm feasibility. It declined with higher electric field strength (V/Cm) and with prolonged shock length. The survival rate of embryos that were fertilized by electroporated sperms and by control sperms (untreated) was not different because 30 millions sperm cells were fertilizing 500 eggs [16].

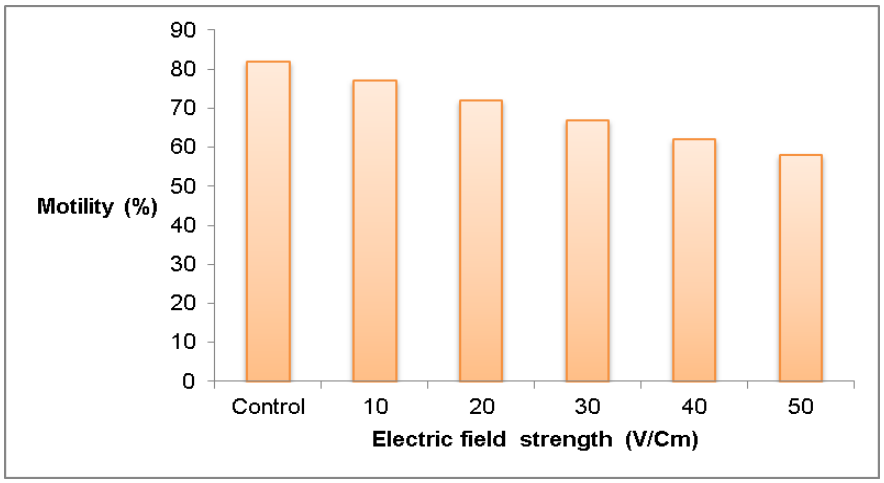

Figure 1. Bar Graphic of Motility of Koi Fish Sperms

b. Viability of Koi fish sperms

Result of observation with Inverted Microscope at 500x magnificence (Figure 2) had shown that dead sperms absorbed dyes because membrane permeability was increasing and facilitating the absorption of dyes. Only few living sperms absorbed the dyes, and therefore, the color was transparent. When sperms mixed with dyes, the living sperms did not absorb dyes or if any, only few of them could absorb it. Dead sperms would absorb great quantity of dyes because sperm's wall permeability was increasing after the death [17]. More understandings required examination on Figure 3. Graphic explained that higher exposure of electric field strength to the examined fish sperms might reduce viability of tested fish sperms. Higher electric field strength could also disturb the membrane of the treated sperms.

According to Partodihardjo [8], 100–2,000 sperms were involved to determine the percentage of sperm viability, and this could be seen in Figure 3. The figure 3 displayed that the highest viability rate was 80% obtained from electric field strength 10 V/Cm whereas the lowest was 59% from electric field strength 50 V/Cm. Sperm viability rate of sperms after electroporation treatment was lower than that of control sperms before electroporation, and the differential was 80%. The reduction of sperm viability rate was possible due to electric shock treatment at higher electric field strength that could damage plasma membrane, thus reducing sperm viability. It was consistent to Dewi et al. [13], sperm viability declined with the increase of electric field strength exposed to sperms. Stronger electric field could produce few number of sperms survived. It was evident because higher electric field strength could disturb sperm's membrane or film permeability, which would reduce metabolism activity and damage sperm cell [1].

Kalkianto [15], previously found that the percentage rate of viability of gold fish sperms was 50 % in average after it was given with electric shock at electric field strength 40 Volts at shock length 0.5 ms. This percentage rate was smaller compared to that of control sperms before treatment because the later was counted for 73.33%. Furizal [19], explained the result of observation on sperm dyeing by stating that viability rate after electroporation was 64.99%, and if compared to viability rate of control sperms (70.58%), this result remained in better category. The viability of post-eletroporated sperms was influenced by electric field strength and shock length during treatment. According to Rubinsky [20], electric current that produced heat could damage cells through electroporation where electric shock influenced cell membrane to transform into permanent pores which risked cells to death.

Statistic measurement on the viability of Koi fish sperms indicated that F-table 5% < F-count < F-table 1 %, meaning that Ho was accepted. It could be said that treatment with electric field strength (V/Cm) in different forms of square wave was not influential obviously to the viability of Koi fish sperms.

The reasons of this finding were some possibilities, such as:

1. Diluter material, respectively fructose 3%, increased the viability but declined the motility time to less significant portion. According to Hidayaturrahmah [21], treating sperms with fructose as diluter for fish sperms could provide energy and nutrient to fish sperms, and ATP energy might increase or elongate sperm motility time.
2. Replication was 3 times. More than 3 replications were assumed producing obviously different results, and therefore, more replications were more accurate the data would be.
3. This research was specified only to Koi fish, and therefore, the fish must be healthy. It was possible that other species could deliver obviously different results.
4. Sperms during stripping must be in good condition and good concentration. According to Adewumi et al. [22], sperm quality might vary depending on various external factors such as feeding schedule and feed quality. Physical characteristic of sperms was white-milk color and viscous.
5. The interval between one treatment and other was too high, respectively 10 V/Cm. If treatment interval was set on 5 V/Cm, the results might be obviously different. According to Cheng et al. [23], result of observation on sperm motility after electroporation at various shock strength and shock length had shown that sperm motility was reducing with the increase of shock voltage.

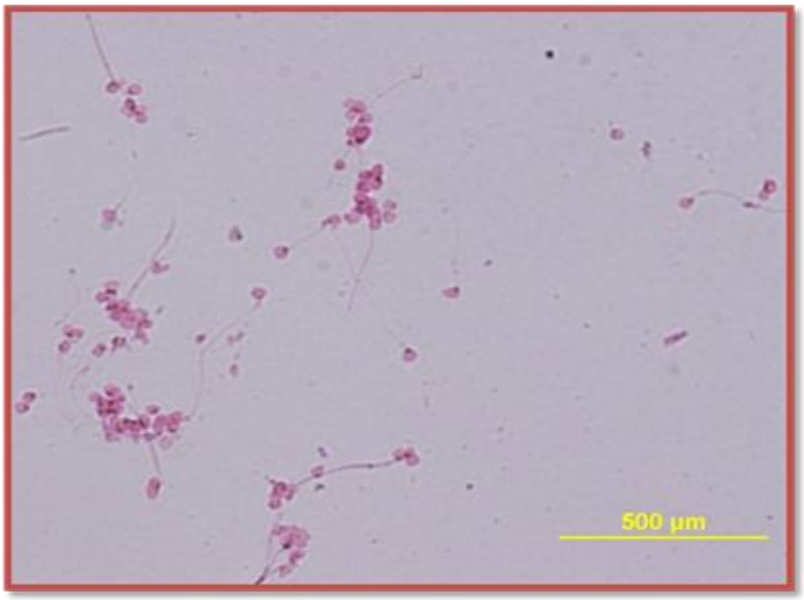

Figure 2. Viability of Koi fish sperms after dyeing with *hematoxin eosin*;
A) Red-colored dead sperms were due to absorbing many dyes; B) Pink-colored living sperms were due to absorbing few dyes.

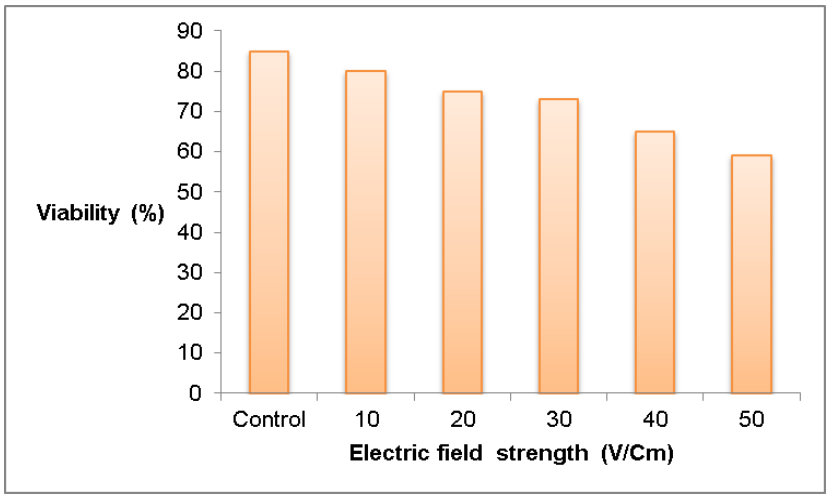

Figure 3. Bar graphic of viability of Koi fish sperms

c. Fertility

To understand the ability of sperms in fertilizing eggs, it was measured through sperm fertility [1]. This research was aimed to acknowledge how many eggs could be fertilized by sperms after exposing sperms with electroporation at electric field strength 50 V/Cm. It was assumed that if highest electric field strength at 50 V/Cm had been useful for egg fertilization, then it was possible that the electroporated-sperms with lower electric field strength 10 V/Cm was also capable to fertilize the eggs.

Graphic above showed that treating sperms with electric field strength 50 V/Cm was producing average rate of fertility counted for 50%. Electric field strength of electroporation at 50 V/Cm had produced higher motility rate and thus, it stimulated higher fertility. Successful egg fertilization by sperms was influenced greatly by sperm motility because higher viability could produce higher fertility. In this condition, sperms needed great energies to fertilize the eggs [21].

High sperm concentration could increase the possibility of fertilization. Fishes that produced hundreds thousands eggs could have high egg concentration, and thus, might need high sperm volume [24]. According to Dacie and Lewis [25], the concentration of fish sperms was ranged about 3.7–11.9 x 10^9 sperms/ml liquid. Sperm density was observed about 40.294 x 10^9 cells/ml liquid.

The dimension size of observational result of trialed fish sperms was displayed in Figure 5. According to Anonymous [26], sperm cells had total length of 50-60 µm. Sperm comprised of two parts, respectively head and tail. Head dimension was 4-5 µm length and 2.5-3.5 µm width. The ratio of length to width was 1.5-1.75 µm.

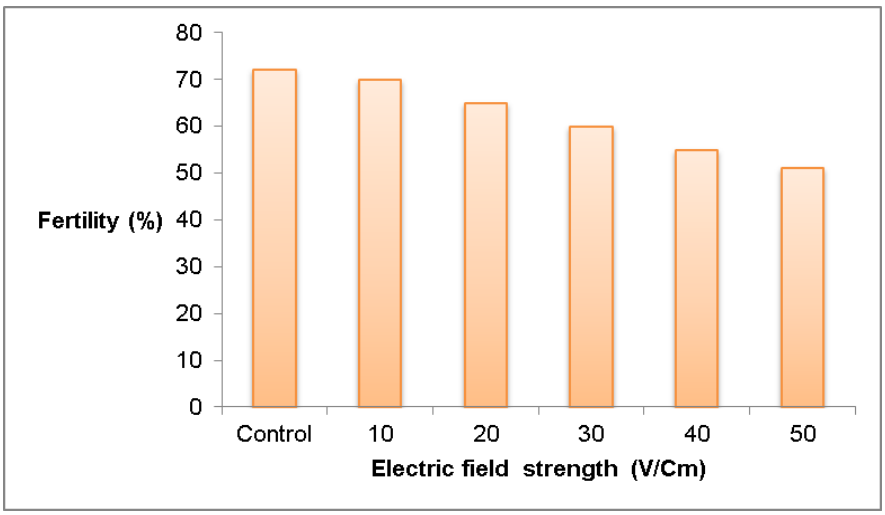

Figure 4. Graphic of Fertility of Koi Fish Sperms

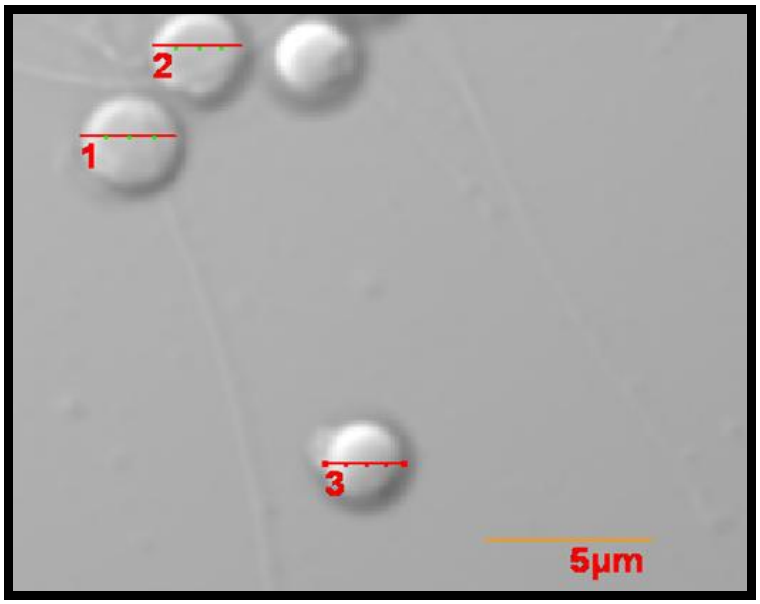

Figure 5. Total length of Koi fish sperms was 15 µm with head width 2.75 µm and tail length 12.5 µm.

CONCLUSION AND RECOMMENDATION

Some conclusions were made from this research, such as:

1. The density of sperm cells was 8.4 x 10^9 cells/ml.
2. The highest motility was 77 % and it was obtained at electric field strength 10 V/Cm whereas the lowest was 58 % attained at electric field strength 50 V/Cm.
3. The highest viability was 80 % and it was obtained from electric field strength 10 V/Cm whereas the lowest was 59 % attained from electric field strength 50 V/Cm.
4. The higher electric field strength was the fewer surviving sperms.
5. Treating sperms with different levels of electric field strength (V/Cm) with different form of square wave was not producing obvious difference on motility and viability of Koi fish sperms.
6. The average rate of fertility of control sperms was 72 %, while that of sperms treated with electric field strength 50 (V/Cm) was 51 %.

It was suggested from this research that:

1. To obtain better motility and viability rates, electric field strength 10 V/Cm should be considered.
2. Further research could be focused on electroporation with square wave method against other fish species.

DECLARATIONS

Acknowledgements

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Authors' Contributions

All authors contributed equally to this work.

Competing interests

The authors declare that they have no competing interests.

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Biomonitoring of Heavy Metals (Cu, Zn, Pb, Cd and Ni) in the West Coast of Peninsular Malaysia Using Giant Mudskipper *Periophthalmodon Schlosseri* (Pallas 1770)

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ABSTRACT

The giant mudskipper *Periophthalmodon schlosseri* (Pallas 1770) is the largest notable species of Malaysian mudskippers and invariable the best known example of resident intertidal fish. This paper present an assessment of heavy metals concentration; Cu, Zn, Pb, Cd and Ni in scales, muscle, bone, gills, operculum, intestine, liver and cartilage of *Periophthalmodon schlosseri* (*P. schlosseri*) with aim of proposing it as a biomonitoring agent in the coastal mudflats. The giant mudskippers; *P. schlosseri* were collected from six sampling sites in the west coast of Peninsular Malaysia and analyzed for heavy metals concentrations; Cu, Zn, Pb, Cd and Ni in scales, muscle, bone, gills, operculum, intestine, liver and cartilage by using an air-acetylene flame atomic absorption spectrophotometer, Perkin Elmer Analyst 800. The levels of heavy metals concentrations in the tissues of *P. schlosseri* varied significantly ($P < 0.05$) with exception of Cu in gills ($P > 0.05$). The studied metals showed different accumulation target in the fish; Cu was observed highest in liver while Zn, Pb, Cd and Ni exhibited their highest concentrations in cartilage. The pattern of metal accumulation was in the order: Zn > Pb > Ni > Cu > Cd, with a similar pattern observed for metal selectivity index (MSI %) value. There was no unique ranking with regards to tissue selectivity index (TSI %) among the tissues, except liver which showed high copper selectivity at all the six sampling sites. However, the result of biota-sediment accumulation factor (BSAF) for Cu and Zn were highest in liver and intestine respectively while the BSAF for Pb, Cd and Ni were highest in cartilage. Results of the present study indicate bioaccumulation of the studied metals in the tissues of giant mudskipper *P. schlosseri* which might suggest it use as a potential bio monitoring agent for heavy metals in the west coast of Peninsular Malaysia.

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Biomonitoring, Heavy metals, *Periophthalmodon schlosseri*, West coast Peninsular Malaysia.

ABBREVIATIONS

AAS: atomic absorption spectrophotometer; **ANOVA:** one way analysis of variance; **BSAF:** biota sediment accumulation factor; **°C:** degree Celsius; **CB:** Chemical concentration in the biota; **cm:** centimeter; **CRM:** certified reference material; **CS:** chemical concentration in the sediment; **EFLE:** easily freely leachable and exchangeable; **DDT:** dichlorodiphenyltrichloroethane; **DDW:** double distilled water; **d/w:** dry weight; **g:** gram; **GPS:** global positioning system; **HNO3:** Nitric acid; **IAEA:** international atomic energy agency; **kg:** kilo gram; **mm:** millimeter; **ml:** milliliter; **mg/L:** milligram per liter; **MSI:** metal selectivity index; **MT:** metallothionein; **NRCC:** national research council Canada; **ND:** not detectable; **nm:** nanometer; **%:** percentage; ***P. schlosseri*:** *Periophthalmodon schlosseri*; **QA:** quality assurance; **QC:** quality control; **SET:** sequential extraction technique; **TSI:** tissue selectivity index; **µg/g:** microgram per gram; **µm:** micrometer

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INTRODUCTION

Fishes are one of the most important and largest groups of vertebrates in the aquatic system, that are globally accepted as excellent organisms for the study of heavy metals pollution in aquatic environment. They have been used as one of the most indicative organisms for assessing metal pollution in freshwater and marine systems [1]. Fish are relatively situated at the top of the aquatic food chain; therefore, they can normally accumulate heavy metals from food, water and sediments [2, 3]. Bioaccumulation of metals in fish can be considered as an index of metal pollution in the aquatic bodies [4, 5] and a useful tool to study the biological role of metals present at higher concentrations in fish [6, 7]. The use of fish as aquatic bio monitor/bio indicator has increase significantly over years because they are sensitive indicators of aquatic changes and possess high tissues metals accumulation capacity. After incorporation into the fish body, heavy metals were distributed among different tissues via a process that depends on biological needs [8] and subsequently transferred to higher trophic levels via food chain. However, metals amassing in tissues reveal the past exposure of fish via water or food [9-11] or sediment which can serve as pollution indicator.

Mudskippers are peculiar-looking amphibious fish that are characteristic for mangrove forests and mudflats [12]; they are members of the subfamily Oxudercinae, tribe *Periophthalmini*, family Gobiidae (Gobies) [13]. They are the key species of the soft bottom intertidal areas and mangrove swamps of the Indo-west Pacific region and tropical African coast [13].

The unique behavior and ecology of mudskippers make them potential bio monitoring agents. Mudskippers inhabiting the coastal ecosystems faces a direct exposure to most of the heavy metals and metalloids that are discharged from power, thermal, desalination and water treatment plants, and leakage from oil wells in dissolved as well as dietary phases [14], they are requisites of an ideal bio monitor as they are able to accumulate metals that are relatively easy to measure, easy to identify, abundant in the study area, size large enough to provide sufficient tissues for analyses on individuals and possess a stationary behavior and do not migrate [15, 16]. In particular, mudskippers are known to accumulate higher concentrations of some toxic compounds (e.g. DDT and some heavy metals) in their tissues, relative to other aquatic and benthic species [17]. The mudskippers showed a very high potential for bioaccumulation of different types of pollutants from coastal waters and are very important for bio monitoring the coastal ecosystems [14].

The giant mudskipper, *P. schlosseri* is one of the notable species of mudskipper that spends much of its time out of water [13] and the largest Malaysian mudskipper. The unique characteristics features of this fish being a dominant force on the mudflat, unintimidated by snakes and birds that harassed its smaller relatives [18], top predator in the food chain, big size, time dwell in water and intertidal coastal mudflats make them a good and interesting research organisms for the study of heavy metals accumulation and environmental monitoring assessment. In fact, their robustness to environmental stressors and tolerance to many contaminants give them the capacity to be chronically exposed to toxicants without significant acute effects, while their relatively low trophic status makes them less prone to biomagnifying toxicants [17]. Therefore, they were chosen in this study because they could enable continuous surveillance of pollutant presence in water and/ or sediment where they live.

Although, several studies demonstrated that fishes are important biological indicator for investigation of heavy metal contamination and health risk to both animals directly and human upon consumptions of aquatic foods in different parts of the world [19-23] but only few counted number of researches are available on heavy metals concentrations in *P. schlosseri*. Therefore, the objective of this study is: to assess the concentrations of heavy metals (Cu, Zn Cd, Pb and Ni) in scale, muscle, bone, gills, operculum, intestine, liver and cartilage of giant mudskipper P. schlosseri and determine its potential as a biomonitoring organism for heavy metals pollution in the coastal mudflat environment of west coast of Peninsular Malaysia.

MATERIAL AND METHODS

Ethical approval

The authors declare that this research followed the ethical guidance for animal research. The study protocol and ethics were approved by Postgraduate studies committee/ supervisory committee, Department of Biological Science, Universiti Putra Malaysia.

Sample collection and preparation

Surface sediments and giant mudskipper *Periophthalmodon schlosseri* were collected from six sampling sites in the west coast of Peninsular Malaysia (Figure 1). The samplings were conducted in August 2008 at Segantang Garam and Minyak Beku, in September 2008 at Sungai Tiga and Sungai Puluh and in March and June 2010 at Bagan Lalang and Kuala Juru, respectively. The coordinates of the sampling sites were recorded with Global Positioning System (GPS); (Garmin OREGON 45OT 850 MB waterproof GPS). The coordinates and description of each sampling site were given in Table 1.

Top 3 - 5 cm surface sediments [24, 25] were collected in triplicates from three different points within a certain area (approximately 1 meter radius) from each sampling site using plastic scoop and placed in separate labeled polyethylene plastic bags. Each sediment sample was instantly placed in ice and transported to the laboratory until further analysis. Fish were collected using trap net at almost the same locations were the sediments were collected and placed in a plastic aquarium containing some sediment and water. Fish samples were transported to Ecotoxicology laboratory, Department of Biological Sciences, Universiti Putra Malaysia, were stomach and intestines were emptied and dissected immediately or put in labeled plastic bags and kept in deep freeze at –20˚C until further analysis.

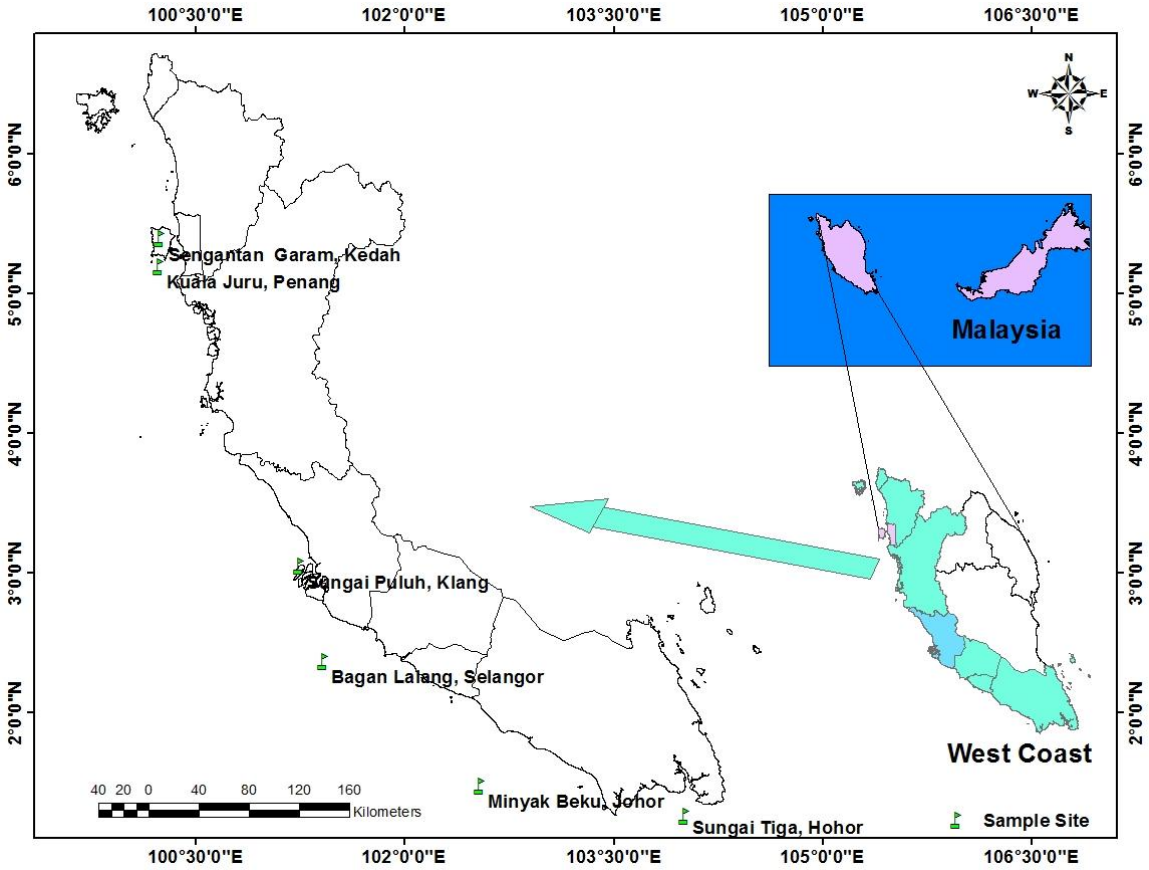

Figure 1. Map of west coast of Peninsular Malaysia showing the six sampling sites.

Table 1. Names, coordinates and description of sampling sites in the west coast of Peninsular Malaysia

Sampling site	Coordinates	Site description
Segantang Garam, Kedah (S.Garam)	N 05° 39.552′ E 100° 23.983′	Jetty, aqua cultural area and paddy field
Kuala Juru, Penang (K. Juru)	N 05° 19.683′ E 100° 22.949′	Industrial area, urbanization and aquaculture
Sungai Puluh, Klang (Sg. Puluh)	N 03° 04.786′ E 101° 23.903′	Jetty receiving domestic wastes and industrial area
Bagan Lalang, Selangor (Bg. Lalang)	N 02° 36.669′ E101° 41.100′	Recreational and agricultural areas
Minyak Beku, Johor (M.Beku)	N 01° 47.746′ E102° 53.395′	Jetty receiving domestic wastes and shipping activities
Sungai Tiga, Johor (Sg.Tiga)	N 01° 25.841′ E 104° 00.281′	Jetty, agricultural and oil plantation

Metal analysis

Sediment samples were dried in the laboratory using an air-circulating oven to a constant dry weight (d/w) at 80˚C. The dried sediment samples were crushed to powder by using a porcelain mortar and pestle then sieved vigorously to produce homogeneity [26], through a 63 µm stainless steel aperture sieve.

Geochemical fractions of Cu, Zn, Pb, Cd and Ni in the sediments were determined by modified sequential extraction technique (SET) as describe by Badri and Aston [27] and Tessier and Campbell [28]. The four-step extraction procedure of sediment handling and analysis employed using SET was described as:

1) Easily, freely or leachable and exchangeable (EFLE) (Fraction 1); 2) acid reducible (Fraction 2); 3) oxidisable organic (Fraction 3) and; 4) resistant fractions (Fraction 4). The mathematical summation of the first three types of fractions constitutes the "non-resistant phase which is closely related to anthropogenic inputs" [27].

Fish samples were removed from the refrigerator and plastic bags, rinsed with double distilled water (DDW) then thawed at room temperature. About 6-10 *P. schlosseri* from each sampling sites with the recorded body length (mm) and weight (g) were dissected on clean plastic material using stainless steel kits and glass equipment. The dissected parts were pooled into eight different parts namely; scales, muscle, bone, gills, operculum, intestine, liver and cartilage. These tissues were chosen because they play a role in metals uptake, bioaccumulation, formation of metal-complexes, storage and detoxification processes. All the eight parts were dried at 80˚C according to Mucha et al. [29] until a constant dry weight. Sample of each dried part was weighed separately (0.5 - 1.0 g) in triplicate and placed in digestion tubes. To each digestion tube 10 ml concentrated nitric acid (AnalaR grade, BDH 69%) was added and placed in a hot block digester unit at 40˚C for 1 hour (h). The temperature was then increased to 140˚C for at least three hours [26]. The digested samples were diluted to 40ml with DDW. The samples were then filtered through filter papers into pill box and the filtrate was stored until metal determination. The filtrates obtained from sediments and biological samples were determined for Cu, Zn, Pb, Cd and Ni by using an air-acetylene flame atomic absorption spectrophotometer (AAS) Perkin Elmer Analyst 800. Standard solutions were prepared from 1000 mg/L stock solution of each metal (BDH-Spectrosol). Standard solutions were prepared from 1000 mg/L stock solution of each metal (BDH-Spectrosol). The wavelengths for each metal were 324.8, 213.9, 283.3, 228.8 and 232.0 nm for Cu, Zn, Pb, Cd and Ni respectively.

The data were presented in µg/g dry weight. Multiple-level calibration standards were analyzed to generate calibration curves against which sample concentrations were calculated. During the period of AAS metal analysis, a quality control sample was routinely included for every 5 - 10 samples. Procedural blanks and quality control samples made from standard solutions for Cu, Zn, Pb, Cd and Ni were analyzed after every 5 - 10 samples to ensure the sensitivity and recovery of the instrument used. The procedures of quality assurance (QA) and quality control (QC) were employed to ensure the validity of the analytical data [30]. All plastics and glassware used were washed with detergent, Deacon 90, rinsed with double-distilled water and soaked in 10% HNO3 for at least 24 h, then rinsed with double distilled water and allowed to dry at room temperature. The QA and QC were controlled by procedural blanks, sample replicates and certified reference material (CRM). The quality of the method was checked with a CRM for soil from International Atomic Energy Agency (IAEA), Soil-5, Vienna; Austria and Dogfish liver DOLT-3 from National Research Council Canada (NRCC) were analyzed. These were checked to accuracy of the digestion method with the certified values supplied by the IAEA and NRCC. To ensure the sensitivity of the Atomic Absorption Spectrophotometer (AAS) and generate calibration curves against which sample concentrations were calculated. The results of similar digested samples analyzed for Cu, Zn, Pb, Cd, and Ni by the flame AAS Perkin Elmer. An analyst 800 showed acceptable recoveries of the metals; about 94% - 107% for soil and 87% - 110% for dogfish liver.

Heavy Metal Assessment Index in *Periophthalmodon schlosseri*

Metal selectivity index (MSI) and Tissue selectivity index (TSI)

MSI is defined as the relative metal-accumulating capacity of a tissue for a particular metal while TSI is defined as the relative tissue-occupying capacity of a metal in a particular tissue. MSI and TSI were calculated according to Nair et al. [31] as follows:

MSI = (absolute concentration of a metal in a tissue ×100)/Total concentration of all metals in that tissue

TSI = (absolute concentration of a metal in a tissue ×100)/Total concentration of that metal in all tissues

Biota sediment accumulation factor (BSAF)

Biota sediment accumulation factor (BSAF) is a parameter defining bioaccumulation of sediment-associated organic compounds or metals in tissues of the organism [32]. In the present study, the metal bioaccumulation in the tissues of giant mudskipper was evaluated by calculating the BSAF, which is defined as the ratio between the metal concentration in tissues and that in the sediment. Heavy metals accumulation in tissues of *P. schlosseri* from the sediments is expressed by the BSAF.

The BSAF is calculated using the following equation:

BSAF = CB/CS

Where; CB is the chemical concentration in the biota [mass of chemical per kg of biota/dry weight (d/w)], while CS is the concentration in the related sediment (mass of chemical per kg of sediment/d/w).

In the present study, the summation of non-resistant geochemical fractions was used as the total metal concentration (µg/g d/w) in sediment due to its bioavailable characteristics to the living organisms. BSAF is used to calculate the ratio of metal found in the organism to that in the sediments based on the amount of metal it accumulated. BSAF was used to classify the tissues of *P. schlosseri* as a macroconcentrator (BSAF > 2), microconcentrator (1 < BSAF < 2) or deconcentrator (BSAF < 1).

Statistical analysis

All statistical analyses of data were carried out using SPSS statistical package programs version 17 and graphs were plotted with Microsoft EXCEL 2007. Data were tested for the basic assumptions of normality and homogeneity of variance in exploratory data analysis in SPSS 17. One way analysis of variance (ANOVA) was calculated, post host comparison was made using Duncan's multiple range test at 0.05 confidence level.

RESULTS

Heavy metals concentrations in surface sediments

The Biota sediment accumulation factor (BSAF) of heavy metals in the tissues of *P. schlosseri,* was calculated by using the non-resistant geochemical fraction of heavy metals in the surface sediments from the six sampling sites in the west coast of Peninsular Malaysia and was presented in Table 2.

Heavy metals in *P. Schlosseri*

The measured metal concentrations in the tissues of *P. schlosseri* were presented in Table 3, as mean ± SE. The highest Cu concentration was observed in liver as 18.61 µg/g dry weights at M.Beku while cartilage recorded the highest concentrations of Zn, Pb, Cd and Ni as 218.28, 37.71, 4.42 and 121.84 µg/g dry weights at Bg. Lalang, Sg. Tiga, K. Juru, and K. Juru respectively. The lowest concentrations of Cu, Zn, Pb and Cd were found in muscle at Sg. Tiga, K. Juru, M. Beku and Sg. Tiga respectively, while liver recorded the lowest concentration of Ni at Bg. Lalang. Zinc concentrations were the highest in all tissues of *P. schlosseri* from the six sampling sites, except Ni concentration in cartilage at K.Juru.

Metal Selectivity Index (MSI) and Tissue Selectivity Index (TSI)

The MSI (%) and TSI (%) values were calculated for the five metals and presented in Tables 4 and 5 respectively. Based on the MSI (%) values, the affinity for metals in the tissues of *P. schlosseri* can generally be ranked in the order Zn > Pb > Ni > Cu > Cd, irrespective of the type of tissue for most of the sampling sites. Zn stands as the metal being accumulated to the greatest extent in all the tissues, except Ni in muscle and cartilage at K.Juru. The highest TSI (%) values for Cu and Pb were recorded in liver and gills respectively while highest TSI values for Zn, Cd and Ni were recorded in cartilage.

Biota sediment accumulation factor (BSAF)

The Biota sediment accumulation factor (BSAF) for Zn, Cu, Pb, Cd, and Ni in the tissues of *P. schlosseri* based on non – resistant sediments concentration are tabulated in Table 6. The BSAFs indicated bioaccumulation of heavy metals in intestine, liver, operculum, cartilage, gills and bone. Liver and intestine recorded the highest BSAF for Cu and Zn respectively, while the highest BSAF for Pb, Cd and Ni were recorded in cartilage.

[a] deconcentrators; [b] microconcentrators; [c] macroconcentrators

Table 2. Mean concentrations (µg/g d/w, n=3) of non-resistant geochemical fraction of Cu, Zn Pb, Cd and Ni in sediments of west coast of peninsular Malaysia.

Sampling site	Non-resistant Geochemical Fraction of Metals				
	Cu	Zn	Pb	Cd	Ni
S. Garam	4.89	25.51	9.04	0.39	6.10
K. Juru	9.57	296.32	0.00	0.00	0.65
Sg. Puluh	8.28	178.27	20.76	0.31	23.42
Bg. lalang	2.13	36.80	6.85	0.40	4.25
M. Beku	6.01	114.28	19.85	0.34	10.72
Sg. Tiga	3.60	60.25	14.08	0.36	6.42

Table 3. Mean metal concentration (µg/g d/w; ±SE) in the tissues of *P. schlosseri* from west coast of Peninsular Malaysia. Statistical significant ($P < 0.05$) between the sampling sites for a given examined metal was indicated by different superscript alphabets.

Sampling site	Tissue	Concentration (µg/g dry weight; ± SE)				
		Cu	Zn	Pb	Cd	Ni
S.Garam (n=10)	Scale	7.30^{d}±0.29	102.58^{c}±0.98	22.86^{c}±1.00	1.55^{b}±0.23	8.20^{ab}±0.13
	Muscle	5.23^{c}±0.37	45.24^{bc}±4.74	2.29^{a}±0.61	0.19^{a}±0.15	1.09^{a}±0.49
	Bone	7.16^{c}±0.06	75.76^{b}±0.79	19.01^{c}±2.00	1.58^{b}±0.24	5.06^{a}±0.15
	Gills	8.24^{a} ±0.29	100.40^{bc}±1.95	14.67^{a}±1.52	0.84^{bc}±0.23	3.98^{a}±0.55
	Operculum	7.49^{c}±0.32	109.54^{cd}±2.05	19.98^{c}±0.34	1.09^{bc}±0.19	6.15^{a}±0.89
	Intestine	16.98^{d}±0.32	152.63^{e}±1.69	11.98^{c}±0.16	0.61^{c}±0.18	5.82^{a}±1.16
	Liver	13.47^{b}±0.26	60.27^{c}±2.74	10.11^{d}±0.43	0.49^{ab}±0.22	2.90^{b}±0.41
	Cartilage	9.96^{c}±0.19	72.60^{c}±1.67	34.51^{d}±0.85	0.08^{a}±0.01	17.11^{b}±1.45
K.Juru (n=6)	Scale	3.50^{c}±0.23	68.30^{a}±5.75	1.57^{a}±0.07	1.17^{b}±0.07	14.90^{c}±4.33
	Muscle	1.72^{b}±0.16	12.79^{a}±11.23	0.43^{a}±0.04	0.11^{a}±0.03	21.00^{c}±0.92
	Bone	3.61^{b}±0.08	63.48^{a}±5.68	3.90^{a}±0.08	1.45^{b}±0.23	29.64^{c}±2.67
	Gills	4.52^{a}±0.06	93.37^{ab}±1.65	7.87^{a}±0.30	0.68^{b}±0.23	9.89^{b}±0.29
	Operculum	3.77^{a}±0.08	96.60^{ab}±4.39	3.13^{a}±0.05	1.29^{cd}±0.09	19.91^{b}±3.15
	Intestine	8.96^{b}±0.41	105.26^{c}±3.39	5.83^{b}±0.08	0.40^{bc}±0.10	89.98^{b}±13.59
	Liver	9.30^{a}±0.92	45.36^{b}±1.19	5.42^{bc}±0.96	1.33^{c}±0.12	5.04^{c}±0.70
	Cartilage	8.50^{c}±0.38	54.10^{a}±1.88	9.32^{a}±0.84	4.42^{b}±0.82	121.84^{c}±5.77
Sg.Puluh (n=10)	Scale	7.09^{d}±0.03	96.65^{c}±1.75	19.14^{bc}±0.38	0.49^{a}±0.20	12.35^{bc}±2.12
	Muscle	5.45^{d}±0.28	44.08^{c}±4.93	2.09^{bc}±1.17	0.20^{a}±0.11	8.74^{b}±0.72
	Bone	6.98^{c}±0.06	73.09^{ab}±2.19	15.21^{b}±1.64	0.55^{a}±0.26	12.70^{b}±0.23
	Gills	7.83^{a}±0.37	95.03^{ab}±3.50	12.09^{a}±0.64	0.28^{ab}±0.18	10.87^{b}±0.84
	Operculum	7.81^{c}±0.21	101.36^{bc}±2.45	15.38^{b}±2.93	0.91^{b}±0.12	15.64^{b}±1.04
	Intestine	15.56^{c}±0.79	80.75^{b}±0.72	10.49^{c}±2.30	0.08^{ab}±0.06	12.13^{a}±1.31
	Liver	14.67^{b}±1.18	34.31^{a}±2.11	7.90^{cd}±0.90	0.40^{ab}±0.20	12.51^{e}±0.85
	Cartilage	8.26^{c}±1.10	58.49^{b}±0.90	23.71^{c}±0.49	0.31^{a}±0.15	17.52^{a}±1.44
Bg.Lalang (n=9)	Scale	2.84^{b}±0.17	101.22^{c}±6.44	3.86^{a}±0.32	1.53^{b}±0.15	4.26^{a}±0.76
	Muscle	1.28^{b}±0.11	62.79^{c}±5.71	1.02^{a}±0.07	0.07^{a}±0.02	0.04^{a}±0.02
	Bone	3.32^{b}±0.22	82.97^{b}±2.12	3.21^{a}±0.02	1.76^{b}±0.18	7.71^{a}±0.22
	Gills	15.77^{a}±11.68	117.90^{d}±1.28	12.73^{a}±9.40	1.40^{c}±0.22	3.75^{a}±0.05
	Operculum	3.84^{b}±0.14	133.76^{e}±3.47	3.10^{a}±0.08	1.42^{cd}±0.08	5.03^{a}±0.44
	Intestine	7.10^{a}±0.58	123.53^{d}±1.10	2.36^{a}±0.48	0.59^{c}±0.15	0.04^{a}±0.01
	Liver	17.83^{c}±0.88	89.39^{d}±0.77	2.43^{ab}±0.26	0.60^{b}±0.06	0.04^{a}±0.01
	Cartilage	8.54^{c}±1.50	218.28^{e}±1.32	8.54^{a}±0.85	3.48^{b}±0.58	10.09^{ab}±1.01
M.Beku (n=10)	Scale	3.78^{c}±0.07	84.08^{b}±1.69	18.32^{b}±0.96	1.16^{b}±0.22	6.23^{ab}±0.14
	Muscle	1.21^{b}±0.18	43.40^{bc}±1.86	0.04^{a}±0.00	1.21^{b}±0.04	0.60^{a}±0.29
	Bone	3.38^{b}±0.07	81.50^{b}±4.05	19.53^{c}±1.19	1.70^{b}±0.32	7.89^{a}±0.88
	Gills	4.25^{a}±0.09	106.01^{c}±2.78	11.55^{a}±0.57	1.40^{c}±0.06	3.70^{a}±0.21
	Operculum	4.17^{b}±0.04	115.21^{d}±1.75	17.31^{bc}±0.90	1.49^{d}±0.07	5.40^{a}±0.13
	Intestine	9.75^{b}±0.00	120.46^{d}±1.10	0.20^{a}±0.01	0.04^{a}±0.01	0.16^{a}±0.06
	Liver	18.61^{c}±1.32	84.87^{d}±2.30	0.04^{a}±0.01	0.23^{ab}±0.20	0.67^{a}±0.06
	Cartilage	5.09^{b}±0.58	85.11^{d}±1.10	20.54^{b}±0.91	1.03^{a}±0.01	6.92^{a}±0.58
Sg.Tiga (n=10)	Scale	1.66^{a}±0.11	68.90^{a}±2.86	35.38^{d}±3.08	0.04^{a}±0.01	6.03^{a}±0.25
	Muscle	0.04^{a}±0.00	36.88^{b}±0.44	20.65^{b}±1.75	0.05^{a}±0.02	0.28^{a}±0.06
	Bone	0.83^{a}±0.75	63.40^{a}±4.97	31.71^{d}±0.44	0.13^{a}±0.09	5.82^{a}±0.99
	Gills	0.85^{a}±0.77	89.41^{a}±5.56	28.06^{b}±2.08	0.04^{a}±0.01	3.47^{a}±0.32
	Operculum	0.17^{a}±0.11	90.18^{a}±4.89	33.52^{d}±1.00	0.04^{a}±0.00	6.22^{a}±0.49
	Intestine	9.39^{b}±0.00	66.28^{a}±1.16	28.45^{a}±0.93	0.05^{a}±0.01	4.46^{a}±0.38
	Liver	10.38^{a}±0.88	61.86^{c}±1.75	29.02^{e}±2.18	0.04^{a}±0.01	6.64^{d}±0.41
	Cartilage	0.48^{a}±0.11	76.02^{c}±1.36	37.71^{e}±0.47	0.04^{a}±0.01	12.42^{b}±0.90

Table 4. Metal selectivity index (MSI) in the tissues of *P. schlosseri*.

Sampling site	Tissue	MSI (%)				
		Cu	Zn	Pb	Cd	Ni
S.Garam	Scale	5.12	71.99	16.04	1.09	5.75
	Muscle	9.68	83.72	4.24	0.35	2.02
	Bone	6.59	69.78	17.51	1.46	4.66
	Gills	6.43	78.36	11.45	0.66	3.11
	Operculum	5.19	75.94	13.85	0.76	4.26
	Intestine	9.03	81.18	6.37	0.32	3.10
	Liver	15.44	69.09	11.59	0.56	3.32
	Cartilage	7.42	54.07	25.70	0.06	12.74
K.Juru	Scale	3.91	76.36	1.76	1.31	16.66
	Muscle	4.77	35.48	1.19	0.31	58.25
	Bone	3.54	62.19	3.82	1.42	29.04
	Gills	3.89	80.26	6.77	0.58	8.50
	Operculum	3.02	77.47	2.51	1.03	15.97
	Intestine	4.26	50.02	2.77	0.19	42.76
	Liver	14.00	68.26	8.16	2.00	7.58
	Cartilage	4.29	27.30	4.70	2.23	61.48
Sg.Puluh	Scale	5.22	71.21	14.10	0.36	9.10
	Muscle	9.00	72.79	3.45	0.33	14.43
	Bone	6.43	67.35	14.01	0.51	11.70
	Gills	6.21	75.36	9.59	0.22	8.62
	Operculum	5.54	71.84	10.90	0.64	11.08
	Intestine	13.07	67.85	8.81	0.07	10.19
	Liver	21.02	49.16	11.32	0.57	17.93
	Cartilage	7.63	54.01	21.89	0.29	16.18
Bg.Lalang	Scale	2.50	89.02	3.39	1.35	3.75
	Muscle	1.96	96.30	1.56	0.11	0.06
	Bone	3.35	83.83	3.24	1.78	7.79
	Gills	10.41	77.80	8.40	0.92	2.47
	Operculum	2.61	90.90	2.11	0.97	3.42
	Intestine	5.31	92.45	1.77	0.44	0.03
	Liver	16.17	81.05	2.20	0.54	0.04
	Cartilage	3.43	87.69	3.43	1.40	4.05
M.Beku	Scale	3.33	74.03	16.13	1.02	5.49
	Muscle	2.60	93.41	0.09	2.60	1.29
	Bone	2.96	71.49	17.13	1.49	6.92
	Gills	3.35	83.53	9.10	1.10	2.92
	Operculum	2.90	80.24	12.06	1.04	3.76
	Intestine	7.46	92.23	0.15	0.03	0.12
	Liver	17.82	81.28	0.04	0.22	0.64
	Cartilage	4.29	71.71	17.31	0.87	5.83
Sg.Tiga	Scale	1.48	61.51	31.59	0.04	5.38
	Muscle	0.07	63.70	35.66	0.09	0.48
	Bone	0.81	62.22	31.12	0.13	5.71
	Gills	0.70	73.39	23.03	0.03	2.85
	Operculum	0.13	69.30	25.76	0.03	4.78
	Intestine	8.64	61.01	26.19	0.05	4.11
	Liver	9.62	57.31	26.89	0.04	6.15
	Cartilage	0.38	60.01	29.77	0.03	9.81

Table 5. Tissue selectivity index (TSI) in the tissues of *P. schlosseri.*

Sampling site	Tissues	TSI (%)				
		Cu	Zn	Pb	Cd	Ni
S.Garam	Scale	9.63	14.27	16.88	24.11	16.30
	Muscle	6.90	6.29	1.69	2.95	2.17
	Bone	9.44	10.54	14.04	24.57	10.06
	Gills	10.87	13.96	10.83	13.06	7.91
	Operculum	9.88	15.23	14.76	16.95	12.22
	Intestine	22.39	21.23	8.85	9.49	11.57
	Liver	17.76	8.38	7.47	7.62	5.76
	Cartilage	13.13	10.10	25.49	1.24	34.01
K.Juru	Scale	7.98	12.67	4.19	10.78	4.77
	Muscle	3.92	2.37	1.15	1.01	6.73
	Bone	8.23	11.77	10.41	13.36	9.49
	Gills	10.30	17.31	21.00	6.27	3.17
	Operculum	8.59	17.91	8.35	11.89	6.38
	Intestine	20.42	19.52	15.56	3.69	28.82
	Liver	21.19	8.41	14.46	12.26	1.61
	Cartilage	19.37	10.03	24.87	40.74	39.03
Sg.Puluh	Scale	9.63	16.56	18.05	15.22	12.05
	Muscle	7.40	7.55	1.97	6.21	8.53
	Bone	9.48	12.52	14.35	17.08	12.40
	Gills	10.63	16.28	11.40	8.70	10.61
	Operculum	10.60	17.36	14.51	28.26	15.26
	Intestine	21.13	13.83	9.90	2.48	11.84
	Liver	19.92	5.88	7.45	12.42	12.21
	Cartilage	11.22	10.02	22.37	9.63	17.10
Bg.Lalang	Scale	4.69	10.89	10.36	14.10	13.76
	Muscle	2.12	6.75	2.74	0.65	0.13
	Bone	5.49	8.92	8.62	16.22	24.90
	Gills	26.06	12.68	34.17	12.90	12.11
	Operculum	6.35	14.39	8.32	13.09	16.25
	Intestine	11.73	13.29	6.34	5.44	0.13
	Liver	29.46	9.61	6.52	5.53	0.13
	Cartilage	14.11	23.48	22.93	32.07	32.59
M.Beku	Scale	7.52	11.67	20.93	14.04	19.73
	Muscle	2.41	6.02	0.05	14.65	1.90
	Bone	6.73	11.31	22.31	20.58	24.99
	Gills	8.46	14.71	13.20	16.95	11.72
	Operculum	8.30	15.99	19.78	18.04	17.10
	Intestine	19.41	16.72	0.23	0.48	0.51
	Liver	37.04	11.78	0.05	2.78	2.12
	Cartilage	10.13	11.81	23.47	12.47	21.92
Sg.Tiga	Scale	6.97	12.46	14.47	9.30	13.30
	Muscle	0.17	6.67	8.45	11.63	0.62
	Bone	3.49	11.47	12.97	30.23	12.84
	Gills	3.57	16.17	11.48	9.30	7.65
	Operculum	0.71	16.31	13.71	9.30	13.72
	Intestine	39.45	11.99	11.64	11.63	9.84
	Liver	43.61	11.19	11.87	9.30	14.64
	Cartilage	2.02	13.75	15.42	9.30	27.39

Table 6. Metals Biota sediment accumulation factor (BSAF) in the tissues of *P. schlosseri* from west coast of Peninsular Malaysia.

Sampling site	Tissues	Cu	Zn	Pb	Cd	Ni
S. Garam	**Scale**	1.49^{b}	4.02^{c}	2.53^{c}	3.97^{c}	1.35^{b}
K. Juru		0.37^{a}	0.23^{a}	0.15^{a}	2.93^{c}	22.89^{c}
Sg. Puluh		0.86^{a}	0.54^{a}	0.92^{a}	1.49^{b}	0.53^{a}
Bg. lalang		1.33^{b}	2.75^{c}	0.56^{a}	4.94^{c}	1.00^{b}
M. Beku		0.63^{a}	0.74^{a}	0.92^{a}	3.44^{c}	0.58^{a}
Sg. Tiga		0.46^{a}	1.14^{b}	2.51^{c}	0.11^{a}	0.94^{a}
S. Garam	**Muscle**	1.07^{b}	1.77^{b}	0.25^{a}	0.48^{a}	0.18^{a}
K. Juru		0.18^{a}	0.04^{a}	0.04^{a}	0.27^{a}	32.27^{c}
Sg. Puluh		0.66^{a}	0.25^{a}	0.10^{a}	0.60^{a}	0.37^{a}
Bg. lalang		0.60^{a}	1.71^{b}	0.15^{a}	0.21^{a}	0.01^{a}
M. Beku		0.20^{a}	0.38^{a}	0.00^{a}	3.59^{c}	0.06^{a}
Sg. Tiga		0.01^{a}	0.61^{a}	1.47^{b}	0.15^{a}	0.04^{a}
S.Garam	**Bone**	1.46^{b}	2.97^{c}	2.10^{c}	4.05^{c}	0.83^{a}
K. Juru		0.38^{a}	0.21^{a}	0.37^{a}	3.62^{c}	45.54^{c}
Sg. Puluh		0.84^{a}	0.41^{a}	0.73^{a}	1.65^{b}	0.54^{a}
Bg. lalang		1.56^{b}	2.25^{c}	0.47^{a}	5.71^{c}	1.82^{b}
M. Beku		0.56^{a}	0.71^{a}	0.98^{a}	5.05^{c}	0.74^{a}
Sg. Tiga		0.23^{a}	1.05^{b}	2.25^{c}	0.37^{a}	0.91^{a}
S.Garam	**Gills**	1.68^{b}	3.94^{c}	1.62^{b}	2.14^{c}	0.65^{a}
K. Juru		0.47^{a}	0.32^{a}	0.75^{a}	1.70^{b}	15.19^{c}
Sg. Puluh		0.95^{a}	0.53^{a}	0.58^{a}	0.85^{a}	0.46^{a}
Bg. lalang		7.39^{c}	3.20^{c}	1.86^{b}	4.52^{c}	0.88^{a}
M. Beku		0.71^{a}	0.93^{a}	0.58^{a}	4.15^{c}	0.35^{a}
Sg.Tiga		0.24^{a}	1.48^{b}	1.99^{b}	0.11^{a}	0.54^{a}
S. Garam	**Operculum**	1.53^{b}	4.29^{c}	2.21^{c}	2.79^{c}	1.01^{b}
K. Juru		0.39^{a}	0.33^{a}	0.30^{a}	3.23^{c}	30.59^{c}
Sg. Puluh		0.94^{a}	0.57^{a}	0.74^{a}	2.74^{c}	0.67^{a}
Bg. lalang		1.80^{b}	3.64^{c}	0.45^{a}	4.59^{c}	1.18^{b}
M. Beku		0.69^{a}	1.01^{b}	0.87^{a}	4.42^{c}	0.50^{a}
Sg. Tiga		0.05^{a}	1.50^{b}	2.38^{c}	0.11^{a}	0.97a
S. Garam	**Intestine**	3.47^{c}	5.98^{c}	1.33^{b}	1.56^{b}	0.95^{a}
K. Juru		0.94^{a}	0.36^{a}	0.55^{a}	1.00^{b}	138.23^{c}
Sg. Puluh		1.88^{b}	0.45^{a}	0.51^{a}	0.24^{a}	0.52^{a}
Bg. lalang		3.33^{c}	3.36^{c}	0.34^{a}	1.91^{b}	0.01^{a}
M. Beku		1.62^{b}	1.05^{b}	0.01^{a}	0.11^{a}	0.01^{a}
Sg. Tiga		2.61^{c}	1.10^{b}	2.02^{c}	0.14^{a}	0.69^{a}
S.Garam	**Liver**	2.75^{c}	2.36^{c}	1.12^{b}	1.26^{b}	0.48^{a}
K. Juru		0.97^{a}	0.15^{a}	0.52^{a}	3.32^{c}	7.74^{c}
Sg. Puluh		1.77^{b}	0.19^{a}	0.38^{a}	1.21^{b}	0.53^{a}
Bg. lalang		8.36^{c}	2.43^{c}	0.35^{a}	1.94^{b}	0.01^{a}
M. Beku		3.10^{a}	0.74^{a}	0.00^{a}	0.67^{a}	0.06^{a}
Sg. Tiga		2.89^{c}	1.03^{b}	2.06^{c}	0.11^{a}	1.03^{b}
S. Garam	**Cartilage**	2.03^{c}	2.85	3.82^{c}	0.20^{a}	2.81^{c}
K. Juru		0.89^{a}	0.18^{a}	0.89^{a}	11.04^{c}	187.17^{c}
Sg. Puluh		1.00^{b}	0.33^{a}	1.14^{b}	0.95^{a}	0.75^{a}
Bg. lalang		4.00^{c}	5.93^{c}	1.25^{b}	11.25^{c}	2.37^{c}
M. Beku		0.85^{a}	0.74^{a}	1.03^{b}	3.07^{c}	0.65^{a}
Sg. Tiga		0.13^{a}	1.26^{b}	2.68^{c}	0.11^{a}	1.93^{b}

Table 7. Heavy metals concentrations (µg/g) in fish species from Malaysia and other regions of the world.

Location	Species	Cu	Zn	Pb	Cd	Ni	Reference
South-western Mediterranean (coast of Sfax)	*Diplodus annularis* *Solea vulgaris* *Liza aurata*	ND-40.81	106-284.90	0.17-5.06	0.10-6.47	0.08-6.42	[76]
Iranian coastline of the Caspian Sea	*Acipenser persicus* *Acipenser stellatus* *Huso huso*	-	11.17-182.26	0.55-18.52	0.001-4.06	-	[81]
Red Sea, Egypt	*Epinephelus sp.,* *Caranx sp.,* *Scarus gibbus,* *Nemipterus japonicus,* *Sardinella sp.,* *Synodus sp.,* *Carangoides bajad,* *Lutjanus bohar,* *Thunnus albacares,* *Gerres oyena,* *Sargocentron spiniferum,* *Siganus rivulatus,* *Lethrinus sp.* *Trachurus mediterraneus*	0.17-18.62	1.17-64.61	0.14-6.93	0.03-8.37	-	[41]
Coastal Waters of Kapar and Mersing, Malaysia	*Arius thalassinus* *Johnius belangeri*	0.66 -55.87	13.12 -739.6	-	0.027-13.35	-	[114]
Kapar Coastal Waters, Malaysia	*Pennahia anea* *Arius maculatus*	0.83 -55.01	17.7-555.8	0.07-1.96	0.02-1.08	-	[80]
Pulicat Lake, North of Chennai, Southeast Coast of India	*Labeo rohita* *Cirrhina mrigala*	BDL-0.19	0.08-0.55	1.06-5.20	0.04-0.15	-	[73]
Coastal waters of Mersing, Malaysia	*Megalaspis cordyla* *Arius thalassinus* *Johnius belangeri*	1.51-26.0	17.54-365.1	0.12-2.03	0.02-6.14	-	[77]
Masan Bay, Korea	*Mugil cephalus , Enedrias nebulosus, Pleuronichthys cornutus,* *Conger myriaster,* *Acanthogobius flavimanus,* *Hexagrammos otakii,* *Sebastiscus marmoratus*	0.18-0.25	6.33-12.90	0.04-0.15	0.01	0.02	[75]
South West Malaysian Coast	*Nemipterus japonicas,* *Chirocentrus dorab,* *Lutjanus sebae,* *Otolithes ruber* *Pampus argenteus*	-	2.33-10.52	0.04-0.26	-	-	[87]

Gulf of Aqaba, Jordan	*Abudefduf saxatili, Chaetodon austricus, Chaetodon fasciatus, Epinephelus fasciatus, Fistularia petimba, Kyphosus sp., Mugil sp, Mulloidichthys auriflama, Parupenus cyclostomus, Polysteganus coeruleopunctatus, Thalassorna sp.*	ND-42.6	1.90-271.70	ND-35.0	ND-10.4	ND-23.0	[115]
Poompuhar coast, India	*Mugil cephalus*	20.48	156.78	ND	ND	0.004	[78]
East and west coast of Peninsular Malaysia	*Decapterus kurroides, Decapterus macrosoma, Megalaspis cordyla, Parastromateus Niger, Scomberoides lysan, Selar crumenophthalmus, Paraplagusia Bilineata, Pomadasys Kaakan, Nemipterus Furcosus, Euthynnus affinis, Rastrelliger kanagurta,* Acanthopagrus latus,	0.86-29.0	17.1-953	0.01-0.88	0.001-33.8	NA	[82]
Victoria Harbour, Hong Kong	*Siganus oramin*	5.7-26.6	66.6-192.9	19.1-94.6	3.3-18.0	-	[83]
Zhejiang Coastal Area, China	*Periophthalmus sericus*	1.31	-	0.064	0.03	19.9	[88]
Sunderban mangrove, India	*Liza parsia*	5.85-28.65	21.25-88.58	-	-	-	[89]
Gulf and Gulf of Oman	*Epinephelus coioides and Lethrinus nebulosus*	0.235-276.0	1.82-240	< 0.001-0.55	<0.001-195.0	<0.01-0.111	[85]
Coastal waters of Uruguay, Uruguay	*Odontesthes sp., Mugil platanus, Micropogonias furnieri, Urophycis brasiliensis, Cynoscion guatucupa, Menticirrhus americanus, and Mustelus schmitti.*	< 0.63-493.0	12.0-214.0	-	-	-	[86]
Coastal Lagoon, Eastern Gulf of California Mexico	*Cathorops fuerthii, Mugil cephalus, Oistithonema libertate, Seriola lalandi, Carcharhinus leucas, Cynoscion xanthulus,*	ND-535.0	6.0-2341.0	0.50-9.20	0.02-165.0	-	[84]

	Galeichthys peruvianus, Lutjanus Colorado and Sphyrna lewini,						
El-Mex Bay, Egypt	*Siganus rivulatus and Sargus sargus*	1.12-44.23	4.03-283.04	1.03-11.97	0.11-2.56	-	[93]
French coast of the Eastern English Channel and Southern Bight of the North Sea	*Pleuronectes platessa, Limanda limanda, Platichthys flesus and Gadus morua*	0.78-52.20	-	ND-0.38	0.004-1.1	-	[92]
Tagus Estuary, Portugal	*Liza ramada, Solea senegalensis and Pomatochistus minutus*	0.94-4.40	18.5-138.2	1.10-8.90	18.5-138.2	-	[74]
Esmoriz-Paramos coastal Lagoon, Portugal	*Liza saliens*	< 2.6-262.1	25.7-88.6	BDL	-	-	[90]
Camargue, French coast	*Anguilla anguilla*	24.0-43.0	104.0-128.0	0.16-0.2	0.04-0.09	-	[91]
West coast, Peninsular Malaysia	*P. schlosseri*	0.04-18.61	12.79-152.63	0.04-37.71	0.04-4.42	0.04-121.84	Present study

ND: Not detectable, BDL: Below detection limit

Table 8. Comparison of heavy metal concentration (µg/g) in giant mudskipper *P. schlosseri* with other species of mudskippers from other geographical regions.

Sample area/country	Fish species	Cu	Zn	Pb	Cd	Ni	Reference
Mai Po Bay, Hong Kong	*Boleophthalmus pectinirostris*	2.08	84.61	1.56	0.012	-	[116]
Coast of Tanzania	*Periophthalmus argentilineatus*	2.90-5.50	148.0-219.0	1.9-20.9	0.2-1.5	1.3-3.1	[117]
Dumai waters, Indonesia	*Periophthalmus sp*	-	-	1.30-2.88	0.01-0.41	4.06-8.95	[118]
Zhejiang Coastal Area, China	*Periophthalmus sericus*	1.31	-	0.064	0.03	19.9	[88]
Intertidal areas, Peninsular Malaysia	*P.schlosseri*	0.8-6.6	16.0-57.8	2.8-14.9	0.4-1.8	-	[119]
Northern coast of Hormuz Strait (Persian Gulf)	*Periophthalmus waltoni*	-	61.94-263.88	2.33-2.50	-	0.35-12.5	[94]
West coast, Peninsular Malaysia	*P. schlosseri*	0.04-18.61	12.79-152.63	0.04-37.71	0.04-4.42	0.04-121.84	Present study

DISCUSSION

The mean concentrations of metals in mudskipper *P. schlosseri* and their standard errors were presented in Table 3. Analysis of variance (ANOVA) followed by Duncan's multiple comparisons analysis showed that significant difference ($P < 0.05$) were observed in tissues metals concentrations in *P. schlosseri* except Cu in gills which shows no significant difference ($P > 0.05$) between the sampling sites.

The tissues of *P. schlosseri* showed different pattern of metals accumulation, but high concentration of Zn and low concentration of Cd were generally observed in the studied tissues.

The highest mean concentration of Cu was recorded in liver at M. Beku. The accumulation of essential heavy metal in the liver is associated with its role in metabolism. Cu is an essential element that is carefully regulated by physiological mechanisms in most organisms [1]. High levels of Cu in hepatic tissues are usually related to a natural binding proteins such as metallothioneins (MT) [33, 34]. It is well known that large amount of metallothionein induction occurs in the liver tissue of fishes. The high levels of Cu in the liver can be ascribed to the binding of Cu to metallothioneins (MT), which serves as a detoxification mechanisms [35, 36] and important role it plays in Cu homeostasis [37].

Non - significant difference ($P > 0.05$) was observed in liver Cu concentration between M. Beku and Bg. Lalang. Liver Cu was higher at these two sampling sites compared to others sites that recorded higher bioavailable Cu, which could be related to the fish size. It was observed that small fish shows higher Cu concentration in liver than big fish. And it is well known that metabolic activity of a young marine animal is normally higher than that of an old individual. Thus, metal accumulation was shown to be higher in younger individuals than the older ones [38, 39]. The mechanisms of neutralization harmful compounds are not developed sufficiently in young organisms. Therefore, larger amounts of toxins can accumulate in their bodies [40]. This result is in agreement with several studies conducted on heavy metals concentrations in different fish species which showed high Cu concentration in liver tissues [8, 41, 42].

The highest concentrations of Zn, Pb, Cd and Ni were found in cartilage. It has been shown that highly mineralized materials like cartilage accumulate, at a varying intensity, both the essential (including Fe, Zn, Cu, Mn) and non-essential (Pb, Cd, Hg) trace elements [43]. Zinc is one of the most important environmental toxicants [44]; yet also perform essential roles in a wide range of biological processes [45]. Zinc is the second most abundant trace element in the body after iron [46]. It functions as a cofactor where many enzymes depends upon it as well as body cells [47]. However, Zinc plays an important part in metabolic processes and the metal is also involved in ossification and acts on cartilage growth especially in young organisms [48, 49].

Lead (Pb) is a persistent heavy metal which has been characterized as a priority hazardous substance [50]. The highest mean concentration of Pb in the present study was observed in cartilage, calcareous tissue has great affinity for Pb, and once bound, Pb is practically immobile. Osteoblasts and chondocytes seem to be important target cells for the toxic effects of Pb [51].

The high concentration of Pb in cartilage is probably due to it higher affinity to osteocalcin than calcium. Increases amount of mineral bound to osteocalcin [52, 53] has shown to interfere with Ca^{2+} signalling in cells by competing for calcium binding sites [54]. Indeed, it has been shown that Pb^{2+} can be carried into cells via Ca^{2+} channels [55, 56]. While the low concentration in muscle and liver could be attributed to metal binding proteins such as metallothioneins which is low in muscle [57] and do not binds Pb in the liver [58].

Cadmium is a non-essential highly toxic and ecotoxic metal [59] with limited biological function [60] and of considerable environmental and occupational concern. It is a common pollutant in surface water and can cause adverse effects on fish and other organisms inhabiting these bodies of water [61, 62]. Cadmium is well known to disturb Ca homeostasis in both fish and mammals [63, 64]. Direct uptake of Cd by fish from the water is mainly in its free ionic form (Cd^{2+}) [65] and the indirect exposure is possible as dietary means when consumer organisms subsequently ingest metals bio accumulated in organisms at a lower trophic level with the potential for effects or bioaccumulation [66]. In fishes, Cd can cause very adverse effects because its blocks sulfhydryl groups in the enzymes and competes for binding sites that are essential for normal enzymes functioning [67]. It competes for binding site with calcium because of their resemblance. Cadmium is believed to share a common transport pathway with Ca^{2+} [64, 68] it seems possible that Cd^{2+} can mimic Ca^{2+} at and in Ca^{2+} channels in order to gain entry into the target cells. Experimental evidence indicates that Cd^{2+} may interact with membrane transporters involved in the uptake of nutritive metals, such as Ca^{2+}, Fe, and Zn, as a means to gain entry into target cells of organs affected adversely by this metal. This uptake has been proposed recently to occur through a mechanism of ionic mimicry [69],

whereby Cd^{2+} mimics the divalent cationic species one or more of these nutritive metals at the binding site of one or more carrier proteins and/or channels that transport these metals. Recently, some studies have shown that when Cd^{2+} enters into the body it interferes with metabolic pathways of some elements including Zn, Cu, Ca, among others and causes significant damage to their biological activities [70].

Nickel is well known contaminant with low rate of bioaccumulation in fish tissues. Nickel is classified as borderline element between hard and soft acid acceptor in chemical reactions with donor atoms. It occurs principally as Ni^{2+} and relatively non-toxic to fresh and marine water fishes but exposure to low level over extended periods may results in a number of toxicological effects. Cartilage recorded the highest mean concentration of Ni. The highest concentration of nickel in cartilage may be attributed to the competitive nature of Ni^{2+} with Ca^{2+} for the same transport mechanism. The metal accumulation in different fish organs depends on their physiological role, behavior and feeding habits, as well as regulatory ability, as reported by Clearwater et al. [71].

In general the accumulation of these metals by the cartilage was due to their calcium mimicking effects which they displaced it from its binding sites. Concentrations of Cd and Ni were found higher in cartilage than sediment, suggesting a higher rate of accumulation of these metals by cartilage, while the lowest concentrations of metals were found in muscle which could be attributed metal binding proteins such as metallothionein which is low in muscle. It's important to mention that accumulation of trace elements in muscle is relatively lower than other tissues due to the fact that muscle does not come into direct contact with the metals as it is totally covered externally by the skin and also it is not an active site for detoxification and therefore transport of trace metals from other tissues to muscle [72]. Metal accumulation in fish are mainly focused on metabolically active tissues and muscle tissues while the metal accumulation in cartilage and its potential use in monitoring programs have been neglected and does not received proper attention. Therefore, at the time of this study no available data on heavy metals concentration in fish cartilage to compare with the present findings.

The results of the present study were comparable to reported metals concentration in the tissues of different fish species from other geographical regions of the world as shown in Table 7. The concentration of Cu in the tissues of giant mudskipper *P. schlosseri* was above the reported concentrations from Pulicat Lake, North of Chennai, Southeast Coast of India [73], Tagrus Estuary, Portugal [74] and Masan Bay, Korea [75], but below the concentrations from Coast of Sfax [76] Coastal Waters of Kapar and Mersing, Malaysia [77], Poompuhar coast, India [78]) and Gulf of Aqaba, Jordan [79]. Compared to the concentrations of Zn reported from Kapar coastal waters, Malaysia [80], Coastal waters of Mersing, Malaysia [77], Iranian Coastline of Caspian Sea [81], East and west coast of Peninsular Malaysia [82], Victoria Harbour, Hong Kong [83], Coastal Lagoon, Mexico [84], Gulf of Oman [85] and Coastal waters of Uruguay [86] the concentration of Zn in the tissues of *P. schlosseri* was low but above South west Malaysia coast [87], Red sea , Egypt [41], Zhejiang coastal area, China [88], Sunderban mangrove, India [89]) and Esmoriz-Paramos coastal Lagoon, Portugal [90]. The concentrations of Pb and Cd in the tissues of the present study were higher than Iranian Coastline of Caspian Sea [81], Kapar coastal waters, Malaysia [80], Pulicat Lake, North of Chennai, Southeast Coast of India [73], Camargue coast, France [91], Southern Bight of the North Sea, England [92] and El-Mex Bay, Egypt [93]. The concentrations of Ni in this study were found higher than all the reported Ni concentrations in Table 7.

Comparison between heavy metal concentrations in giant mudskipper *P. schlosseri* with other species of mudskippers from various geographical regions (Table 8.) revealed higher concentrations of Cu, Zn, Pb, Cd and Ni in *P. schlosseri* than in other species of mudskippers with exception of Zn at Northern coast of Hormuz Strait, Persian Gulf [94]. Concentration of Cu, Zn, Pb and Cd were found higher in the present study than in *P. schlosseri* from intertidal areas at Morib and Remis in Peninsular Malaysia.

The calculated MSI (%) values in most of the studied tissues indicated Zn as the metal with highest value. Zinc is an essential micronutrient in all marine organisms and a cofactor in nearly 300 enzymes; it is therefore not surprising the concentration of this metal was found highest in all the examined tissues. Although fish bio accumulate Pb from seawater in proportion to its concentration in solution, Pb is not very bio available or toxic to marine animals [95], the metal (Pb) was ranked second based on MSI (%) in this study. It was observed that, fish have the ability to accumulate heavy metal in their tissues and organs in different amounts. These differences result from different affinity of metals to fish tissues, different uptake, deposition and excretion rates [96]. The differences in concentration of metals in different parts of an organism could be attributed to the tendency of metals to bind to various molecular groups found within the cells of organisms as well as the degree of exposure to metal as influenced by its metabolic characteristics and position in the food chain [97]. Zinc, Pb and Cd indicated high MSI values in muscle, whereas Cu and Ni showed high MSI value in liver and cartilage respectively. High accumulation of heavy metals in muscles may be due to its strong binding with cysteine residues of metallothionein [98] and the feeding habit of the fish. High

concentration of heavy metals in muscles were reported by different authors; Cd [99], Zn and Pb Abdallah [100], many studies have attributed high metal accumulation in muscle to the feeding habit of the fish.

The tissue selectivity index (TSI) has not shown any unique ranking with regards to the studied tissues except for liver which showed high copper selectivity at all the six sampling sites. The specific tissues in which certain metals can be retained depend on the properties of the element, metabolic turnover and the state of the organism [101]. Furthermore, tissue specific accumulation has been proposed as a key biomarker to assess the effect of the chronic exposure of metals in aquatic organisms [102].

Based on the BSAF values, most of the tissues of *P. schlosseri* accumulated higher Cd and Ni and could be classified as macro concentrators of these metals. Liver record the highest BSAF value for Cu while intestine recorded the highest BSAF for Zn. It is generally accepted that heavy metal uptake occurs mainly from water, food and sediment. Digestive tissue is observed as one of the major routes for uptake of metal in diet or sediment by aquatic organism [103]. It has been suggested that diet is a much more significant contributor for nutritionally essential metals such as Cu and Zn [104].

Many researchers have stated that dietary Zn is the fundamental reason for increased Zn in marine fish [105]. Miller et al. [106] has reported that the higher Cu accumulation in liver of rainbow trout increased as the Cu concentration increased in the diet.

Even though there was no clear evidence about Cu and Zn dietary but according to Bordajandi et al. [107], the diet has remarkable role in the bio concentration process for some metals, mainly for Cu and Zn. Liver is an active organ in fish and high amount of Cu accumulation can possibly be attributed to the involvement of liver in detoxification and removal of toxic substances circulating in the blood stream [108]. Metabolic tissues such as liver, intestine and kidneys that are involved in the process of digestion, accumulate trace metals more than any other tissues [109-111]. The highest BSAF for Pb, Cd and Ni were recorded in cartilage. The variations in heavy metals contents in fish tissues were attributed to affinity of tissues to the metals and variation in heavy metals content in the environment. Other factors, such as sex and size may also influence metal bioaccumulation [112, 113]. Nickel has the highest BSAF values and was therefore considered as the most bio accumulated of all the metals studied. While liver, intestine and cartilage showed greater capacity for metal bioaccumulation than other tissues analyzed in this study. It could be suggested based on BSAF values that, liver and intestine were good accumulators of Cu and Zn respectively while cartilage for Pb, Cd and Ni. The non-essential metals especially Cd and Ni were higher than the essential metals i.e. Cu and Zn in the tissues of *P. schlosseri*, because essential metals like Cu and Zn are carefully regulated by physiological mechanisms in most organisms [101]. On the other hand, the non-essential metals compete for calcium binding sites and they are not regulated by the organisms. Based on the BSAF values, most tissues of *P. schlosseri* accumulated higher Cd and Ni and could be classified as macro concentrators of these metals.

CONCLUSION

The present study was carried out to provide information on the use of giant mudskipper *P.schlosseri* as a biomonitoring agent of heavy metals in the coastal environment. Results obtained from the study indicated high metal accumulation in liver, intestine and cartilage of giant mudskipper *P.schlosseri*. It was concluded that *P.schlosseri*, can accumulate metals in higher concentrations than the quantity in its surrounding environment and it might be considered as a bio monitor of heavy metals pollution in marine coastal environment. However, there is need for further research on heavy metals content in giant mudskipper in order to make them excellent organisms in Eco toxicological research.

Authors' Contributions

Prof. Dr. Ahmad Ismail participated in the design of the research, supervision and intellectual corrections of the manuscript. Tijjani Rufa'i Buhari has participated in design and planning of the experiments, data collection, analyses, interpretation and drafting of manuscript. All authors of this research paper have directly participated in the planning, analysis of the study and have approved the final version submitted.

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Competing interests

The authors declare that they have no competing interests.

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Two-Component Mesh Repair of Medium-Sized Ventral Hernias

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ABSTRACT

In order to reduce the frequency of adhesions which are accompanying hernial repair procedure, various types of combined prostheses are offered. At the same time many researchers are still searching for a more profitable combination of synthetic materials. Experimental studies were performed on 8 rabbits. Animal weight was varying from 2.2 to 3 kg. General endotracheal anesthesia was performed. We developed a two-component prosthesis model consisting of two layers, firs layer (lower) was a polytetrafluoroethylene (PTFE) and was placed in the abdominal cavity and the second (upper) was a prolene layer and located above the muscular aponeurosis of the anterior abdominal wall. The absence of adhesions of the visceral peritoneum after establishing PTFE prosthesis was determined by a macroscopic method. On the 10th day, the formation of a thin capsule covering the lower prosthesis was visually observed. Morphological studies have shown that the use of a two-component prosthesis does not affect the course of the wound regeneration process and the wound healing time, the upper polypropylene film is integrated with the surrounding tissues and neovascularization is observed already by the 14th day.

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Keywords

Ventral hernia, Synthetic PTFE prosthesis, Two-component mesh for ventral hernia repair, Intraperitoneal prosthesis, Intra-abdominal prosthesis adhesions.

INTRODUCTION

The choice of the surgical treatment volume, and an abdominal wall repair type is still a discussable issue in giant hernias. Postoperative ventral hernia (POVH) repair is also a topic of ongoing discussion [1]. Not all types of surgeries using synthetic mesh are able to provide the basic principle of modern hernial surgery – and specially its main principle - absence of tissue tensioning. The use of synthetic prostheses has led to a rather high percentage of wound complications (seroma, fistula of the anterior abdominal wall, suppuration) and the appearance of new complications that had not occurred before in autoplasty: migration of the prosthesis into the abdominal cavity or lumen of hollow organ, the development of intestinal fistula on the basis of arosie intestinal wall of the implant, the formation of cysts in the area of the implant, rejection of the implant, etc. [2, 3].

Intra-abdominal location of the mesh can create serious problems. Depending on the method of plastics and mesh type, and the adhesions formation is observed in 80% of patients [4, 5]. Adhesions of the internal organs with the mesh can cause serious complications such as chronic abdominal pain, intestinal obstruction, strangulation, intestinal fistula [6, 7].

In order to reduce the incidence of adhesions, various combined prostheses were offered, as well as the searching for a combination of synthetic materials with biological, such as the amniotic membrane, xenopericardium. In addition, there are situations where the necessary an anti-adhesive coating synthetic material due to economic constraint and the health system, and the patients themselves are absent.

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Aim of the study was to conduct an experimental observation of the anterior abdominal wall tissue reaction as well as the abdominal cavity organs reaction to implantation of two-component prosthesis in animals.

MATERIAL AND METHODS

This study was conducted at the department of «General Surgery» at the Republican Specialized Surgery Centre named after academician V.V. Vakhidov. In all experiments part with animals we adhered to Interdisciplinary Principles and Guidelines for the use of animals.

Characteristics of the applied prosthesis:

Polypropylene mesh (parieten, prolene, promesh). Polypropylene was introduced into production in 1954. It was synthesized from ethylene with the addition of methyl groups to the molecules. The first experimental work on the use of polypropylene was published by Usher et al. in 1958 [8]. Polypropylene mesh consists of monofilament threads, so they cause a much smaller inflammatory reaction. The risk of infectious complications when using polypropylene is also significantly lower. This is due to the fact that micro-organisms are not colonized on monofilament threads, as in a number of cases it is observed when using knitted and woven synthetic materials.

Polytetrafluoroethylene (e-PTFE) was synthesized in 1938 and was widely used in engineering and medicine, widely called - Teflon. Mesh which made with polytetrafluoroethylene, have both positive and negative properties. On the one hand, they are strong, elastic, practically do not decompose in tissues, do not cause allergic reactions, soft enough to touch, do not cause adhesions and adhesions to internal organs, so they can be used intraperitoneally; Easy to sterilize, because they are since the withstand autoclaving. On the other hand, they have quite large pores (10 μm), which are easy to get micro-organisms (Staphylococcus diameter – 1 micron) and can't get macrophages (diameter of 18-35 microns) and the blood (diameter 15-20 μm). Therefore, phagocytosis within these mesh much more difficult. This leads to the possibility of suppuration and rejection of mesh.

Experimental studies were performed on 8 rabbits weighing 2.2 to 3 kg. Endotracheal anesthesia was used. We have developed a two-component prosthesis model consisting of two types of implants, the lower PTFE (polytetrafluoroethylene) located in the abdominal cavity and the upper (prolen) located above the aponeurosis. Both prostheses are connected along the length by a prolene suture conditionally simulating the "white line" (Figure 1).

After a median laparotomy, the animals were implanted combined prosthesis (Figure 2). The lower leaf of the design was fixed by nodal U-shaped sutures to the rectus abdominal wall muscles. The lateral margins of the prosthesis are set at a depth of up to 2 cm along the entire perimeter of the defect. Thus, the bottom sheet (PTFE) delimits the internal organs of the abdominal cavity from the top sheet. The last (polypropylene) has been used to strengthen the anterior abdominal wall, and located above the aponeurosis with fixation by nodal or continuous sutures without suturing the defect (Figures 3 and 5).

Ethical approval

The review board and ethics committee of Surgery Institution approved the study protocol and experimental study

RESULTS

Dynamic monitoring of the wound process was carried out. Postoperative wounds in experimental animals healed by primary tension. On the 10th, 20th and 30th day, by overdosing anesthetics, the animals were removed from the experiment. The absence of adhesions of the visceral peritoneum with the PTFE prosthesis was determined by a macroscopic method. The loops of the intestine lie freely. On the 10th day, the formation of a thin capsule covering the lower denture was visually observed (Figure 5). The top sheet (polypropylene) of the structure is integrated with a connective tissue. As evidenced by the germination of the cells of the prosthesis (Figure 6) Samples of the tissues of the anterior abdominal wall with the endoprosthesis were subjected to morphological examination.

The study by light microscopy of samples from the area of abdominal wall plasty with an integrated use of PTFE mesh and discovered that the contact structures of PTFE and the grid components they do not prostrate to the changes, indicating their interaction, leading to structural changes (Figure 7) Contact of PTFE with the tissues of the aponeurosis and muscles does not appeal any pathological reactions (Figure 8) Light-optical studies show that in the muscle tissue large cavities are formed, usually round-oval. Sometimes they merge with each other, forming large

fields in which a homogeneous eosinophilic content is determined, which is the remains of the mesh structures exposed to the organic substances used during wiring and coloring of the tissue and sections. In the rounded cavities, the remnants of the filaments from which the link is formed are often determined. At the border of the muscle with the aponeurosis and in the thickness of the aponeurosis itself, the cavities formed by the mesh fragments often have an irregular shape and often merge with each other. In their lumen, a homogeneous weakly eosinophilic substance and individual fragments of the mesh fibers are also determined (Figures 9 and 10).

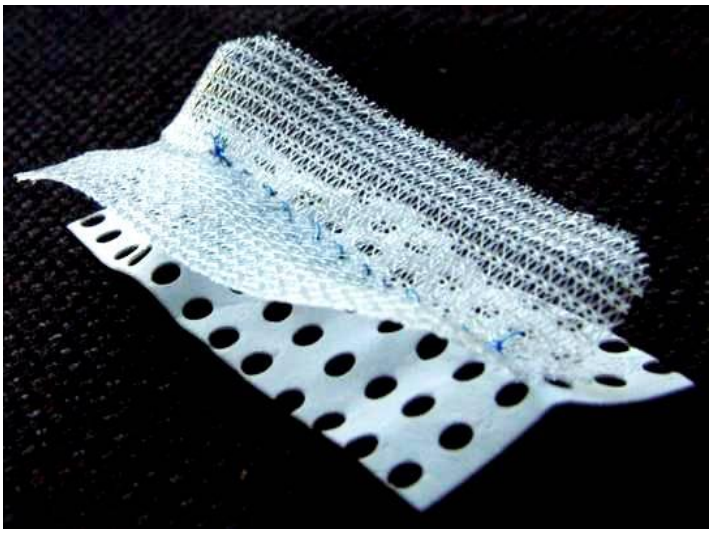

Figure 1. Model (design) two-component prosthesis.

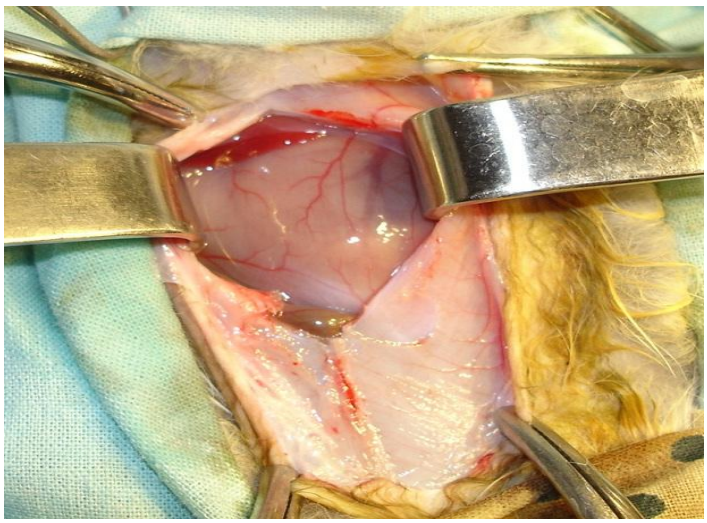

Figure 2. The median laparotomy

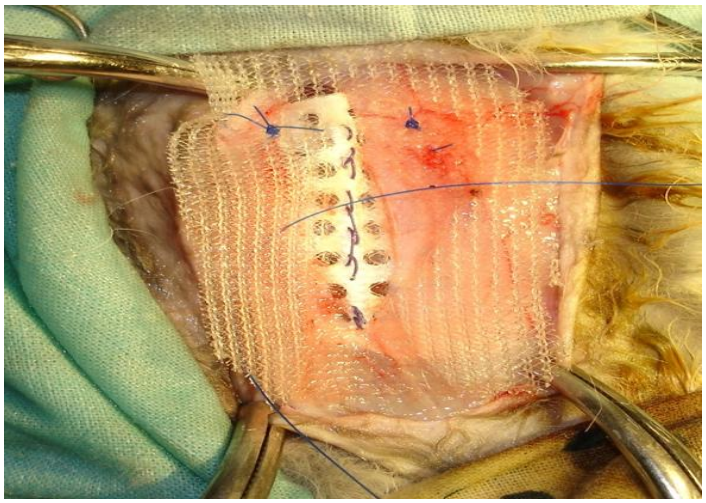

Figure 3. A method for implanting a two-component construction

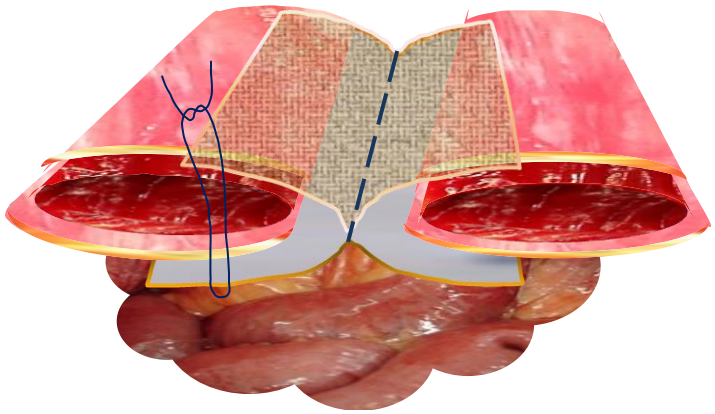

Figure 4. Scheme of fixation of a two-layer prosthesis to the anterior abdominal wall

Figure 5. Encapsulated PTFE at 10th day after surgery

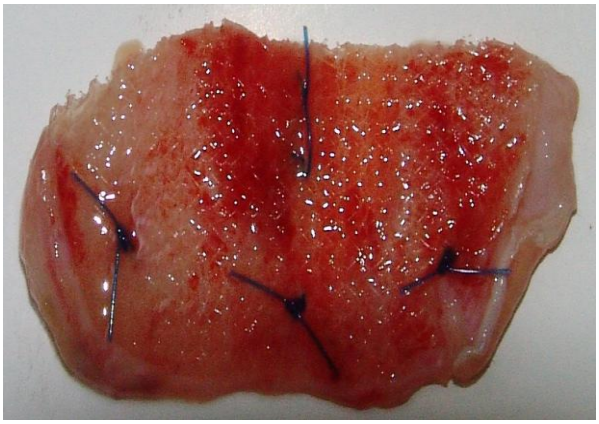

Figure 6. Propylene prosthesis cells, sprouted surrounding tissues

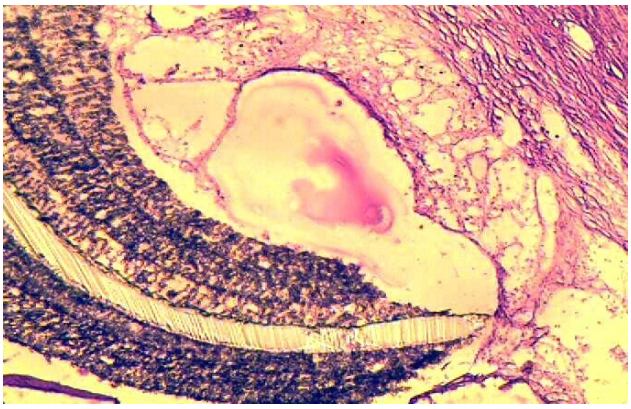

Figure 7. Contact fragments of a prolene mesh and a film. H-E 10x10

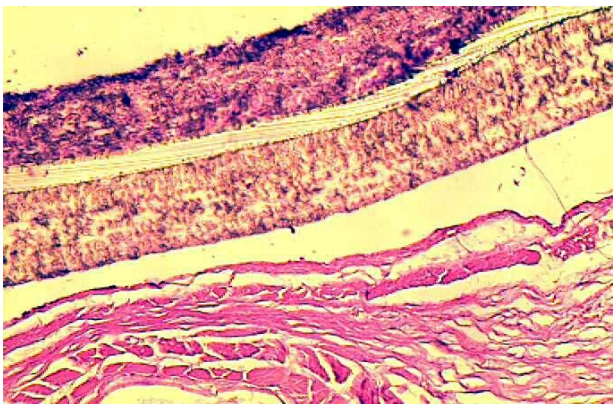

Figure 8. Contact the film with the muscles. H-E10x10.

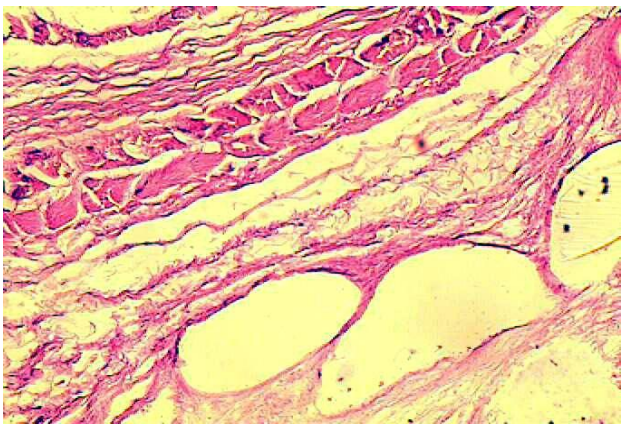

Figure 9. Isolated and fused with each other with remnants of mesh. H-E10x10.

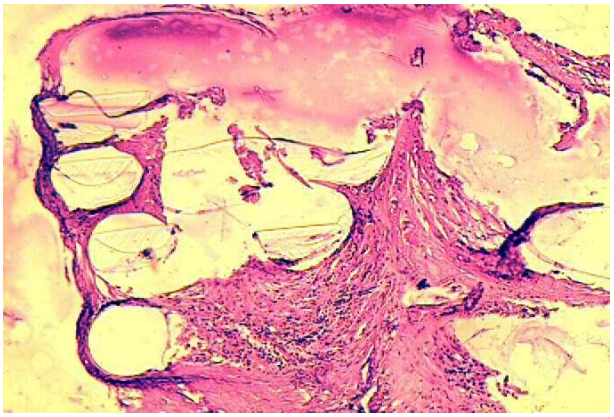

Figure 10. Cavities with traces of the mesh a rounded cavity at the border of the muscle and aponeurosis. H-E10x10.

DISCUSSION

The most commonly used in practice prosthesis is made from polypropylene because of their relative cheapness, availability, almost unchanged with time strength. The resolution of the conference of herniologists, held in Moscow in 2006, was to consider the technique of sublay as a method of choice. It is believed that the plastic sublay method, in the presence of a large omentum, isolating the prosthesis from the internal organs, is most reliable [9, 10].

In a situation where it is impossible to separate the prosthesis, the contact of the polypropylene mesh with the abdominal organs can cause the formation of intraperitoneal adhesion [11]. The polytetrafluoroethylene (PTFE) prosthesis used for sublay plastic has a number of disadvantages: it has low resistance to infection and when the wound infection develops, the prosthesis needs to be removed, the pore size of 10 μm does not ensure through penetration by the connective tissue that covers the prosthesis from the outside and does not provide reliable fixation. Insignificant pore size, high hydrophobicity, negative electric charge prevents cellular ablution, promotes the persistence of bacteria, as they are protected in cells from destruction by macrophages.

Therefore in the experimental work held by Zaikov et al. [12] used combined polypropylene mesh prostheses with biological coatings (amniotic membrane and modified xenopericardium). At the same time, the absence of intra-abdominal adhesion was observed in comparison with the use of a combination of polypropylene mesh with synthetic coatings (polyoxyalkanoate and experimental absorbable polymeric membrane), which was accompanied by the formation of adhesions between the prosthesis and the abdominal organs [13].

The opposite results in the experiment were observed by Sidelnikov and Mikheev [13] using a combined endoprosthesis (amniotic membrane with a polypropylene mesh). A macrophage granuloma was formed around the polypropylene after 3 months and the inflammatory reaction was chronic. The combined endoprosthesis was encapsulated after a month and its fusion with the abdominal cavity organs (the omentum) took place. Where the author concludes that a combined endoprosthesis from an amniotic membrane and a polypropylene mesh for purposes of hernioplasty is unsuitable, since it contributes to a prolonged inflammatory reaction, repair of own tissues is untenable [14].

Biosynthetic mesh [15, 16] have certain advantages compared with synthetic mesh. They are resistant to infection, contribute to remodeling, because they synthesize their own collagen. Animal studies have shown a marked decrease in the adhesion level matrixes, their strength and area, the risk of bacterial infection, and the matrix for angiogenesis and the synthesis of their own collagen [17]. However, recommendations for methods of hernioplasty using these materials have not been developed to date.

A wide distribution for ventral hernias with a hernia gate size> 10 cm has received composite prostheses. Using a combined VentrioTM Hernia Patch (consisting of a resorbable ring (polydioxanone, polytetrafluoroethylene (ePTFE) and polypropylene), 119 patients achieved good results. Complications were observed in the form of: serome in 4.2%, intestinal impossibility in 1.7%, in two patients it was necessary to remove the prosthesis due to its infection [17].

Another type of Composix Kugel Mesh prosthesis is also made up of ePTFE and polypropylene, with a memory ring for convenience and self-alignment of the prosthesis in the abdominal cavity. Greenberg [18] reports the use of this prosthesis in 138 patients with good results. But the literature describes the cases of complications after the implantation of Composix Kugel, such as infection of the prosthesis, the appearance of intestinal fistulas due to the arising defects of the supporting ring and its implementation into the intestinal loops [19].

According to the Food and Drug Administration (FDA) of the United States, from 1996 to 2004 there were 13 intestinal complications [20]. As of January 2007, the number of Composix Kugel Mesh withdrawn products exceeded 100,000 units. Of these, the FDA received 34 reports of a ring break, 21 of which caused serious damage to patients and one resulted in death. Another type of combined mesh is Parietex ® and Proceed® [21]. These nets consist of a nonabsorbable material (polyester, polypropylene), and the lower layer (visceral) in Parietex ® is covered with collagen, and. Proceed® is covered by cellulose oxide. Experimental studies conducted by Winny et al. [22], on the basis of macroscopic and histological results, proved that the use of Parietex ® nets or. Proceed ® does not significantly reduce the development of intestinal adhesions with the prosthesis. In comparison with these data, the use of UltraPro ® in combination with 4DF gel shows a significantly higher ability to prevent adhesion ($p < 0.0001$) [20].

Another pilot study was aimed at comparing the tendency for adhesions of four surgical mesh which available on the market, polypropylene (Marlex®), polyglactin 910 (Vicryl®), polypropylene with poliglecaprone (UltraPro®) and polyester with a collagen layer (Parietex®) Analysis of the results showed that none of the four mesh had antiadherent properties, although polyglactin 910 Mesh (Vicryl®) showed a lower incidence of adhesions [22].

CONCLUSION

Based on the above, it can be concluded that there are no safe prosthetic mesh for intraperitoneal implantation, and the available mesh for hernioplasty lead to more or less intra-abdominal complications.

Thus, the conducted experimental and morphological studies have shown that the use of a two-layer prosthesis does not affect the course of the wound process and the healing time of the wound. Macroscopic studies showed a lack of adhesion of the visceral peritoneum with PTFE mesh in animals. 30 day film is covered with a thin connective tissue film. As seen from microscopy, the upper polypropylene film is integrated with the surrounding tissues and neovascularization is observed by the 14th day. Double prosthetics simulates the white line of the abdomen and does not cause any structural changes and pathological reactions with the tissues of the aponeurosis, muscles and visceral peritoneum.

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Authors' Contributions
All authors contributed equally to this work.

Competing interests
The author declares that they have no competing interests.

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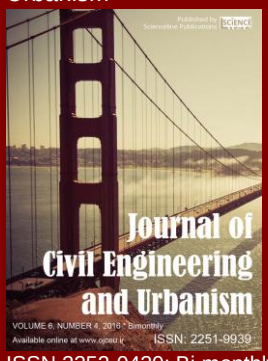

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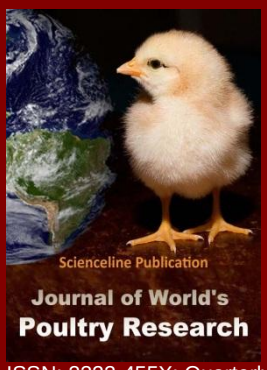

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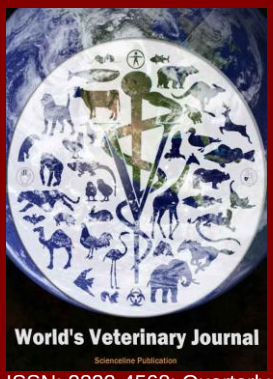

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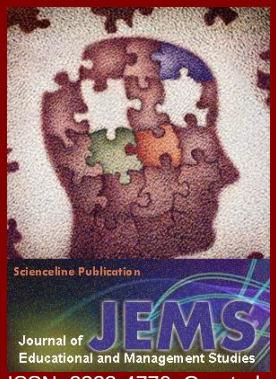

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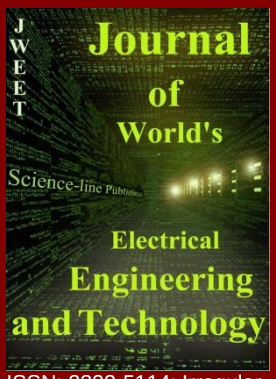

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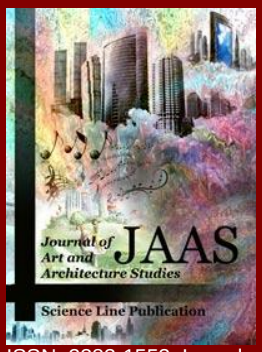

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